Prostatic Intraepithelial Neoplasia: Progression, Regression, a Model for Prostate Cancer?

This paper looks closely at High Grade Prostatic Intraepithelial Neoplasia, HGPIN, and its progression and regression. It has been assumed by many over the past thirty years or more that HGPIN is a precursor for prostate cancer, PCa. However that view is now held somewhat in doubt for a variety of reasons. One is the fact that it is not uncommon for HGPIN to either progress to PCa or regress to totally benign cell structure. We examine here some of the current literature on this topic and attempt to structure a model for HGPIN from the perspective of activated pathways. Clearly HGPIN is morphologically as well as chemically different from benign cells in that the HGPIN cells have changed structure including large nucleoli and expansive intra ductal growth. They also often exhibit PTEN anomalies as well as other pathway anomalies. However they do regress to a fully benign state. The question is therefore, is HGPIN an unambiguous precursor to PCa or is HGPIN and its changes a window into understanding and controlling PCa? In this paper we review what is known concerning HGPIN, much of which is contradictory, and we also attempt to create a somewhat agreed to pathway model for progression and regression.

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PROSTATIC INTRAEPITHELIAL NEOPLASIA: PROGRESSION, REGRESSION, A MODEL FOR PROSTATE CANCER?

Abstract

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Contents

1 Introduction 7
1.1 Key Questions 7
1.2 An Example 8
1.3 Summary of Prostate States 9
1.4 Regression 11
1.4.1 NSAID Regression 11
1.4.2 Androgen Deprivation Therapy Regression 12
1.4.3 mTOR Inhibition 12
1.5 Overview 13
2 HGPIN Characterization 15
2.1 Basic Structure and Function 16
2.2 PIN 18
3 Some HGPIN Models 20
3.1 The Goldstein Model 20
3.2 Other Models 27
4 Cancer and Stem Cells 28
4.1 The Stem Cell Theory 29
4.2 Progression and Regression 32
4.3 Implications of Stem Cells in HGPIN 35
5 Progression and Genetic Drivers 36
5.1 Genes and HGPIN 36
5.1.1 PIA 38
5.1.2 PIN 38
5.2 HGPIN Genes 41
5.2.1 GSTP1 41
5.2.2 CDKNA1B:p27 44
5.3 Other Key Genetic Players 46
5.3.1 MYC 46
5.3.2 PTEN 49
5.3.3 EGFR, ERBB and Neu 51
5.3.4 NKX 3.1 53
5.3.5 NF-κB 54
5.3.6 AR 56

5.3.7 GRP78 61
5.3.8 TPMRSS2-ERG 64
5.3.9 mTOR 70
5.3.10 FOXO 75
6 Epigenetic Factors 79
6.1 miRNA 79
6.1.1 miRNA Production and Action 79
6.1.2 siRNA and miRNA 81
6.1.3 Dynamics of miRNA 81
6.1.4 miRNA and Cancer 82
6.1.5 MiRNA and Stem Cells 85
6.2 Methylation 86
7 Putative Pathways; Activation and Deactivation 91
7.1 Global Cancer Pathway Models 91
7.2 Specific Pathway Elements 93
7.3 Gene Specificity and Frequency 94
8 Enzymatic Reactions and Pathways 98
8.1 Example Reaction 98
8.2 PCa Reaction Models: A Subset 104
8.3 HGPIN and GSTP1 104
8.4 Total Pathways 104
8.5 Specific Pathways 105
8.6 Reaction Kinetics 107
1.1.1 Enzyme Reactions 107
8.6.1 Michaelis- Menten Model 108
8.6.2 Basic Kinetics and Reactions 114
1.1.2 Sigmoidal Reaction 114
8.6.3 Synthesis and Degradation 115
8.6.4 Negative Feedback 115
8.6.5 Activator Inhibitor 116
8.6.6 Substrate Depletion 117
9 Pathway Controls 119
9.1 Cell Growth and Its Metabolic Control 121
9.2 Pathway Dynamics 122

9.3 Pathways, Gene Expression Measurement, Metastasis 124
9.3.1 Dana Farber Study 125
9.3.2 PTEN Suppression 126
9.3.3 Cyclin D 128
9.3.4 SMAD4 129
9.3.5 SPP1 132
9.3.6 Analysis 133
9.3.7 Gene Tables 136
10 Pathway Models 144
10.1 Boolean Networks 144
10.2 Bayesian Networks 147
10.2.1 Bayesian Analysis 148
10.2.2 Microarrays 151
10.2.3 Bayesian Network Summary 154
10.3 State Dynamic Networks 155
11 Identification, Regression and its Drivers 160
11.1 Identification 160
11.1.1 Network Model 160
11.1.2 Measurement Model 162
11.2 Measurements 166
11.3 Inversion Approaches 168
11.3.1 CIE Approach 169
11.3.2 Newton Steepest Descent 170
11.3.3 Kalman Filter 171
11.3.4 The Matched Filter Approach 174
12 Conclusion 178
13 Appendix A Grading 180
13.1 Cancer 180
13.2 Grading 180
13.2.1 Gleason 1 180
13.2.2 Gleason 2 and 3 181
13.2.3 Gleason 4 182
13.2.4 Gleason 5 183
13.2.5 Gleason Summary 184

13.3 Models From Grading 185
14 Appendix B Cell Cycle 187
15 Appendix C: Other Key Pathways 191
15.1 WNT 191
15.2 Hedgehog 192
15.3 Notched 193
16 References 196

1 INTRODUCTION

There has been an extensive amount of literature claiming that high grade prostatic intraepithelial neoplasia, HGPIN, is a precursor to prostate cancer, PCa. The research has gone as far as delineating genetic changes which ultimately lead to metastatic PCa. However, at the same time it is not uncommon for HGPIN to regress and totally disappear. This would seem to counter the theory of genetic change and resulting morphological change of the prostate acini cells.

Moreover there have been many murine models of HGPIN which have been induced with specific genetic changes in specific pathways which lead inexorably to PIN and then to PCa. Likewise there have been many microarray analyses of HGPIN demonstrating the presence or absence, enhancement or deactivation, of the same or similar genes. Yet again there is at time spontaneous remission.

Thus it begs the question; what causes the remission of HGPIN? Is it possibly akin to the remission seen in certain cancers, a remission generated by an immune response effect, as discussed by Rosenberg. Or is it a pathway apoptosis that occurs as a natural course of having aberrant genes?

1.1 KEY QUESTIONS

Let us begin with what we assume is known:

1. HGPIN is driven by underlying progressive and non-changeable changes in the genetic structure of benign cells in the prostate glands.

2. There is a putative association between HGPIN and PCa, reflected in an increased incidence of PCa when HGPIN is present.

3. PCa like most other cancers is characterized by the clonal model, namely one cell becomes aberrant and all other cancers cells are daughter cells of the aberrant clone.

4. PCa is known to result via a set of genetic changes resulting in the cell growth outside of the gland and the creation of malignant glandular structures wherein additional genetic changes occur and result in a less structured morphology and then metastasis.

5. HGPIN regression is seen. This means that the HGPIN cells totally disappear resulting in a purely benign appearance of the prostate glands. It begs the question of; do they cells die or are they attacked and destroyed or is there some reversion mechanism? PIN is a proliferation, so any continuation of cell existence would imply at best a morphological change of say the nucleus and nucleoli but not the total cell count, namely the clustering of many cells in the gland. Thus in regression we do not know what happens or how.

Thus these observations pose the following questions:

1. What causes the disappearance of multiple clusters of HGPIN? Is it apoptosis of some form, an immune response, a genetic switch, or something else?

2. Has there been any extensive studies of HGPIN regression to understand how it arises?

3. If HGPIN regression is based upon some to-be-understood mechanism, can that same mechanism be applied in some form to PCa?

4. Does HGPIN, which is regressionable, have certain cell surface markers which are presentable to the immune system and thus enable enhanced immune responses.

5. Is there a stem cell created when PCa evolves and is PIN lacking in such a stem cell?

The literature demonstrates how to create PIN. There are a few presentations on how to regress PIN[1]. However the nexus of forward PIN progression and backward PIN regression is not complete. We attempt herein to review this in some detail and then to place it in a structure for further analysis and study.

As a natural extension to these questions we can then ask similar ones regarding PCa. How does PCa progress and what are the pathway dynamics related to that progression.

1.2 AN EXAMPLE

Let us begin with a simple example. A 68 year old male is examined due to an increase in PSA from 1.5 to 2.3 in a one year period. The DRE is normal but there is a family history of a first degree relative who died from an aggressive PCa, at 79 years of age. Re-measuring the PSA from two independent sources yields values of 1.8 and 1.9 two months after the raised PSA. A 14 core biopsy is performed and the results are as follows:

A. Prostate, right apex, biopsy: Benign prostatic glands and stroma.
B. Prostate, left apex, biopsy: Prostatic intraepithelial neoplasia, high grade, focal. Glandular hyperplasia of prostate.
C. Prostate, left peripheral zone, biopsy: Prostatic intraepithelial neoplasia, high grade, focal, Glandular hyperplasia of prostate.
D. Prostate, right peripheral zone, biopsy: Benign prostatic glands and stroma.
E. Prostate, transition zone, biopsy: Prostatic intraepithelial neoplasia, high grade, focal Glandular hyperplasia of prostate.

After an eight month period PSA was measured again and this time it was 2.0. A second biopsy was performed using 16 cores. The results are:

A. Prostate, right apex, needle core biopsy: Benign prostatic tissue with very focal and mild acute inflammation.

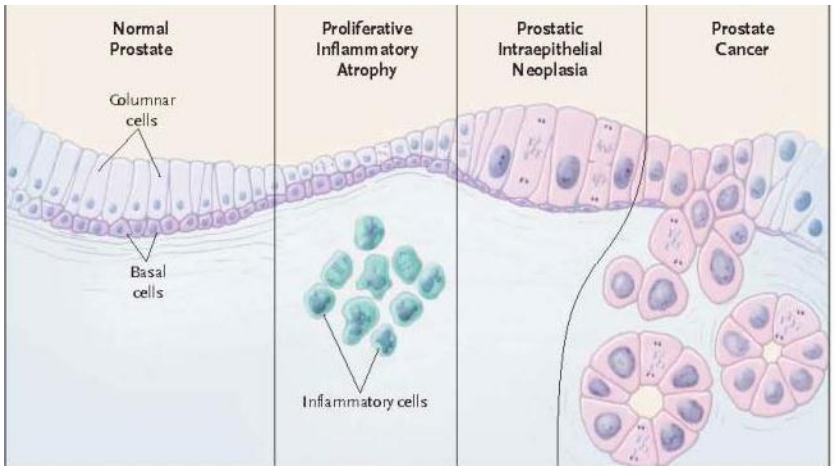
[1] Narayanan et al using NSAID.

B. Prostate, left apex, needle core biopsy: Benign prostatic tissue.
C. Prostate, right mid, needle core biopsy: Benign prostatic tissue.
D. Prostate, left mid, needle core biopsy: Benign prostatic tissue.
E. Prostate, right base, needle core biopsy: Benign prostatic tissue.
F. Prostate, left base, needle core biopsy: Benign prostatic tissue.
G. Prostate, transition zone, needle core biopsy: Benign prostatic tissue.

This is a clear case of total HGPIN regression. The question then is, how common is this and what is its cause, and if regression can be obtained how might it be achieved clinically?

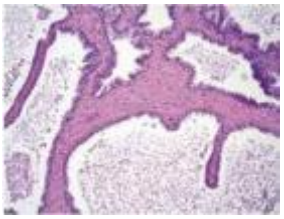
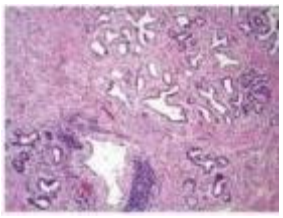
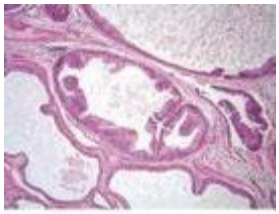
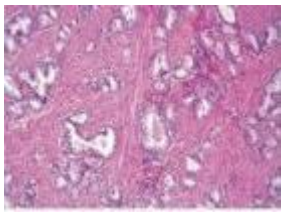
1.3 SUMMARY OF PROSTATE STATES

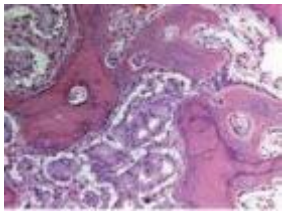
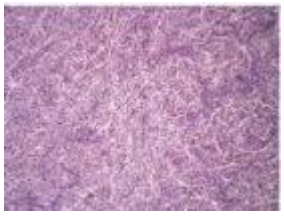
There is a general agreement, with of course many exceptions, as to the progression of prostate pathology and its related causes. A graphic from a recent NEJM article is shown below:



Not the progression from normal prostate with basal and luminal cells and then through PIA and then PIN and finally PCa. The PIN demonstrates a complex but contained development of cells. As one moves o PCa, that is when the cells move away from the existing gland, and they are for the most part luminal cells establishing de novo glandular like structures.

An excellent tabular summary from Taichman et al follows:

Disease State	*Histology*	*Details*
Normal Prostate		Large glands with papillary infoldings that are lined with a 2-cell layer consisting of basal and columnary secretory epithelial cells (luminal) with pale cytoplasm and uniform nuclei. Susceptibility genes or events related to hereditary PCa: RNASEL: regulates cell proliferation through the interferon regulated 2-5 oligoadenylate pathway ELAC2/HPC2: Loss of function of tRNA-3 processing endoribonuclease MSR1: Macrophage scavenger receptors process negatively charged macromolecules.
PIA		Atrophic glands have scant cytoplasm, hyperchromic nuclei and occasional nucleoli and are associated with inflammation Susceptibility genes or events: NKX3: Allelic loss of homeobox protein allowing growth of prostate epithelial cells PTEN: Allelic loss of phosphatase and tensin homolog allowing decreased apoptosis and increased cell proliferation. CDKN1B: Allelic loss of cyclin dependent kinase inhibitor p27 allowing increased cell proliferation
PIN		Intermediate to large size glands with proliferation changes contained within the gland and having nuclear abnormalities that resemble invasive carcinoma. Susceptibility genes or events: GSTP1: Hypermethylation of the upstream regulatory region inactivates the Pi class gluthionine S transferase enzyme which detoxifies carcinogens. Hepsin: Increased expression of this serine protease leads to increased invasiveness and disruption of the basement membrane. AMACR: Increased expression results in increased peroxisomal b-oxidation of branched chain fatty acids from red meat thereby increasing carcinogen exposure. TMPRSS2: Fusion of this androgen regulated gene with ETS family of transcription factors in late stages of PIN results in in increased breakdown of the extracellular matrix. Telomerase: Activation leads to maintenance of telomere length and immortalization of cells.
Prostate Cancer		Small irregular glands with cells having abnormal nuclei and nucleoli and lacking basal cells. Susceptibility genes or events: MYC: Overexpression leads to cell proliferation and transformation RB: Loss of expression leads to cell proliferation and transformation

Disease State	*Histology*	*Details*
Metastatic PCa		Nests of cancer cells within the bone Susceptibility genes or events: TP53: Mutation results in loss of multiple tumor suppressor functions E-cadherin: Aberrant expression leads to increased invasive and metastatic phenotype NM23: loss of this NDP kinase leads to increased metastasis EZH2: Histone methyltransferase PcG protein whose activation causes repression of genes that suppress invasion and metastasis
AR PCa		Cancer cells that grow in androgen depleted environment Susceptibility genes or events: AR: may remain active through amplification, phosphorylation by other steroids or non-androgen growth factors BCL2 Increased expression leads to protection from apoptosis Stem cells: potential repopulation by progenitor cells

Note in the above, Taichman et al make mention of the separate gene elements that are putatively assumed to have caused the subsequent event. These genetic changes then will become a key factor in how we view PIN transitions.

Also note in the above, it implies a set of sequences of genetic changes that moves from benign to malignant. The question then is; if a genetic change is necessary for a morphological change, then is the genetic change reversible or are the genetically changed cells killed off by some other process, and if so what process?

To understand this question, and hopefully set a path to answering it, we lay out the known elements in the path towards malignancy, look at the gene maps and dynamics, and then attempt to establish a model for examining the dynamic processes which move the cell forward to malignancy or backwards towards a benign state.

1.4 REGRESSION

There has been some discussion of regression in the literature. We examine briefly three possible means here. However, there does not seem to have been any detailed clinical study or models, murine or otherwise, which have been used to ascertain the details surround the regression issue. As we have seen above the current general understanding is that HGPIN is a clear and unambiguous predecessor of HGPIN, albeit regression is evident.

1.4.1 NSAID Regression

An interesting paper by Narayanan et al describes their work using NSAIDs as a means to reduce and in some cases eliminate PIN. They used specifically celecoxib and exisulind as the NSAID and they demonstrated that the use of these drugs did reduce PIN lesions. Now exactly why this happened one cannot determine. The authors present the factual results without any further

interpretation. In addition there would not seem to be any rational explanation based upon the above overviews.

1.4.2 Androgen Deprivation Therapy Regression

In the paper by Kang et al they indicate that ADT, androgen deprivation therapy does reduce PIN[2]. They state:

Our results demonstrate that ADT does cause PIN regression, and that there is heterogeneity in this effect with respect to hormonal duration. We propose for future prospective, multi-centered, randomized trials in which ADT impact on PIN is characterized further….However PIN response to ADT was not uniform as 16% of patients with ADT longer than 6 months had residual PIN, suggesting variable sensitivity of PIN to ADT.

Kang et al also noted in another paper:

Eighteen patients initially diagnosed with PIN who had no ADT were identified, and 28 with PIN who had ADT were also assessed. All patients who had had no ADT had residual PIN, whereas seven of 28 receiving ADT had no residual PIN (P=0.043). The evaluation of ADT between responders and nonresponders showed a statistically significant association between PIN regression and the duration of ADT (P<0.001). However, the response of PIN to ADT was not uniform, as 16% of patients on ADT for >6 months had residual PIN, suggesting variable sensitivity of PIN to ADT.

1.4.3 mTOR Inhibition

The mTOR gene can be activated by the Akt gene which in turn can be activated by the suppression of the PTEN gene. This is but a small segment of a pathway. mTOR then

Thus there seems to be an ability to eliminate PIN via ADT. In this case there is some clear pathway dependence. mTOR is short for "mammalian target for rapamycin"[3]. mTOR when positively enhanced by activation can result in cell growth by the up-regulation of protein synthesis. Akt regulates mTOR via the negative regulation of an intermediate pathway element the gene product TSC2 which inhibits mTOR.

By inhibiting TSC2 the inhibition of mTOR is reduced and in fact mTOR expression and actions are increased. It is this change which Majumder et al used to create PIN.

Majumder et al state that they were able to revers PIN in murine models by managing mTOR pathways. The use of rapamycin was e reasonable approach for pathway control. Akt induced PIN was totally controlled by mTOR and reversal allowed regression of the PIN.

[2] http://meeting.ascopubs.org/cgi/content/abstract/24/18_suppl/4648

[3] Bunz, pp 192-194.

The above three are a few of the known mechanisms related to regression. There may be many others yet to be determined but the existence of these may assist in understanding the possible options.

1.5 OVERVIEW

Let us now provide a simple overview of the development of models. We develop it in the following manner:

First, we look at the histological structure of PIN and PCa. Cell changes occur and the changes morphologically are dependent upon the expression of or lack thereof of certain genes. The linking of morphology and gene expressions seems to fall short at this stage. Thus the nexus is missing.

Second, we look at some simple models for the development of HGPIN. As we have stated, the reason for this is twofold. First HGPIN is often assumed to be a natural precursor of PCa and as such one can assume that genetic changes necessary for PCa are first seen in HGPIN. Second we know that HGPIN can suddenly regress and the cells revert to benign state. If that is the case and indeed it is one may ask if the genetic changes were the cause also of the regression or was there some exogenous cause. We focus primarily on the Goldstein et al model because it demonstrates both HGPIN and PCa and the relationship to morphological and genetic changes.

Third, we examine the cancer stem cell, CSC, model. The CSC is an interesting paradigm which may explain the less than rapid growth of certain cancers. PCa may be dominated in many cases by indolent slow reproducing CSC. Understanding the dynamics of the CSC is therefore essential.

Fourth, we look at the many specific genetic drivers such as PTEN and the other first and second order products in the pathway chain. This is an extensive discussion which we will rely upon to build pathway models.

Fifth, we examine the epigenetic factors such as miRNA and methylation. These may be the most significant factors in cell change and genetic expression alteration that we see in PCa progression.

Sixth, we present and examine in some high level detail the many complex pathway models currently presented.

Seventh, we examine the various models for reaction kinetics. This will be essential when we attempt to model the dynamics. The classic approaches are significant and their simplifications are useful. By looking at linear models we often can find reasonable insight but it is often by examining the nonlinear models that we can ascertain the tipping points with more clarity.

Eighth, we examine pathway controls, that is what components such as PTEN play the most significant role.

Ninth, we look at the three dominant modeling techniques; Boolean, Bayesian, and System model using reaction rates and complex time varying differential equations. We do not in this analysis examine the spatial models (as initially developed by Turing and detailed by Murray).

Tenth, we examine how the constants in these models may be obtained by means of system identification methods. We have accomplished this in other pathway systems and we believe it is directly applicable here as well.

2 HGPIN CHARACTERIZATION

HGPIN is represented by morphological changes in prostate cells in the acinar or glandular locations. It generally is a complex set of growth patterns of new cells whose morphological appearance is similar to but not identical to the existing cells in the gland. The new cells clearly have form and shape that demonstrates pre-malignant morphology, with enlarge and prominent nucleoli.

From the paper by Putzi and DeMarzo we have:

T*he high-grade form of prostatic intraepithelial neoplasia (PIN) has been postulated to be the precursor to peripheral zone carcinoma of the prostate. This is based on zonal co-localization, morphologic transitions, and phenotypic and molecular genetic similarities between high-grade PIN and carcinoma. Although high-grade PIN is thought to arise from low-grade PIN, which in turn is thought to arise in normal or "active" epithelium, little is known whether truly normal epithelium gives rise to PIN or whether some other lesion may be involved.*

Focal atrophy of the prostate, which includes both simple atrophy and postatrophic hyperplasia, is often associated with chronic, and less frequently, acute inflammation. Unlike the type of prostatic atrophy associated with androgen withdrawal/ blockade (hormonal atrophy), epithelial cells in simple atrophy/postatrophic hyperplasia have a low frequency of apoptosis and are highly proliferative. In addition, hormonal atrophy occurs diffusely throughout the gland and is not usually associated with inflammation.

To simplify terminology and to account for the frequent association with inflammation and a high proliferative index in focal atrophy of the prostate, we introduced the term "proliferative inflammatory atrophy" (PIA).

In a similar manner in a review paper by O'Shaughnessy et al on multiple intraepithelial neoplasia the authors state the following regarding HGPIN:

The evidence that PIN is a morphological and genetic precursor to prostate cancer is extensive and conclusive ...

When examined microscopically, PIN lesions are characterized by collections of proliferative prostatic epithelial cells confined within prostatic ducts that exhibit many morphological features of prostate cancer cells, including architectural disorganization, enlarged cell nuclei and nucleoli. ...

In addition to the similarity of the cellular morphologies of HGPIN and invasive lesions, evidence that HGPIN is a precursor of prostatic adenocarcinoma includes the multifocality of both lesions and the presence of carcinoma in foci of PIN; among older men, foci of PIN are found in 82% of prostates with carcinoma but in only 43% of normal prostates.

PIN is frequently located in the peripheral zone of the prostate, the site at which 70% of prostatic carcinomas occur. Additional similarities include enhanced proliferative activity of both PIN and carcinoma (3-fold that of benign tissue), cytokeratin immunoreactivity, lectin binding, and loss of blood group antigen with both PIN and carcinoma.

Prevalence of PIN and its temporal association with invasive cancer are illustrated by the known 40–50% PIN incidence in men 40–60 years of age, evolving into the 40–50% incidence of prostate cancer in men 80 years of age. Autopsy data reveal that PIN lesions appear in the prostates of men in their 20s and 30s in the United States, preceding the appearance of prostate cancer lesions by as many as 10 years …

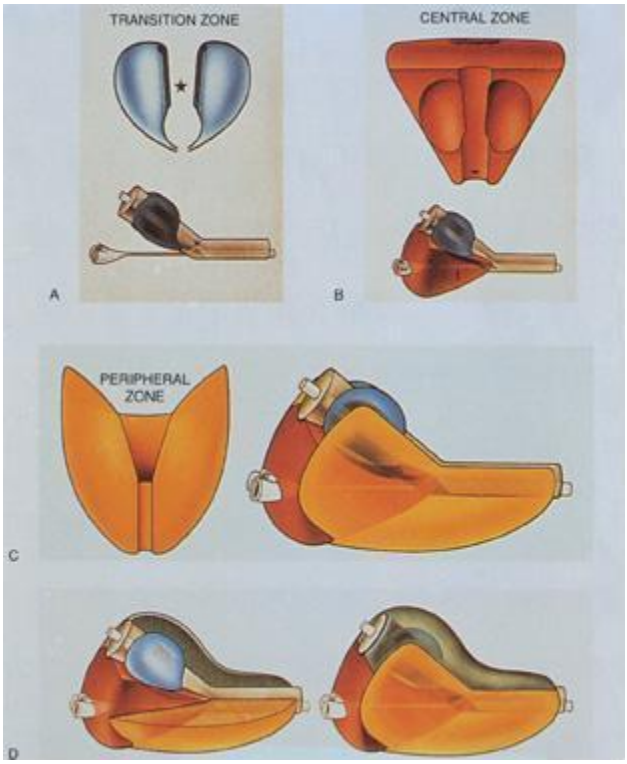
African-American men, who are at higher risk of prostate cancer mortality, appear to have a greater extent of PIN at any given age. PIN and prostate cancer lesions share a number of somatic genome abnormalities, including loss of DNA sequences at 8p and increased GSTP1 CpG island DNA methylation, among others.

Finally, transgenic mouse strains prone to developing prostate cancers typically develop PIN lesions in advance of the appearance of invasive cancer. PIN lesions are always asymptomatic and cannot currently be diagnosed or detected by any reliable means other than examination of prostate tissue histologically. In autopsy studies, the incidence and extent of PIN increases with age, as does the incidence of prostate cancer.

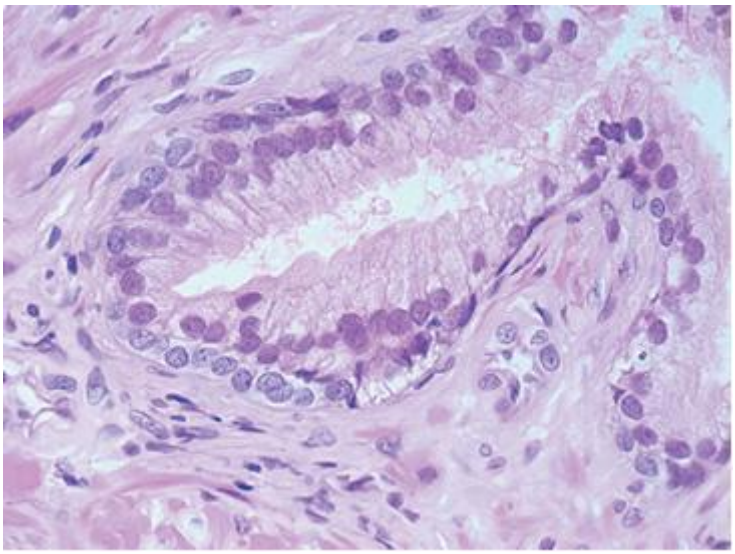
Notwithstanding the correlation, there does not seem to be causality. In addition, the authors do indicate that HGPIN can be reduced but they seem to fail to speak to the issue of total remission without any treatment. The question is therefore, is PIN a precursor of PCa? If it is or is not, is PIN the result of a genetic change as has been postulated by many? It would seem clear that the existence of remission of PIN would imply that it is not at all necessarily a precursor and furthermore that it is not necessarily a genetic change for all PIN. That is can there be a morphological PIN that is genetic and not remissionable and one which is remissionable. Remissionable implies the existence of apoptosis, that is a natural cell death or perhaps a cell death due to some immune response.

2.1 BASIC STRUCTURE AND FUNCTION

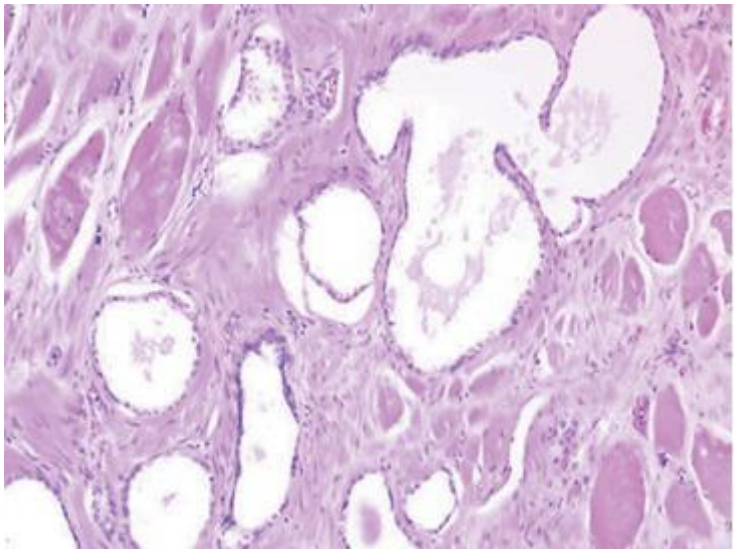
The basic structure of the prostate is shown below. It consists of three major zones; peripheral (dominant zone), central zone which is around the urethra), and the transition zone.



The cellular structure is depicted below. There are approximately 35-50 glands in the prostate, mostly in the peripheral zone and the glands have a lumen in which the prostatic secretions flow, and the glands have basal cells and luminal cells as shown below. The basal cells are dark and the luminal cells are somewhat lighter. Between the cells is the stroma which includes the blood flow from veins and arteries, the muscle and other stroma elements. Simply stated, the prostate is a collection of the basal/luminal glands scattered about veins, arteries, muscles and nerves.

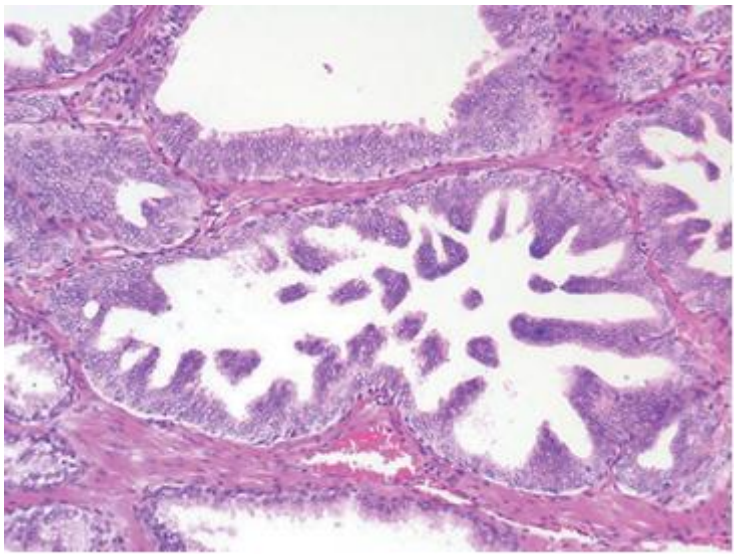


The figure below depicts a second view of the prostate glands. Again this is with HE stain and under low magnification. The basal cells are clearly see with their dark stains and the luminal stand above them. The stroma is fairly well articulated in this slide.



2.2 PIN

Prostatic Intraepithelial Neoplasia, PIN, is a growth within the normal glands of more cells than should normally be there. The slide below depicts high grade PIN, HGPIN. Note the PIN in the center shows significant cell growth in the existing gland as compared to the gland at the bottom which shows normal thinner growth.



The PIN shows papillae which are shooting out within the gland and there is also significant basophillic staining of the papilla cells whereas the normal gland has limited staining of the luminal cells.

The key question is one of whether PIN is a precursor to PCa. Many articles state that it is but when one looks at the data there is still a significant area of doubt.

3 SOME HGPIN MODELS

There has been an extensive amount of work in trying to create HGPIN from normal prostate cells. There are questions as to what cells the HGPIN derives from, for example basal or luminal, and then there are questions as to what genetic changes result in PIN. As with so many parts of the puzzle there are no single set of answers. We start with the recent Goldstein model and use it as a basis. Then we look at other models and specific genes expressed. We defer until later the issue of pathways.

3.1 THE GOLDSTEIN MODEL

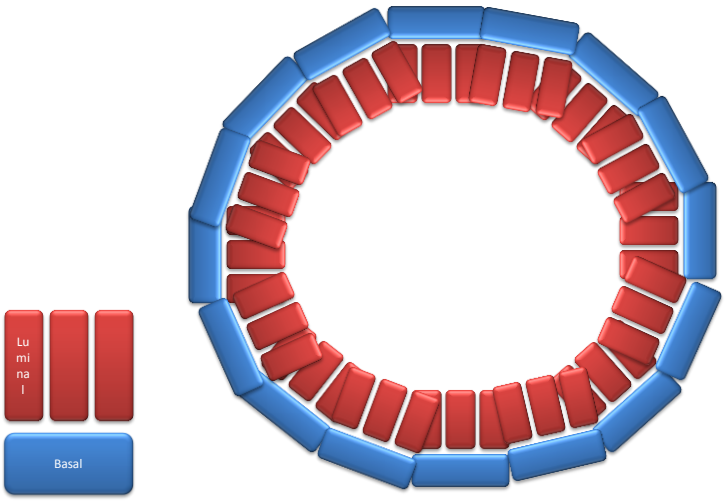
A novel set of experiments on prostate cancer were based on the work by Goldstein et al at UCLA. Understanding this work is useful in understanding both HGPIN and PCa. Goldstein et al demonstrate that one set of elements in the intracellular pathways if disturbed in a certain manner can result in morphological changes that first become HGPIN and then mode to PCa. The essential usefulness of this work is that it allows for a demonstrable relationship first between genetic change and histological change and second that changes in pathway elements lead to progression.

Simply what they did was to take two types of prostate cells, the basal and the luminal, tag them with surface tags, inject them into a mouse, and saw that only the basal cells grew, then they added two genes encoding for putative cancer pathways, and they saw that the basal cells grew to basal and luminal, like PIN, and then finally they added an AR, androgen receptor gene, and voila, prostate cancer. Result, showing how a specific pathway can generate cancer.

Let us go back and look at this a bit more.

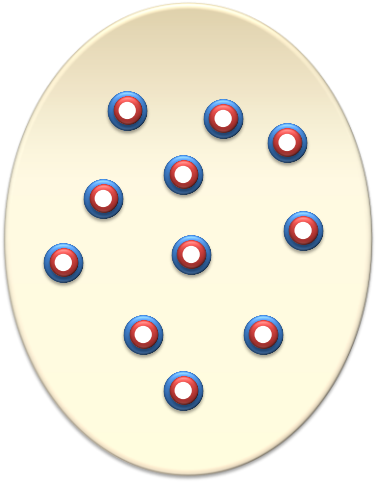
1. First the prostate has cell collections which act as glands with basal cells at the base and luminal cells on top. The luminal cells secret to the gland, the luminal space. This we show below.

Normal Prostate Gland

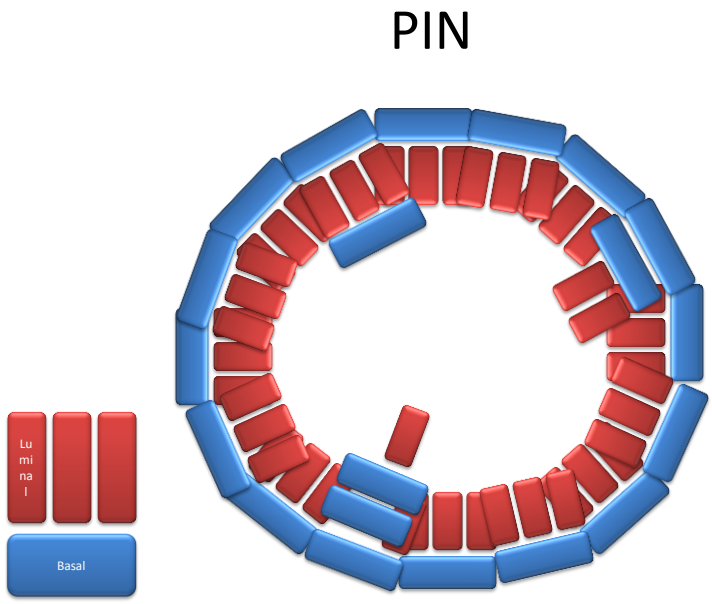


2. The normal prostate looks like what we show below, about 35-50 of these glands, and then surrounding material of muscle, blood supply, nerves, and lymphatics. The glands stand apart and they secret fluids into the lumen, the open parts of the gland. In between is the stroma composed of nerves, blood vessels and other connective tissues.

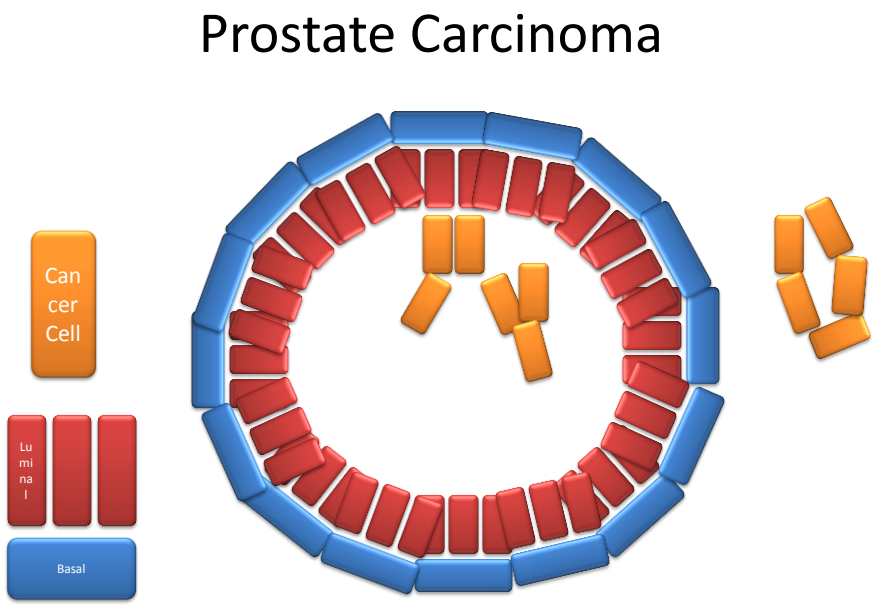
Normal Prostate



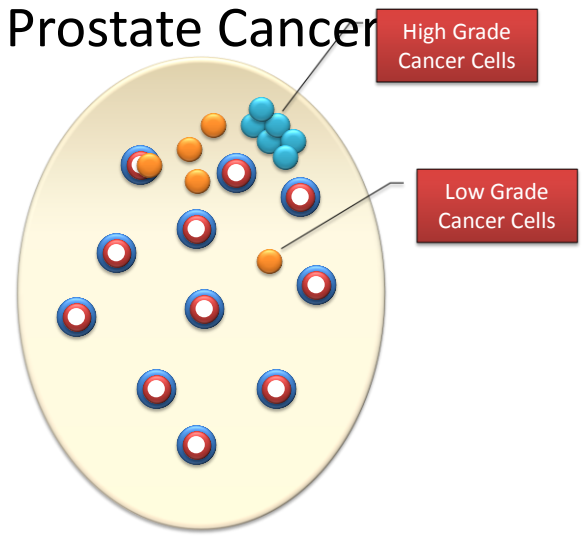
3. Now sometimes we see PIN, prostatic intraepithelial neoplasia, which is a growth of normal cells but not where they are to be. We may see the basal cells growing outwards and even some more luminal cells as well. The sign may be an increase in PSA since we have more luminal cells but the percent free PSA may stay high since the luminal cells are health ones. We show this below:



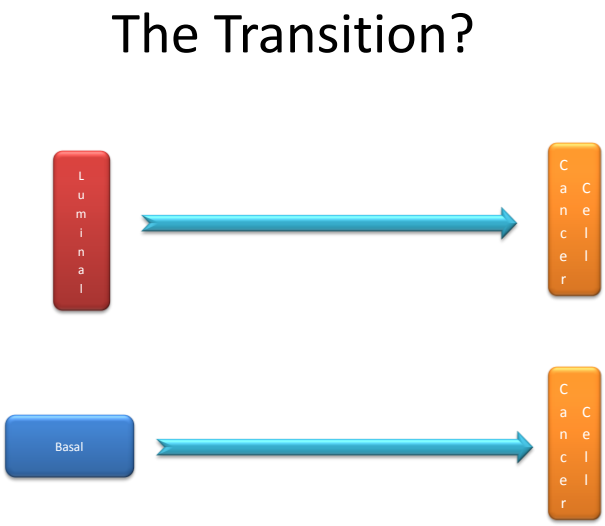
4. Then we may get prostate cancer, PCa, where the luminal cells types start to appear and grow without bound. The question is, where did these cells come from, other luminal cells or basal cells, or what. This is the question that the authors addressed with this elegant experiment. There is also the key question of whether it is just one cell that starts it or if the changed basal cells grow and if the environment switches many on over time. The latter effect is similar to that which has been observed in melanoma. Below we show what happens next,



Looking at the prostate as a whole we then may see what appears below. Namely we may see low grade cancer cells and then clusters of high grade cancer cells, this leads to the Gleason grading system.



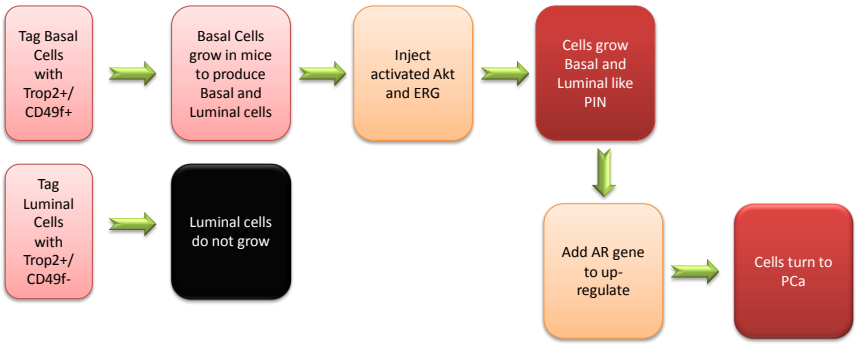
5. Thus the question posed by the authors was the one which asks from what cell does cancer begin? Their answer suggests the basal cell.



6. Pathways have been studied for PCa extensively and we shall discuss them in some detail.

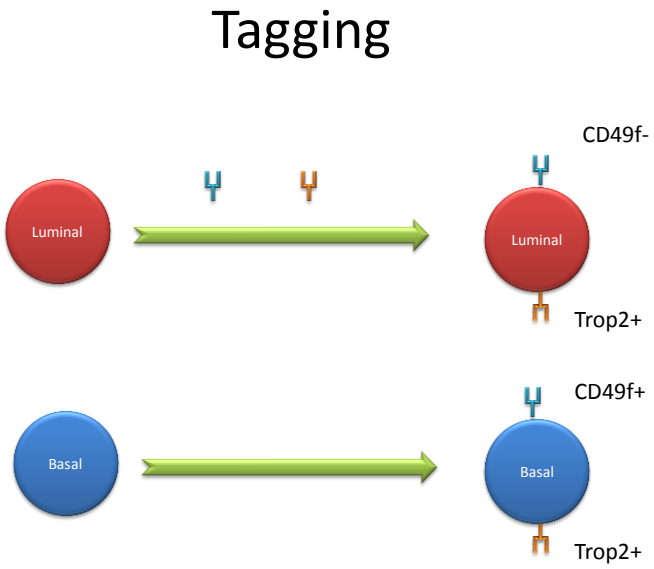
But the authors took a simple approach and looked at three genes in the putative pathway process. This is shown below:

Goldstein Process



First they showed that only basal cell proliferate into both basal and luminal. Then they added ERG and Akt genes known as key in the pathways, and they obtained PIN, and then they added AR, the androgen receptor to drive the previous two genes and the result was PCa.

They were able to keep track of basal and luminal cells by tagging them with cell surface markers, as shown below. Basal was positive for both and luminal positive for one and negative for another, a good example of tracking the cells as the transform.



As to the two initial genes we have:

(i) Akt: There are in humans three genes in the "Akt family": Akt1, Akt2, and Akt3. These genes code for enzymes that are members of the serine/threonine-specific protein kinase family. Akt1 is involved in cellular survival pathways, by inhibiting apoptotic processes. Akt1 is also able to induce protein synthesis pathways, and is therefore a key signaling protein in the cellular pathways that lead to skeletal muscle hypertrophy, and general tissue growth. Since it can block apoptosis, and thereby promote cell survival, Akt1 has been implicated as a major factor in many types of cancer.

(ii) ERK: Extracellular signal regulated kinases, ERK, are protein kinase signaling molecules involved in the regulation of meiosis, mitosis, and postmitotic functions in cells.

This study still leaves several open questions:

1. Is the clonal theory of cancer still standing or can a single cell transform and then induce other cells via chemical signaling.

2. Is the basal cell the only one. There appears to be some issues here and the review article looks at these.

3. Is PIN an artifact or a precursor. Clinically men with PIN have a slightly higher risk of PCa but not a substantially higher as would be argued in this model. In fact men with PCa do not always have PIN and men with PIN do not always get PCa.

4. Is this just an artifact pathway, the true pathway, one of many pathways.

5. If we can duplicate pathways can we than better control the disease.

6. What does this tell us about detection and staging.

3.2 OTHER MODELS

The Goldstein et al model is but one of several which have taken this approach. There are others and the results are not always consistent. Two of them are discussed as follows:

1. Yen et al (2003) have reported on a murine model which demonstrated that by implanting c-Myc genes into a mouse that it resulted in murine PIN and then shortly thereafter PCa. Yen et al also shown loss of NKX3.1, a tumor suppressor gene, which is putatively involved in PCa as well as PIN. NKX3.1 is a 8p21 gene whose function is to generate the Homeobox protein[4]. It is known to be suppressed in familiar prostate cancer and in the case of Yen it is reduced in its expression as well.

2. Lawton and Witte discuss the generation of PIN by means of lentivirus infection via an siRNA which is a knock out for PTEN.

[4] Pecorino, Cancer, p 114.

4 CANCER AND STEM CELLS

As Weinberg notes, there is the theory of clonal development of cancer which states that the cancer cells are pluripotent and have developed from a single source and that they have the capability of reproducing and do so in an autonomous manner[5]. Then there is the theory of the cancer stem cell, the theory which states that there is the equivalent of a stem cell as we know in blood cells, which have the capability but that the majority of malignant cells do not necessarily have that capacity.

The NCI presents an excellent summary of Cancer stem cell, CSC, research[6]:

The theory of the cancer stem cell (CSC) has generated as much excitement and optimism as perhaps any area of cancer research over the last decade. Biologically, the theory goes, these cells are distinct from the other cells that form the bulk of a tumor in that they can self-perpetuate and produce progenitor cells, the way that traditional stem cells do. The progenitors' job is then to repopulate tumor cells eradicated by treatments such as chemotherapy or radiation.

But for all the attention and fanfare CSC research has received, the findings reported to date are far from clear-cut, investigators acknowledge. For example, most of the studies that have identified human CSCs have used mouse xenograft assays and cells from only a small number of human tumor samples, making it difficult to draw firm conclusions. In addition, other researchers haven't always been able to replicate initially reported findings. And while these tumor-initiating cells, as they are also called, have been described as being a rare class, several studies have found that the number of cells that can form tumors in these mouse experiments is actually quite large, suggesting that perhaps CSCs aren't such a privileged breed.

In other words, the idea of just what cancer stem cells are, and their role in different cancers, appears to be changing.

"The [stem cell] model has not been adequately tested in most cancers," said Dr. Sean Morrison, who directs the Center for Stem Cell Biology at the University of Michigan. "I think that there are some cancers that do clearly follow a cancer stem cell model…But it will be more complicated than what's been presented so far."

They continue:

Unlike the random or "stochastic" model dominant in cancer research, which holds that nearly any cancer cell has the potential to form a tumor, the cancer stem cell model is one of a hierarchical organization, with the pluripotent cancer stem cell sitting ready and able to amass all of the components of the original tumor.

[5] Weinberg, Cancer, pp 416-417.

[6] http://www.cancer.gov/ncicancerbulletin/072710/page4

It's also thought, with some experimental evidence to support it, that CSC pluripotency allows these cells to adapt and to resist chemotherapy, radiation therapy, and even current molecularly targeted therapies. If true, then these treatments may not harm the most lethal tumor cells, those that can lead to a recurrence with the production of a new set of progenitors.

Despite numerous studies published in the last 16 years that identified CSCs for different cancers—including colon, brain, pancreatic, and breast cancer—the consensus among researchers seems to be that the evidence is strongest for the first cancer in which a population of tumor-initiating cells was discovered, acute myeloid leukemia (AML), as well as for other blood cancers.

The existence of CSCs in PCa has been examined and as with many cancers is still open for discussion. However as we shall discuss later the CSC model does have certain interesting uses in the progression and metastasis of cancer. For example:

Cell Proliferation: If we assume that the CSC is the dominant cell that proliferates and all others do not, albeit being cancer cells themselves, then the growth of PCa in terms of cells is complex but one can then more easily explain indolent PCa.

Metastasis: We know that metastasis occurred by lymphatic and hematological means. However PCa cells, non-CSC PCa cells may break loose and yet not result in classic metastasis. The issue then is one where it may be necessary for the CSC to move by these means.

Many other such issues will arise and we discuss the CSC idea here and we return to it later in the work.

4.1 THE STEM CELL THEORY

In an NIH report they define cancer stem cells as follows:

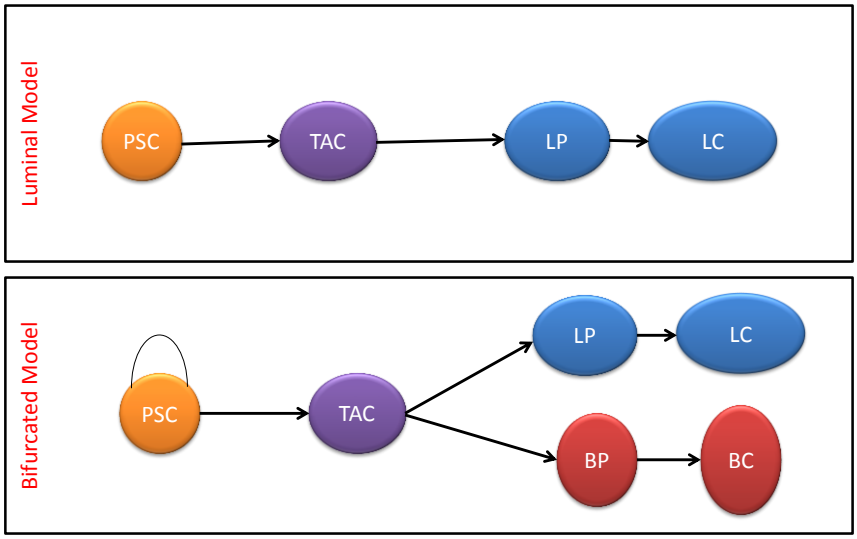
A consensus panel convened by the American Association of Cancer Research has defined a CSC as "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor." It should be noted that this definition does not indicate the source of these cells—these tumor-forming cells could hypothetically originate from stem, progenitor, or differentiated cells.

As such, the terms "tumor-initiating cell" or "cancer-initiating cell" are sometimes used instead of "cancer stem cell" to avoid confusion. Tumors originate from the transformation of normal cells through the accumulation of genetic modifications, but it has not been established unequivocally that stem cells are the origin of all CSCs.

The CSC hypothesis therefore does not imply that cancer is always caused by stem cells or that the potential application of stem cells to treat conditions such as heart disease or diabetes, as discussed in other chapters of this report, will result in tumor formation. Rather, tumor-initiating cells possess stem-like characteristics to a degree sufficient to warrant the comparison with stem cells; the observed experimental and clinical behaviors of metastatic cancer cells are highly reminiscent of the classical properties of stem cells.

The stem cell theory, and there seems now to be significant evidence of its validity in prostate cancer, is principally that the clonal theory has merit to a point but that the development is more complex and the cancer stem cell plays a critical role in fostering growth of the cancer cells, most of which has less aggressive a growth characteristic if any at all.

Lawson and Witte present a recent overview of this concept as applied to the prostate and PCa. Recent studies apparently indicate that the cancer stem cells, CSC, are necessary to sustain later stages of the development of the malignancy. Only a small subpopulation of the cancer cells, the CSC population, has a demonstrated ability to maintain the malignancy as well. Lawson and Witte present two theories of this CSC process. One is called the stochastic theory which is that all cells are equally malignant. The other theory, the one for CSC, called the hierarchical theory is that only the CSC has the ability to multiply. These two are graphically depicted below. The CSC or in this case the PSC, prostate stem cell, yields a TAC, or transition amplifying cells, then yield progenitor cells, LP or BP, and then finally a luminal or basal cell. This is slight contrast to the Goldstein model. This model applies for both benign as well as cancer cells, at least as viewed by Lawson and Witte.



12/24/2010 Lecture 3 A Simple Model 19

Now if one looks at the CSC theory, then we see a CSC has progeny, and yet those progeny may not have the ability to multiply. Thus the explosive exponential growth of cancer is not as clear in a CSC model, because almost all of the progeny of the CSC are no reproducing progeny. Thus the growth models for a CSC based malignancy are more complex and are dependent on limited CSC reproduction and non-CSC reproduction. However the CSC model also argues for there being some CSC support for the progeny which are not CSC. The dynamics of cell growth then becomes quite complex here, for the stem cells replicate themselves at a slow rate but are replicating other cells at a higher rate. However the other cells do not replicate themselves they

just go through a standard cell process. If the cells are benign then they go through apoptosis as seen in red blood cells and the skin keratinocytes.

We quote Lawson and Witte as follows:

Models of prostate epithelial differentiation. The traditional model for prostate epithelial differentiation proposes that PSCs residing in the basal cell layer give rise to intermediate, transit-amplifying cells that produce large numbers of terminally differentiated secretory luminal cells …. This model implies a linear differentiation scheme in which basal and luminal cells comprise one lineage and basal cells are essentially luminal cell progenitors …

This hypothesis is supported by the existence of cells of intermediate phenotype that express both basal- and luminal cell–specific cytokeratins in both fetal and adult stages of prostate development … Intermediate cells can also be identified in in vitro cultures of primary prostate epithelium … Several studies have also suggested basal cells can differentiate into luminal cells in vitro … Alternative theories for prostate epithelial differentiation propose basal and luminal cells may represent separate epithelial lineages … This is similar to prevailing models for epithelial differentiation in the mammary gland, a tissue that is anatomically and functionally analogous to the prostate …

Now there have been several others who have examined the stem cell model for PCa. Another of recent merit is that of Hurt et al. They summarize their work as follows:

Recent evidence supports the hypothesis that cancer stem cells are responsible for tumor initiation and formation. Using flow cytometry, we isolated a population of CD44+CD24- prostate cells that display stem cell characteristics as well as gene expression patterns that predict overall survival in prostate cancer patients. CD44+CD24- cells form colonies in soft agar and form tumours in NOD/SCID mice when as few as 100 cells are injected.

Furthermore, CD44+CD24- cells express genes known to be important in stem cell maintenance, such as BMI-1 and Oct-3/4. Moreover, we can maintain CD44+CD24- prostate stem-like cells as non-adherent spheres in serum-replacement media without substantially shifting gene expression. Addition of serum results in adherence to plastic and shifts gene expression patterns to resemble the differentiated parental cells.

Thus, we propose that CD44+CD24- prostate cells are stem-like cells responsible for tumor initiation and we provide a genomic definition of these cells and the differentiated cells they give rise to. Furthermore, gene expression patterns of CD44+CD24- cells have a genomic signature that is predictive of poor patient prognosis. Therefore, CD44+CD24- LNCaP prostate cells offer an attractive model system to both explore the biology important to the maintenance and differentiation of prostate cancer stem cells as well as to develop the therapeutics, as the gene expression pattern in these cells is consistent with poor survival in prostate cancer patients.

Jordan et al characterize cancer stem cells as having three characteristics:

1. Self-Renewal: at the end of mitosis of the stem cell, either one or both retain all the characteristics of the parent. The stem cell goes through a mitotic doubling and when it does it always retains one or two stem cell daughters.

2. Capability to generate multiple lineages. This means that a stem cell can generate offspring which can become anyone of many cell types.

3. Potential to proliferate extensively. The cell can keep replicating, it has no limitation within reason and thus contains the elements ultimately for metastasis.

A normal stem cell may mutate to a cancer stem cell or a normal progenitor cell may morph back to a cancer stem cell.

As Delarbra et al state:

Although monoclonal in origin, most tumors appear to contain a heterogeneous population of cancer cells. *This observation is traditionally explained by postulating variations in tumor microenvironment and coexistence of multiple genetic subclones, created by progressive and divergent accumulation of independent somatic mutations.*

An additional explanation, however, envisages human tumors not as mere monoclonal expansions of transformed cells, but rather as complex tridimensional tissues where cancer cells become functionally heterogeneous as a result of differentiation.

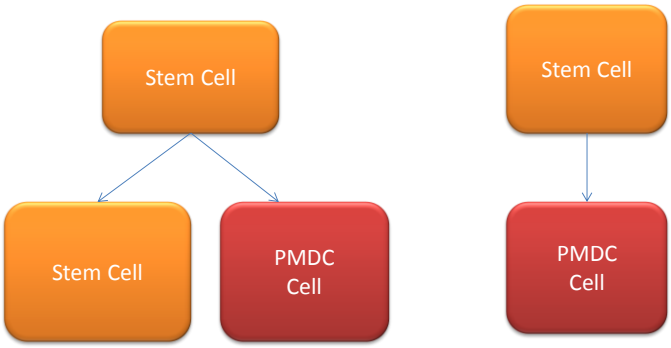
According to this second scenario, tumors act as caricatures of their corresponding normal tissues and are sustained in their growth by a pathological counterpart of normal adult stem cells, cancer stem cells.

The statement starts with the accepted monoclonal hypothesis and then departs to a polyclonal alternative view. It retains the CSC, cancer stem cell, paradigm for solid tumors as well. In the context of HGPIN we see a change in the cells and we have heard the argument that they have made one or several of the unchangeable steps towards PCa. Thus using the CSC theory one would expect that it would be from one or several of these cells that PCa would arise. In addition, we could assume that there is no unique pathway mutations or changes which result in PCa but a plethora of them. Simply stated, cancer is complex, it finds ways to migrate forward no matter what the path.

4.2 PROGRESSION AND REGRESSION

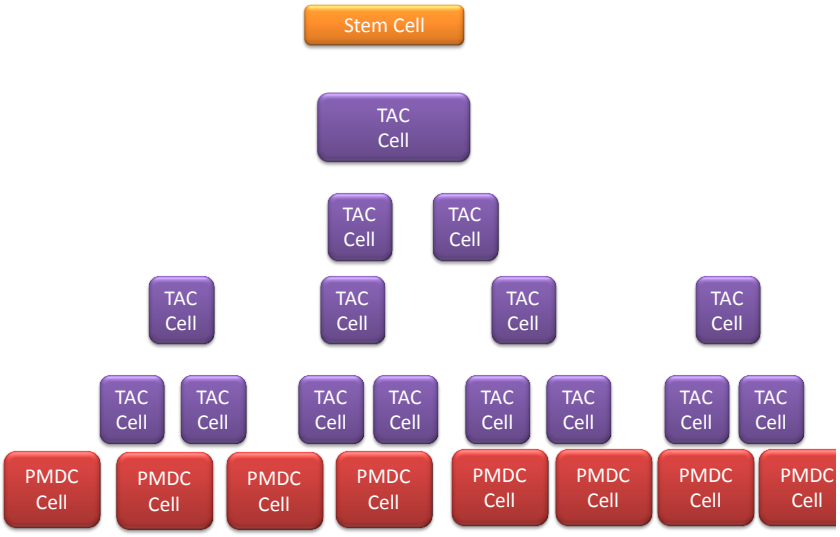
We first relook at the progression and regression dynamics. The key driver for the analysis herein has been the regression often seen in HGPIN. Knowing that most likely the methylation of GSTP1 has given rise to development of PIN we then ask what gives rise to its regression and why have the HGPIN cells themselves not only stopped growing but have disappeared. Again we have seen this in melanomas, and this is also the Rosenberg effect in certain sporadic cancer regressions.

To look more closely we first return to the stem cell model for cancer which we developed earlier. The stem cell theory states that there are a certain number of cancer stem cells which in turn may replicate themselves but also create what are termed post mitotic differentiated cells. Not really stem cells but cells which exhibit the phenotypic characteristics of a cancer cell. One of the questions one may pose is do these PMDC exhibit a different genotypic character as well or are they controlled by some epigenetic factors. We show these examples below;



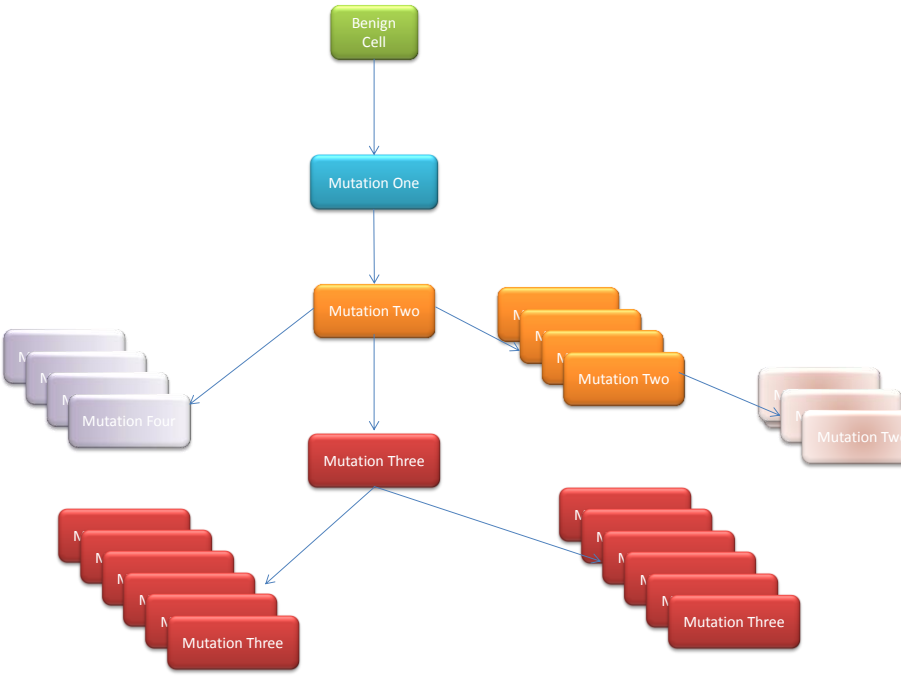
PMDC Cell: Post Mitotic Differentiated Cancer Cell

Now we can also see as Weinberg has noted (Weinberg p 419) that a progression may occur in a somewhat more complex mechanism as we depict below. Now from the stem cell arises Transit Amplifying Cells and then the PMDC.

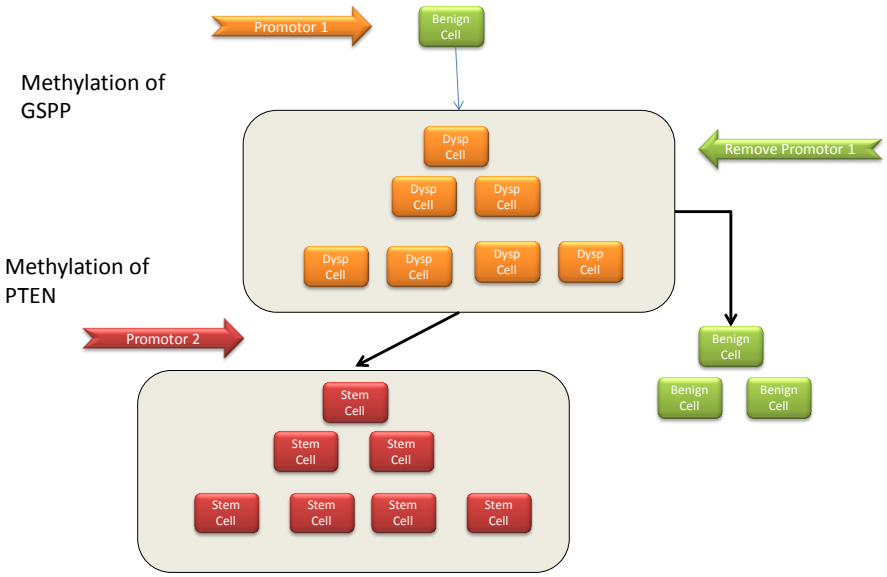


TAC: Transit Amplifying Cells

Now in reality there may be multiple genetic hits which give rise to the stem cell, the pluripotent self-replicating core of a cancer. The Figure below provides a generic profile, namely we may see many genetic changes, some leading to cancer as in mutation 3 below and others just wandering off into self-replicating cells but not with a malignant tendency.



Finally when we return to the HGPIN model we see the benign cell migrating to a dysplasia, say HGPIN, and then to a malignant cell, but then there is the regression back to a benign cell. The question is then; what pathway elements takes us one way and what elements take us back. And what happened to the dysplastic cells? Did they just die, apoptosis, or were they scavenged?



4.3 IMPLICATIONS OF STEM CELLS IN HGPIN

The stem cell theory stipulates that there exists a set, possibly small, of self-sustaining cells, the stem cells, which provide for the continuing growth of the tumor mass. Thus the question is, does a stem cell exist in the case of HGPIN?

5 PROGRESSION AND GENETIC DRIVERS

We will first consider several of the specific genes and their impact on the development of HGPIN and in turn PCa. In the next section we move on to the pathways and the interaction amongst and between the genes.

The genes and their resulting proteins fall into the following general categories[7]:

Oncogenes: Oncogenes are mutated forms of a normal gene called a proto-oncogene. The proto oncogene is norm ally controlling or regulating the growth and the regulation of a cell. When changed it results in loss of control or excess growth and expansion. Some typical oncogenes are RAS, C-MYC, CDK4, ERBB2, MET, PIK3CA amongst many. For example C-MYC is a transcription gene, it facilitates DNA transcription. Normally its function is working is a controlled manner and transcription and the products are produced in a regulated concentration. However C-MYC can undergo changes which cause its amplification.

One manner of amplification is the via amplification, a process where copies of the gene can be made and thus its product, c-myc protein, gets created at a greater concentration. Another change in C-MYC could be that of it being translocated and moved near another gene which amplifies the output as is seen in some leukemias.

Translocations: The movement of genes en masse from one chromosome to another is a translocation. The most prominent is the Philadelphia chromosomal translocation found in chronic myelogenous leukemia. In the case of PCa the translocation gene is the TMPRSS2:ERG fusion, which is in a sense a translocation since the intron between the two gene is removed and they are then fused.

Tumor Suppressor Genes: These genes are controllers of a set of processes key to the maintenance of the normal integrity of the cell. Tumor suppressor genes are frequently inactivated by mutations and it is their loss of function which results in cell instability and thus cancer. Typical Tumor Suppressor Genes, TSP, are TP53, or as it is known p53, which controls cell growth. PTEN is a second, and a key player in PCa. BRCA1 and BRCA2 are genes which are predominant in breast cancer. CDKN2A (also known as P16, INK4) is a key player in melanoma.

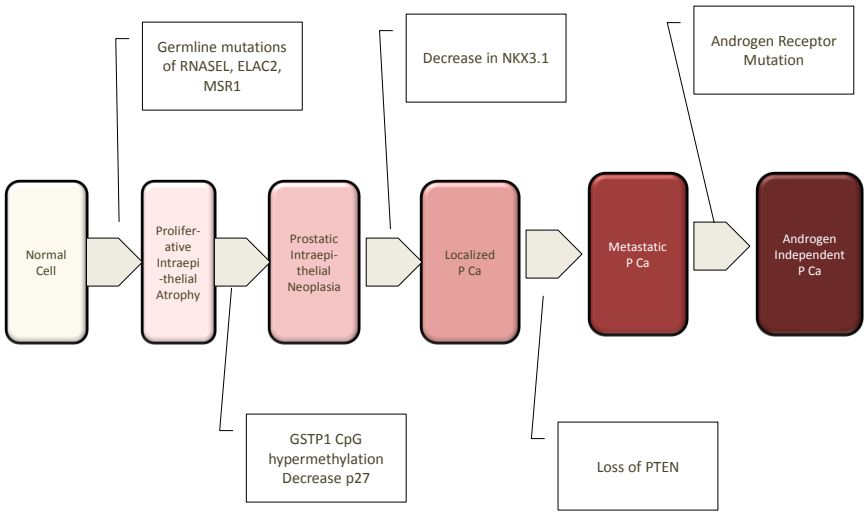
In this section we concentrate on those genes as characterized above which have the most significant role to play in HGPIN and PCa.

5.1 GENES AND HGPIN

We now look at the putative genetic changes from benign prostate cells to HGPIN. The arguments in the literature for the most part state that the process is a result of genetic changes and that furthermore, implied but not expressly stated, these changes are immutable and they

[7] We will refer to Bunz for many of these descriptions.

naturally progress from HGPIN to PCa. It should be noted that there does not appear to be any work indicating the regression of HGPIN as regards to specific genes or subsequently specific pathways. We start from the paper by Nelson et al (2003) where they argue that the genes we discuss below are the ones leading to full androgen independent PCa. We start with a modification of the process as described by Nelson et al. below:



From: Nelson W et al. N Engl J Med 2003;349:366-381

12/22/2010 17

They argue that to reach HGPIN it is necessary to go through a PIA step, proliferative intraepithelial atrophy, step and then full HGPIN. We now go through the first two steps and examine the genes related thereto. It should be recalled, however, that the Nelson model is but one of several. It is not as simple as the Vogelstein model for colon cancer. We shall return to a collection of these models later in this paper.

Thus we can reach the following conclusions from Nelson et al:

1. To reach PIA we need germline alterations of RNASEL, ELAC2, and MSR1.

2. To reach HGPIN we need hypermethylation of GSTP1 and a decrease in p27.

The steps towards PCa we will also discuss briefly. There are a few, clearly NKX3.1 is essential as is loss of PTEN and amplification of c-myc. The questions we ask are:

1. Can we get HGPIN without PIA as an initial step. Is hypermethylation alone needed. Also given the Goldstein model, where does Akt and PTEN suppression play in this process.

2. Is PIA regressible itself and if so is it due to apoptosis of the PIA cells and if so what causes the apoptosis, is it a normal cell death or exogenous to the cell say from the immune system.

3. If hypermethylation of GSTP1 is necessary for HGPIN, what causes its regression. What is one to make of the Goldstein model of Akt and the trans-located ERG TMPRSS change as well.

These are but a few of the issues which need be addressed.

5.1.1 PIA

The understanding of the development of PIA is still fragmentary.
PIA is the purported first step. To Nelson, this requires first a germline mutation of RNASEL, ELAC2 and MSR1. Let us examine each of these in turn.

RNASEL: A gene which encodes a widely expressed latent endoribonuclease that participates in an interferon inducible RNA-decay pathway that is thought to degrade viral and cellular RNA.

What mutations, what causes the mutations, and what the cell response is to the mutations is still an open issue.

5.1.2 PIN

Now PIN is a more complex issue. The problem here is twofold. First, looking at human HGPIN and then performing microarray analysis one obtains many different profiles of genes expressed, under-expressed, or over-expressed. Second, there are murine models and other models in which specific genes are activated, changed or deactivated and where the relationship between murine models do not map one to one onto the human facts.

Morphologically and histologically we can identify HGPIN. It is simply the overgrowth within benign glands or acini of new cells whose characteristics appear to be neoplastic but whose growth is constrained. We see this in other areas such as melanoma in situ where the melanocyte may leave the basal layer and migrate to the upper epidermis or where the melanocyte may for clusters at the junction which are larger than say a junctional nevus, but true malignancy is not identifiable.

Let us briefly look at the genes involved in PCa. First there are the susceptibility genes which set the stage and although not drivers do facilitate the process. These are shown below, the Prostate-Cancer–Susceptibility Genes.

Gene	Location	Alterations*	Phenotypic Consequences
RNASEL	1q24–25	Base substitutions leading to Met1Ile, Glu265X, and Arg462Gln alleles Four-base deletion at codon 157 leading to premature protein truncation at codon 164	Encodes endoribonuclease that participates in an interferon-inducible 2',5'-oligoadenylate– dependent RNA-decay pathway *RNaseL-/-* mice have diminished interferon-a antiviral activity
ELAC2	17p11	Base insertion leading to premature termination 67 amino acids after codon 157; base substitutions leading to Arg781His, Ser217Leu, and Ala541Thr alleles	Unknown
MSR1	8p22	Base substitutions leading to Arg293X, Pro36Ala, Ser41Tyr, Val113Ala, Asp174Tyr, Gly369Ser, and His441Arg alleles	Encodes subunits of class A macrophage- scavenger receptor *Msr-A*$_i$/$_i$ mice have an increased sensitivity to serious infection with *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli,* and herpes simplex virus type 1
AR	Xq11–12	Polymorphic polyglutamine (CAG) and polyglycine (GGC) repeats	Encodes androgen receptor, an androgen- dependent transcription factor Different polymorphic alleles may be associated with different transcriptional transactivation activities
CYP17	10q24.3	Base substitution in transcriptional promoter (T˚C transition leading to new Sp1 recognition site)	Encodes cytochrome P-450c17a, an enzyme that catalyzes key reactions in sex-steroid biosynthesis
SRD5A2	2p23	Base substitutions leading to Val89Leu and Ala49Thr alleles	Encodes the predominant 5-a-reductase in the prostate, converts testosterone to dihydrotestosterone

Now we can look at the genes modified that result in PCa. We list them below as the Somatic Gene Alterations in Prostate Cancer:

Gene	Location	Alterations	Phenotypic Consequences	Initiates
GSTP1	11q13	CpG island hypermethylation (decreased expression)	Encodes carcinogen-detoxification enzyme *Gstp1/2-/-* mice show increased skin tumorigenesis when exposed to topical carcinogen	PIN
NKX3.1	8p21	Allelic losses (decreased expression)	Encodes a prostate-specific homeobox gene essential for normal prostate development *Nkx3.1+/-* and *Nkx3.1-/-* mice manifest prostatic hyperplasia and dysplasia	Localized PCa
PTEN	10q23.31	Allelic losses, mutations, probable CpG island hyper-methylation (decreased expression, function, or both)	Encodes a phosphatase active against protein and lipid substrates *Pten+/-* mice have prostatic hyperplasia and dysplasia Prostatic intraepithelial neoplasia develops in *Pten+/-Nkx3.1+/-* and *Pten+/-Nkx3.1-/-* mice Prostate cancer with a poor prognosis develops in *Pten+/-TRAMP* mice	Initiates Metastatic PCa
CDKN1B	12p12–13	Allelic losses (decreased expression)	Encodes p27, a cyclin-dependent kinase inhibitor *Cdkn1b-/-* mice have prostatic hyperplasia Prostate cancer develops in *Pten+/-Cdkn1b-/-* mice	Reduced levels of p27, a cyclin-dependent kinase inhibitor encoded by the *CDKN1B* gene, also are common in prostate cancers, particularly in prostate cancers with a poor prognosis
AR	Xq11–12	Amplification, mutations (increased expression, altered function)	Encodes androgen receptor *Pb-mAR* transgenic mice have prostatic hyperplasia, and prostatic intraepithelial neoplasia develops in them	Androgen Independent Metastatic PCa

Yet to get to HGPIN Nelson et al argue we need hypermethylation of GSTP1 and a decrease in p27. Let us look at these two separately.

In the paper by Jeet et al (2010), the authors describe a set of genes which relate to HGPIN in detail based upon single trans-genic mice:

Models	*Genes regulated*	*Prostate phenotype*
Hormone receptors	Androgen receptor	HGPIN
	Retinoic acid receptor α/γ	Squamous metaplasia and pre-neoplastic lesions
	Estrogen receptorα/β	No marked phenotype
Growth factors and receptors	FGF8b	HGPIN
	FGFreceptor1	PIN with reversible hyperplasia
	FGF7	Prostate epithelial dysplasia
	FGFR2iiib	Hyperplasia/dysplasia
	IGF-1	PIN and spontaneous tumor growth
	TGFR-β	PIN and invasive adenocarcinoma
	HER-2/Neu	PIN and invasive carcinoma
Tumor suppressors, cell cycle, and signaling pathways	p53Rb	PIN with reduced apoptotic potential Focal hyperplasia
	Nkx3.1	Hyperplasia followed by PIN
	H-Ras	LGPIN and intestinal metaplasia
	APC	PIN and invasive adenocarcinoma
	Pten	PIN and metastatic adenocarcinoma
	Bcl-2	No overt phenotype
	Akt-1	Focal regions of PIN
	C-MYC	PIN and locally invasive adenocarcinoma
Genomic instability	Eco RI c-fos	HGPIN No significant pathology
Composite transgenic mice	Ink4a/Arf+/−/Pten+/−	Rapid growth of PIN lesion
	Nkx3.1/Pten	PIN and metastatic spread of invasive tumors to lymph nodes
	Pten+/−/Akt1−/−	Akt1−/− repressed prostate tumor growth
	Pten+/−/p27kip1−/−	Rapid progression of invasive carcinoma
	Pten−/−/p53−/−	Early onset of invasive tumors
	PTEN+/−/TRAMP	Increased rate of tumor development
	P53−/−/Rb−/−	Highly metastatic adenocarcinoma
	Pten+/−/FGF8b	Metastatic adenocarcinoma
	Bcl-2/TRAMP	Multi step prostate carcinogenesis

In the above studies there is a set of regressions noted. However, the study does make clear that there are multiple effects with limited consistency. They then continue to detail PCa genetic elements in the following Table.

Prostate phenotype	*Models*	*Genetic disruptions*
Hyperplasia	PB-FGF7(PKS)	FGF7
	C3(1)-bcl-2PSA-CRExNkx3.1f/f	Bcl-2Nkx3.1
	PB-FGFRiiib	FGFRiiib
	C3(1)-Polyomavirus Middle T	Polyomavirus middle T gene
	MMTV-wap	Whey acidic protein gene Retinoblastoma
	PB-Cre+/Rb loxp/loxP	
PIN	ARR2PB-FGFR1	FGFreceptor1
	BK5-IGF1	IGF-1
	ARR2PB-myc-PAI	Myc
	MPAKT model	Akt1
	PSA-Cre+xNkx3.1+/flox	Nkx3.1
	PB-RAS	H-Ras
	PB-Cre4xPtenloxp/loxp	PTEN
	PB-Eco RI	ECO:RI
	LPB-Tag/PB-Hepsin	Hepsin,p53,Rb
	TRAMP	p53,Rb
	LADY	p53,Rb
HGPIN	PB-m AR	Androgen receptor
	ARR2PB-FGF8b	FGF8b
	PB-Cre4xPtenloxp/loxp	Pten
	MMTV-Crex PTEN loxp/loxp	Pten
Locally invasive adenocarcinoma	C3(1)-SV40T/t	p53,Rb
	PB-Cre+xAPCflox/flox	APC
	PB/Neu	HER-2/Neu
Metastatic carcinoma	PSP-KIMAP	p53,Rb
	Cryptidin-2/SV40T	p53,Rb
	Fetal Gγ-globin	p53,Rb
	TRAMP	p53,Rb
	PTEN+/−/TRAMP	Pten,p53,RB
	P53−/−/Rb−/−	p53,Rb
	Nkx3.1−/−/Pten+/−	Nkx3.1,Pten
	Pten+/−/FGF8b	Pten,FGF8b

Notwithstanding the complexity of the many elements above, the results do seem to bear some consistency on two issues; first, HGPIN has some regressible models, second, there are a common set of genes which do lead to PCa.

5.2 HGPIN GENES

For HGPIN there are two genes of focus, GSTP1 and p27, the product of the gene CDKNA1B. We examine them as best understood today. The key question we keep in the back of our minds are what causes the change and then what causes a remission. Do all HGPIN have these genetic changes?

5.2.1 GSTP1

One of the first steps in the development of HGPIN it is alleged is the methylation of GSTP1 (GSTP1[8] (glutathione S-transferase pi 1, 11q13)). Glutathione S-transferases (GSTs) are a family

[8] see http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&cmd=retrieve&dopt=default&rn=1&list_uids=2950

of enzymes that play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. Based on their biochemical, immunologic, and structural properties, the soluble GSTs are categorized into 4 main classes: alpha, mu, pi, and theta. This GST family member is a polymorphic gene encoding active, functionally different GSTP1 variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility to cancer, and other diseases.

The methylation of sections of DNA has been seen as a major contributor to cancer formation. Regarding that of GSTP1, the work by Phe et al and that of Gonzalgo et al as well as Brooks et al demonstrate how the methylation is achieved and what the impact is. We will summarize their work herein.

As Deutsch et al state:

The GSTP1 enzyme acts as an reactive oxygen species scavenger. Inactivation of GSTP1 could lead to inefficiency During chronic inflammation processes, inflammatory cells generate oxidative reactive species that can produce genomic changes such as point mutations, deletions, and rearrangements.[35] Chronic inflammation involves repeated tissue damage and regeneration in the presence of highly reactive oxygen species that can interact with DNA. GSTP1 is both overexpressed and hypermethylated in about 10% of PIA.

The overexpression of GSTP1 and COX2 found in PIA suggest inflammatory and oxidative stresses in these cells.[73] A prostate-cancer progression model has been proposed, in which PIA is a precursor to prostate cancer via an intermediate stage of high-grade PIN. Like high-grade PIN and carcinoma, PIA occurs frequently in the peripheral zone of the prostate gland, and is often found in close proximity to carcinoma. Chromosome 8 changes have been found in microdissected samples of PIA at a frequency similar to that of high-grade PIN and prostate carcinoma.

Many PIA cells have decreased expression of P27, the cyclin-dependentkinase inhibitor, and highly express BCL2 as in high-grade PIN. PIA frequently shows morphological transition to PIN[7] and high-grade PIN occupies an intermediate position in the tumorigenic process with an intermediate phenotype harboring anomalies found in prostate cancer (table 3). However, these models are speculative without solid data confirming a defined role for either COX2 or GSTP1 as initiators of this process.

Deutsch et al (2004) also contains the following Table:

	Healthy Prostate	*PIA*	*High-grade PIN*	*Prostate cancer*
COX2	+	+++	+	+
Morphological changes	0	–/+	+++	+++
BCL2	+	+	+++	+++
PCNA, KI67*	+	++	+++	+++
RER mutations	4%	..	22%	40%
Telomerase	36%	16%	73%	..
P27	86%	..	59%	44%

The contention is that there is a substantial change as one goes from benign to HGPIN. The reduction in p27 is significant and we discuss that later. The BCL2 and PCNA increased expression is interesting but it appears not to have been of continuing interest.

As DeMarzo et al states about prostate inflammation:

What is the source of prostatic inflammation? In most cases, the cause of prostatic inflammation is unclear. Various potential sources exist for the initial inciting event, including direct infection, urine reflux inducing chemical and physical trauma, dietary factors, estrogens, or a combination of two or more of these factors. Furthermore, any of these could lead to a break in immune tolerance and the development of an autoimmune reaction to the prostate.

However, there seems to be a consensus that stress, inflammation, oxidative stress, and the like has a precipitating set of factors for HGPIN. Donkena et al detail the oxidative stress arguments and Nelson et al detail those on inflammation. Deutsch et al provides an excellent summary of all the environmental and genetic factors. Palapattu et al provide an alternative view on the inflammation drivers. Ramoutar and Brumaghim details the use of antioxidants as means to counter these effects.

Specifically Brooks et al (1998) state:

In all prostate tissue specimens examined by immunohistochemistry, normal prostatic epithelia, particularly the basal cell layer, demonstrated the expression of GSTP1 immunoreactive polypeptide. All cancerous epithelia were devoid of GSTP1 expression…

Indeed, basal cells in each of the 60 high-grade PIN lesions could be identified by their abundant staining for GSTP1. Dysplastic epithelia (characterized by prominent nucleoli, increased nuclear size, and multilayering of cells) demonstrated a complete absence of GSTPl expression. Lack of expression occurred whether the PIN focus was adjacent to (30 cases) or far removed from (30 cases) prostate adenocarcinoma…

High-grade PIN lesions were evaluated for GSTP1 expression and GSTP1 promoter methylation because of their purported role as a prostate cancer precursor lesion.

Low-grade PIN (grade 1) has a more uncertain relationship to prostate cancer. Low-grade PIN is not associated with increased risk of prostate cancer on subsequent biopsy. Histological

diagnosis of low-grade PIN is poorly reproducible even by experienced pathologists (10, 11). Many question whether low-grade PIN is related to high-grade PIN or prostate adenocarcinoma, or whether it is merely a normal epithelial variant.

High-grade PIN, on the other hand, is characterized by cytological and nuclear morphometric changes characteristic of carcinoma. Nearly 80% of prostates removed for adenocarcinoma harbor histological PIN lesions accompanying the primary tumor. Detection of high-grade PIN on prostate needle biopsy is associated with a 50% chance of detection of cancer on subsequent biopsy…

It is fair to state that the numbers in the 1998 Brooks et al article as stated above for HGPIN and PCa have dropped dramatically over the past decade or more.

5.2.2 CDKNA1B:p27

This gene protein, p27, encoded by gene CDKNA1B, encodes a cyclin-dependent kinase inhibitor, which shares a limited similarity with CDK inhibitor CDKN1A/p21. It is considered a tumor suppressor gene along with p53, PTEN and NKX3.1 (see Chen and Sawyers in DeVita, Cancer).

p27 is a cyclin-dependent kinase inhibitor and Cdkn1b-/- mice, deficient of p27, are shown to have prostatic hyperplasia. Prostate cancer is shown to develop in Pten+/- and Cdkn1b-/- mice. The encoded protein binds to and prevents the activation of cyclin E-CDK2 or cyclin D-CDK4 complexes, and thus controls the cell cycle progression at G1. p27 is considered a tumor suppressor gene which can lose its effect early and be one of the drivers for HGPIN.

The degradation of this protein, which is triggered by its CDK dependent phosphorylation and subsequent ubiquitination by SCF complexes, is required for the cellular transition from quiescence to the proliferative state. p27 is a gene (located at 12p13.1-p12) involved in the control of kinase chains in the cell and controls cyclin for cell reproduction[9]. In a recent paper by Majumder et al (2008) they state:

Transgenic expression of activated AKT1 in the murine prostate induces prostatic intraepithelial neoplasia (PIN) that does not progress to invasive prostate cancer (CaP). In luminal epithelial cells of Akt-driven PIN, we show the concomitant induction of p27(Kip1) and senescence. Genetic ablation of p27(Kip1) led to down-regulation of senescence markers and progression to cancer. In humans, p27(Kip1) and senescence markers were elevated in PIN not associated with CaP but were decreased or absent, respectively, in cancer-associated PIN and in CaP. Importantly, p27(Kip1) up-regulation in mouse and human in situ lesions did not depend upon mTOR or Akt activation but was instead specifically associated with alterations in cell polarity, architecture, and adhesion molecules. These data suggest that a p27(Kip1)-driven checkpoint limits progression of PIN to CaP.

[9] Also known as CDKN1B cyclin-dependent kinase inhibitor 1B (p27, Kip1) see http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene&cmd=retrieve&dopt=default&rn=1&list_uids=1027

Weinberg describes the function of p27 in the cyclin-CDK complexes that regulate cell reproduction[10]. Also the work of Yang et al describes the reduced expression of p27 as a major factor in the development towards PCa.

As DeMarzo et al state:

In terms of molecular modes of action, ***p27 functions as an inhibitor of cell-cycle progression by inhibiting the activity of cyclin–cyclin dependent kinase complexes in the nucleus. Interestingly, p27 levels are generally reduced but not absent in human proliferative inflammatory atrophy (PIA), prostatic intraepithelial neoplasia (PIN) and prostate cancer****. The fact that p27 levels are not lost entirely (or biallelically inactivated by mutations) in cancer might be explained by recent findings that indicate that cytoplasmic p27 levels, which are increased by signalling through the MET receptor tyrosine kinase, are required for cell migration in response to hepatocyte growth factor signalling through MET and in response to increased cyclin D1 levels. Therefore, although high levels of nuclear p27 can prevent cell-cycle progression, cytoplasmic p27 might be required for optimal tumor cell motility, which is a key feature of malignant transformation and tissue repair.*

There is the issue therefore as to what function p27 plays across many cancers. As Singerland and Pagano state:

CDKN1B cyclin-dependent kinase inhibitor 1B (p27, Kip1)

p27 is a cell cycle inhibitor whose cellular abundance increases in response to many antimitogenic stimuli. In this review, we summarize the current knowledge on p27 function and its regulation by synthesis and by ubiquitin-mediated degradation. Importantly, p27 degradation is enhanced in many aggressive human tumors. The frequency with which this is observed suggests that loss of p27 may confer a growth advantage to these cancers.

Thus across many types of cancer p27 is down regulated and allows uncontrolled growth since its control on the cyclins is removed.

In a recent paper by Taylor et al they conclude regarding murine models treated with 9-cis retinoic acid (9cRA), which had been shown to reduce the incidence of HGPIN in the murine models, that :

1. p27Kip1 deficiency increases prostate epithelial cell proliferation
2. p27Kip1 deficiency promotes prostate carcinogenesis
3. 9cRA suppresses prostate carcinogenesis irrespective of p27Kip1 expression
4. 9cRA was unable to suppress hormone-induced prostate carcinogenesis
5. 9cRA inhibits prostate cell proliferation and induces cellular senescence

They then conclude:

[10] Weinberg pp 268-279.

Our data indicate that p27Kip1 deficiency in PEC promotes cell proliferation in an age-dependent manner and increases cellular response to hormone stimulation. p27Kip1 haplo-insufficiency and deficiency stimulate MNU-induced prostate carcinogenesis, suggesting that PIN in human prostate lacking partial or total p27Kip1 expression may have a higher potential to progress and develop malignant phenotype than lesions with wild type p27. The efficacy of 9cRA in suppressing PIN is apparently not p27-dependent, indicating that in potential clinical trials, 9cRA may affect pre-malignant and tumor cells that differentially expressed p27Kip1. The induction of CS by 9cRA in PIN suggests that this biomarker could be used as a potential biomarker of response in clinical trials for the prevention and treatment of prostate cancer.

Thus a reduction in p27 expression is clearly related to HGPIN. The issue is what causes this underexpression. We will see later the prominent role p27 plays in the control but the details of its underexpression seem yet to be clarified.

As Deutsch et al state:

P27 is an inhibitor of the CDK2-cyclin E complex, thus regulating cell-cycle progression through the G1 cell cycle phase. A low concentration of P27 has been associated with the Gleason score and aneuploidy in studies based on primary prostate-cancer samples. Low expression of P27 is associated with higher recurrence and poor disease-free survival rates. One study found down-regulation of P27 in high-grade PIN samples, supporting the fact that P27 down-regulation could occur at early stages of carcinogenesis.

The important role of P27 in the development of the healthy prostate gland has been shown in p27 knockout mice, which have an enlarged prostate gland. The inactivation of one Pten allele and one or both alleles encoding p27 in mice was shown to accelerate spontaneous neoplastic changes showing that Pten and p27 have a pivotal and cooperative role in prostate cancer cell suppression. Moreover, this study strongly supports the theory of multistep carcinogenesis in prostate cancer, with the crucial relevance of a two-hit process (P27 and PTEN) occurring along the transformation process.

5.3 OTHER KEY GENETIC PLAYERS

We now examine several of the key genes in the development of prostate cancer. This list is not exhaustive but it does present the major players in the resulting uncontrolled spread of the disease. Although not necessarily part of HGPIN development, they do occur along the way. One question could be, does the development of HGPIN establish a path for these genes to also have their changes in expression.

5.3.1 MYC

Myc or specifically c-Myc, is a powerful gene element which induces cell growth. c-Myc is so strong promoter of cell proliferation and growth. c-Myc is a transcription factor which is essential in the growth and expansion of the cell.

In the paper by Iwata et al the authors examine its influence during the development of PIN. They state:

Lo-MYC and Hi-MYC mice develop prostatic intraepithelial neoplasia (PIN) and prostatic adenocarcinoma as a result of MYC overexpression in the mouse prostate[1]. However, prior studies have not determined precisely when, and in which cell types, MYC is induced. Using immunohistochemistry (IHC) to localize MYC expression in Lo-MYC transgenic mice, we show that morphological and molecular alterations characteristic of high grade PIN arise in luminal epithelial cells as soon as MYC overexpression is detected.

These changes include increased nuclear and nucleolar size and large scale chromatin remodeling. Mouse PIN cells retained a columnar architecture and abundant cytoplasm and appeared as either a single layer of neoplastic cells or as pseudo-stratified/multilayered structures with open glandular lumina—features highly analogous to human high grade PIN.

Also using IHC, we show that the onset of MYC overexpression and PIN development coincided precisely with decreased expression of the homeodomain transcription factor and tumor suppressor, Nkx3.1. Virtually all normal appearing prostate luminal cells expressed high levels of Nkx3.1, but all cells expressing MYC in PIN lesions showed marked reductions in Nkx3.1, implicating MYC as a key factor that represses Nkx3.1 in PIN lesions.

To determine the effects of less pronounced overexpression of MYC we generated a new line of mice expressing MYC in the prostate under the transcriptional control of the mouse Nkx3.1 control region. These ''Super-Lo-MYC'' mice also developed PIN, albeit a less aggressive form. We also identified a histologically defined intermediate step in the progression of mouse PIN into invasive adenocarcinoma. These lesions are characterized by a loss of cell polarity, multi-layering, and cribriform formation, and by a ''paradoxical'' increase in Nkx3.1 protein. Similar histopathological changes occurred in Hi-MYC mice, albeit with accelerated kinetics.

Our results using IHC provide novel insights that support the contention that MYC overexpression is sufficient to transform prostate luminal epithelial cells into PIN cells in vivo. We also identified a novel histopathologically identifiable intermediate step prior to invasion that should facilitate studies of molecular pathway alterations occurring during early progression of prostatic adenocarcinomas.

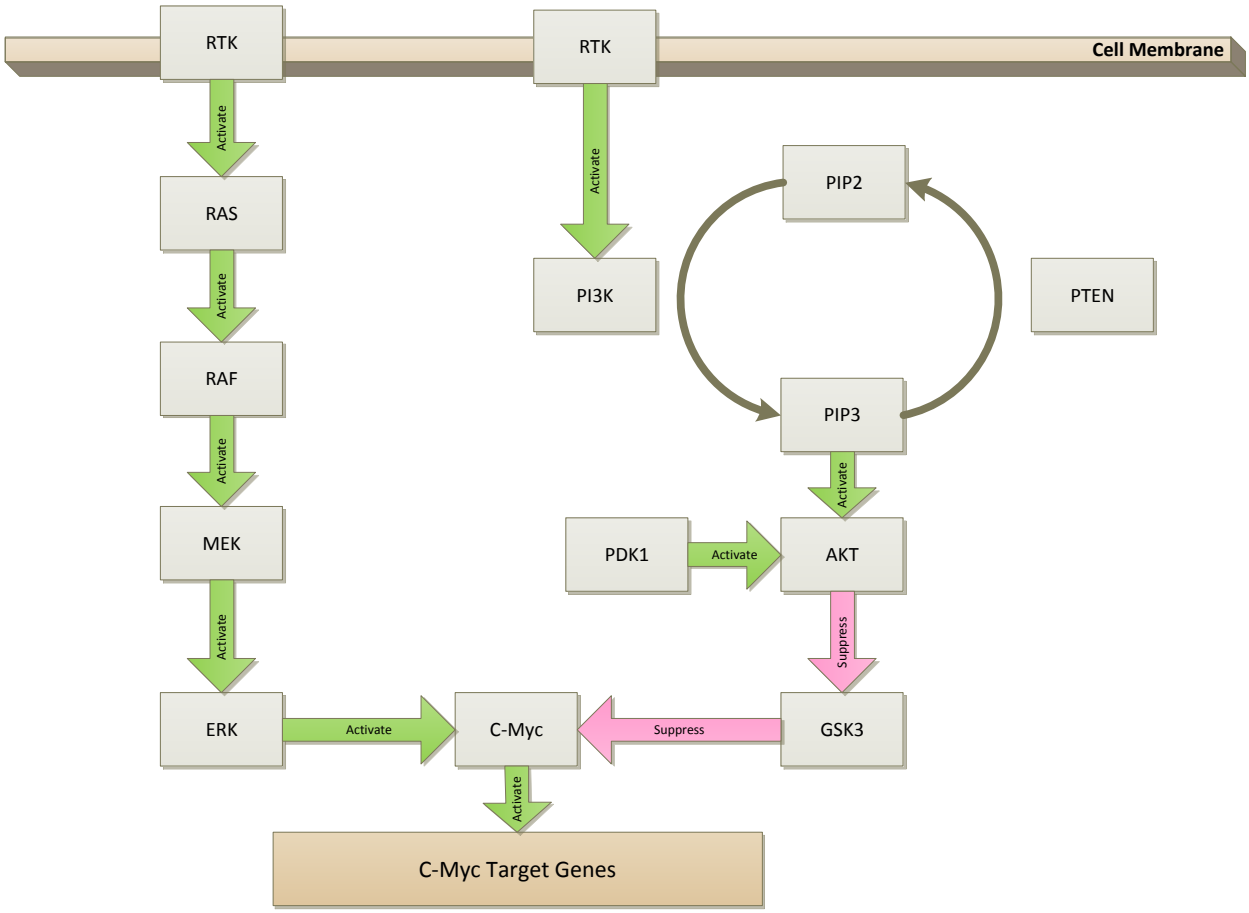
In the following graphic we depict the influence elements on c-Myc. This is a complex system of interlinking genes which when expressed in the correct manner can slow cell over expansion. The chart below is a modification from Bunz (p. 203) and it shows the gross characteristics of this control path. PTEN is a key element in control. What this does not show are two key elements, and indirectly a third.

First it does not show the fact that these are protein concentrations at work, one influencing the other and so forth. There is a feedback mechanism missing.

Second, it does not portray the temporal elements, namely this is a static gross representation of the influencing factors as if done in some generic snapshot. I fact the concentrations are time

varying and it is this time variation which when combined with the feedback loops renders certain instabilities leading to malignancy, namely uncontrolled growth.

Third, it fails to show the other genes and specifically the feedback mechanism of these genes. Namely PTEN is influence by these.



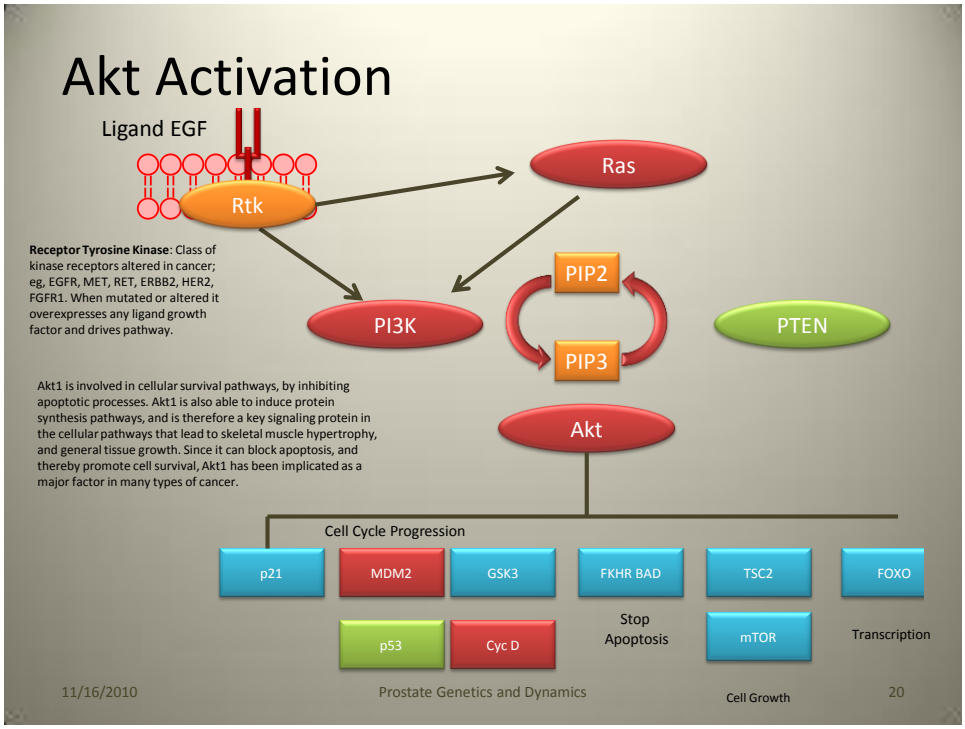
As Deutsch et al state:

MYC is one of the earliest genes to be expressed in the prostate gland after androgen stimulation. Transgenic mice that overexpress Myc develop prostate neoplasia. The importance of MYC overexpression in prostate cancer has been corroborated by the discovery of an increased copy number of the portion of 8q containing MYC in prostate-cancer samples, and particularly in advanced cases.

Only 6% of primary tumours show a gain of 8q sequences, whereas this change is seen in 89% of tumor recurrences after hormonal therapy.
MYC (a downstream target of AR) has been shown to be regulated by the AR and to be needed for AR-dependent and AR-independent growth. These data suggest that MYC might be involved in the development of androgen-independent prostate cancer, including that resulting from an increase in AR signalling.

5.3.2 PTEN

PTEN has become a key gene in the development of prostate cancer. It controls a pathway leading up to c-myc control and once PTEN is lost the PCa can be considered as very aggressive. Its loss results in an activation of Akt and then c-myc causing uncontrolled cell growth. The pathway is shown below:



As Jelovac and Park state[11]:

*The phosphatase and tensin homolog gene (*PTEN*) is a tumor suppressor located on the human chromosome 10q arm and is an important mediator of carcinogenesis in a variety of human malignancies. By the strictest definition, a tumor suppressor is a gene whose loss confers an increased lifetime risk of developing tumors. The most illustrative examples of genes that fulfill this criterion are those associated with familial cancer syndromes whereby heritable inactivation of 1 allele and subsequent increased tumor risk is passed along to each generation in an autosomal-dominant fashion.*

Using this as a framework, PTEN *is a bona fide tumor suppressor gene in that heritable germline mutations have been described in Cowden syndrome (CS), giving rise to a number of human tumors and cancers, most notably thyroid and breast cancers. As is the paradigm of tumor suppressor genes, affected patients with CS inherit 1 mutant inactive copy of* PTEN *from either parent, and the ensuing loss of the second allele results in tumor formation with subsequent genetic events that eventually lead to cancer. Although there are notable exceptions to this model, most heritable cancer syndromes are believed to adhere to this pattern.*

[11] http://jama.ama-assn.org/content/304/24/2744.full

From the work of McMenamin et al we have the slides below. Here is a case where PIN is still expressing PTEN but as we increase the grade of PCa we see the elimination of PTEN expression. Thus we can say that PIN is a state prior to PTEN suppression and a corollary may be that PCa aggressiveness is reflective of loss of PTEN and activation of Akt pathway.

The authors state in McMenamin et al state:

PTEN expression in prostate tissue.

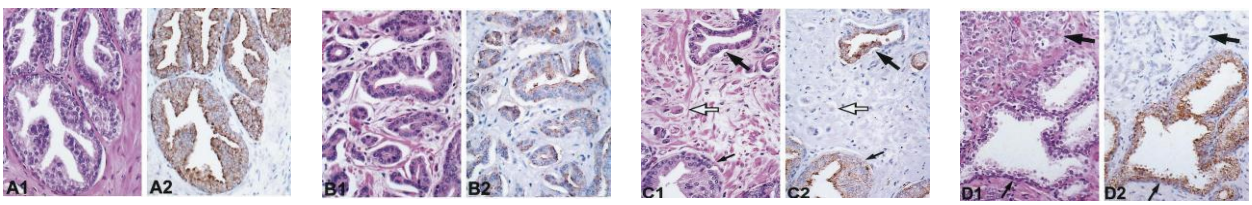
A, PIN.
A1, H&E-stained section of PIN.
A2, positive staining for PTEN in PIN.

B, prostate cancer (Gleason score, 3 + 3= 6).
B1, H&E-stained section.
B2, positive staining for PTEN in prostate cancer.

C, an example of heterogeneous expression of PTEN in a case of prostate cancer (Gleason score, 3 + 5= 8).
C1, prostate cancer. Gleason grade 3 cancer (large black arrow), Gleason grade 5 cancer (open arrow), and PIN (short black arrow). H&E-stained section.
C2, Gleason grade 3 prostate cancer stains positively for PTEN (see large black arrow). In contrast, Gleason grade 5 prostatic carcinoma is negative for PTEN expression (open arrow). PIN shows positive staining for PTEN (short black arrow).

D, benign prostate acinus with surrounding prostate cancer (Gleason score, 4 + 3= 7).
D1, H&E-stained section. Small arrow, benign prostatic glandular epithelium; large arrow, prostatic adenocarcinoma).
D2, positive staining for PTEN in benign prostate tissue and absence of staining for PTEN in prostate cancer.

Now look at A below and we see typical PIN, namely the overgrowth of the acinus with luminal cells and the closing of the lumen itself. The nucleoli structure is not show at this magnification. We can see in the PTEN stained slide the strong reflection of PTEN activity. PTEN is generally active in PIN and as is shown below it can become inactive in PCa.



Normal *PIN* *PCa* *PCa*

Now the slide B is the PIN slide above is a lower grade PCa. Note the growth of new glandular structures and the elimination of the connective stroma. The new glands have varying shapes yet

for the most part retain some form. The PTEN expression is shown on the right and it demonstrates that the cells still express PTEN.

The following in C is PTEN expression in ever increasing Gleason levels of PCa. In C we can see on the slide 1 the clusters of small PCa cells and then when stained for PTEN the large clusters exhibit PTEN but the small ones no longer do. Somewhere they have lost PTEN expression capability. The exact mechanism of this loss does not yet seem to be apparent. We see the same in slide D below. PTEN expressed in PCa clusters which have form and then none in the more sporadic clusters.

In the Doctoral thesis by Hermans he states:

PTEN is most frequently inactivated in several sporadic human cancers including, gliobastomas, endometrial cancer, and prostate cancer, either by mutation, homozygous deletion or promoter methylation.

Loss of one PTEN allele without inactivation of the second allele is also found in prostate cancer. This suggests that PTEN haplo-insufficiency plays a role in tumorigenesis. Loss of PTEN is detected, in 39-68% of primary prostate cancer samples and in 23% of PIN lesions. Loss of one copy of the 10q region, where PTEN maps, might also be explained by the presence of a second tumor suppressor gene located nearby PTEN.

We have screened 14 genes mapping in a ~3 Mbp region around PTEN for mRNA expression alterations in prostate cancer xenografts and cell lines. The frequency of complete PTEN inactivation reported in clinical prostate cancer is highly variable. This might partially be due to the different techniques used to detect complete inactivation of PTEN and to the different stages of disease analyzed.

The major mechanism of complete PTEN inactivation is by homozygous deletion. Most common point mutations produce a premature stop codon. Other frequent mutations are point mutations in the active site of the phosphatase domain of PTEN. Complete inactivation of PTEN is most often detected in metastatic prostate cancer, up to 60%, and less frequently in primary tumours, in ~15%. In prostate cancer PTEN is the most frequently mutated tumor suppressor gene found to date.

5.3.3 EGFR, ERBB and Neu

From the paper of Deutsch et al (2004) we have:

ERBB2 is a receptor tyrosine kinase that belongs to the epidermal growth-factor receptor family. Overexpression of ERBB2, which is seen in 20–30% of breast and ovarian cancers, is the result of gene amplification and is associated with a poor prognosis. In prostate cancer, the assessment of ERBB2 overexpression has been more difficult (procedural differences, and variability of tissue fixation protocols). Overexpression of ERBB2 has been associated with progression to androgen-independence in prostate cancer cells.

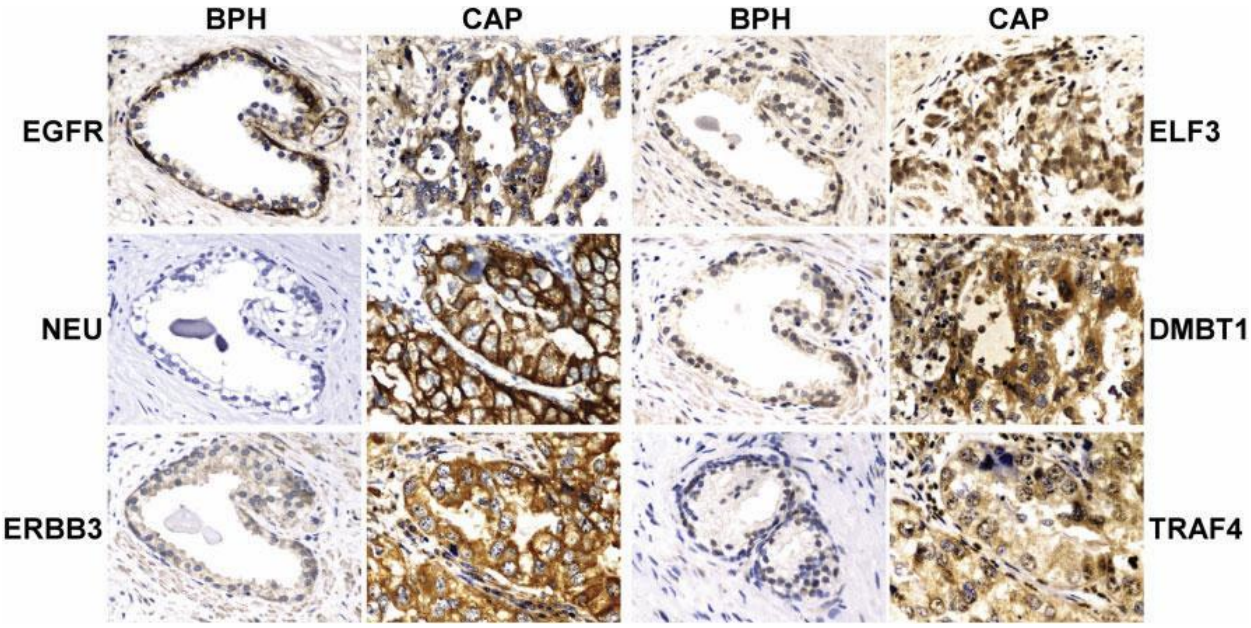
First, as discovered in SCID mice with the prostate cancer xenograft LAPC (Los Angeles prostate cancer), ERBB2 is overexpressed in androgen-independent cells. Second, overexpression of ERBB2 in androgen-dependent prostate-cancer cells activates the AR in a ligand independent ligand independent fashion, conferring androgen-independent growth on these cells. FISH studies of primary prostate cancer samples suggest that ERBB2 amplification and ERBB2 overexpression are significantly correlated with DNA content, advanced stage, and high-grade lesions.

The PI3K pathway is one of the most important signalling pathways activated by receptor tyrosine kinase. The PI3K pathway controls several important biological functions, such as cell growth regulation, apoptosis, and migration. As well as androgen signalling, which plays an essential part in the survival of prostate-cancer cells, the PI3K pathway is another important survival signal for these cells.[52] *The androgen-signalling and P13K pathways can compensate for each other in regulating the growth of prostate cancer cells. Since androgen treatment can rescue cells from apoptosis induced by PI3K inhibitors, these data suggest that the pathways are interconnected.*

Furthermore, the PI3K pathway interferes with AR mediated prostate-cancer cell survival and could change the stability of the AR protein. In addition, the IGF1 receptor can activate the PI3K pathway, resulting in phosphorylation of AR. The enhancement of AR transcriptional activity in the presence of epidermal growth factor (EGF) or IGF1 shows the synergistic interactions between these pathways.

Conversely, androgens promote overexpression of the EGF receptor, which is essential for androgen-induced proliferation and survival.

In a paper by Li et al they demonstrate the expression of EGFR. NEU and ERBB. We reproduce below the results from the Li et al paper:



5.3.4 NKX 3.1

NKX3.1 is a gene and Nkx3.1 its protein which is putatively a tumor suppressor gene which is primarily prostate specific. As c-Myc tends to regulate the transcription of many genes, Nkx3.1 regulates the control mechanism for the prostate cells. Even more specifically it has been argued that Nkx3.1 regulates the luminal cell growth by Iwata et al. Yet Goldstein et al would argue from similar murine models that use of up-regulated ERG translocations with Akt activation, namely putatively suppressing PTEN, allows for basal cell growth rather than luminal cell transformation.

It has been observed that diminished expression of NKX3.1 (8p21) is associated with prostate cancer progression in humans, and in mice, loss of nkx3.1 leads to epithelial cell proliferation and altered gene expression patterns….Loss of heterozygosity of 8p21 is observed in a high percentage of intraepithelial prostatic neoplasia and early carcinoma lesions, strongly implicating this region in the initial stages of prostate carcinogenesis. The importance of NKX3.1 as a dose-dependent regulator of prostate epithelial cell growth is strongly supported by analyses of nkx3.1 knockout mice . Homozygous nkx3.1 mutant mice develop prostate epithelial hyperplasia and dysplasia that progresses with age , and lesions with histologic features strongly resembling human prostatic intraepithelial neoplasia develop in homozygous mice between 1 and 2 years of age . Importantly, both hyperplasia and prostatic intraepithelial neoplasia-like lesions also occur in a significant proportion of nkx3.1 heterozygous mutants

The question then is, is NKX3.1 a true tumor suppressor gene? Despite that loss of function of Nkx3.1 predisposes to prostate cancer, it is not sufficient for tumorigenesis.(Shen 2003) Moreover, while one allele of NKX3.1 is lost by means of chromosomal deletion in PIN and prostate cancer, the other allele does not undergo mutational inactivation, although protein expression is epigenetically down-regulated or lost. These features, along with the relatively subtle consequences following forced expression of Nkx3.1 in prostate cancer cells are not consistent with activities of "classic" tumor suppressor genes, such as p53, Rb, or Pten. Instead, Nkx3.1 appears to act more like a tumor modulator, serving as a regulator of differentiation, which in turn prevents cancer initiation. In this regard, further analyses of NKX3.1 can provide important insights into the relationship between regulation of differentiation and carcinogenesis.

As Iwata et al have observed:

The prevailing model of NKX3.1 expression in human prostate cancer suggests that while the protein may decrease in PIN lesions, it is much more commonly decreased in invasive adenocarcinomas, and nearly completely lost in most, if not all, metastatic prostate adenocarcinomas … there was a variable decrease in expression of Nkx3.1 in PIN lesions, and that Nkx3.1 was virtually completely lost in invasive adenocarcinomas… several observations from our group regarding NKX3.1 differ from this prevailing view. First, in a previous report, while reductions of NKX3.1 protein occurred in PIN lesions and some adenocarcinomas, the reductions were relatively minor and virtually all invasive adenocarcinomas retained significant levels of NKX3.1 protein …

More recently we have found that the majority of very high grade (Gleason score 8–10) localized prostate cancers … retain high levels of expression of NKX3.1 protein. In the present study we found that, as compared to high grade PIN, the staining for Nkx3.1 protein actually increased substantially in pre-invasive cribriform PIN/CIS lesions and in early invasive adenocarcinomas, and these levels correlated inversely with levels of MYC expression.

These results indicate the Nkx3.1 may be dynamically regulated during progression of this disease. … It is possible, therefore, that Nkx3.1 expression in invasive prostatic acini in MYC-driven mouse prostate cancers may represent a recapitulation or caricature of the process of stromal invasion/branching morphogenesis in development, and, that Nkx3.1 may facilitate this process. …

Lei et al. found that forced restoration of Nkx3.1 expression in Pten null epithelium led to decreased cell proliferation, increased cell death, and prevention of tumor initiation .. They further showed that Nkx3.1 was required to engage the p53 pathway, indicating that reduced Nkx3.1 expression can itself abrogate p53 signaling.

These findings raise the interesting possibility that the reduction in Nkx3.1 seen upon the induction of MYC in the mouse prostate prevents the induction of p53 induced apoptosis, thus facilitating MYC's ability to transform these cells. Additional studies in which Nkx3.1 expression is kept at high levels during induction of MYC in prostate epithelium will be required to address this question further. We do not know precisely how MYC is regulating Nkx 3.1 protein expression, …"

Specifically Iwata et al state:

Since MYC may downregulate Nkx3.1 at the level of transcription …, it is possible that elevated MYC itself may be responsible for down-regulating Nkx3.1 expression.
In effect, this implies that MYC controls NKX3.1 and thus up-regulated MYC results in a down regulated NKX3.1. If NKX3.1 is controlling prostate stability then its overall regulation is via MYC. Controlling and suppressing MYC would control and up-regulate NKX3.1 and thus stabilize prostate growth. The complete pathway for this gene does not seem to be complete at this stage. Its importance is well defined however.

5.3.5 NF-κB

NF-κB is a transcription factor that resides in the cytoplasm. It is called Nuclear Factor and was identified by David Baltimore as an enhancer factor for the κ chain of Ig light chain in B lymphocytes. When activated it moves to the nucleus and is a transcription factor in activating over 400 genes. It is activated by a large number of stimuli and its action of a large gene set causes significant DNA activity. NF-κB appears on 10q24 and is somatic and acts in a dominant manner.

In a recent paper by Zhang et al they state:

The majority of tumors progressing during androgen deprivation therapy (referred to here as androgen deprivation- resistant prostate cancer or ADRPC) express higher levels of AR transcript and protein suggesting that a marked increase in AR expression is a critical event in therapy resistance…

Recent studies also demonstrate that increased AR expression is both necessary and sufficient to convert prostate cancer growth from a hormone therapy-sensitive to a resistant state in xenograft models.. Since AR mRNA levels are often increased in ADRPC without gene amplification,…

it is likely mediated by transcription factors and transcription regulating signal transduction pathways that are altered during progression.

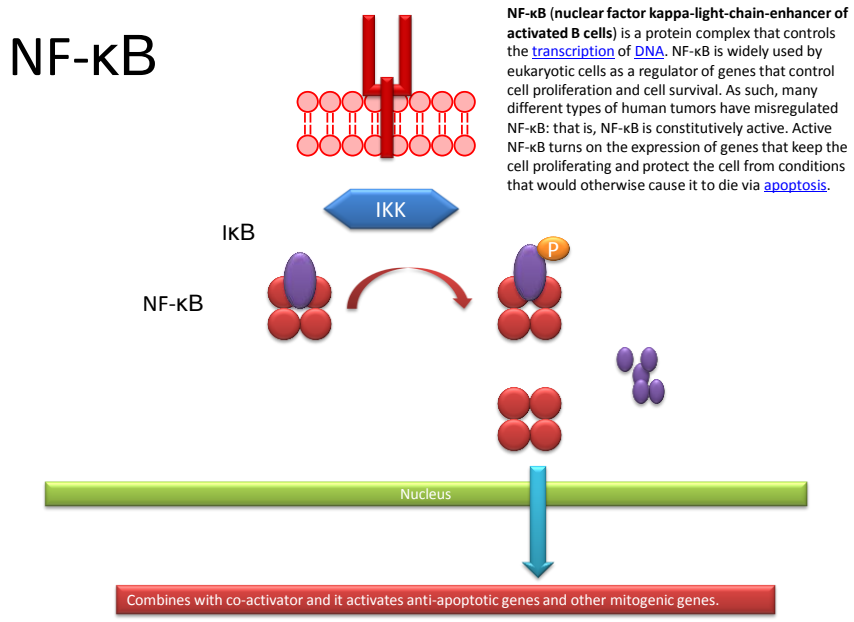
Nuclear Factor (NF)-κB is a family of transcription factors composed of homo- and hetero-dimers initially identified as an enhancer binding protein for the immunoglobulin light chain in B lymphocytes…

Zhang continues:

Several studies have examined the expression of NF-κB in human prostate cancer and its relationship to clinical features of the disease. NF-κB/p65 is overexpressed in prostatic intraepithelial neoplasia and cancer compared with benign epithelium. Nuclear levels of NF-κB/p65 correlate with NF-κB-dependent expression of BclII, cyclin D1, matrix metalloproteinase-9, and vascular endothelial growth factor.

Recent work indicates that NF-κB/p65 expression is predictive of biochemical recurrence in patients with positive surgical margins after radical prostatectomy and nuclear localization of NF-κB is increased in prostate cancer lymph node metastasis and can be used to predict patient outcome. These results demonstrate that NF-κB/p65 is frequently activated in human prostate adenocarcinoma and expression may be related to progression.

We now depict this putative pathway based upon the work of Kwang and Aggarwal. This is shown below. Activated NF-κB is clearly an activator of an anti-apoptosis process in the nucleus. The paper by Huang et al shows that blockade of NF-κB is an effective suppressor of angiogenesis, invasion and metastasis of prostate cancer.



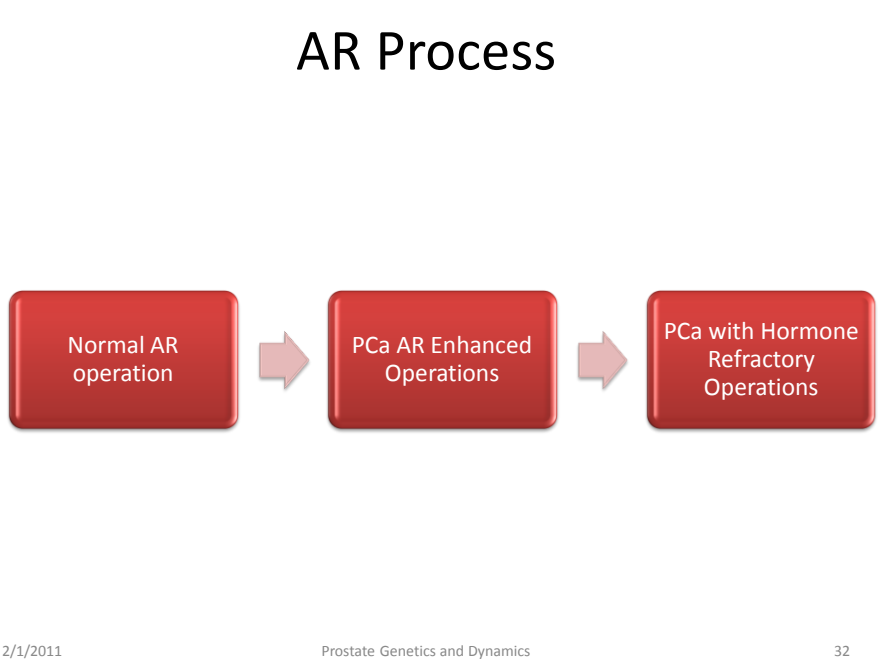
5.3.6 AR

The Androgen Receptor, AR, is located on Xq12. Androgens mediate a wide range of developmental and physiological responses and are especially important in male sexual differentiation and pubertal sexual maturation, the maintenance of spermatogenesis, and male gonadotropin regulation. The principle steroidal androgens, testosterone and its metabolite DHT (5-Alpha-Dihydrotestosterone), mediate their biological effects predominantly through binding to the AR (Androgen Receptor), an androgen-inducible member of the nuclear receptor super-family of transcription factors.

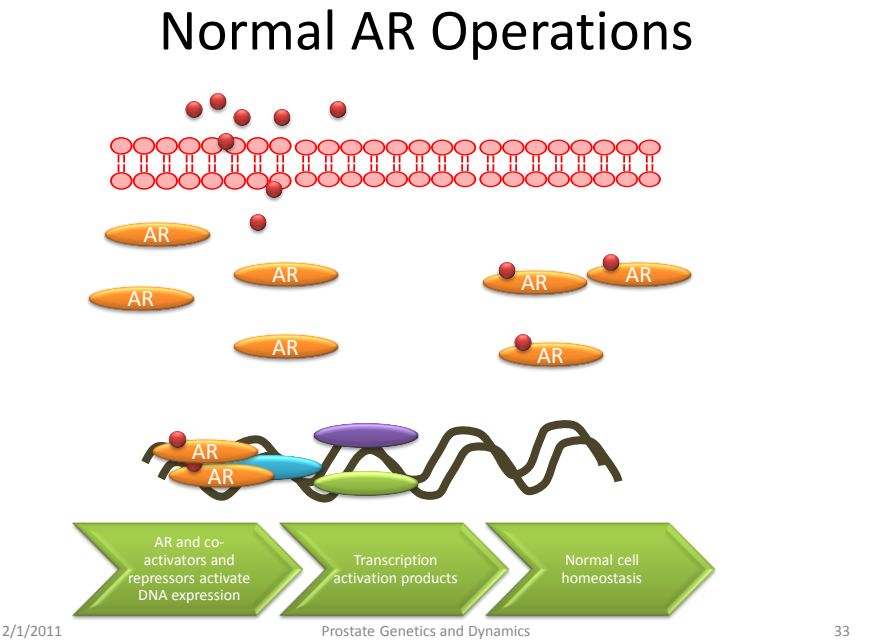
The normal function of the Androgen Receptor is as follows:

1. Testosterone enters the cell
2. If 5-α-Reductase is present the testosterone is converted to dihydrotestosterone, DHT.
3. The DHT then binds with the AR and the entity undergoes a transformation and releases heat shock proteins, HSPS
4. Then there is a phosphorylation
5. The AR translocates to the nucleus where it dimerizes, and there is DNA binding.
6. Target genes are then transcribed.

AR mediates transcription of proteins which are essential for normal development. However as PCa progresses there is at first normal AR operation, then it is enhanced, and then the PCa which was dependent upon the AR function can become independent of it altogether. We depict that process below.

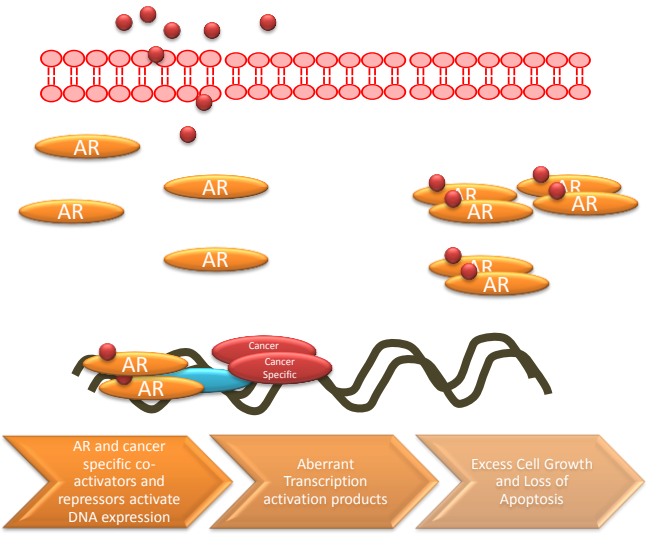


In normal AR operations, we show below the Testosterone coming into the cell and then it binds with the AR. It is this normal bonding which gives the AR the ability to manage a significant portion of the normal growth of the prostate cell. We use the graphics from Turner (2010) as modified below:



In the case of PCa we see the AR playing the role of excess growth enhancer.

Cancer and AR Operations



2/1/2011 Prostate Genetics and Dynamics 34

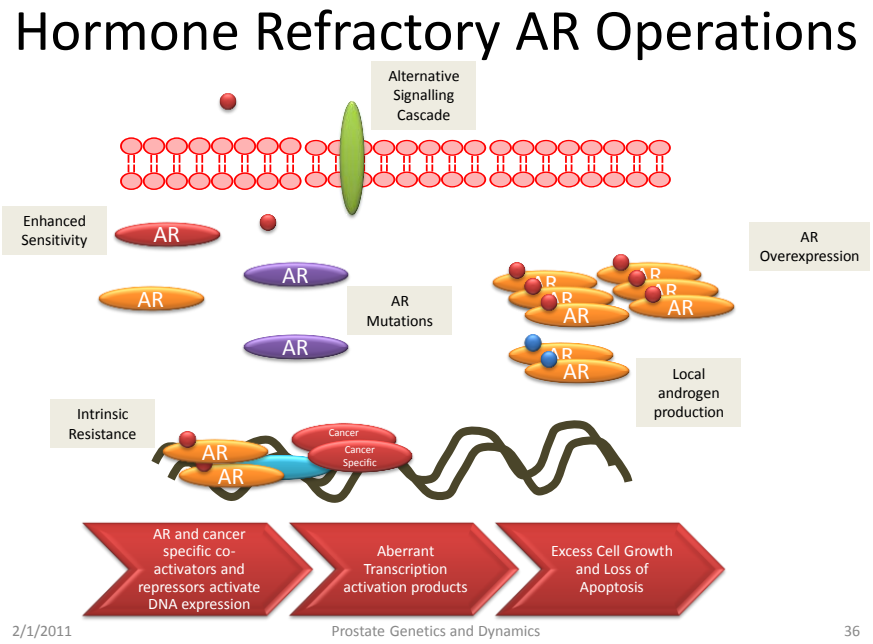
As is best understood, the progression towards AR resistant PCa follows the path shown below.

Hormone Refractory Options

Enhanced AR Sensitivity	AR Mutations	Alternative Signalling Cascades	AR Overexpression	Local Androgen Expression	Intrinsic Resistance
•The reduced androgens interact with increased ARs so that the efficiency is increased	•The ARs are mutated so that they are more efficient	•The signalling channels on the cells surface may be modified	•The ARs may have an ability to excessively produce excess receptors	•Local internal cytoplasmic androgen production may occur	•Cancer cells may be those with low intrinsic resistance

2/1/2011 Prostate Genetics and Dynamics 35

When the cell becomes refractory to AR functions, there may at first be AR overexpression and then a set of PCa specific receptors develop which result in metastatic grown as depicted below.



The recent work by Niu et al and that of Vis and Schroder describe in detail many of the specifics of the operation of the AR as currently understood. As regards to some details on specific pathway expressions the work of Nantermet et al (2004) states:

The androgen receptor (AR), when complexed with 5-dihydrotestosterone (DHT), supports the survival and proliferation of prostate cells, a process critical for normal development, benign prostatic hypertrophy, and tumorigenesis. However, the androgen-responsive genetic pathways that control prostate cell division and differentiation are largely unknown.

To identify such pathways, we examined gene expression in the ventral prostate 6 and 24 h after DHT administration to androgen- depleted rats. 234 transcripts were expressed significantly differently from controls ($p < 0.05$) at both time points and were subjected to extensive data mining. Functional clustering of the data reveals that the majority of these genes can be classified as participating in induction of secretory activity, metabolic activation, and intracellular signaling/signal transduction, indicating that AR rapidly modulates the expression of genes involved in proliferation and differentiation in the prostate.

Notably AR represses the expression of several key cell cycle inhibitors, while modulating members of the ***wnt and notch signaling pathways****, multiple growth factors, and peptide hormone signaling systems, and genes involved in MAP kinase and calcium signaling. Analysis of these data also suggested that* ***p53 activity is negatively*** *regulated by AR activation even though p53 RNA was unchanged. Experiments in LNCaP prostate cancer cells reveal that AR*

inhibits p53 protein accumulation *in the nucleus, providing a post-transcriptional mechanism by which androgens control prostate cell growth and survival. In summary these data provide a comprehensive view of the earliest events in AR-mediated prostate cell proliferation in vivo, and suggest that nuclear exclusion of p53 is a critical step in prostate growth.*

The authors continue:

AR induces cell proliferation and apoptosis in part because of its effects on cell-cell communication, particularly the stromal-epithelial interaction . As expected, the insulin-like growth factor (IGF-1) signaling system, which plays an essential role in prostate growth, was regulated at the level of ligand (IGF-1 was induced), extracellular binding protein (IGF-BP3 was repressed), and receptor (the IGF-1 receptor-1 exhibited biphasic expression). Also as expected, epidermal growth factor, which is induced by androgens in the prostate epithelium was upregulated . In addition to these well studied factors, several genes with potentially novel roles in the prostate were identified .

These include the transforming growth factor-2 (TGF-2) secretory partner latent TGF-binding protein- 1 (Ltbp1), which was repressed. Although the role of TGF-proteins in growth repression has been documented (e.g. Ref. 44), latent TGF-binding protein 1 function in the prostate has not been extensively studied, although its expression might be frequently reduced in PCa. DHT also repressed granulin/epithelin (Grn), a cysteine-rich growth factor expressed throughout the reproductive tract that regulates growth in multiple epithelial cell types.

Given the role of Grn in certain epithelial neoplasias, it would be interesting to examine its expression in PCa. Finally, DHT led to the down-regulation of ephrin-A1/B61 (Efna1), a ligand for the Eph receptors expressed in various epithelia. EFNA1 regulates cell growth and inhibits tumor angiogenesis; its function in the prostate is unknown.

In short, other than p53 suppression, and a collection of other genes, there is not significant addition to what is already known. The Chen and Sawyers discussion ends with:

Despite the fact that AR occupies such a central role in prostate physiology and pathology, there ***is little insight into the direct AR target genes responsible for disease progression****. One very intriguing possibility, based on the high frequency of TMPRSS2-ETS fusion, is that the primary effect of antiandrogen therapy is to reduce expression of this presumed oncogene. Even though AR is overexpressed in more advanced stages of prostate cancer, recent profiling studies indicate that many AR target genes are actually expressed at lower levels in high-grade and metastatic lesions.*

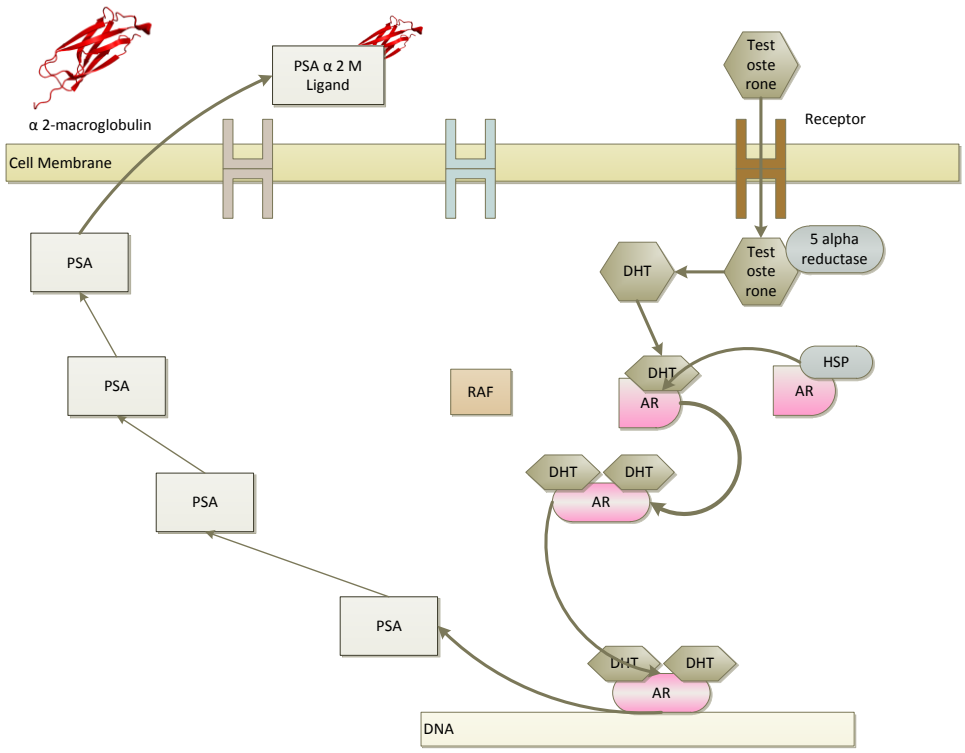
This finding is consistent with older data that cancers with a high Gleason grade often produce lower levels of PSA and other markers of differentiation. Therefore, the relationship between the AR pathway and castrate resistance may also reflect the differentiation state of late-stage tumors. A small subset of very aggressive prostate cancers (small cell variant) does not express AR.

Clearly the importance of the AR is critical in PCa as it progresses and yet as noted above the full pathway development is still lacking.

5.3.7 GRP78

In the normal course of prostate cell homeostasis, PSA is produced by the binding of Testosterone to the ligand on the luminal cell and the resulting transformation to DHT and its binding to the Androgen Receptor which acts in turn as a transcription factor for PSA. PSA exists mostly in complexed and inactive form; bound to alpha 2 macroglobulin and alpha 1 antichymotrypsin. 40% of the bound is with alpha 2 macroglobulin. Half-life PSA is 2.2-3.2 days. The baseline may takes weeks to return to normal after certain procedures.

We show this process below (See Feldman and Feldman, 2001):



In the above the PSA is released and then is free, it may bind with alpha 2 macroglobulin or with alpha 1 antichymotrypsin. In a benign configuration there is generally 35% of the PSA remaining free and unbound.

Now in a recent paper by Misra et al (2011) they discuss the impact of GRP78 in cancerous cells. In a recent write-up of the work the authors state[12]:

[12] http://www.eurekalert.org/pub_releases/2011-01/dumc-rlw011311.php

Using human prostate cancer cells in a laboratory culture, the team found that an antibody reacts with a cell surface receptor called GRP78 on the cancer cells to produce more PSA. The PSA arises inside of the cancer cell and then moves outside of the cell, where it can bind with the same antibody, called alpha2-macroglobulin (α2M).

The PSA forms a complex with the antibody that also binds to the GRP78 receptor, and that activates several key pathways which stimulate cancer cell growth and cell movement and block cell death.

The study bolsters the case for measuring PSA as a marker of tumor progression, as well as for monitoring for α2M antibody levels.

That is the release of GRP78 enforces the release of PSA, bound to alpha-2-M which increases PSA total and reduces PSA free. The GRP78 is then an added marker for excess cell growth, and thus a putative marker for PCa and putatively PSA total and percent free than has substantially increased significance for monitoring PCa.

Also this is a positive feedback loop, the link to the enhancement of Akt then is an enhancement of cell proliferation and growth even with the negative influence of PTEN if present.

GRP78, a well characterized chaperone in the endoplasmic reticulum, is critical to the unfolded protein response. As Lee (2007) states:

The glucose-regulated protein GRP78, also referred to as BiP (immunoglobulin heavy-chain binding protein), was discovered in the late 1970s together with GRP94 and GRP58 as cellular proteins induced by glucose starvation. Residing primarily in the ER, GRP78 belongs to the HSP70 protein family, which plays critical roles in the stress of oncogenesis.

In addition to facilitating proper protein folding, preventing intermediates from aggregating, and targeting misfolded protein for proteasome degradation, GRP78 also binds Ca^{2+} and serves as an ER stress signaling regulator. In nonstressed cells, GRP78 binds to ER transmembrane sensor proteins PERK, IRE1, and ATF6 and maintains them in an inactive form. When unfolded proteins pull GRP78 away from them, these pathways are activated, sending signals to the nucleus to trigger the UPR.

As Pootrakul et al state:

The glucose-regulated proteins (GRP) were initially identified as such in transformed chick embryo fibroblasts growing in glucose-deprived medium. The most well studied member of the GRP family is Grp78, a 78-kDa protein also recognized as immunoglobulin heavy-chain binding protein (BiP).

Normal functions of Grp78, which resides in the endoplasmic reticulum (ER) lumen, include proper folding and assembly of other polypeptides leading to formation of functional proteins, retention of unassembled precursors to the ER, targeting mis-folded protein for degradation, ER Ca^{2+} binding, and the regulation of trans-membrane ER stress inducers. The involvement if

Grp78 in enhanced cell survival is suggested by the remarkable elevation of GRP78 transcription rates under various stress conditions.

Recently, Grp78 has been shown to directly interact with intermediates of the apoptotic pathway, blocking caspase activation, where Grp78induction results in increased cell survival and inhibition of apoptosis.

More recently, it has been identified on the cell surface, where it has many roles. On cancer cells, it functions as a signaling receptor coupled to proproliferative-antiapoptotic and promigratory mechanisms.

In the current study, Misra et al (2011) demonstrate that:

1. Ligation of prostate cancer cell surface GRP78 by its natural ligand, activated α_2-macroglobulin (α_2M*),

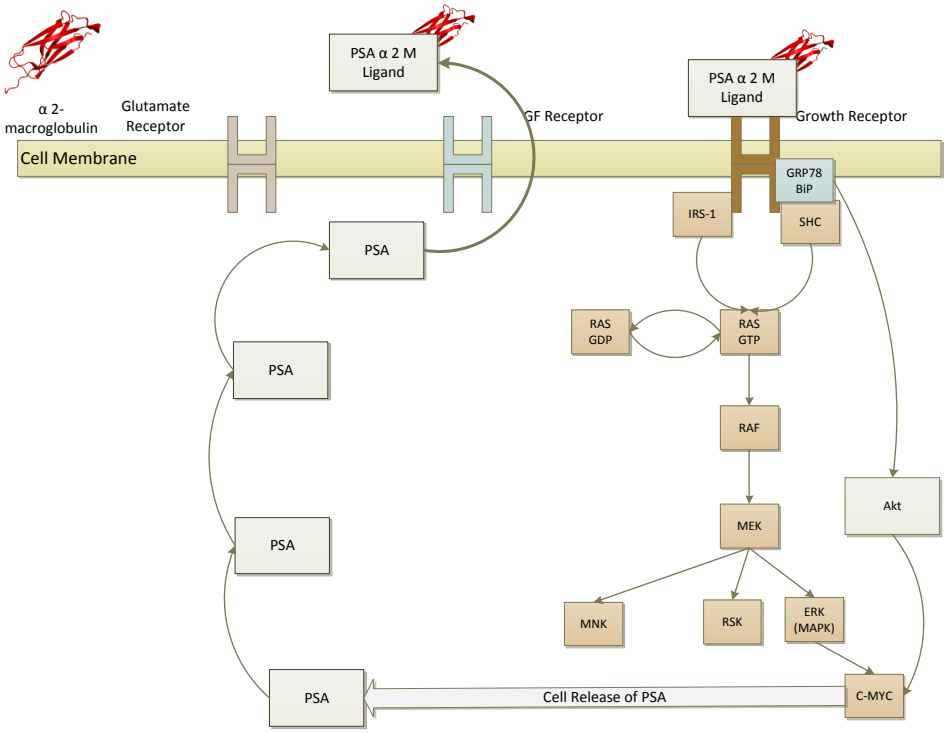
2. Results in a 2–3-fold up-regulation in the synthesis of prostate-specific antigen (PSA).

3. The PSA is secreted into the medium as an active proteinase, where it binds to native α_2M.

4. The resultant α_2M·PSA complexes bind to GRP78, causing a 1.5–2-fold increase in the activation of MEK1/2, ERK1/2, S6K, and Akt,

5. Which is coupled with a 2–3-fold increase in DNA and protein synthesis.

PSA is a marker for the progression of prostate cancer, but its mechanistic role in the disease is unclear. The present studies suggest that PSA may be involved in a signal transduction-dependent feedback loop, whereby it promotes a more aggressive behavior by human prostate cancer cells. We demonstrate this below:



5.3.8 TPMRSS2-ERG

One of the few known fusions or translocations involved in PCa is the TMPRSS2 and ERG fusion. It is seen in PCa as well as HGPIN. Its presence is known to be a marker for highly aggressive PCa.

5.3.8.1 ETS Family

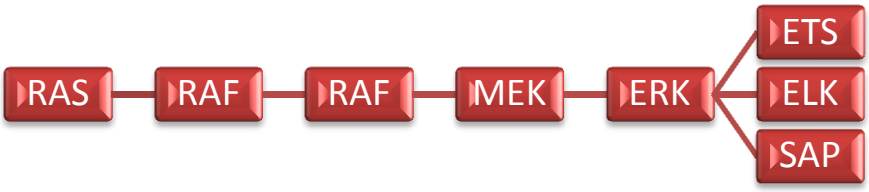
The ETS family of genes are positive or negative regulators of gene expression. They can up or down regulate expression. They are named for the initial gene discovered, the E26 Transforming Sequence, where E26 was the oncogene v-ets characterized in 1986 of an avian transforming virus called E26. It is also called the erythroblast transforming specific family, as discussed by Zong et al.

The ETS family is a large family of over 20 such genes, and we will focus on ERG specifically. The Table below is from Watson et al.

	Subgroup	Name	Unigene Name	Alternative Names	Locus	Size
1	ETS	ETS1	ETS1		11q23.3	441
2		ETS2	ETS2		21q22.3	469
3	ERG	ERG2	ERG		21q22.3	462
4		FLI1	FLI1	ERGB	11q24.1-q24.3	452
5		FEV	FEV		2q36	238
6	PEA3	PEA3	ETV4	E1AF, PEAS3	17q21	462
7		ERM	ETV5		3q28	510
8		ER81	ETV1		7p21.3	458
9	ETV	ER71	ETV2	ETSRP71	19q13.12	370
10	TCF	ELK1	ELK1		Xp11.2	428
11		SAP1	ELK4		1q32	431
12		NET	ELK3	SAP2, ERP	12q23	407
13	GABP	GABP α	GABPA	E4TF1	21q21.3	454
14	ELF1	ELF1	ELF1		13q13	619
15		NERF	ELF2	NERF1, NERF2, EU32	4q28	581
16		MEF	ELF4	ELFR	Xq26	663
17	SPI1	SPI1	SPI1	PU.1, SFPI1, SPI-A	11p11.2	264
18		SPIB	SPIB		19q13.3-q13.4	262
19		SPIC	SPIC		12q23.2	248
20	TEL	TEL	ETV6		12p13	452
21		TEL2	ETV7	TEL-B	6p21	264
22	ERF	ERF	ERF		19q13	548
23		PE-1	ETV3	METS	1q21-q23	250
24	PDEF	PDEF	SPDEF		6p21.3	335
25	ESE	ESE1	ELF3	ESX, JEN, ERT, EPR1	1q32.2	371
26		ESE2	ELF5		11p13-p12	255
27		ESE3	EHF	ESEJ	11p12	300

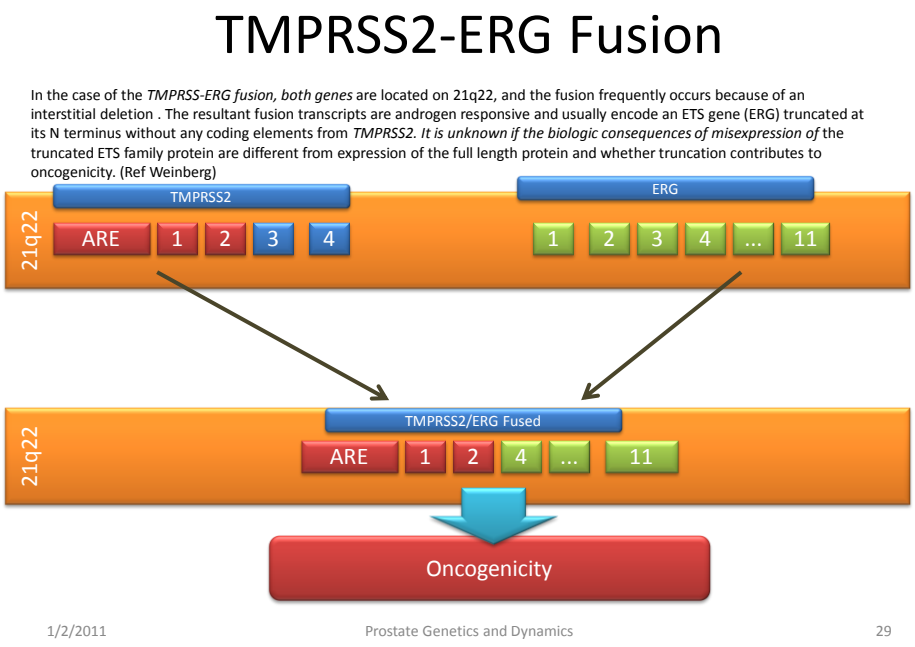
The ERG gene was first presented in the paper by Reddy et al in 1987. There the authors identified it and set it in the ETS family.

From Weinberg, we see that the ETS are transcription factors driven by the RAS/RAF pathway along with other such factors.

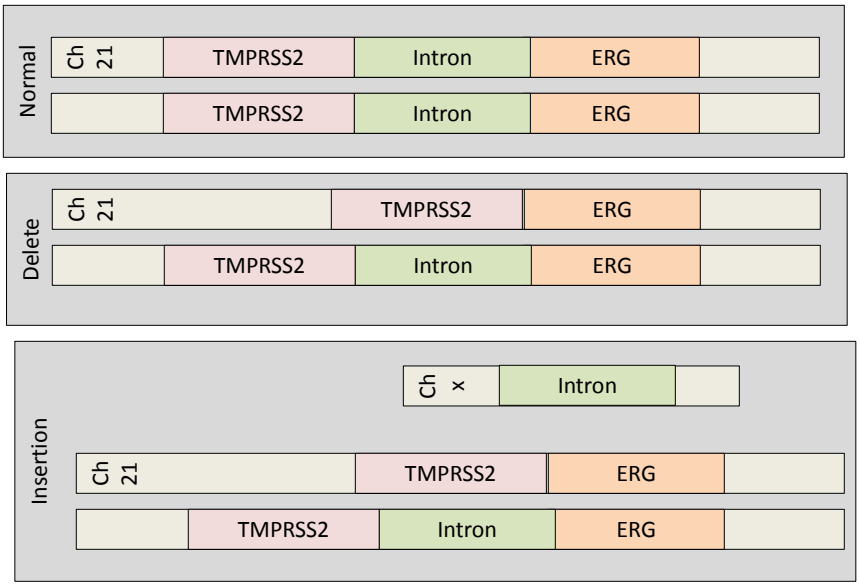


5.3.8.2 TMPRSS2 and ERG Fusion

The fusion of TMPRSS and ERG is another genetic promoter of PCa and it is the primary translocation gene seen.



Tomlins et all discuss the various conjectures regarding the fusions. The graphic below is based upon Tomlins et al. The example below shows the normal state on 21 and then the deletion, the intron is just removed, and then an insertion where the intron is removed but inserted elsewhere. See also the work by Mani et al (2009) regarding the gene fusions in general as applied to PCa. Also the work by Demichelis et al (2009), Marucci et al (2007) Iljin et al (2006) and Esgueva et al 2010) for extensions of this description.



It is thus the fused gene that cause the problem acting as an oncogene. This is unlike the other processes, for here we actually have genetic changes in location. The intron is 3Mb long so it is a nontrivial deletion. Unlike a methylation of a base pair element this requires substantial genetic change.

As the work of King et al state:

These data suggest that TMPRSS2-ERG is insufficient to initiate prostate neoplasia and that cooperating oncogenic lesions are required. Two relatively common abnormalities in human prostate cancer are PTEN loss and MYC amplification, both of which have pathogenic roles in genetically engineered mouse models

In a 2005 paper by Tomlins et al the authors discuss the fusion of the two genes, TMPRSS2 and ERG and the prevalence of this fusion in PCa. They relate the translocation and fusion of the genes in CML where BCR-ABL is fused to create a new gene, with an associated translocation, and then discuss the juxtapositioning of promoter and enhancers of one gene being juxtaposed to a proto-oncogene. Using a technique calls Cancer Outlier Profile Analysis, COPA, they had managed to isolate the fused product of TMPRSS2 and ERG in PCa. This is a fusion on 21q22. See also the work by Rubin and Chinnaiyan (2006) on the COPA analysis.

In the work of Esgueva et al the authors indicate that this fusion has several distinct features:

1. Murine models with overexpressed ERG with and without PTEN loss show a neoplastic phenotype.

2. ERG and histological features have been correlated. This is detailed in the paper by Mosquera et al (2007).

3. Specific pathways have been shown to have been rearranged, especially estrogen signalling.

4. Somatic copy number alterations have been found to be increased in ERG enhance PCa.

5. ERG rearranged PCa have highly negative outcomes.

6. ERG rearranged response to abiraterone is different.

The conclusion that Esgueva et al then reach is that ERG rearrange PCa is a different clinical class.

5.3.8.3 TMPRSS2:ERG HGPIN

There have been several studies on the relationship of this fusion to HGPIN. In Mosquera et al they state:

Given the more aggressive nature of TMPRSS2-ERG prostate cancer, the findings of this study raise the possibility that gene fusion-positive HGPIN lesions are harbingers of more aggressive disease. To date, pathologic, molecular, and clinical variables do not help stratify which men with HGPIN are at increased risk for a cancer diagnosis. Our results suggest that the detection of isolated TMPRSS2-ERG fusion HGPIN would improve the positive predictive value of finding TMPRSS2-ERG fusion prostate cancer in subsequent biopsies.

The authors then continue regarding HGPIN:

In the United States, approximately 1,300,000 prostate biopsies were done in 2006 with the detection of 234,460 new cases of prostate cancer. The incidence of isolated high-grade prostatic intraepithelial neoplasia (HGPIN) without carcinoma ranges from <1% to 16% , and the risk of finding carcinoma on subsequent biopsies is 10% to 39% [median risk of 24% (6)] depending on the time of repeat biopsy and number of cores.

A decline in the predictive value of HGPIN for prostate cancer to 20% in contemporary needle biopsies is most likely due to extended biopsy techniques that yield higher rates of cancer detection. Both HGPIN and prostate adenocarcinoma share molecular anomalies, including telomere shortening, RAR hypermethylation, allelic imbalances, and several chromosomal anomalies and c-myc amplification. Overexpression of p16, …, and altered proliferation and apoptosis in HGPIN and prostate cancer have also been shown…In particular, the TMPRSS2-ERG gene fusion prostate cancer is associated with higher tumor stage and tumor-specific death or metastasis . Two recent studies have shown the presence of TMPRSS2-ERG gene fusion in 20% of HGPIN lesions…

In a detailed study of murine models, Zong et al have concluded further the following:

1. *ERG Overexpression in Adult Murine Prostate Cells Results in Epithelial Hyperplasia and Focal PIN Lesions.*

2. *ERG-Transduced Prostate Glands Display a Skewed Cell Lineage Composition with Loss of Cytokeratin 5 (CK5)-Positive Basal Cells and Increased CD49f Expression in Luminal Cells.*

3. *ERG Overexpression Induces Up-Regulation of c-Myc and c-Jun Protein in Primary Prostate Epithelia.*

4. *Combined ERG Overexpression and p53 Deletion in Prostate Epithelia Does Not Result in Invasive Adenocarcinoma.*

5. *ERG Collaborates with Aberrant PI3K Pathway to Promote PCa Progression. Deletion of the tumor suppressor PTEN occurs in 68% of human PCas and results in activation of the PI3K pathway. We demonstrated that increased PI3K signaling via shRNA-mediated PTEN knockdown or overexpression of an activated form of AKT in murine prostate cells causes PIN lesions in the tissue-regeneration model. In this study, we combined overexpression of ERG and activated AKT and found that grafts derived from co-infected adult prostate cells weighed 2–3 times more than grafts generated from AKT or ERG overexpression alone. In contrast to AKT-induced PIN lesions, the prostate glands that simultaneously overexpressed ERG and AKT/GFP exhibited a cribriform growth pattern with cell crowding and embedded acini. The cells in these proliferative foci exhibited nuclear atypia, evidenced by hyperchromatic nuclei, mitotic figures, nuclear contour irregularity, and enlargement. These findings suggest that high levels of ERG protein collaborate with constitutively activated AKT kinase, leading to the development of invasive PCa.*

6. *High Levels of ERG Fully Transform Primary Prostate Cells Through Synergy with Enhanced AR Signaling. AR is commonly mutated or amplified in human PCa, and the AR pathway is the most extensively studied pathway in PCa because of its role in late-stage hormone-refractory PCa. Given that up-regulation of ETS transcription factors is mainly driven by the androgenresponsive TMPRSS2 promoter in most samples of human PCa, it is reasonable to hypothesize that both ETS overexpression and AR signaling coexist in malignant prostate epithelial cells.*

As we have discussed before, the subsequent work by Goldstein et al took this a step further and in murine models demonstrated the development of PIN and then PCa. However, the murine model is not exactly projectable to the human. In addition, there is no viable reverse path from HGPIN to benign cells. In fact the work of Demichelis et al indicate that watchful waiting, the proverbial do nothing strategy, is somewhat effective except in TMPRSS2:ERG fusion cases. However, the determination of the gene fusions is currently not common in prostate biopsies.

5.3.8.4 TMPRSS2:ERG Pathways and Control

There currently is limited pathway modeling of this fusion effect. We demonstrated the Weinberg ETS model and there is work by Yu et al showing AR control effects but no clear definitive pathway models seems to exist. A similar analysis of the AR driving of the ERG promoters is performed by Dobi et al (2010). Dobi et al conclude:

Expression of the ERG proto-oncogene, is activated in 50-70% of prostate tumors by androgen receptor (AR) mediated signals due to the fusion of AR regulated promoters (primarily TMPRSS2 and to a lesser extent SLC45A3 and NDRG1) to the ERG protein coding sequence.

Our previous studies of quantitative expression levels of ERG or TMPRSS2-ERG fusion transcripts have noted that relatively low or no ERG expression in prostate tumors significantly associated with progressive disease. Here, we have tested the hypothesis that ERG expression levels in prostate tumor cells reflect AR transcriptional regulatory function in a given biological context of the tumor progression.

Therefore, tumors with lower ERG may represent a subset with attenuated AR signaling. Expression of ERG and other AR regulated genes were evaluated …. Overall, ERG expression pattern was similar to that of other AR regulated genes. Strikingly low frequency of ERG expression was noted in PD tumor cells (30%) in comparison to WD tumor cells (80%), suggesting for subdued AR function in a significant fraction of tumors with genomic alterations of ERG. By integrating ERG into a panel of defined AR target genes, we developed a cumulative AR Function Index (ARFI), which if validated may have future potential in stratifying patients for targeted therapy on the basis of overall AR functional status in primary tumors….

Taken together, the ARFI approach reported here, if developed further has potential to stratify prostate tumors on the basis of in vivo functional status of AR which could lead to development of new paradigms in the treatment selection of patients for androgen ablation or other therapies. For example patients with ARFI positive versus ARFI negative/attenuated tumors may be identified in early stages of disease and latter may be more responsive to non-androgen ablation focused strategies.

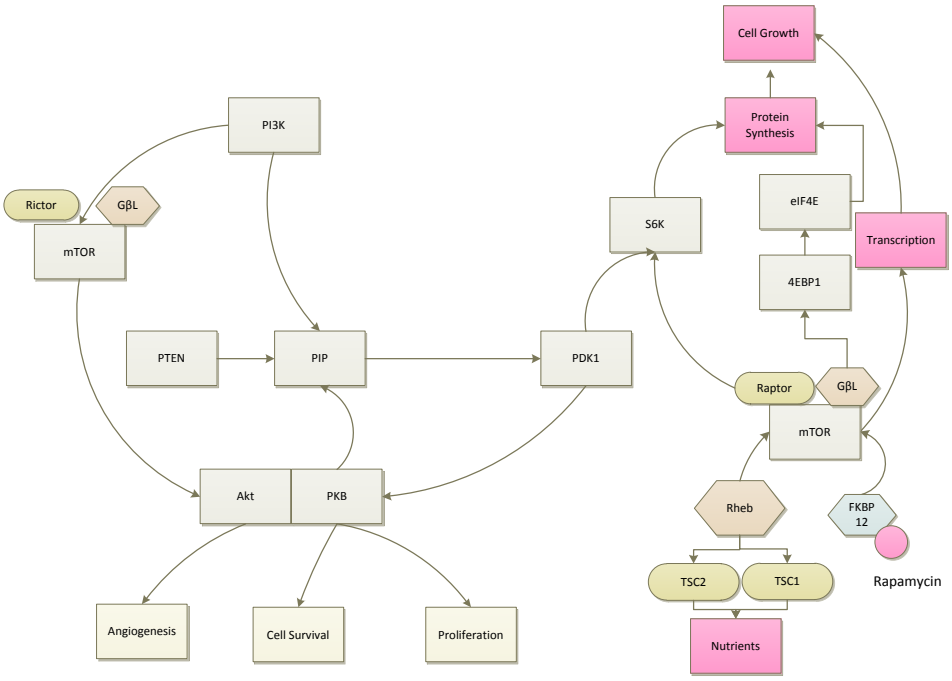
Along similar lines patients with ERG gene fusion but not expressing ERG may not benefit from a potential ERG targeted therapy. Alternatively patients with varying degree of ARFI positivity may need different androgen ablation therapy strategies. Finally, association of low or no ERG in a large percentage of poorly differentiated tumors appears to be either reflection of attenuated AR signaling in tumors harboring ERG fusions or a distinct class of tumors without ERG alterations.

Clearly the ERG fusion plays a significant role in PCa. The AR effects are critical and the overall ETS pathway architecture is also a controlling element. However there is no clear and well defined path and the mechanism for the fusion seems also to be now understood at this time.

5.3.9 mTOR

mTOR, the mammalian target of rapamycin, is a gene product (1p36.2) is a protein which acts in a critical manner in interconnecting the genetic circuits in mammals, and especially man. It fundamentally controls glucose transport and protein synthesis. The pathway depicted below is a modification of the graphic from Weinberg (p 785) which shows mTOR in its two modes, one with Raptor assisting and one with Rictor. The Rictor/mTOR mode activates the Akt pathway via the placement of a phosphate and the manages the protein synthesis portion. The inclusion of

rapamycin will block the Raptor/mTOR path and reduce the protein synthesis and cell growth portion. The inhibitory effect on Akt/PKB by rapamycin is assumed to be the main factor in its anti-cancer effects.



Looking at the complexity of the mTOR pathway it presents an interesting one for addressing PCa. Kinkaide et al (2008) indicate:

Among the major signaling networks that have been implicated in advanced prostate cancer are the AKT/mammalian target of rapamycin (AKT/mTOR) and MAPK pathways. Indeed, deregulated expression and/or mutations of the phosphate and tensin homolog tumor suppressor gene (PTEN) occur with high frequency in prostate cancer, leading to aberrant activation of AKT kinase activity as well as its downstream effectors, including the mTOR signaling pathway. In addition, many prostate tumors display deregulated growth factor signaling, which may result in activation of MAPK kinase 1 (MEK) kinase and ultimately ERK MAP.

Notably, previous studies have demonstrated that the AKT/mTOR and MAPK signaling pathways are alternatively and/ or coordinately expressed in advanced prostate cancer and function cooperatively to promote tumor growth and the emergence of hormone- refractory disease. These observations formed the basis for our hypothesis that targeting these signaling pathways combinatorially may be effective for inhibiting tumorigenicity and androgen independence in prostate cancer.

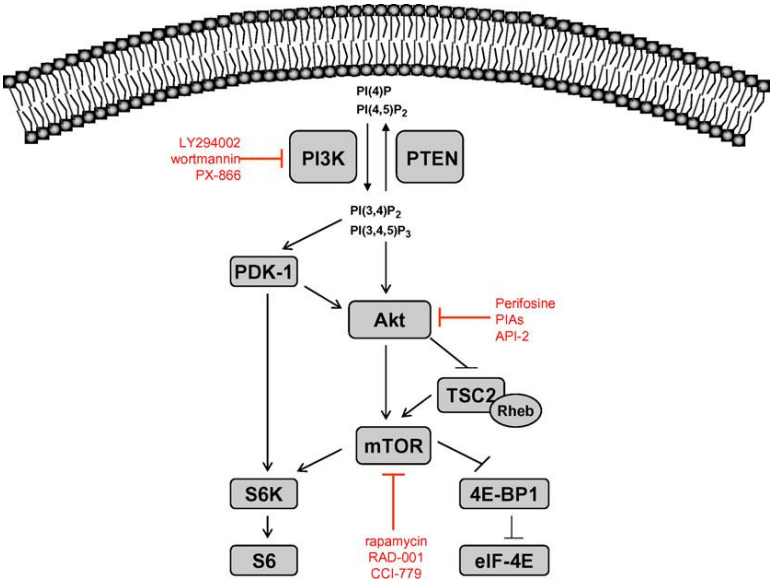
Kinkaide et al also demonstrate the creation of HGPIN via their work. This represents another pathway of HGPIN to PCa.

LoPiccolo et al state:

The PI3K/Akt/mTOR pathway is a prototypic survival pathway that is constitutively activated in many types of cancer. Mechanisms for pathway activation include loss of tumor suppressor PTEN function, amplification or mutation of PI3K, amplification or mutation of Akt, activation of growth factor receptors, and exposure to carcinogens. Once activated, signaling through Akt can be propagated to a diverse array of substrates, including mTOR, a key regulator of protein translation. This pathway is an attractive therapeutic target in cancer because it serves as a convergence point for many growth stimuli, and through its downstream substrates, controls cellular processes that contribute to the initiation and maintenance of cancer.

Moreover, activation of the Akt/mTOR pathway confers resistance to many types of cancer therapy, and is a poor prognostic factor for many types of cancers. This review will provide an update on the clinical progress of various agents that target the pathway, such as the Akt inhibitors perifosine and PX-866 and mTOR inhibitors (rapamycin, CCI-779, RAD-001) and discuss strategies to combine these pathway inhibitors with conventional chemotherapy, radiotherapy, as well as newer targeted agents. We (show) how the complex regulation of the PI3K/Akt/mTOR pathway poses practical issues concerning the design of clinical trials, potential toxicities and criteria for patient selection.

LoPiccolo et al show the more simplified pathway as follows:

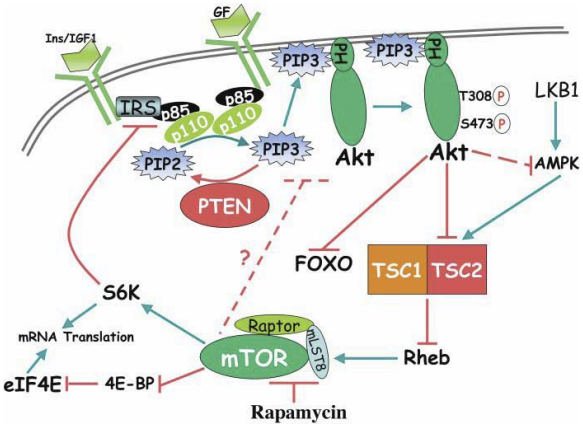


As we have shown with the more complex Weinberg model, here mTOR and PTEN play a strong role in the overall control. The authors show the points of possible control. The complexity of the pathways will be a challenge. It is less an issue of size complexity than a feedback and instability complexity. E Nelson et al (2007) have demonstrated similar results as well.

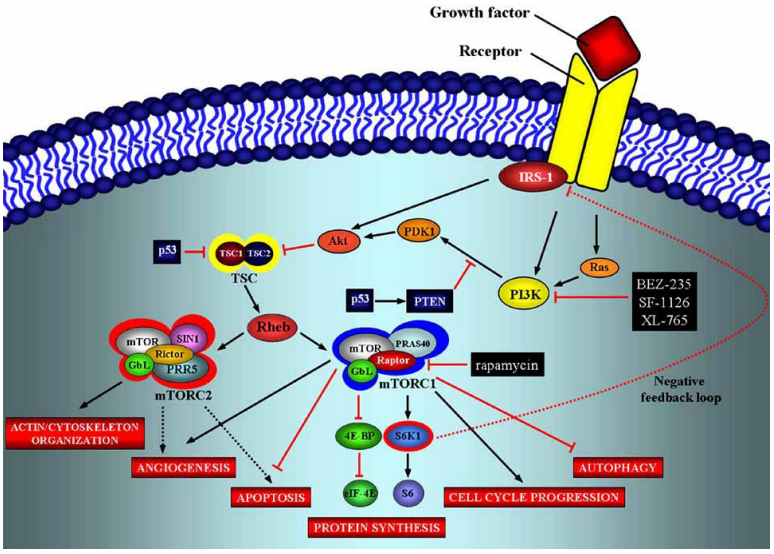
Other researchers have also posited other simple models. We demonstrated the one by Hay as has been stated:

The downstream effector of PI3K, Akt, is frequently hyperactivated in human cancers. A critical downstream effector of Akt, which contributes to tumorigenesis, is mTOR. In the PI3K/Akt/mTOR pathway, Akt is flanked by two tumor suppressors: PTEN, acting as a brake upstream of Akt, and TSC1/TSC2 heterodimer, acting as a brake downstream of Akt and upstream of mTOR.

In the absence of the TSC1/TSC2 brake, mTOR activity is unleashed to inhibit Akt via an inhibitory feedback mechanism. Two recent studies used mouse genetics to assess the roles of PTEN and TSC2 in cancer, underscoring the importance of Akt mTOR interplay for cancer progression and therapy.



An additional model has been proposed by Baldo et al (2008) which we depict below:



The Baldo et al model is quite similar to the Weinberg model shown initially. It clearly demonstrates the overall controlling influence of mTOR. As Baldo et al state:

There is a great body of evidence supporting consideration of the mTOR signaling system as an important network in cell regulation, differentiation and survival . mTOR is a sensor of mitogen, energy and nutritional levels, acting as a "switch" for cell-cycle progression from phase G1 to phase S.

The antibiotic Rapamycin, a potent mTOR inhibitor, has been known to the National Cancer Institute and recognized for its potential anticancer properties since the 1970s. The observation that cell lines from different cancer types exposed to low doses of Rapamycin underwent cell-cycle arrest in phase G1, provided the basis for considering mTOR as a target for cancer therapy.

Development of mTOR inhibitor compounds has proceeded empirically due to the lack of understanding of the precise molecular targets and the required dose of the new compounds . The development of Rapamycin analogs ("Rapalogs"), but also of other, structurally different, mTOR inhibitors, was directed at the selection of specific cancer type sensitivity and an optimization of pharmaceutical forms.

To give an example, Temsirolimus revealed clinical responses in patients with renal cell carcinoma in advanced stage. Temsirolimus was approved by the FDA on May 2007 for this therapeutic use and is being investigated in clinical trials for other cancer types (breast cancer, lymphoma, renal cancer, glioblastoma); significantly there are a considerable number of clinical studies involving mTOR inhibitors currently active worldwide…

The mTOR pathway controls cell size and cellular proliferation*…nutrient metabolism, mRNA translation and cell survival control. Disruption of TOR leads to early embryonic death in flies and mammalian cells, indicating mTOR plays an important role in regulating cell survival. … deregulation of several mTOR components leads to modified cell proliferation patterns and, on the other, that many mTOR components are deregulated in several human cancers.*

… ***Therefore, inhibition of mTOR leads to slowing or arrest of cells in the G1 phase****. Translational control may have an important role in the balance of cell survival and death, and hence for apoptosis. Importantly, components of mTOR are deregulated in some human cancers, for example, breast and colon. Alteration of PI3-K/Akt is frequently observed in head and neck cancer .*

PTEN, a phosphatase that acts on PIP3 to convert it to PIP2, normally regulates the mTOR pathway negatively, and shows decreased activity in some tumors. A strong relation seems to exist between the sensitivity to the effect of Rapamycin and PTEN loss or deregulation. PTEN is frequently mutated in several cancers and in cancer-like syndromes like Cowden and Proteus syndromes…

Loss of PTEN function can occur in 26-80% of endometrial carcinomas, …recent studies of human prostate cancer have shown that loss of PTEN is strongly associated with more aggressive cancers. The relationship between PTEN status and sensitivity to rapalogs has been questioned by several investigators. Some attention has recently been dedicated to the role of the mTORC2 complex in the mTOR pathway.

In fact this complex, believed until recently to be completely insensitive to the effect of Rapamycin, after long-term exposure to Rapamycin is able to prevent mTOR-mediated Akt phosphorylation and the activation of the mTOR pathway. Another component, the TSC1/TSC2 complex located upstream of mTOR, is predicted to integrate signals derived from nutrients, cellular energy status and hypoxia into a common growth regulatory signal to the mTORC1 complex.

As Easton and Houghton state:

Proteins regulating the mammalian target of rapamycin (mTOR), as well as some of the targets of the mTOR kinase, are overexpressed or mutated in cancer. Rapamycin, the naturally occurring inhibitor of mTOR, along with a number of recently developed rapamycin analogs (rapalogs) consisting of synthetically derived compounds containing minor chemical modifications to the parent structure, inhibit the growth of cell lines derived from multiple tumor types in vitro, and tumor models in vivo.

Results from clinical trials indicate that the rapalogs may be useful for the treatment of subsets of certain types of cancer. The sporadic responses from the initial clinical trials, based on the hypothesis of general translation inhibition of cancer cells are now beginning to be understood owing to a more complete understanding of the dynamics of mTOR regulation and the function of mTOR in the tumor microenvironment. This review will summarize the preclinical and clinical data and recent discoveries of the function of mTOR in cancer and growth regulation.

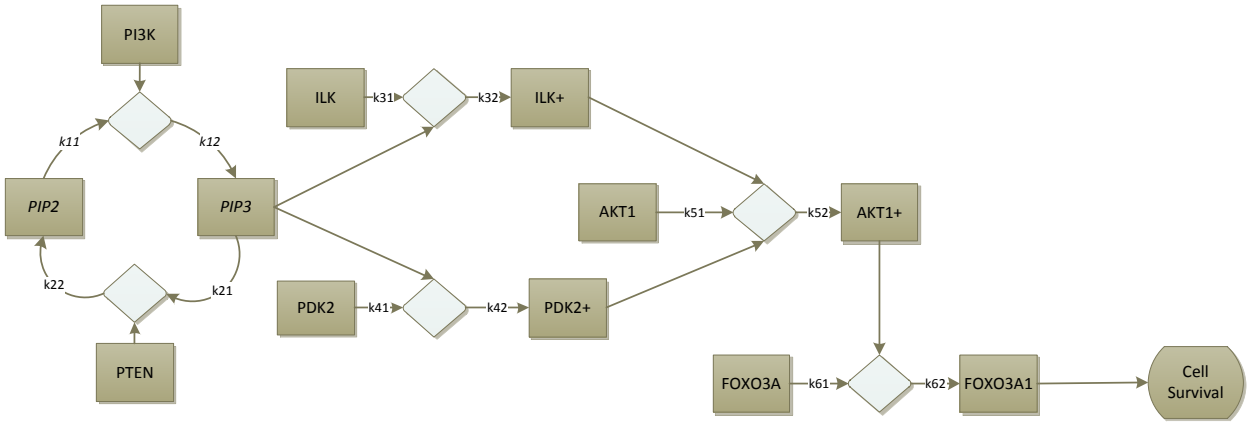
5.3.10 FOXO

The FOXO gene, specifically FOXO3a, forkedhead box zero gene, is located at 6q21 in humans and is a key nuclear transcription regulator. It has the ability to mediate cell cycle arrest, DNA repair, apoptosis and as such acts in many ways like a tumor suppressor gene. Loss of the FOXO gene activity may lead to uncontrolled cell growth. Also impairment or suppression of FOXO can result in impaired DNA repair capabilities as well. In a normal situation a reduced level of FOXO in a cell would lead to normal cell death however in cancerous cells this is no longer the case. As Lam et al state the FOXO molecule is key to the regulation of normal cell homeostasis. Although mutations in FOXO are not common it is the FOXO function controlled via PI3K and PTEN that often are of interest.

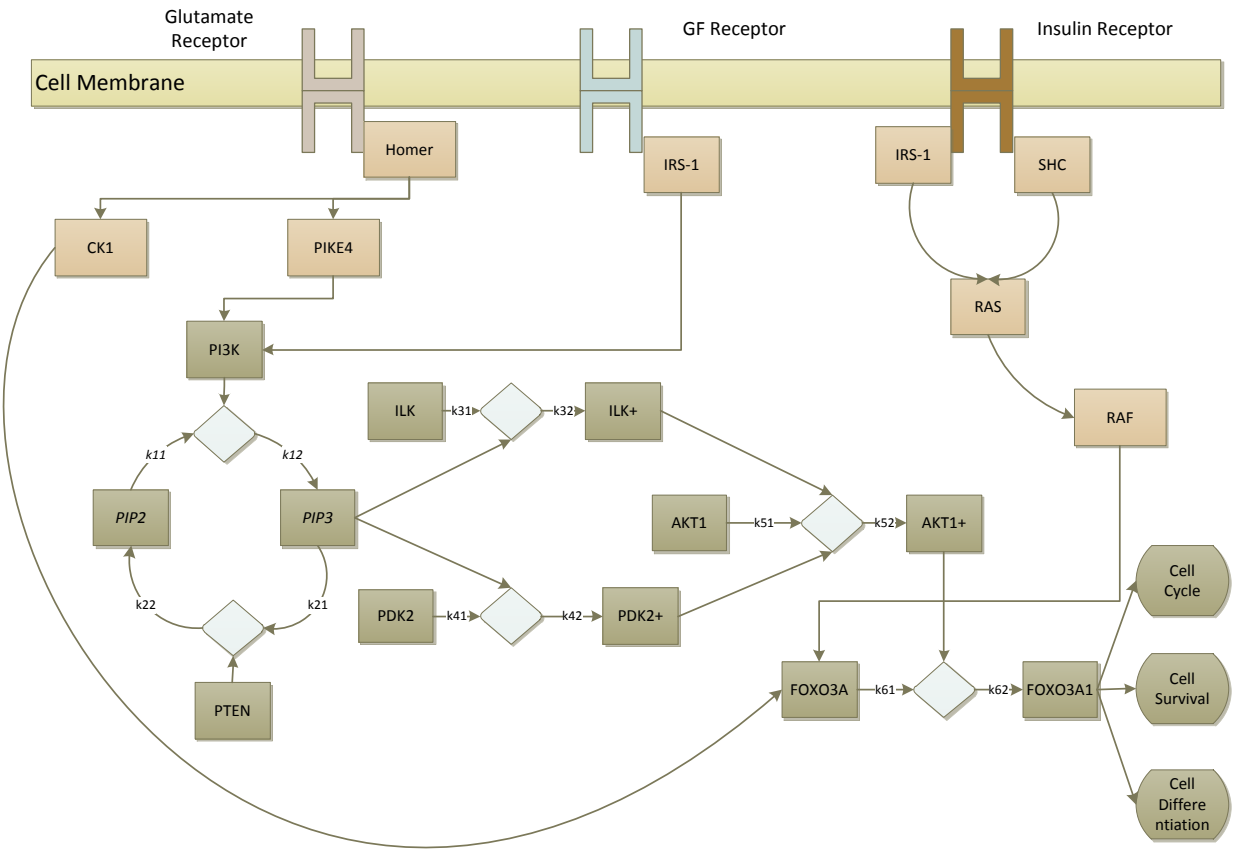
As noted by van der Heide et al, FOXO is a major player in pathways activated by Glutamate and insulin. We will depict that detail later. However the nexus to the insulin activator may also provide a connection to the role that inflammation may have in PCa and especially Type 2 Diabetes and its related hyperglycemia.

FOXO is a key element in the PI3K pathway and has its control facilitated by such elements as PTEN, growth factors, insulin and glutamate. As Essaghir et al state, in the absence of growth factors, FOXO remains in the nucleus and FOXO up-regulates genes which inhibit cell cycle such as p27 KIP1 and p21 WAF1. It also promotes apoptosis via the Fas ligand, Bim and TRAIL, and decreases oxidative stress. As a blocker of cell growth therefore FOXO is often considered as a tumor suppressor. There has been a recent interest in dealing with the FOXO gene directly as a way to control certain cancers as discussed by Yang et al (2010).

One view of the FOXO pathway is shown as follows:



However we can also add the receptors which are drivers of the internal elements. We do that as follows. This shows the multiple ligan responses, with limited detail regarding reactions. We have taken the pathway we have analyzed elsewhere and included it as a core element of the FOXO control mechanism.



FOXO is a facilitator gene, it facilitates homeostasis of the cell. However it is regulated by many genes above it which are often inhibited in their normal functions in a cancer cell.

As Lam et al state:

The PI3K signal transduction pathway critically regulates cell proliferation, differentiation and apoptosis. Perturbation in the PI3K signalling pathway is strongly implicated in the pathogenesis of many diseases, including heart and neural diseases, autoimmune/inflammatory disorders, cancer and the development of chemo- and endocrine-resistance in tumor cells.

Constitutive activation of the PI3K pathway, a hallmark of many cancers, is commonly a consequence of enhanced expression of genes that encode either class I PI3K subunits or PKB (protein kinase B) or is a result of genetic mutations that inhibit negative regulators of the pathway. For example, somatic deletions or mutations of PTEN (phosphatase and tensin homologue deleted on chromosome 10), an antagonist of the PI3K pathway, have been identified in a large proportion (12–60%) of human tumours of different tissue origins.

They continue:

In mammals, the ability of FOXO factors to mediate cell-cycle arrest, DNA repair and apoptosis makes them attractive candidates as tumor suppressors. Loss of FOXO function can lead to uncontrolled cell proliferation. Furthermore, reduced ability to repair damaged DNA due to

impaired FOXO activity may also result in genomic instability and carcinogenesis. Finally, a deficiency in FOXO proteins in abnormal and damaged cells that would normally undergo programmed cell death may result in tumor development and expansion.

FOXO transcription factors control cell proliferation and survival by regulating the expression of genes involved in cell-cycle progression [e.g. $p27_{Kip1}$, p130(RB2), cyclin D1/2 and Bcl-6 (B-cell lymphocytic leukemia proto-oncogene 6)] and apoptosis [e.g. Bim, Fas ligand, TRAIL (tumor-necrosis-factor-related apoptosis inducing ligand) and Bcl-X_L. Thus one way by which PKB and the related SGK promote cell survival is by phosphorylating FOXOs, which results in their sequestration in the cytoplasm away from cell death-inducing genes. PKB phosphorylation also reduces the DNA-binding ability of FOXO and enhances its degradation.

Common FOXO target genes that mediate apoptosis include bNIP3 and BCL2L11, which encode the pro-apoptotic Bcl-2 family members, bNIP3 and Bim. Furthermore, FOXOs also indirectly down-regulate the expression of the pro-survival Bcl-2 family member Bcl-X_L by inducing the expression of the transcriptional repressor Bcl-6 . In neurons, FOXO3a triggers cell death circuitously by inducing the expression of Fas Ligand, which triggers programmed cell death through the death receptor pathway.

Thus FOXO control is a strategic part of controlling cell growth and stability.

6 EPIGENETIC FACTORS

Epigenetic modifications are considered hereditable changes in gene expression occurring when there is no true underlying change in the DNA[13]. Epigenetic silencing of tumor suppressor genes is one of the most significant contributors to cancer development. Recent summaries by Esteller provides an excellent medical update of the broad reach of epigenetics in clinical medicine.

As Esteller states:

Classic genetics alone cannot explain the diversity of phenotypes within a population. Nor does classic genetics explain how, despite their identical DNA sequences, monozygotic twins or cloned animals can have different phenotypes and different susceptibilities to a disease. The concept of epigenetics offers a partial explanation of these phenomena. First introduced by C.H. Waddington in 1939 to name "the causal interactions between genes and their products, which bring the phenotype into being" epigenetics was later defined as heritable changes in gene expression that are not due to any alteration in the DNA sequence.

The best-known epigenetic marker is DNA methylation. The initial finding of global hypomethylation of DNA in human tumors was soon followed by the identification of hypermethylated tumor-suppressor genes, and then, more recently, the discovery of inactivation of microRNA (miRNA) genes by DNA methylation.

These and other demonstrations of how epigenetic changes can modify gene expression have led to human epigenome projects and epigenetic therapies. Moreover, we now know that DNA methylation occurs in a complex chromatin network and is influenced by the modifications in histone structure that are commonly disrupted in cancer cells.

We look at three epigenetic factors; micro RNAs, Methylation, and repressor and activator genes which are not in the path. We discuss briefly the first two elements here and then we look at the third when we assemble the model.

6.1 MIRNA

This section discusses the micro RNA process and its impact on PCa. Micro RNAs, miRNA, are small single stranded RNAs which when in the cytoplasm may often bind to other RNA on complement binding sites and thus change or incapacitate the mRNA to which it binds from being translated into a protein. Craig Mello was awarded the Nobel Prize in 2006 for the discovery and his Nobel Lecture provides an excellent overview of the early stages of miRNA investigation.

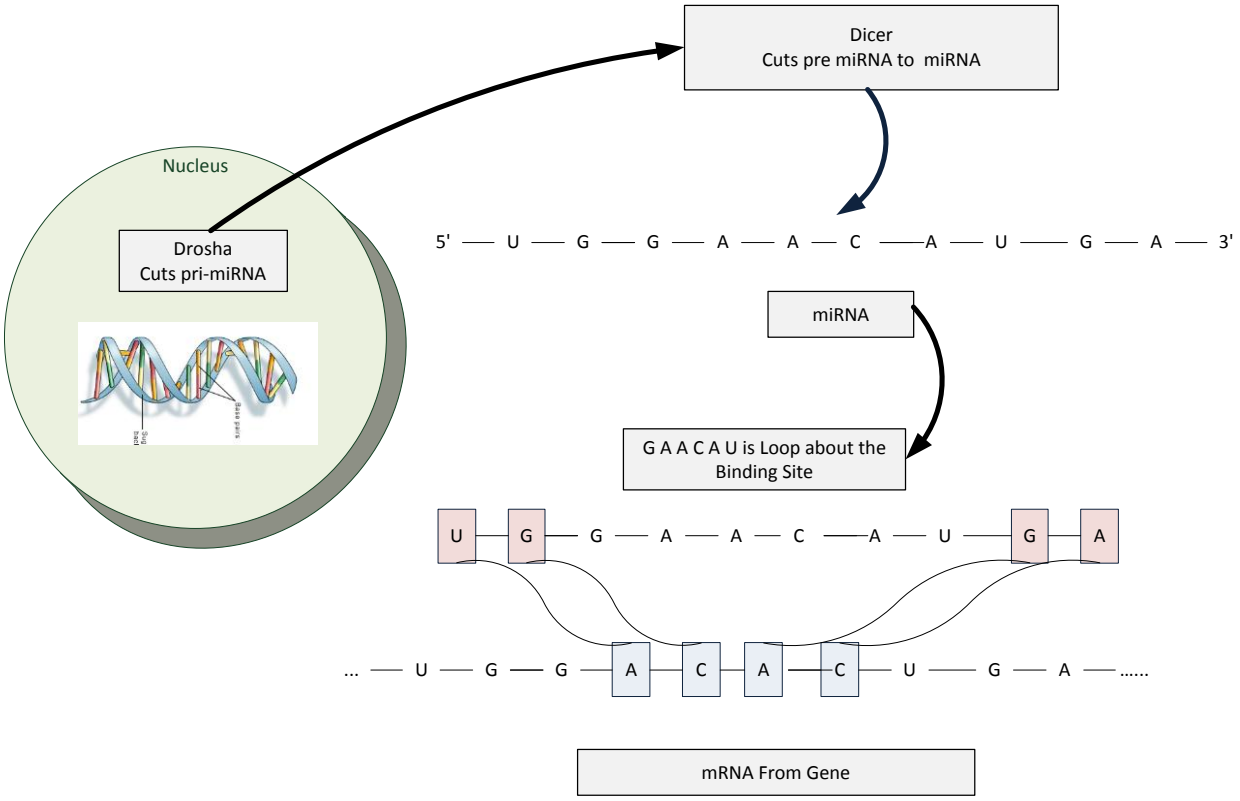
6.1.1 miRNA Production and Action

[13] See Pali and Robertson (2007)

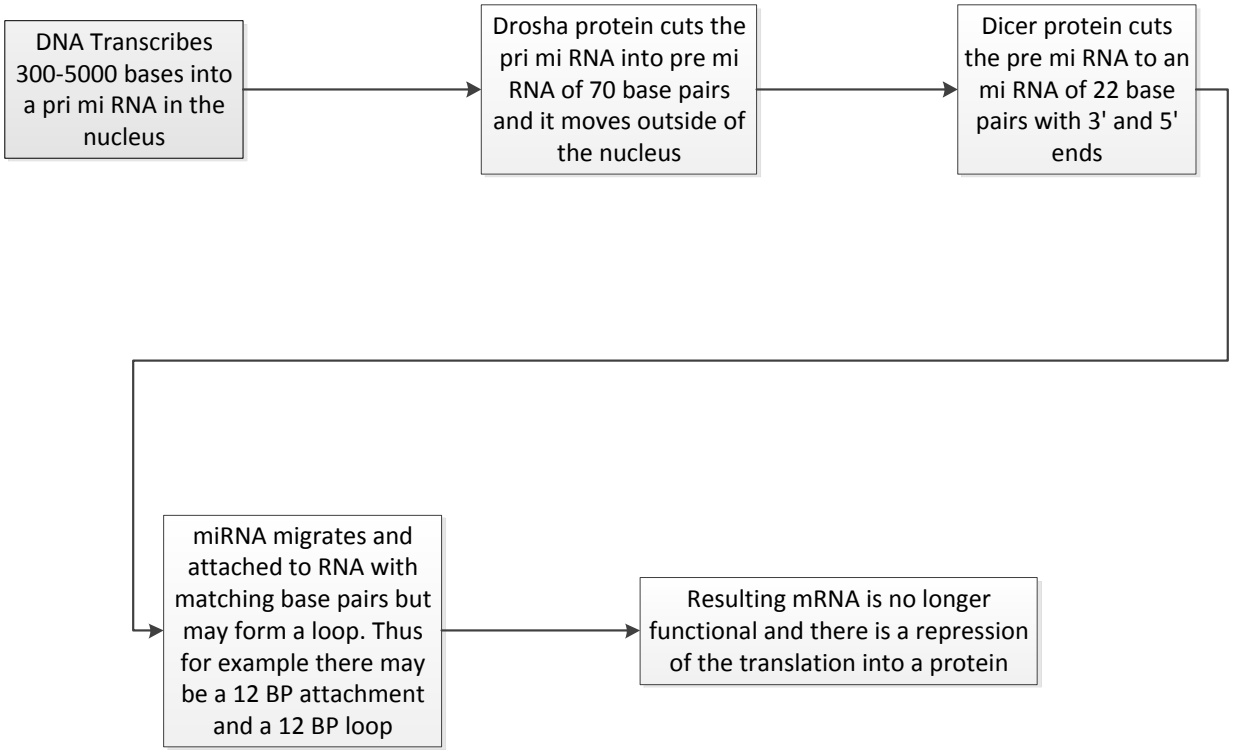
We now briefly examine the miRNA production and action. This is graphically demonstrated below. From segments of the DNA, segments not containing genes, a long segment called a pri-miRNA is generated and it is then cut to a shorter segment called a pre-miRNA and transported to the cytoplasm outside of the nucleus. Then another protein called Dicer cuts up the pre miRNA into about 22 base single-stranded pair segments which are the miRNA,

Then as we show below the small miRNA can bind to mRNA at complement sites, and in fact the binding may allow for a loop which extends out from the binding sites composed of non-complement base pairs. This binding then inactivates the mRNA and prevents its translation to a protein.

The process continues as follows (See Garcia and Miska in Appasani):



The process is described in some detail below. Here we describe the steps one at a time as is currently understood (an alternative view of this is in the paper by He and Hannon, 2004).



It is also possible for the miRNA to target more than one mRNA since the miRNA may bind in its complement binding with many other such sites on other mRNAs. It is currently not clear what the affinity of binding is for an miRNA and any possible mRNA.

Also miRNA may be obtained from introns as well as exons. The former is called intronic and the latter called exonic. Now the exonic miRNA goes through the pri and preprocess whereas the intronic miRNA is cut directly to a pre miRNA segment (see Ying et al in Appasani).

miRNAs have been identified and currently there are well over 1,000. They are named in a simple numerical order such as miRNA 34.

6.1.2 siRNA and miRNA

miRNA is a single stranded product of the process above. An alternative double stranded product is called small-interfering RNA or siRNA. siRNA usually trigger mRNA degradation whereas miRNA may cause degradation or suppression of translation to proteins. For this section we shall not focus a great deal on the siRNA functions.

6.1.3 Dynamics of miRNA

Now there may be some dynamics associated with this miRNA process as well. The model above assumes a simple one to one matching of miRNA and mRNA. However the generation of the two RNAs can be continuous and we should be looking at the concentrations. Thus is we define:

[miRNA] to be the concentration of the miRNA

and

[mRNA] the concentration of the targeted mRNA

then we have a dynamic process. Namely we can see a process such as follows:

If [miRNA] < [mRNA] then there will be excess mRNA and its product protein P will have a [P] >0. Otherwise the miRNA will bind to all mRNA and there will be no resultant protein.

One may view miRNA as a buffer agent which controls the [P] of its associated [mRNA]. One can see in dynamic form the following model:

$$\frac{d[miRNA_i]}{dt} = K_{mi,i}[Pro_{mi,i}]$$

and

$$\frac{d[mRNA_i]}{dt} = K_{m,i}[Pro_{m,i}] - \kappa_i[miRNA_i]$$

where

$[Pro]$ = *Concentration of related promoter*

Now since the binding is not necessarily 1:1, namely the miRNA may bind to several mRNA, then we may want to expand the above as follows:

$$\frac{d[miRNA_i]}{dt} = K_{mi,i}[Pro_{mi,i}]$$

and

$$\frac{d[mRNA_i]}{dt} = K_{m,i}[Pro_{m,i}] - \sum_{n=1}^{N} \kappa_{i,n}[miRNA_{i,n}]$$

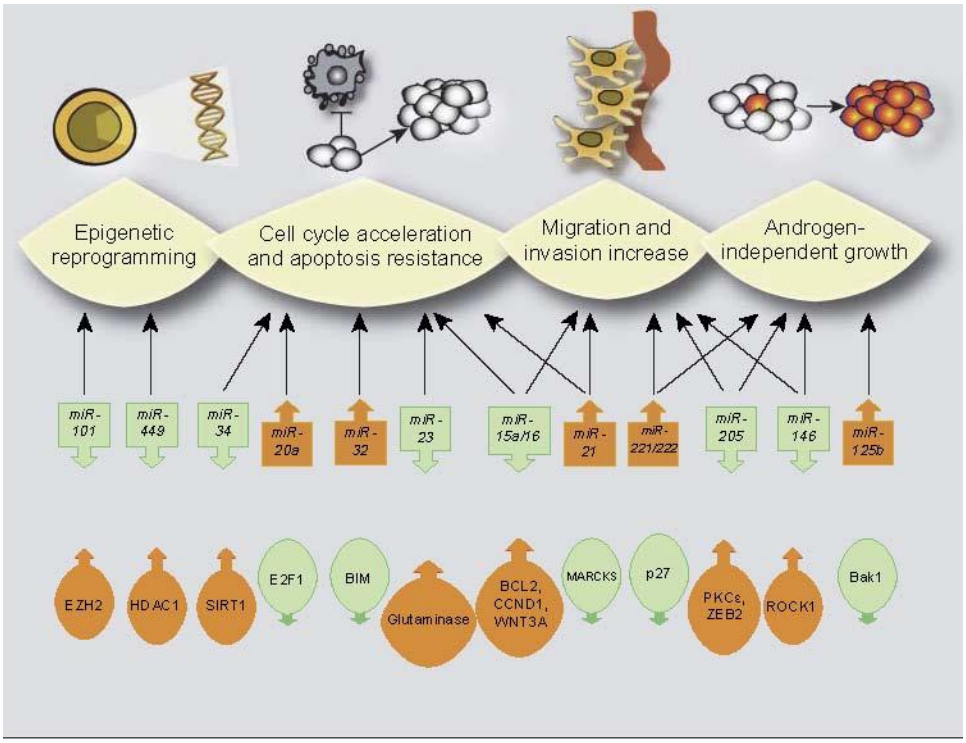
where

$[Pro]$ = *Concentration of related promoter*

6.1.4 miRNA and Cancer

There has been a great amount of research regarding the impact of miRNA on cancer and especially on PCa. miRNAs may downregulate tumor suppressor genes such as PTEN. This has been seen in miRNA 21. Colin and Croce have provided several review article regarding miRNA and their influence on cancers. They argue that miRNA alterations are heavily involved in the initiation of many cancers. Their focus had been on CLL, chronic lymphocytic leukemia, and its initiating miRNAs, miR 15 and miR 16. Coppola et al (2010) provide a detailed summary of miRNAs and PCa.

The graphic from Coppola et al is shown below where it depicts a collection of miRNAs which impact various parts of the PCa process.



For example miR34 can cause the activation and recapitulate p53 which in turn induces cell cycle arrest and apoptosis. Loss of the miR34 can result in the impairment of the p53 control of apoptosis and permit the cells to proliferate. Coppola et al perform a detailed analysis of all of the above related miRNAs and their resultant impact on PCa. miR-21 up-regulation leads to PTEN loss and thus is an oncogene.

Recent work by Poliseno et al have shown that PTEN can be down regulated via miR-106b. It had already been known that PTEN could be down-regulated by miR-22, miR-25 and miR-302. Their work demonstrated that miR-22 and miR-106b are overexpressed in PCa miR-106b is an intronic miRNA. The work of Poliseno thus has demonstrated a proto-oncogenic miRNA dependent network that regulates PTEN and thus can have a significant role in initiating PCa.

Micro RNAs are regulators of mRNA, the post transcriptional result which is then used to generate via translation the operative protein. Currently there are nearly 1,000 identified miRNAs. They are generally 22 nucleotides long, short segments, and they usually target specific mRNA and silence it. Each one of the miRNA may act upon many mRNAs.

As He and Hannon state:

Non-coding RNAs participate in a surprisingly diverse collection of regulatory events, ranging from copynumber control in bacteria[1] to X-chromosome inactivation in mammals[2].MicroRNAs

(miRNAs) are a family of 21–25-nucleotide small RNAs that, at least for those few that have characterized targets, negatively regulate gene expression at the post-transcriptional leve.

Members of the miRNA family were initially discovered as small temporal RNAs (stRNAs) that regulate developmental transitions in Caenorhabditis elegans[6]. Over the past few years, it has become clear that stRNAs were the prototypes of a large family of small RNAs, miRNAs, that now claim hundreds of members in worms, flies, plants and mammals.

The functions of miRNAs are not limited to the regulation of developmentally timed events. Instead, they have diverse expression patterns and probably regulate many aspects of development and physiology. Although the mechanisms through which miRNAs regulate their target genes are largely unknown, the finding that at least some miRNAs feed into the RNA INTERFERENCE (RNAi) pathway has provided a starting point in our journey to understand the biological roles of miRNAs.

miRNAs are simple yet complex entities and key players in the epigenetics which control gene expression.

It is clear from the above that miRNAs can positively and negatively impact many elements in the pathways we have considered in HGPIN and PCa. Coppola et al review several of the key ones. For example:

- miR-146: Down regulates the AR.
- miR-34: Can recapitulate p53 resulting in apoptosis and arrest.
- miR-23: can result in c-myc overexpression and cell proliferation.

In a recent paper by Poliseno et al they have identified several others:

- miR-106b: Down-regulates PTEN and triggers PIN in murine models.
- miR-22, miR-25, miR-302: Down-regulating of PTEN.

Similarly the papers by Petrocca et al and that by Calin and Croce detail many of the miRNAs and their impacts on many cancers. As seen in the above graphic these are but a few in the overall targeting of PCa control genes. As Coppola et al state:

The hypothesis that miRs can be regarded as new broad-spectrum oncogenes or tumor suppressor genes has opened a revolutionary field of research with exciting diagnostic and therapeutic perspectives.

The compelling hint of a widespread miR deregulation in cancer pathogenesis came from the analysis of the genomic distribution of 186 miR. In this study, it was demonstrated that more than half of them mapped in cancer-associated genomic regions, namely in chromosomal sites prone to deletions, amplifications or recombinations. These aberrations can result in miR down- or up-regulation, conferring selective advantages to mutated cells.

Additional mechanisms of miR deregulation include altered expression of miRs as a consequence of excessive or deficient processing; aberrant transcription of the precursors by epigenetic silencing of miR promoters or as a result of the activity of oncogenic transcription factors; and more rarely, point mutations in mature miRs or in target sequences that can interfere with normal target recruitment

The problem that we will have in any modeling of HGPIN and PCa is not only do we have issues regarding the somewhat well-known genes but the impact of the epigenetic factors is unknown, complex, and possibly random.

Furthermore miRNAs can act in a positive or negative manner depending upon the cell and the activated networks in the cell. From Croce (2009) we have:

Importantly, miRNAs should not be described as oncogenes or tumor suppressor genes, unless the tissue or cell type involved in their action is specified. For example, miR-221 and miR-222 target an oncogene, KIT, and inhibit the growth of erythroblastic leukaemia[30], and therefore function as tumor suppressors in erythroblastic cells. but they also target at least four important tumor suppressors −phosphatase and tensin homologue (PTEN), p27, p57 and tissue inhibitor of metalloproteinases 3 (TIMP3) −and function as oncogenic miRNAs by suppressing these tumor suppressors in various human solid tumours[31 (TABLE 1)]. Therefore, before describing an miRNA as a tumor suppressor or an oncogene, it is necessary to specify in which cell or tissue, as cellular context is crucial for the function of miRNAs….

Recent work on miR-34 has demonstrated its impact on p53 (Rokhlin et al) and the fact that miR-34 significantly mediates the role of p53 in apoptosis in AR dependent PCa.

6.1.5 MiRNA and Stem Cells

As we have indicated elsewhere, the concept of the cancer stem cell has received significant attention. There has also been a great deal of work on the area of linking miRNAs and the stem cell model for PCa. In a recent work by Liu et al (2011) the authors demonstrate the nexus between miR-34a and its ability to inhibit PCa stem cells by directly repressing CD44. They observe that cancer stem cells have been observed in many solid cancers by using the fact that CD44 adheres to the cell surface. PCa stem cells with enhance clonogenic and tumor initiating and metastatic capacities are often enriched with CD44+ cell population. The work of Liu et al demonstrated that the administration of miR-34a to PCa cells inhibited PCa metastasis and inhibited PCa regeneration. This is one of the first uses of miRNA as a tumor suppressor.

In a recent paper by Xia (2008) the author states:

The key characteristics of stem cells are that they are capable of self-renewal and differentiation. The mechanisms by which stem cells maintain self-renewal and differentiation are complicated. In the past years, protein-coding genes had been broadly investigated in stem cell self-renewal and differentiation. Recent studies indicate miRNAs as one of the most abundant classes of post-transcriptional regulators proved to be crucial in a wide range of biological processes, which suggest that miRNAs may also play essential roles in stem cell self-renewal and differentiation.

Disruption of Dicer function in murine ESs influences miRNA processing and greatly impairs their ability to differentiate …

Cancer stem cells (CSCs) are the cells within a tumor that possess the capacity to self-renew and to produce the heterogeneous lineages of cancer cells that comprise the tumor. CSCs can thus only be defined experimentally by their ability of self-renewal and tumor propagation.

The implementation of this approach explains the use of alternative terms in the literature, such as "tumor-initiating cells" to describe putative CSCs. …

The identification of growth and differentiation pathways responsible for CSC proliferation and survival will help in the discovery of novel therapeutic targets. Previous studies have shown that many signal pathways may participate in regulating CSC functions, including Wnt/β-catenin, Notch, and Sonic hedgehog homolog (SHH). The canonical Wnt cascade has emerged as a critical regulator of stem cells and activation of Wnt signalling has also been associated with various cancers …

CSC maintenance is dependent on β catenin signaling. Moreover, because Wnt/β-catenin signalling is not essential for normal epidermal homeostasis, such a mechanistic difference may thus be targeted to eliminate CSCs and consequently eradicate squamous cell carcinomas. It is therefore hypothesized that inhibition of Wnt signaling may provide an effective way to reduce the unwanted stem cell renewal which results in cancers.

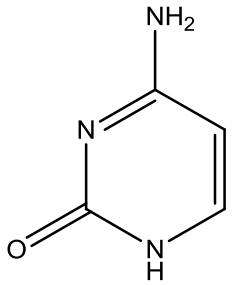
Inhibition of Wnt signalling may prove to be an effective road to inhibit the uncontrolled cell renewal that drives cancer. Acting as novel and pivotal regulators of protein-encoding genes, miRNAs will have great potential in regulating CSCs' biological functions by targeting CSCs-related signal pathway molecules.

The impact of further understanding the modulating role of miRNAs and PCa will play a significant role in the development of analytic tools for PCa. The problem one all too often finds in understanding cancer models is that each step forward reveals new elements which were unknown and not readily understood. Thus incorporating the stem cell paradigm and the double edged sword of the miRNA elements will be challenging.

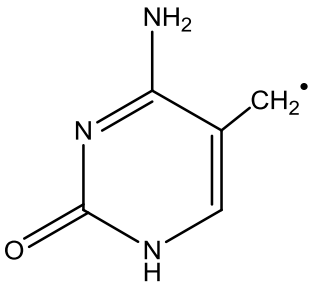
6.2 METHYLATION

DNA methylation is a process whereby the cytosine is changed by the insertion of a methyl group on the 5 carbon of the ring. It is a process which is epigenetic and can dramatically modify gene expression. In fact many of the methylation issue in humans are also common to plants, see the work by Zuberman. There has been a great deal of work demonstrating the impact of methylation on cancer progression. Specifically the recent summary by Herman and Baylin, that of Palii and Robertson, that od Robertson and Wolffe, Strathdee and Brown, Calin and Croce.

Basic cytosine is shown below. It has two NH groups at opposite poles and a single oxygen.



Now when the 5 carbon is replaced by a methyl group we obtain the form below. This is methylated cytosine.



+

As is stated in the paper by Miranda and Jones:

DNA methylation is a covalent modification in which the 5₀ position of cytosine is methylated in a reaction catalyzed by DNA methyltransferases (DNMTs) with S-adenosyl-methionine as the methyl donor.

In mammals, this modification occurs at CpG dinucleotides and can be catalyzed by three different enzymes, DNMT1, DMNT3a, and DNMT3b.DNAmethylation plays a role in the long-term silencing of transcription and in heterochromatin formation.

As an epigenetic modification, DNA methylation permits these silenced states to be inherited throughout cellular divisions.

We continue with the discussion in Mirand and Jones as follows:

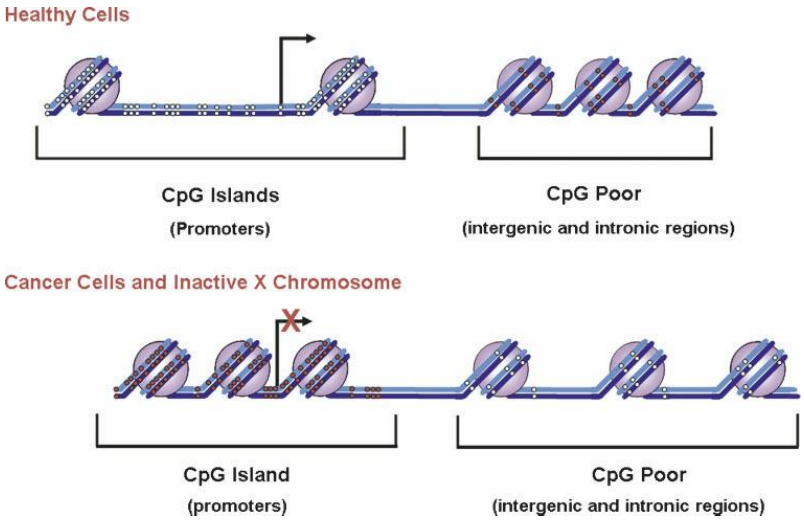
Silencing of genetic elements can be successfully initiated and retained by histone modifications and chromatin structure. However, these modifications are easily reversible making them make poor gatekeepers for long-term silencing. Therefore, mammalian cells must possess an additional mechanism for prolong silencing of these sequences. An important component of this process is DNA methylation. DNA methylation is a stable modification that is inherited throughout cellular divisions.

When found within promoters, DNA methylation prevents the reactivation of silent genes, even when the repressive histone marks are reversed. This allows the daughter cells to retain the same expression pattern as the precursor cells and is important for many cellular processes including the silencing of repetitive elements, X-inactivation, imprinting, and development.

We now present a key Figure from Miranda and Joner regarding the methylated reading of DNA. They state regarding the Figure below:

Chromatin structure of CpG islands and CpG poor regions in healthy cells and during cancer. In healthy cells, CpG islands are generally hypomethylated. This allows for an open chromatin structure. However, the CpG poor regions found in repetitive elements within the intergenic and intronic regions of the genome are methylated and thereby maintain a closed chromatin structure. In cancer and on the inactive X chromosome many CpG islands become methylated, forcing these regions into a closed chromatin structure.

When CpG islands located within promoters are methylated, the corresponding genes are persistently silenced. In contrast, the CpG poor regions become hypomethylated allowing for an open chromatin structure.



As Robertson states:

It is now clear that the genome contains information in two forms, genetic and epigenetic. The genetic information provides the blueprint for the manufacture of all the proteins necessary to create a living thing while the epigenetic information provides instructions on how, where, and when the genetic information should be used.

Ensuring that genes are turned on at the proper time is as important as ensuring that they are turned off when not needed.

The major form of epigenetic information in mammalian cells is DNA methylation, or the covalent addition of a methyl group to the 5-position of cytosine predominantly within the CpG dinucleotide. DNA methylation has profound effects on the mammalian genome.

Some of these effects include transcriptional repression, chromatin structure modulation, X chromosome inactivation, genomic imprinting, and the suppression of the detrimental effects of repetitive and parasitic DNA sequences on genome integrity.

Robertson then proceeds to detail the genes impacted by hypermethylation. We summarize them below:

Gene	*Function*
pRb	Regulator of G1/S phase transition
p16 INK4a	Cyclin-dependent kinase inhibitor
p15 INK4b	Cyclin-dependent kinase inhibitor
ARF	Regulator of p53 levels
hMLH1	DNA mismatch repair
APC	Binds b-catenin, Regulation of actin cyto-skeleton?
VHL	Stimulates angiogenesis
BRCA1	DNA repair
LKB1	Serine/threonine protein kinase
E-cadherin	Cell ± cell adhesion
ER	Transcriptional activation of estrogen-responsive genes
GSTP1	Protects DNA from oxygen radical damage
O6-MGMT	Repair/removal of bulky adducts from guanine
TIMP3	Matrix metallo proteinase inhibitor
DAPK1	Kinase required for induction of apoptosis by g interferon
p73	Apoptosis?, structurally similar to p53

Regarding PIN, the one which is most concern is the GSTP1 gene and its suppression allowing for DNA damage from inflammation and oxygenation damage.

In the context of cancer generation and progression, the epigenetic effect of hyper and hypo methylation are best described by Esteller:

The low level of DNA methylation in tumors as compared with the level of DNA methylation in their normal-tissue counterparts was one of the first epigenetic alterations to be found in human cancer.

The loss of methylation is mainly due to hypomethylation of repetitive DNA sequences and demethylation of coding regions and introns −regions of DNA that allow alternative versions of the messenger RNA (mRNA) that is transcribed from a gene. A recent large-scale study of DNA methylation with the use of genomic microarrays has detected extensive hypo-methylated genomic regions in gene-poor areas.

During the development of a neoplasm, the degree of hypomethylation of genomic DNA increases as the lesion progresses from a benign proliferation of cells to an invasive cancer.

Three mechanisms have been proposed to ex-plain the contribution of DNA hypomethylation to the development of a cancer cell:

(i) generation of chromosomal instability,

(ii) reactivation of transposable elements, and

(iii) loss of imprinting.

Under methylation of DNA can favor mitotic recombination, leading to deletions and translocations, and it can also promote chromosomal rearrangements. This mechanism was seen in experiments in which the depletion of DNA methylation by the disruption of DNMTs caused aneuploidy. Hypomethylation of DNA in malignant cells can reactivate intra-genomic endo-parasitic DNA.

7 PUTATIVE PATHWAYS; ACTIVATION AND DEACTIVATION

We can now progress to aligning the gene products in an organized fashion, call pathways. The cell undergoes a continual process of generating various proteins and interacting with its environment through sending out proteins and by receiving and acting on them. The input-output process many in most cases be via ligands on the surface. During the normal homeostatic state of a cell, it is just acting like a small production factory reading its DNA and creating products. Changes may occur during this homeostatic state resulting from the attachment of miRNA, micro RNAs, or through the methylation of the cytosines on certain DNA strands. This then may result in a change during the static state.

The cell also undergoes changes in the dynamic state of mitosis.

Pathways as currently understood and presented represent the sets of gene expressions, namely the proteins produce by genes, and their gross effects on one another. For example PTEN on Akt and then the effects on the gene, such as c-Myc on expression enhancements. In reality the gene is continually producing the proteins and their concentrations are often the drivers via the kinetics of reactions. Some proteins are catalysts to reactions to occur or to be blocked. Thus the true measures should be [PTEN], the concentration of PTEN, rather than just an on or off state. We shall discuss this later.

7.1 GLOBAL CANCER PATHWAY MODELS

Models for pathway elements and dependencies have been developed. The pathway models show:

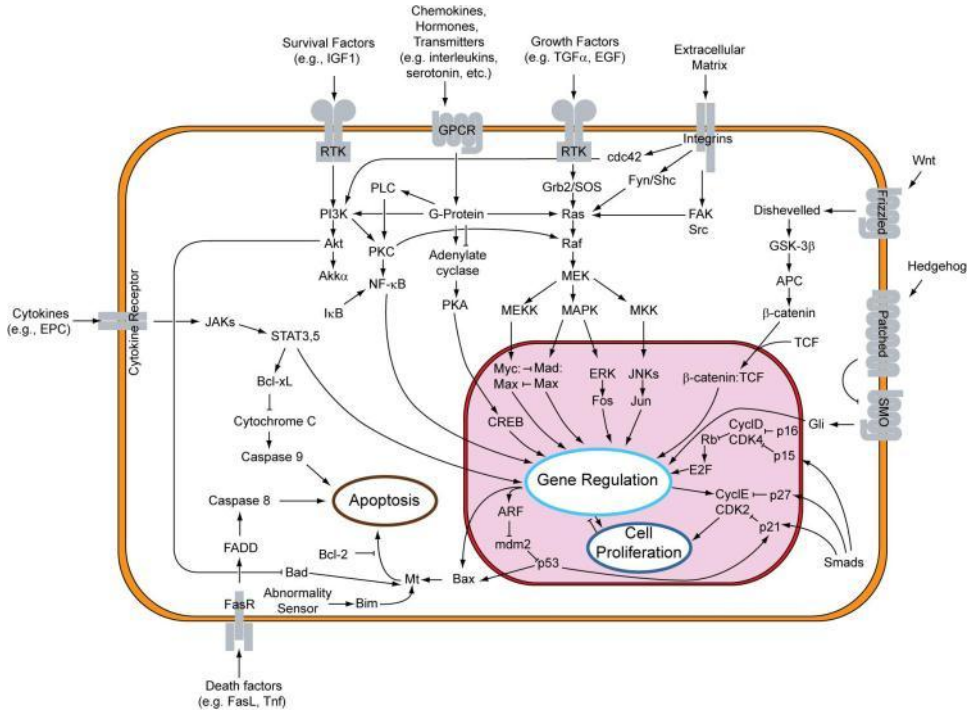
(i) the cell wall and external signalling, including the possible ligands and receptors which can effect cell growth.

(ii) the cell proteins and their control proteins which carry messages from the cell surface to the nucleus,

(iii) the transport proteins across the cell nucleus to the DNA and the genes themselves,

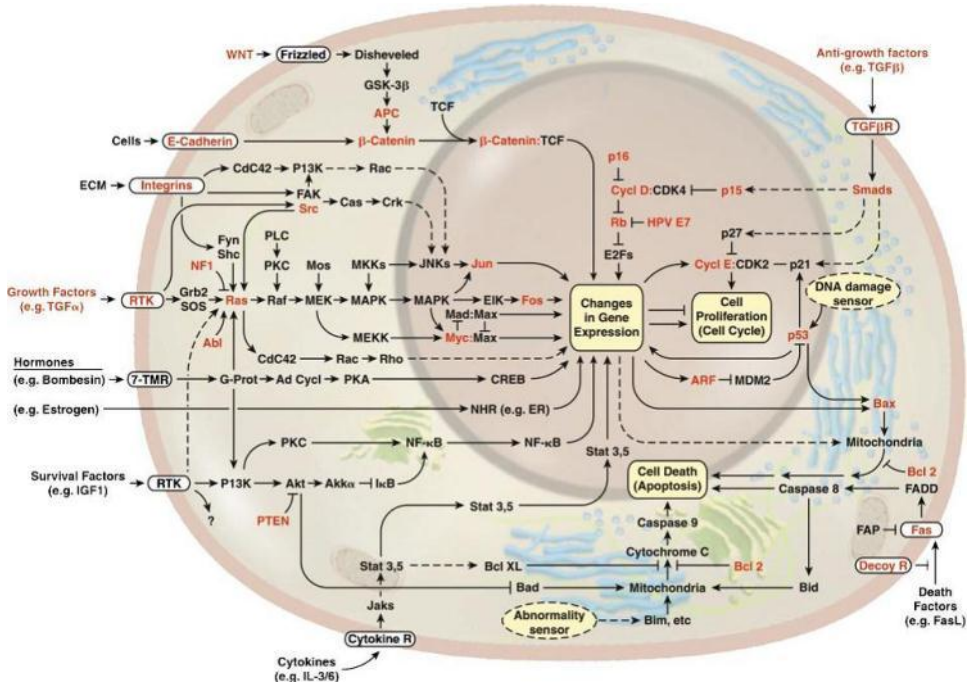
(iv) specific gene regulation proteins and specific cell cycle regulation proteins within the nucleus itself.

One of the standard set of pathways are presented below:



The end states of apoptosis versus proliferation are demonstrated. The issue is both gene activation, a binary issue of being present or absent, versus level of expression, how frequently is it being transcribed, are two separate but equally important considerations.

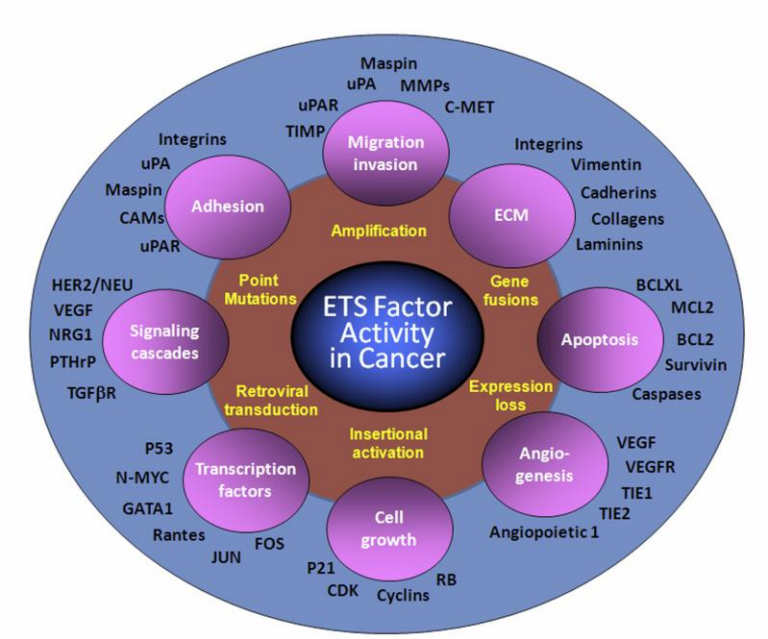
We show a second detailed view below:



What is important to understand is that the above graphics are representative and fail to demonstrate the dynamics and the impact of protein concentrations and feedback on the evolution of a cell.

7.2 SPECIFIC PATHWAY ELEMENTS

A view of one set of factors, the ETS factors, by Watson et al depicts another view centered on a specific set of genes as seen below:



It should be noted that we did discuss the TMPRSS2 and ERG fusion, a fusion with an ETS gene, yet neither is depicted in the pathways shown above although they have a major role to play in PCa. On the other hand we see from above that they affect many of the elements in that set of pathways.

One need just look at two factors below; first the TPMRSS2-ERG fusion and second loss of PTEN. The fusion is also noticed in PIN almost 20% of the time and in PCa almost 60% of the time. Is this causal or a result of the change? PTEN is not present in PIN but it is in PCa 60% of the time. The question that one must keep in the back of their minds is what is the dynamic model for this to occur.

The following Table is from Chen and Sawyers in DeVita and lists what they perceive as the key genes in the controlling pathways. We summarize them below and we have characterized them earlier.

Name	*Type*	*Location*	*PIN Frequency*	*PCa Frequency*
GST π Loss Glutathione S-transferases	Hypermethylated Gene		50%	90%
TPMRSS2:ERG Translocation	Translocation	21q22	0-20%	50-60%
PTEN Loss	Loss	10q23	0%	60%
Akt (Akt1)	Pathway	14q32.32		
NKX3.1	Tumor Suppressor Homeobox TF	8p21	60%	85%
AR	Up-regulate	Xq12		
C-Myc	Transcription Factor	8q24		~90%
NF-κB	Heterodimer of p65/p50 Transcription Factor	10q24		
Wnt	Pathway			
Hedgehog	Pathway			
Notched	Pathway			

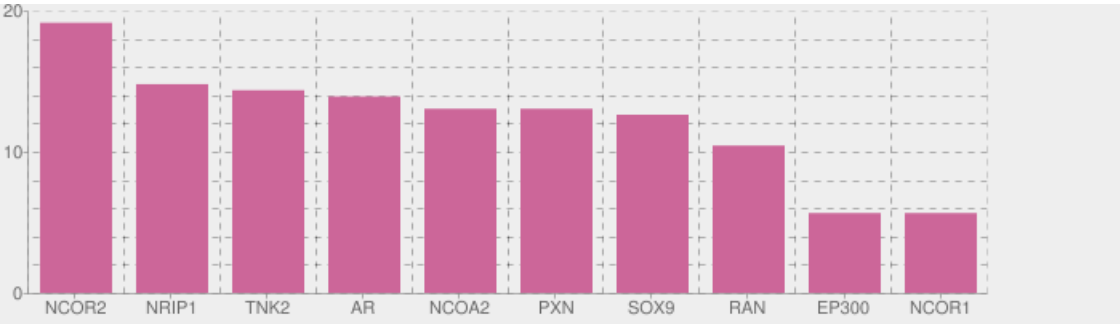
7.3 GENE SPECIFICITY AND FREQUENCY

There has been a great deal of study of many genes which have been altered and thus reregulated in PCa. We rely upon the MSKCC portal which allows us to study their database of genes and PCa. We present a few cases here as examples and then we focus on a few which have received the greatest attention. The genes affected in the androgen pathway are shown below from the MSKCC genomics data portal.[14] Specifically for the following cases we show the percent which have specific genes altered:

Case 1: Gene Set / Pathway is altered in 52.61% of all cases.

- Pathway: Prostate Cancer: AR Signaling (10 genes)
- Total number of input genes: 10
- Case Set: Prostate All: All prostate cancer samples (230 samples)
- Total number of cases selected: 230

[14] http://cbio.mskcc.org/cancergenomics-dataportal/index.do#heat_map

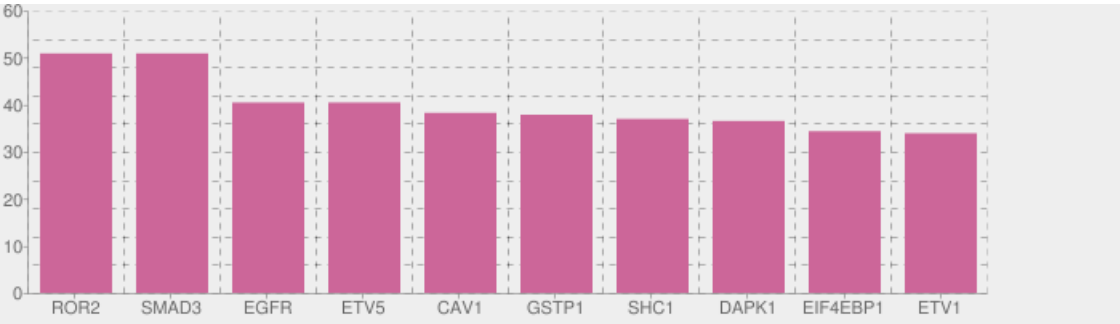


Case 2: Gene Set / Pathway is altered in 80.87% of all cases.

- Pathway: Prostate Cancer: Sequenced (145 genes)
- Total number of input genes: 145
- Case Set: Prostate All: All prostate cancer samples (230 samples)
- Total number of cases selected: 230

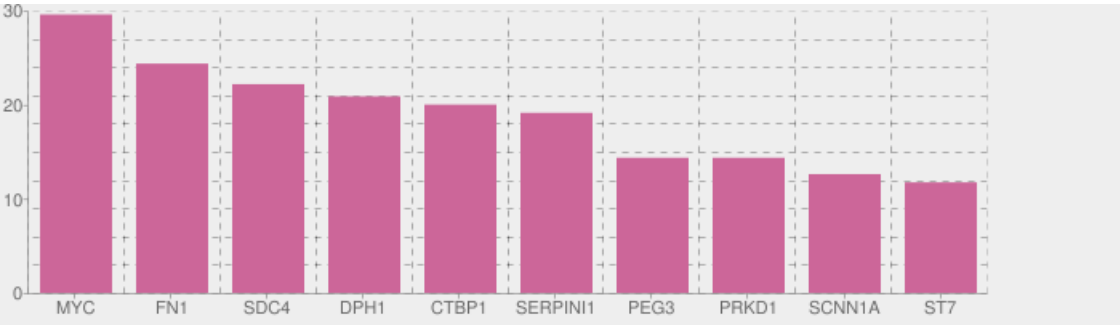
Case 3: Gene Set / Pathway is altered in 81.74% of all cases.

- Pathway: Prostate Cancer: Prostate cancer pathway (280 genes)
- Total number of input genes: 280
- Case Set: Prostate All: All prostate cancer samples (230 samples)
- Total number of cases selected: 230



Case 4: Gene Set / Pathway is altered in 65.65% of all cases.

- Pathway: Prostate Cancer: Down-regulated by androgen (19 genes)
- Total number of input genes: 19
- Case Set: Prostate All: All prostate cancer samples (230 samples)
- Total number of cases selected: 230

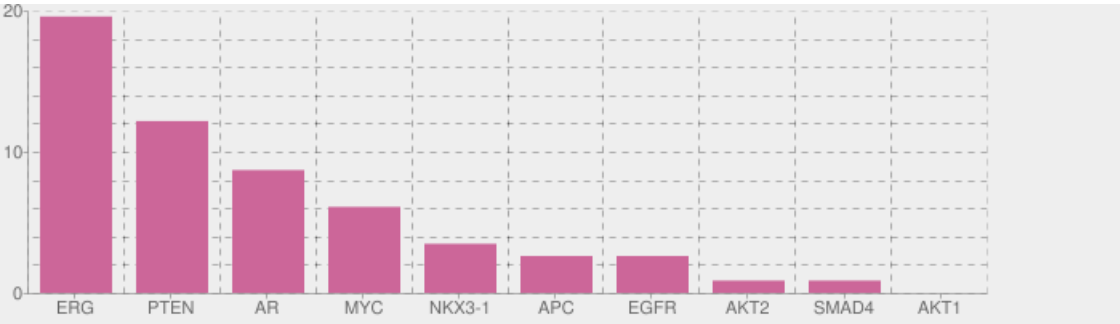


Case 5: Gene Set / Pathway is altered in 38.7% of all cases.

- Pathway: Custom
- Total number of input genes: 11
- Case Set: Prostate All: All prostate cancer samples (230 samples)
- Total number of cases selected: 230

Case Id	ERG	PTEN	AR	MYC	NKX3-1	APC	EGFR	AKT2	SMAD4	AKT1	TMPRSS2	Gene Set
NUMBER OF CASES WITH ALTERED GENE	45	28	20	14	8	6	6	2	2	0	0	89
% OF CASES WITH ALTERED GENE	19.57 %	12.17 %	8.7 %	6.09 %	3.48%	2.61 %	2.61%	0.87%	0.87%	0%	0%	38.7 %

We depict this data below:



We will focus on a few of the above specific genes; ERG, MYC, PTEN, AKT, AR, and TMPRSS2.

It is essential to remember the Dougherty discussion, namely one must not rely solely upon the gene and its presence, but one must understand how it functions, its relationship between itself and other genes, and the issues regarding over and under expression, and finally the issue of mutations and transpositioning. Also it is essential to keep the roles of miRNA in mind. Causality and coincidence must be determined in the development of models.

8 ENZYMATIC REACTIONS AND PATHWAYS

In the pathways in the cells one faces not just a simple, albeit structurally complex, network of gene products, but a collection of dynamic enzymatic interactions. We first provide an example, then proceed through the panoply of different reactions and then try to apply the theory to a somewhat complex pathway leading to PCa.

8.1 EXAMPLE REACTION

Let us begin with a simple example, the PTEN reaction. We will examine several varying descriptions and then focus on a specific model. The issues here will be fundamentally the following:

1. What is the linkage from gene product to gene product?

2. What is the reaction from gene product to gene product? Is it a simple conversion from one to another, or is it an enzymatic process, and if so what type of enzymatic process.

3. If there are enzymatic reactions and if we know or can assume a form of the enzymatic reaction, then how can we measure the reactions in such a manner so as to determine the constants and validate them.

4. If the reaction is temporal, what are the temporal dynamics? Are there cycles.

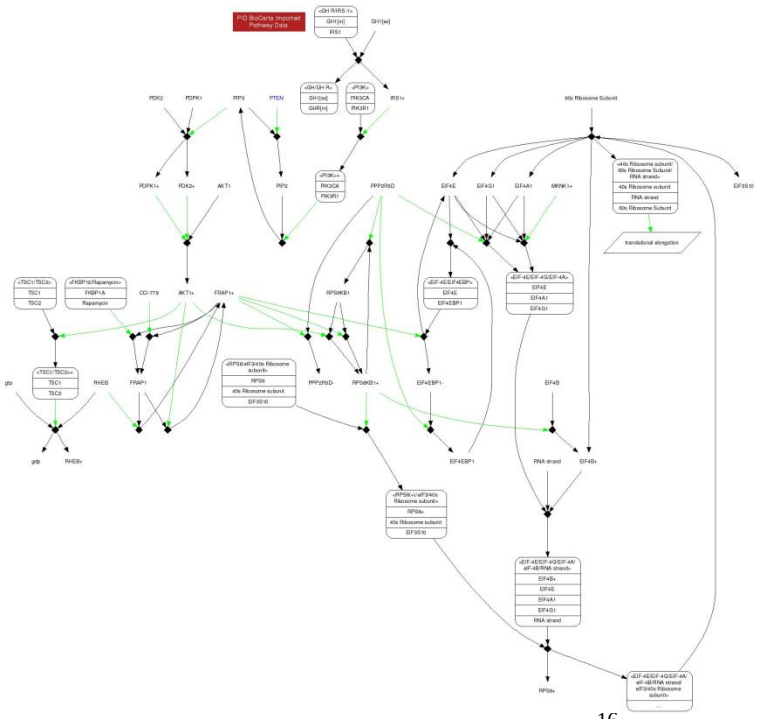
5. Is the reaction also spatial, namely do the cells communicate between each other in such a manner so as to communicate changes across the cell matrix? We have examined this phenomenon in other cell environments and there has been recent studies of this phenomenon in melanoma cells. The question is do they also function on PCa and if so what are the dynamics?

6. If we can determine the temporal characteristics then can we look for cycles?

7. If we can determine the dynamics and models, what are the remaining causative factors which initiate the process. For example, what causes the loss of PTEN. We can determine what happens when it is lost, but is it a methylation as some have suggested, and if so is it permanent?

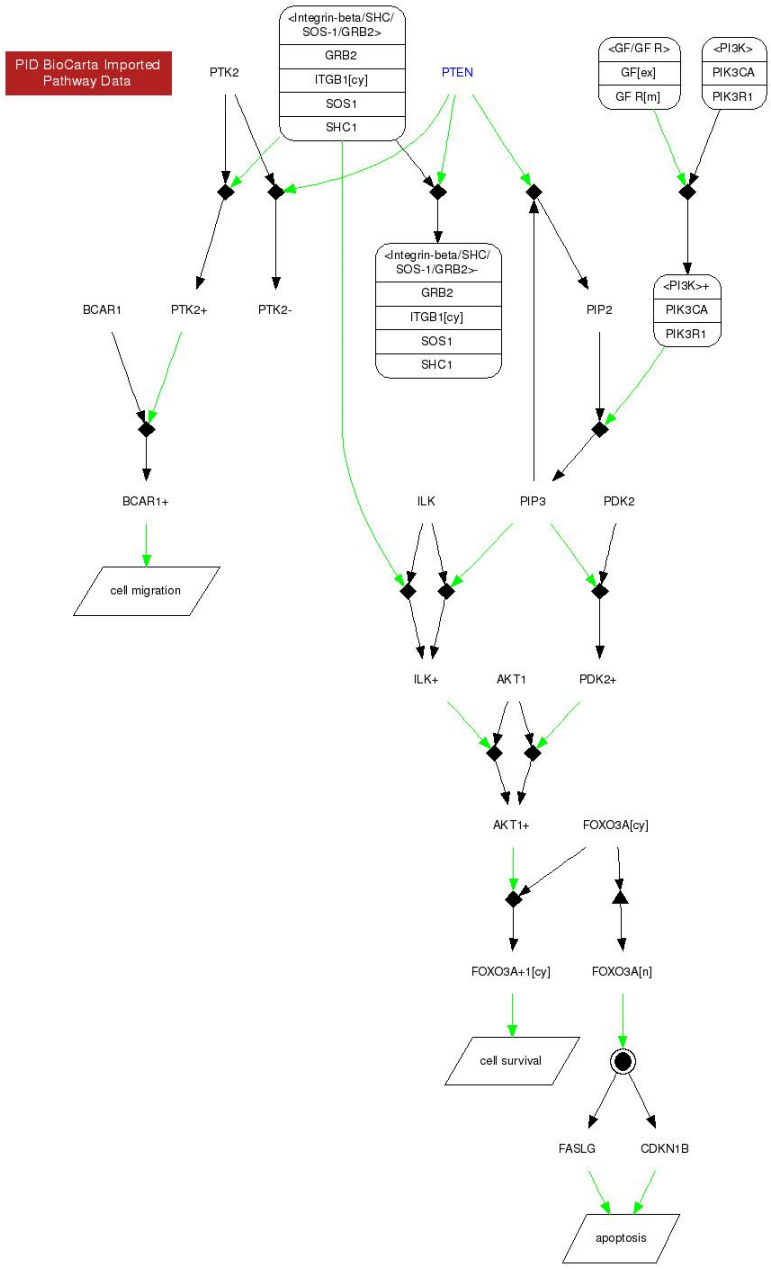
First we examine the pathway and its control of mTOR and cell death. Below is the NCI PTEN control pathway with mTOR[15]. This version is a quite complex pathway.

15 http://pid.nci.nih.gov/search/pathway_landing.shtml?what=graphic&jpg=on&pathway_id=100101&source=2&output-format=graphic&ppage=1&genes_a=5728



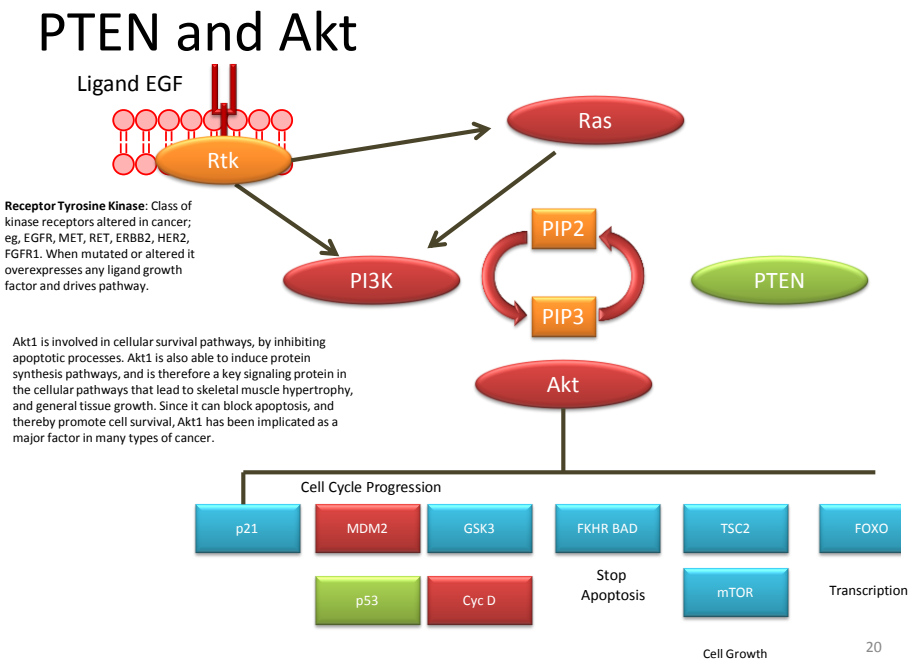
A second example is PTEN controlling apoptosis is also available[16]. We depict this network below:

16 http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=100058&source=BioCarta&genes_a=5728&genes_b=&what=graphic&jpg=on&ppage=1

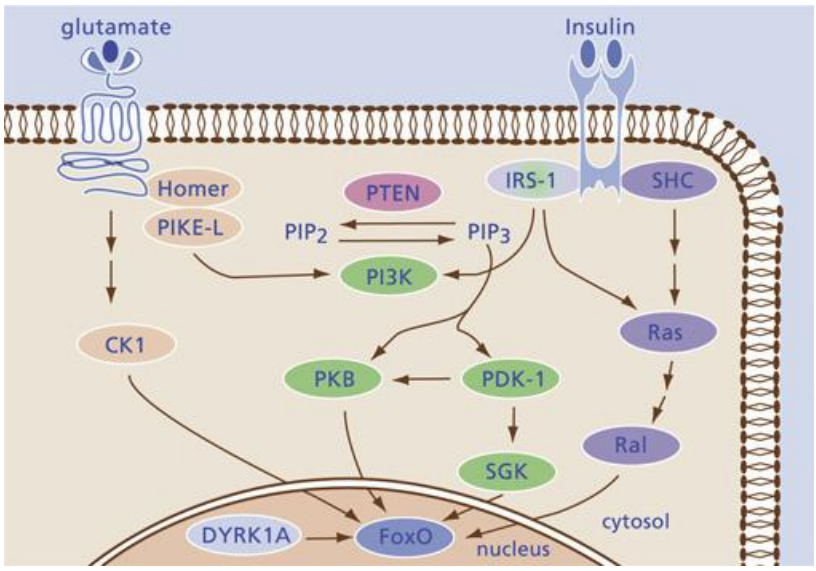


Each step is a reaction and each reaction is facilitated by some enzyme, other protein, which leads to the end result. However since the enzymes are in time varying concentrations and the reactions are temporal and dependent on the specific concentrations, we have a definable but complex dynamic system.

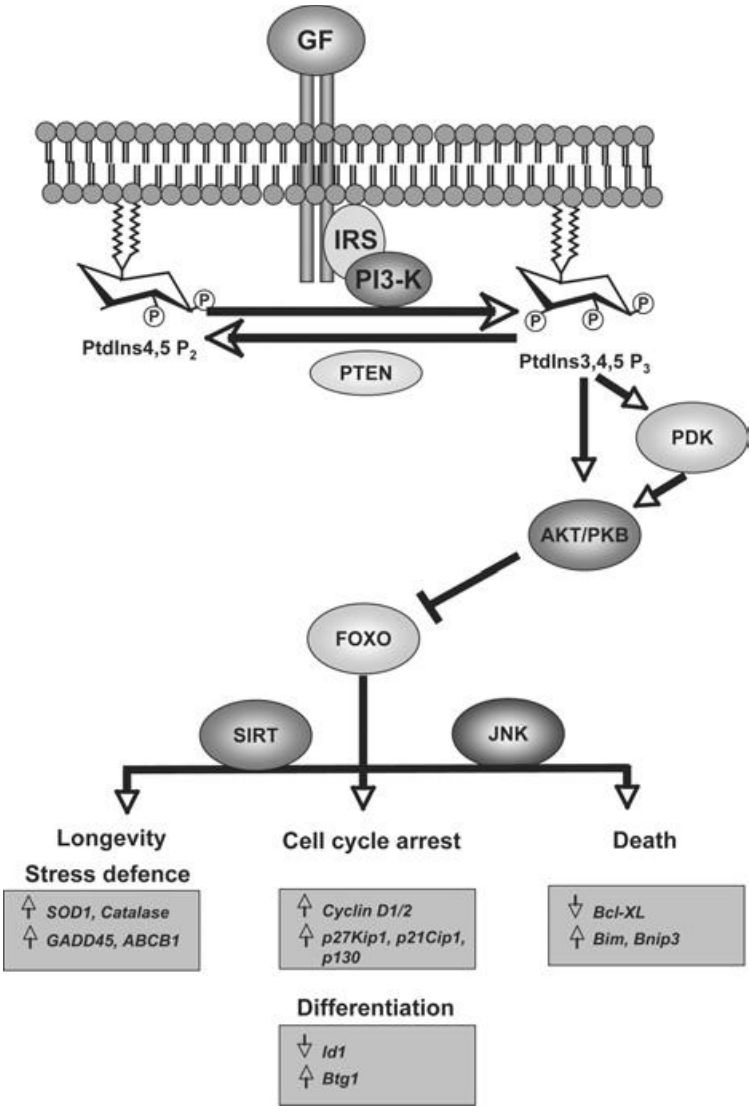
The above reaction can be simplified as follows:



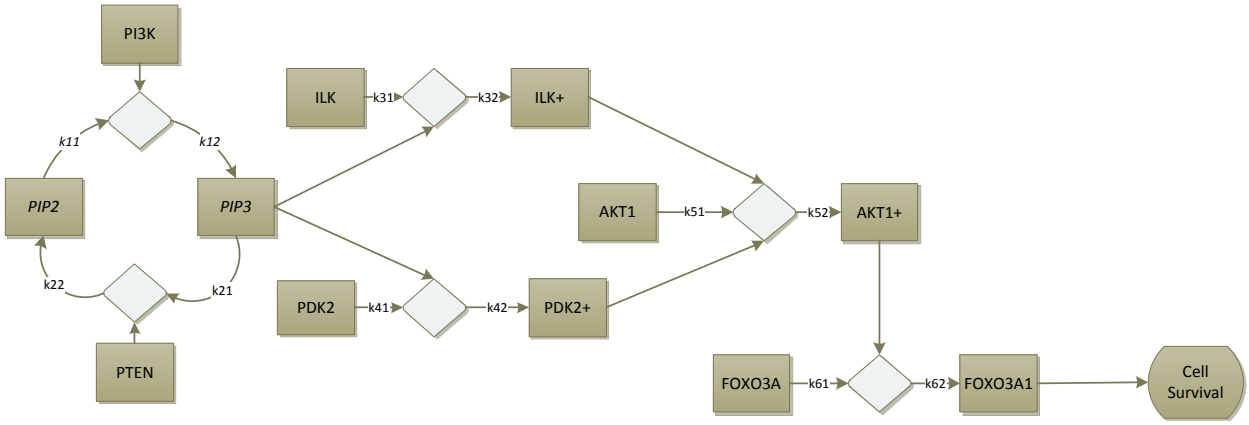
Another view of the pathway is by van der Heidde et al;



Also there is another view in Lam et al:



These simplifications are descriptive at best but fail to demonstrate a clear set of reactions, enzymatic, and otherwise. We can show these reactions in the diagram as follows:



These reactions can be described by dynamic reaction models using standard forms. We rely upon Klipp et al for this formalism. We use [X] as the concentration of a product X and we assume that v is some well determined gross reaction rate which may be dependent upon many factors. For simplicity we defer detailed descriptions of the v until necessary. Now we can write a set of reactions using the approach detailed in Klipp et al to show:

$$\frac{d[PIP2]}{dt} = v_{12} - v_{21}$$
$$\frac{d[ILK]}{dt} = v_{31}$$
$$\frac{d[ILK+]}{dt} = v_{13}$$
$$\frac{d[PDK2]}{dt} = v_{41}$$
$$\frac{d[PDK2+]}{dt} = v_{14}$$
$$\frac{d[AKT1]}{dt} = v_{51}$$
$$\frac{d[AKT+]}{dt} = v_{15}$$
$$\frac{d[FOXO3A]}{dt} = v_{61}$$
$$\frac{d[FOXO3A1]}{dt} = v_{16}$$
$$\frac{d[Cell\ Survival]}{dt} = v_{7}$$

The reaction rates, v, are in several cases determined by the enzymatic concentrations of the prior reactions such as:

$$v_{21} = k_{21}[PTEN]$$
$$v_{12} = k_{12}[PI3K]$$
$$v_{42} = k_{42}[PIP3]$$
$$v_{51} = k_{51}[ILK+] + k_{52}[PDK2+]$$
$$v_{61} = k_{61}[AKT1+]$$

where we would have to further analyze the k factor dependence on the constituents of the reaction. The resulting dynamics are quite complex but analyzable. It is immediately evident that oscillations are possible as well as possible instabilities.

The question then is, what are the dynamics of this reaction and given the dynamics of the reaction what are the stabilities or instabilities.

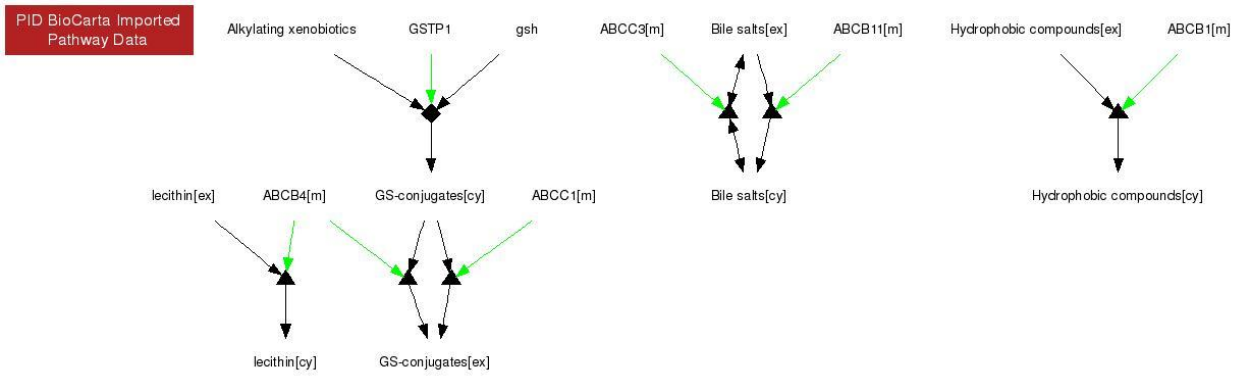
One can assume that if the cell is in some form of homeostatic equilibrium, that perhaps the concentrations of the enzymes, protein, are fluctuating back and forth from some stable set of values. Then, if a perturbation occurs, such as a methylation of PTEN, we seek to determine the new stable points if any exist and the time characteristics of the shifts in those points. An alternative question is how does one measure the time constants and furthermore how does one validate the model employed.

8.2 PCA REACTION MODELS: A SUBSET

There are many pathways associated with HGPIN and PCa. We discuss a few here in order to develop a few models and structures. The pathway data is from the MSKCC web site which details most of the current gene details on PCa as we use the reference of Pestell and Nevalainen also which includes details on most of the specific parts of the pathway.

8.3 HGPIN AND GSTP1

The first is the GSTP1 pathway which by becoming methylated is considered the first if not one of the first steps towards HGPIN and in turn PCa.



We can now proceed to the other pathways.

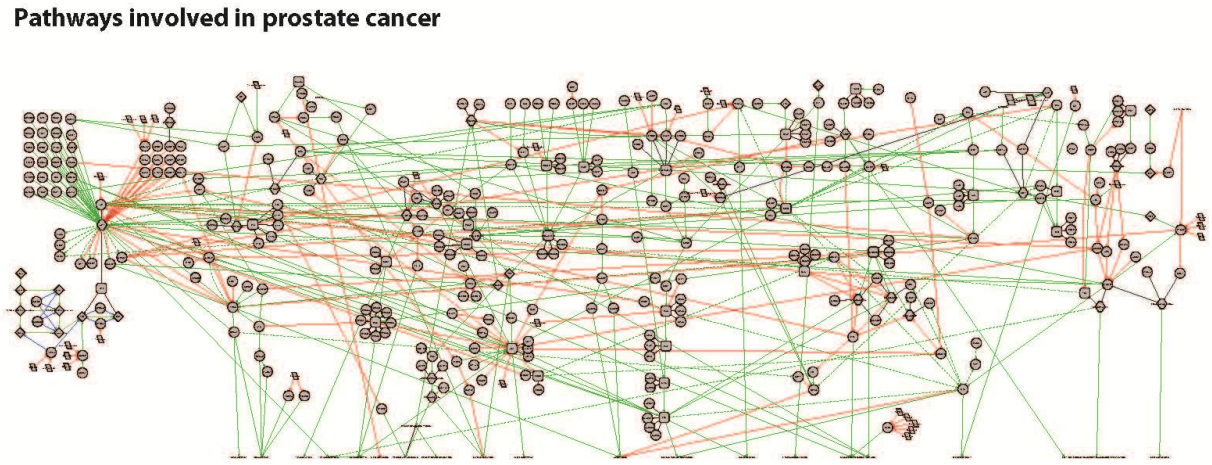
8.4 TOTAL PATHWAYS

MSKCC has published a comprehensive PCa pathway map which we show below[17]:

[17] http://cbio.mskcc.org/cancergenomics/prostate/pathways/prostate_cancer_pathways.pdf

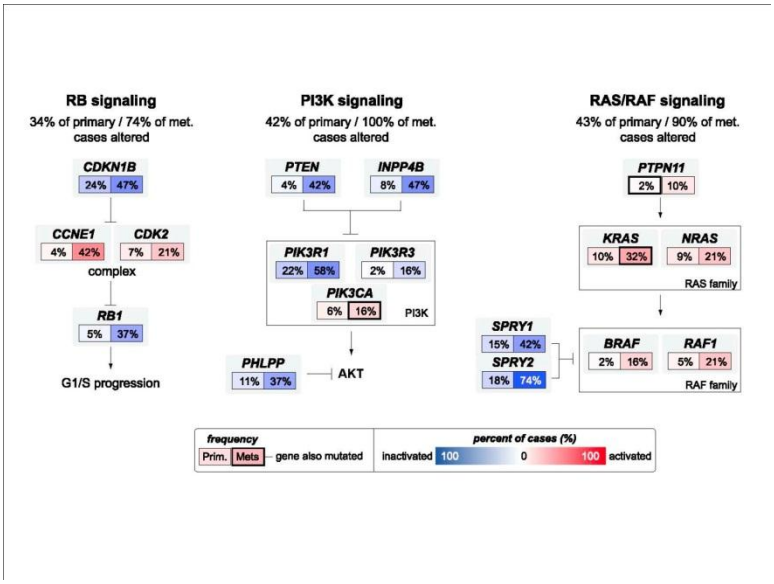
In addition the MSKCC

Specific Pathways



8.5 SPECIFIC PATHWAYS

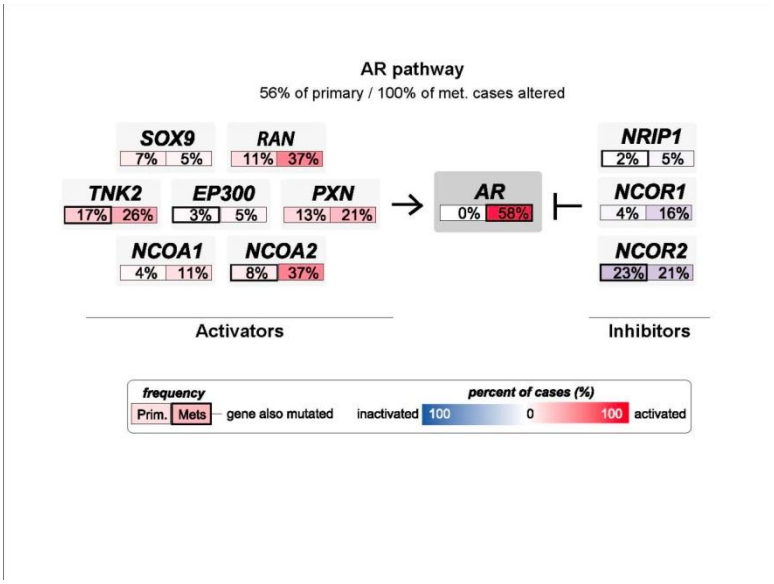
In addition the MSKCC site presents details on specific pathways as relates to PCa. We present them as follows. First we depict the major gene and gene products and then show what percent of the PCa cells exhibit modifications of those specific genes. For example in the PTEN related pathway, 42% of the PCa mets exhibit PTEN loss and in met PCa we have 100% of the cells exhibiting some loss in some element of the pathway. BRAF is activated in 16% of mets, KRAS in 32%. CDKN1B is repressed in 47% of mets.



The immediate conclusion from the above is that there is no clear pattern of what is repressed or activated. There is not a clear and unambiguous pattern of steps which define PCa or met PCa.

In a sense this lack of intensity of any one or a set of genes should be a concern. It infers that there very well may be multiple pathways for the development of PCa and mets related thereto.

The following shows the results for the AR pathway. Here we see that the AR is overexpressed in 58% of met PCa.



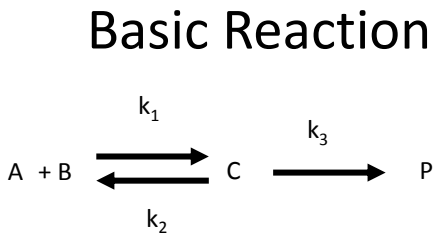
8.6 REACTION KINETICS

Reaction kinetics is a powerful set of chemical dynamics that make the cell function. In this section we review several of the models for reaction kinetics including the Michaelis-Menten model[18]. To start we know that there is a change in a concentration C of some substance and that the concentration may be resulting from one or several competing processes. The following is a general statement of that result;

$$\frac{dC}{dt} = [Synthesis] - [Degradation] - [Phosphorylation]$$
$$+[Dephos] - [Binding] + [Release] + etc$$

1.1.1 Enzyme Reactions

Now we must provide some specifics to this model to determine its form in detail. The Figure below depicts a typical reaction. This shows how the rate of reaction occurs.



C is the intermediate. Assume that the first reaction is in equilibrium. Assume first reaction is so fast and the second is so slow that first is in equilibrium. Then:

$$K = \frac{[C]}{[A][B]}; \quad and \quad K = \frac{k_1}{k_2}$$

$$\frac{d[P]}{dt} = k_3[C] = k_3 K[A][B] = k[A][B] \quad where \quad k = \frac{k_1 k_3}{k_2}$$

Now if we assume that we have the same reaction but there exists a constraint on production, the change in the reaction dynamics is as shown below.

[18] See p. 111 Murray.

Basic Reaction II

Now consider same reaction, but do NOT assume that the first reaction provides an unlimited amount and assume that there is some form of competition. Then we have:

$$\frac{d[C]}{dt} = k_1[A][B] - k_2[C] - k_3[C]$$

assume that we look at the steady state, derivative equals zero;

$$[C] = \frac{k_1[A][B]}{k_2 + k_3}$$

We will use these basic concepts in the following. We begin with the Michaelis-Menten model and its reactions...

8.6.1 Michaelis- Menten Model

Let us begin with a simple enzyme reaction. We start with a source S and a product P with an enzyme E. The reaction is as fo**l**lows:

We first note that this reaction is what controls the production of an anthocyanin in the secondary pathway. Namely E is the enzyme and P the resulting product. However, one can also state that the same reaction would occur when the activator and repressor genes produce their proteins and they then modulate the up or down production of the target gene, the one producing E. The intertwining of all of these control and modulation processes is essential if we are to understand the coloring of the flower.

Let us take a simple example from botany, namely plant color. Plant colors result from the concentrations of a set of anthocyanins. The concentration of each type in a mix will result in what we see, and seeing a color one may invert the result and attempt to ascertain the anthocyanins present. What drives the production of anthocyanins is the presence of enzymes,

ones for each anthocyanin path. The enzymes are the direct protein products of the plants gene. Thus, we need to understand this reaction since when we look at plant colors we are effectively looking at anthocyanin concentrations, and more importantly the target protein, enzyme or gene productions controlling the secondary pathways. If we were to focus on say the protein E, then the more E the more P. Conversely the less E the less P. This domain is where we must look. In most enzyme reactions we have always assumed that E was excessive. That E could not be exhausted and that given the available E the reaction as above proceeds. This may not be the case here, thus the range of evaluating the solution must be somewhat expansive.

Note that in an enzyme reaction the enzyme E facilitates the reaction and does not end up in any way being part of the product. In fact the enzyme has remained intact at the end of the reaction. Thus the enzyme concentration between the free enzyme and bound portion remains constant. That will be a critical fact in modeling this reaction.

We denote the following as measure of concentrations for this reaction:

s=[S], e=[E], p=[P], c=[SE]

for the respective concentrations. We can now, from the law of mass action, write four equations for the four concentrations. They are as follows[19]:

$$\frac{ds}{dt} = -k_1 es + k_{-1} c$$
$$\frac{de}{dt} = -k_1 es + (k_{-1} + k_2) c$$
$$\frac{dc}{dt} = k_1 es - (k_{-1} + k_2) c$$
$$\frac{dp}{dt} = k_2 c$$

we assume that the initial conditions are as follows:

$$s(0) = s_0, e(0) = e_0, c(0) = 0, p(0) = 0$$

From the above differential equations we note the following:

1. p(t) can be calcula6ted if c(t) has been calculated

2. If we add the equations for e and c we find that:

$$\frac{de(t)}{dt} + \frac{dc(t)}{dt} = 0$$

[19] See Murray p. 310.

Thus we are left with two differential equations:

$$\frac{ds}{dt} = -k_1 s + (k_1 + k_{-1})c$$
$$\frac{dc}{dt} = k_1 e_0 s - (k_1 + k_{-1} + k_2)c$$

Remember in this notation c is the bound enzyme and source combination, namely SE in the center of the total reaction. Now we want to solve these two equations for s and c. This will yields the results also for e and p since they are as defined as above. We follow Murray by now normalizing the equations. We also note that Murray calculates the change in S not the change in E. Our focus is the change in E over time and space, from that we can obtain the change in S as well. We note from above that we could just as well use the two equations:

$$\frac{ds}{dt} = -k_1 s + (k_1 + k_{-1})c$$
$$\frac{de}{dt} = -k_1 e_0 s + (k_1 + k_{-1} + k_2)c$$

and then solve for s and e. Now for the normalizations we define:

$$\tau = k_1 e_0 t, u(\tau) = \frac{s(t)}{s_0}, v(\tau) = \frac{c(t)}{c_0}$$
$$\lambda = \frac{k_2}{k_1 s_0}, K = \frac{k_{-1} + k_2}{k_1 s_0}, \varepsilon = \frac{e_0}{s_0}$$

Note that we have use ε as the ratio of initial enzyme to initial source. The use of this will become clear since we are assuming that this is small number since the enzymes concentration is small as compared to the source. This assumption must be looked at closely for this reaction. Then we obtain the following set of equations:

$$\frac{du}{d\tau} = -u + (u + K - \lambda)v$$
$$\varepsilon \frac{dv}{dt} = u - (u + K)v$$
$$u(0) = 1$$
$$v(0) = 0$$

These are nonlinear differential equations which we must solve. However the equations have a singularity which must be dealt with before proceeding. If we want to make the ratio ε small then we need to redefine certain factors. We do this as follows:

$$\sigma = \frac{\tau}{\varepsilon}$$
$$u(\tau,\varepsilon) = U(\sigma,\varepsilon)$$
$$v(\tau,\varepsilon) = V(\sigma,\varepsilon)$$

which yields:

$$\frac{dU}{d\sigma} = -\varepsilon U + \varepsilon(U + K - \lambda)V$$
$$\frac{dV}{d\sigma} = U - (U + K)V$$
$$U(0) = 1$$
$$V(0) = 0$$

This transformation eliminates the singularity about 0. The steady state can be shown as follows:

$$[ES] = \frac{k_1}{k_2 + k_3}[E][S]$$

now let the total enzyme be as follows:

$$[E] + [ES] = [E]_0$$

since only small amount enzyme added, the free free substrate is almost the total substrate, eg S, then;

$$[ES] = \frac{k_1[E]_0[S]}{k_2 + k_3 + k_1[S]}$$

and we can show that the steady state implies:

$$\frac{d[P]}{dt} = k[E]_0 \text{ where we have } k = \frac{k_3[S]}{K_M + [S]}$$

and we define:

$K_M \frac{k_2 + k_3}{k_1}$ *as Michaelis-Menten constant*

The above defines the Michaelis-Menten uptake formula. Note the inclusion of the k term which is the rate limiting factor we will see again and again.

There are many other types of reactions and we have discussed them elsewhere. The issue we want to focus on here is that the enzyme is in a reaction of the form where we have a definable time change of enzyme based upon a definable model. That is if [C] is the concentration of an enzyme involved in an enzymatic model we will have a reaction of the type:

$$\frac{\partial[C]}{\partial t} = F([C], t)$$

where F is definable by the reaction. The function F may also consider concentrations of the reactant source and product materials as well. We will use this model in the next section.

$$K = \frac{[C]}{[A][B]};$$

$$and \quad K = \frac{k_1}{k_2}$$

$$\frac{d[P]}{dt} = k_3[C] = k_3 K[A][B] = k[A][B] \quad where \quad k = \frac{k_1 k_3}{k_2}$$

A model for the enzyme reactions in a competitive environment has been employed. A method to solve for the Turing space the diffusion model A model to apply the results to a single anthocyanin The ability to apply to multiple anthocyanin The ability to determine the analysis and the synthesis problem

The Turing model has been discussed earlier. What Turing proposed was that there was some chemical whose concentration made something one way or another. That this something then diffused throughout the organism in some manner and if it was greater in one part than a threshold the morphology was one way and if less the morphology was another. He had no underlying basis in the current understanding of genetics to put details to his models. We now have that detail.

We know that if we have an activator protein on a secondary pathway then that protein will cause the pathway to become active and create the secondary product, an anthocyanin. The more of that protein we have, the greater it concentration, the more secondary product we can get. This is P is the controlling protein concentration, we have:

$$\frac{\partial P_n(x,t)}{\partial t} = H(P_n, A_n, R_n, x, t) + D_P \nabla^2 P_n(x,t)$$

We show such pathways below. In the above pathway we have a complex but modellable set of interactions. They are characterized by:

When there are multiple A-R interactions then they add and the net result is an overlapping of the anthocyanin pathway products. The overlays can be shown to create the typical patters in the Petit list. The model allows for an analysis of any tessellated product and also provides a basis for determining what products are achievable as well as how to achieve them, at least at the genetic level. Now we want to build on this model. First we must look at the dynamics of the activator and repressor genes and then we look at the dynamics of the controlling enzyme. Remember that the activator suppressor genes produce products which control the colorant gene. Let us now look at a single cell and look at the tempero-spatial dynamics of the concentrations of the products of the activator and repressor genes, A and S respectively. We assume we have a model as shown below:

Where in this model we have sets of genes and each has activators and repressors. Each gene may activate a separate pathway as we have shown. First we write the model for the controlling enzyme:

$$\frac{\partial P_n(x,t)}{\partial t} = H(P_n, A_n, R_n, x, t) + D_P \nabla^2 P_n(x,t)$$

In the above we show the concentration for the controlling enzyme in a cell for path n. It has a function H which results from a Michaelis-Menten pathway mechanism which we described earlier. From the Michaelis-Menten analysis before we have, if we assume some separate A, R process:

$$H(P,S,SP) = [+k_1 PS - (k_{-1} + k_2)C]Q(A,R)$$

where we had defined PS and C as before and where Q is a function of A and R which either turns on or off the process creating the P reactant. That is if A>R we have a reaction and otherwise we do not.

Thus P is also affected by concentrations of activator and repressor genes, A and R respectively, but in a binary manner. Second, now we write the general model for the activator and repressor product concentrations. As we have just discussed, the pathway activating protein is either on or off. If on we can then calculate its intensity and if off it is irrelevant. For the activator we have:

$$\frac{\partial A_n(x,t)}{\partial t} = F(A_n, R_n, x, t) + D_A \nabla^2 A_n(x,t)$$

and for the suppressor we have:

$$\frac{\partial R_n(x,t)}{\partial t} = G(A_n, R_n, x, t) + D_R \nabla^2 R_n(x,t)$$

Here we have A and R as the relative concentrations of the products of the Activator and Repressor genes. The F and G functions are the mass balance functions for this mix and the additional loss or gain come from the diffusion term. Here we assume that A and R may diffuse at different rates and this fact is key to the oscillations in space and in turn to the tessellation.

8.6.2 Basic Kinetics and Reactions

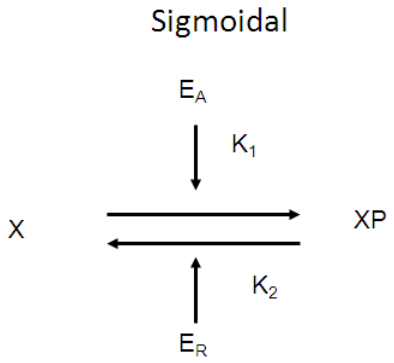
We can now look at a general set of reactions. We assume some reactant X with a concentration [X] and they concentration may increase by means of several different processes. We provide a generic example which is hardly inclusive:

$$\frac{d[X]}{dt} = [Synthesis] - [Degradation] - [Phosphorylation]$$
$$+[Dephosphorylation] - [Binding] + [Release] + etc$$

Now we want to describe a set of such models for the subsequent analysis. Understanding each provides a basis for understanding an integral whole.

1.1.2 Sigmoidal Reaction

We now need a model for the interaction functions. We choose the model provided by Conrad and Tyson in Szallasi et al which is termed the phosphorlation-dephosphorlation model or the sigmoidal model[20]. We show its network below. Here we use the enzyme approach with one enzyme, the activator moving the production of the product enzyme and another the repressor enzyme driving the process backward. As we have done with the enzyme case we assume limited amounts and thus we have the
denominators in the equation.



In the above reaction we look at the concentration of X. Now X increases via k2 and decreases via k1. However both ER and EA enhance those reactions.

[20] There are a multitude of models here.

This is the mathematical model we have deployed again using the same reference. This as we have said assumes that we have some form of enzymatic limiting reaction.

$$\frac{d[X]}{dt} = -E_A g([X]) + E_S h([X])$$

and

$$g([X]) = \frac{k_1[X]}{K_{m1} + [X]}$$

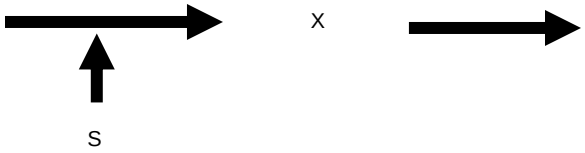
and

$$h([X]) = \frac{k_2\left(x_T - [X]\right)}{K_{m2} + x_T - [X]}$$

The above are also normalized concentrations. We rely upon the recent summary by Baici and the work of McMurray, Schnell as well as Szallasi and his co-authors. This yields a solution for concentrations which is non-linear and which exhibits instabilities and jumps.

8.6.3 Synthesis and Degradation

This is an example from Szalassi et al and considers a mRNA, S, which encodes a protein X. We look at the sole reactant and that its reaction is positively driven via S and decays in a manner consistent with the concentration of the reactant X. The reaction is below:



The dynamics of the linearized reaction is also below.

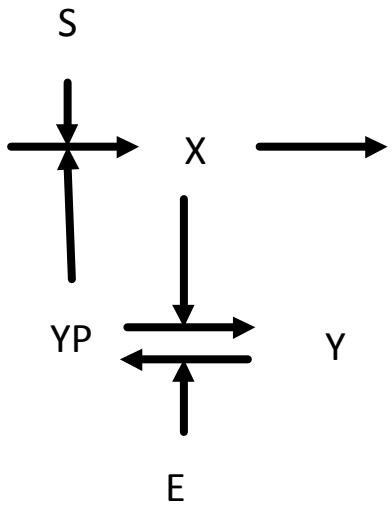
$$\frac{d[X]}{dt} = k_1[S] - k_2[X]$$

Now this reaction is quite simple and it shows exponential growth to a saturation level dictated by S. Here S is the sole outside driver and there is only degradation of [X] due to internal mechanisms.

8.6.4 Negative Feedback

The negative feedback model is one of the simpler models as well. We show it below. Here we have two reactions and both affect the other,

Negative Feedback



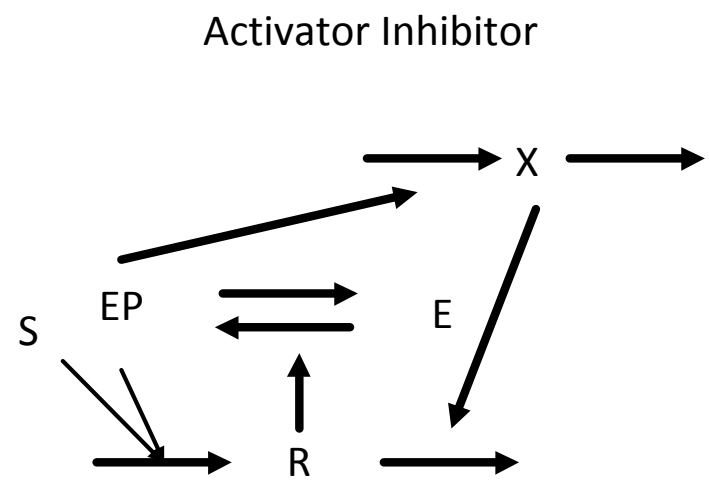
The model then is as follows:

$$\frac{d[X]}{dt} = k_1[S] - k_2[YP][X]$$

$$\frac{d[YP]}{dt} = \frac{k_3[x][[x](y_T - [YP])}{K_{m3} + y_T - [YP]} - \frac{k_4[E][YP]}{K_{m4} + [YP]}$$

8.6.5 Activator Inhibitor

This process has an internal process which activate two processes one of which activates the second.



Here we have a complex process. A substrate S drives the reaction of R as does the enzyme bonded EP and R drives E to EP in a positive loop and EP drives X which in turn drives R to completion.

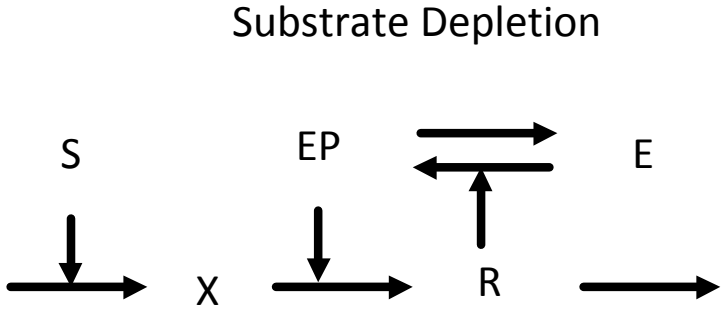
The dynamics of the process are shown below:

$$\frac{d[R]}{dt} = k_0[EP] + k_1[S] - k_2[X][R]$$
$$\frac{d[X]}{dt} = k_3[EP] - k_4[X]$$

Like the other complex processes this shows cyclic instability.

8.6.6 Substrate Depletion

The following shows a process which is initially activated by S then by EP and then the EP is itself activated by the R reactant.



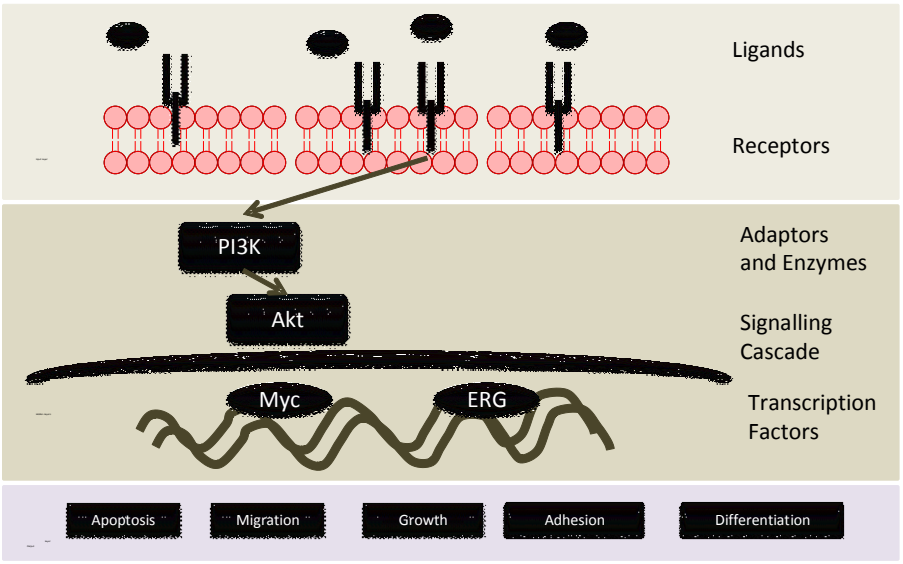
Here we have S producing X and then X produces R which enhances EP which enhances X and the result will be some depletion of S. The dynamics of the process are shown below:

$$\frac{d[X]}{dt} = k_1[S] - \left(k_0^{'} + k_0[EP]\right)[X]$$
$$\frac{d[R]}{dt} = \left(k_0^{'} + k_0[EP]\right)[X] - k_2[R]$$

This system has significant dynamic instabilities. The nature of these instabilities will depend upon the constants and the amount of [S] in the substrate. Note that unlike some earlier models we have limited the dynamics to generally linear in nature, namely first order in each variable.

9 PATHWAY CONTROLS

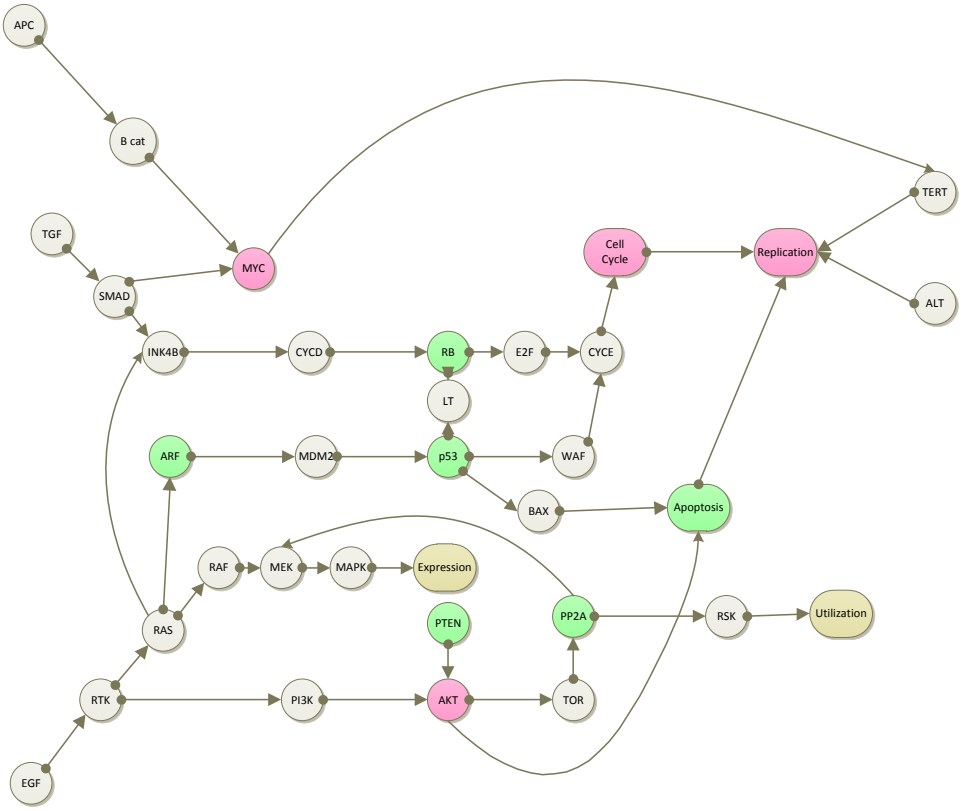
The pathway models are one in which one looks at a cell and examines what causes changes in the cell, growth or death, movement and relationship between cells. The figure below graphically depicts some of the key issues. The cell communicates to the outside via ligands and receptors, and these signal can then be transmitted to the cell nucleus via various chains of signalling mechanism. The signals effectors are most often proteins and these proteins result from genes being expressed in the nucleus. There are also proteins such as c-myc which depending on concentration can effect cell growth and replication, and there are the cyclin kinases which push the cell through the reproduction cycle.



The process can be viewed in two levels; normal cell metabolism of generating proteins via transcription and cell replication, namely the doubling of cells.

As a metric, there are 25 million cells in the human undergoing division per second. There are 50-100 trillion (million million) cells in the human body. Thus every second 0.5/million cells per second are dividing. Clearly that is not the case of every cell. Red blood cells live about 90 days and skin cells have a shorter lifetime. It is estimated that PCa cells double every two years but there can be a great deal of uncertainty in that number. For example if we have a PCa tumor mass of say 10 million cells, and we double to 20 million in 2 years that is 5 million in a year or about 2 cells per second. Recall that we have in the body a cell mitotic process of 25 million per second where a possible PCa is only a ten millionth of that number.

The current view of pathways is a connected map of interactions, activating and suppressing, resulting in certain actions by a cell. The typical example by Holmes is below. We have simplified it a bit.



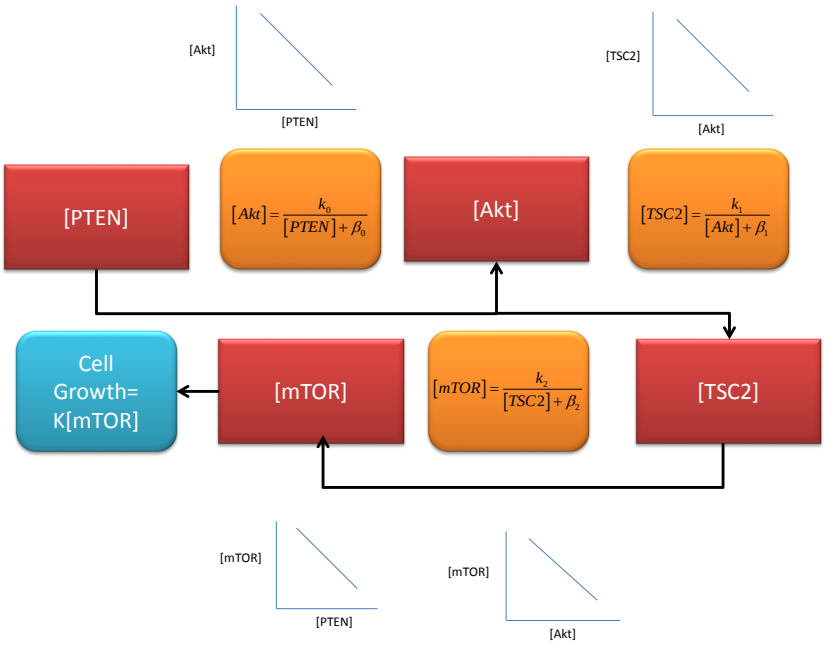
We have shown a few key genes and their products such as PTEN, MYC, p53, AKT and RB. The Holmes model is a graphical connective model and has been used by Milo, Shen-Orr and others. The issues we have with such is that it is necessary to have such a map yet the issues of concentrations, time, and feedback are missing. For example in the above we have PTEN sitting by its lonesome. One may ask what drives PTEN? Is its loss a total loss of the gene or a suppression or just a lowering of concentration with some complex mechanism of enzymatic reactions? To be able to answer these questions we clearly need a much more complex model.

As has been stated, we can summarize some of the key genes involved in PCa as follows:

Gene	*Proposed function*
Mutations causing decreased activity	
MS	Anti-infectious, scavenger receptor
RNASEL	Anti-infectious, apoptosis
ELAC2	Metal-dependent hydrolase
Promoter hypermethylation resulting in gene silencing	
GSTP1	Carcinogen detoxification
Loss of heterozygosity and point mutation	
PTEN	Cell survival and proliferation
***TP53* (also *P53*)**	Cell survival and proliferation, genome stability
Loss of heterozygosity and haplo insufficiency	
NKX3-1	Cell differentiation and proliferation
CDKN1B (P27KIP1)	Cell proliferation
Point mutations	
COPEB* (also *KLR6)	Transcription regulator
AR	Cell proliferation, survival, and differentiation
Amplification	
AR	Cell proliferation, survival, and differentiation
Overexpressed at mRNA and protein level	
HTERT	Cell immortality
HPN	Transmembrane protease
FASN	Fatty-acid synthesis
AMACR	Fatty-acid metabolism, branched chain
EZH2	Transcription repressor, cell proliferation
MYC	Cell proliferation
BCL2	Cell survival
Polymorphisms affecting prostate cancer risks	
AR	Cell proliferation, survival, and differentiation
CYP17	Androgen metabolism
SRD5A2	Androgen metabolism

9.1 CELL GROWTH AND ITS METABOLIC CONTROL

One of the first elements of control is via the metabolic processes that control cell growth. It was in 1926 that Warburg proposed that cancer cells have a metabolic process which was anaerobic instead of aerobic like health cells. This premise then forced researchers to examine pathways which were significantly different that those in benign cells. A recent study by Levine and Kuter demonstrates in summary form how this applies to the cancer cells, many of which are core to PCa.



$$\frac{d[PTEN]}{dt} = unknown$$

$$\frac{d[Akt]}{dt} = f_1([PTEN],[Akt],t)$$

$$\frac{d}{dt} =$$

$$\frac{d[mTOR]}{dt} = f_3(,t)$$

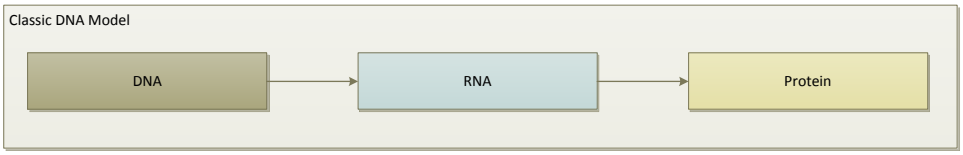
$$\frac{d[Cell\ Growth]}{dt} = g([mTOR])$$

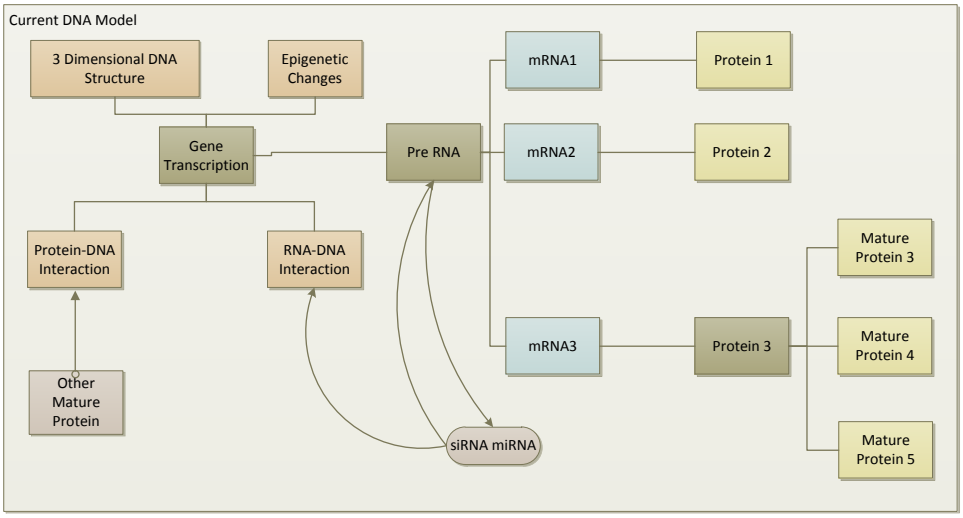
We can expand this model depending on the complexity of the networks.

9.2 PATHWAY DYNAMICS

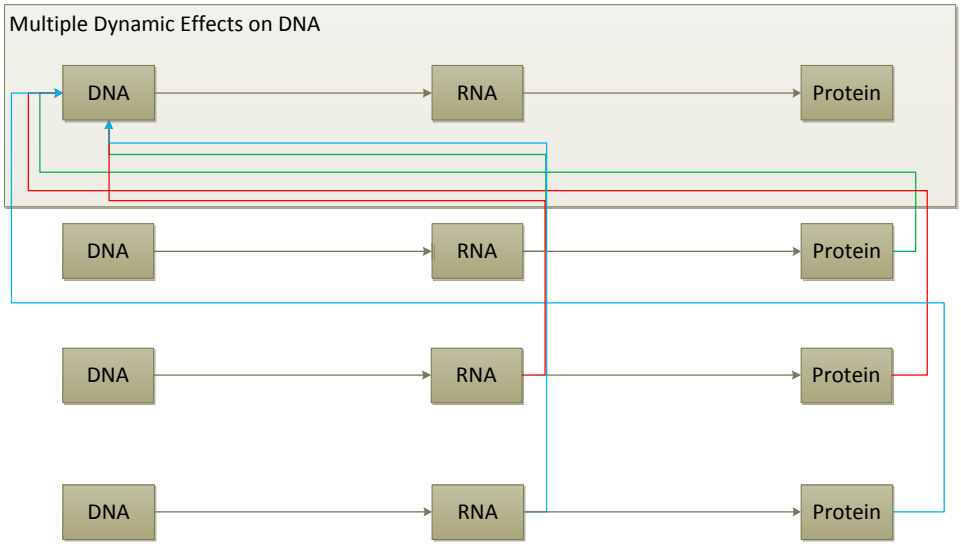
We now look at the evolution of pathways and how they work. Below we show a modified graphic based upon the work of Feero et al. On the top we show the classic Watson-Crick model. A simple progression from DNA to RNA to Protein. Then below we show how the concept has evolved to the way most see it today. Note that it still lacks the three factors we discussed earlier;

concentration, time factors, and full feedback. There is some feedback implied but a limited amount.





Now we can show some of the feedback mechanisms. This is in the graphic below:



Note that we have done this but for one gene segment. Remember here the DNA segment refers to a specific gene and the RNA the RNA resulting from that gene.

There are many models of pathway development and description. A simple approach is given in Bolouri which is basically an on-off type network switch design. He uses methods which have

some dynamics but is limited. In the book by Wang we find other similar alternatives. There is some excellent work in Szallasi et al and we have used it in the past. In McGarty (2008) we developed models which expanded the dynamics and allowed for the identification issue.

9.3 PATHWAYS, GENE EXPRESSION MEASUREMENT, METASTASIS

We have argued before that progress in cancer will develop as we better understand not just the histological appearance of the cells but the genetic expressions therein as well. Indeed many attempts at this have been taken and some seem to be bearing fruit. We look here at some recent work done at Dana Farber with regard to prostate cancer. The approach taken was to look at a large set of cells and then using massive microarray processing attempt to stratify the most significant markers related to metastasis. This is a useful example of understanding genes and their expression. However, our concern with their approach also highlights the overall lack of global knowledge and understanding of the genetic dynamics and linkages controlled by pathways. We use this example as a current step which explains the need for understanding the entire process and avoiding what Dougherty has commented on as the problem of just processing massive amounts of data and resolving on something which may or may not be the right thing.

One of the questions one frequently asks is how do we determine from a biopsy the eventual aggressiveness of PCa. This is a difficult question since we know for example that the loss of PTEN is often an ominous sign. It often pretends an already metastasized PCa, albeit without any way of determining where it has metastasized. In this section we look at a recent suggested test which would augment the histological analysis of Gleason scoring. The test proposed by the team at Dana Farber, of "Farber", entails looking at gene profiles and then using them in a prognostic manner[21]. We discuss this approach, which we have argued for in general before, and discuss its implications and present an alternative manner in which such tests in general may be analyzed.

The gene and gene products which were targeted are shown in the following Table:

Gene/Gene Product	*Function*	*Location*
Pten	*Pten controls the Akt pathway which if not controlled will lead to excessive cell growth.*	*10q23.3*
Smad4	SMAD4 controls the G1 to S transition.	*18q21.1*
SPP1	SPP1 is involved in immune cell activation, wound healing, and bone morphogenesis and plays a major role in regulating mineralization processes in various tissues. Increased SPP1 expression is often associated with pathological calcification.	*4q21.1*
CyclinD1	*Cyclin D is a control with CDK4 and CDK6 of the transitions in the G1 to S stage of mitosis. Lack of control of Cyclin D will allow for uncontrolled cell growth.*	*11q13*

We examine some of the issues related to this study and then discuss some new questions arising from it.

[21] http://www.dana-farber.org/abo/news/press/2011/dana-farber-researchers-identify-molecular-predictor-of-metastatic-prostate-cancer.html

Basically what this study has done is looked at the genomic content of a cell, a malignant cell, and it has tried to ascertain what the degree of potency for metastasis the cell may have. If it is an indolent cell then perhaps a wait and see attitude may prevail. If, however the profile indicates the potential for aggressive growth then surgery should be the option, or some other form of treatment eradicating the cells which hopefully are localized.

The approach by the researchers at the Farber seem to be to examine large samples and then using standard statistical techniques focus on a small targeted gene product set and if the expression of those genes is significantly over or under expressed then one can say with reasonable confidence that the aggressive treatment is warranted.

However this study does not seem to approach this study from a dynamical approach or an approach which relies on the essential pathways relating genes in the homeostasis of the cell.

9.3.1 Dana Farber Study

In the aforementioned recent Dana Farber research study the results state[22]:

"In the current study, researchers began with the well-established fact that prostate cancers without (sic) a working copy of the *Pten* gene tend to remain fairly idle and don't trespass beyond the prostate gland itself[23]. Researchers theorized that the loss of *Pten* in turn activates a collection of genes — a pathway — functioning to constrain the tumor's growth and invasion. If that pathway was shut down, they reasoned, the tumor would begin to break loose from the prostate and spread insidiously through the body.

Using computational biology techniques to analyze gene activity in mouse prostate cancer cells with inactive *Pten*, the investigators found a few pathways that seemed to play a constraining role. One, known as TGFβ-SMAD4 (for some of the genes that comprise it), was particularly intriguing as this pathway had been implicated in the metastasis of other tumor types in the past. When researchers conducted confirmatory molecular signaling studies to see what happens when *Pten* is knocked out of commission, signaling in the TGFβ-SMAD4 pathway "shot through the roof," DePinho says, suggesting that the pathway had sprung into action.

When researchers generated mice whose prostate cells lacked both *Pten* and the *Smad4* gene, the animals developed large, fast-growing tumors that spread to their lymph nodes and beyond. Guided by these insights, they then examined whether something similar was happening in human prostate cancers.

[22] http://www.dana-farber.org/abo/news/press/2011/dana-farber-researchers-identify-molecular-predictor-of-metastatic-prostate-cancer.html also see Nature paper http://www.nature.com/nature/journal/vaop/ncurrent/full/nature09677.html

[23] We believe that this is a mis-statement. PTEN inactivation is known in metastatic PCa and thus we suspect that they are misquoted. The remainder of the article enforces this belief.

Comparing the gene expression profiles of indolent versus aggressive mouse prostate cancers, they found about 300 genes that distinguished the two groups. "We then categorized them for known functions," DePinho says. "We were encouraged to see that the top functional category were genes playing that have roles in cell division and movement" — actions that are needed for cancer cells to grow and spread with lethal consequences.

The researchers conducted an elaborate series of experiments to identify the genes most closely linked to the aggressive biology of prostate cancer. Among the hundreds of genes analyzed, two such genes stood out: *SPP1* and *CyclinD1*, both of which, intriguingly, are close working partners of *Smad4*.

The four-gene signature — *Pten*, *Smad4*, *SPP1*, and *CyclinD1* — showed its effectiveness as a predictive tool for survival when researchers drew on data from the Physicians' Health Study, which has been tracking the health of thousands of U.S. physicians for nearly 30 years. When the investigators screened prostate cancer samples from study participants for the four-gene/protein signature, it was more accurate in predicting the ultimate course of the illness than conventional methods were.

"By integrating a variety of techniques — computational biology, genetically engineered model systems, molecular and cellular biology, and human tissue microarrays — we've identified a signature that has proven effective in distinguishing which men with prostate cancer are likely to progress and die from their disease and those who are not," DePinho remarks. "Efforts are already underway to use this knowledge to develop a clinical test — which we hope will occur within a year or so — that will enable doctors and patients to make more accurate treatment decisions and avoid unnecessary aggressive interventions which adversely impact on quality of life and deplete over-extended healthcare resources. This science holds potential to illuminate a long-sought answer for optimal management of this complex disease."

Thus we look again at the pathways. Our interest is in those pathways which effect:

1. *Pten,*
2. *Smad4,*
3. *SPP1, and*
4. *CyclinD1*

9.3.2 PTEN Suppression

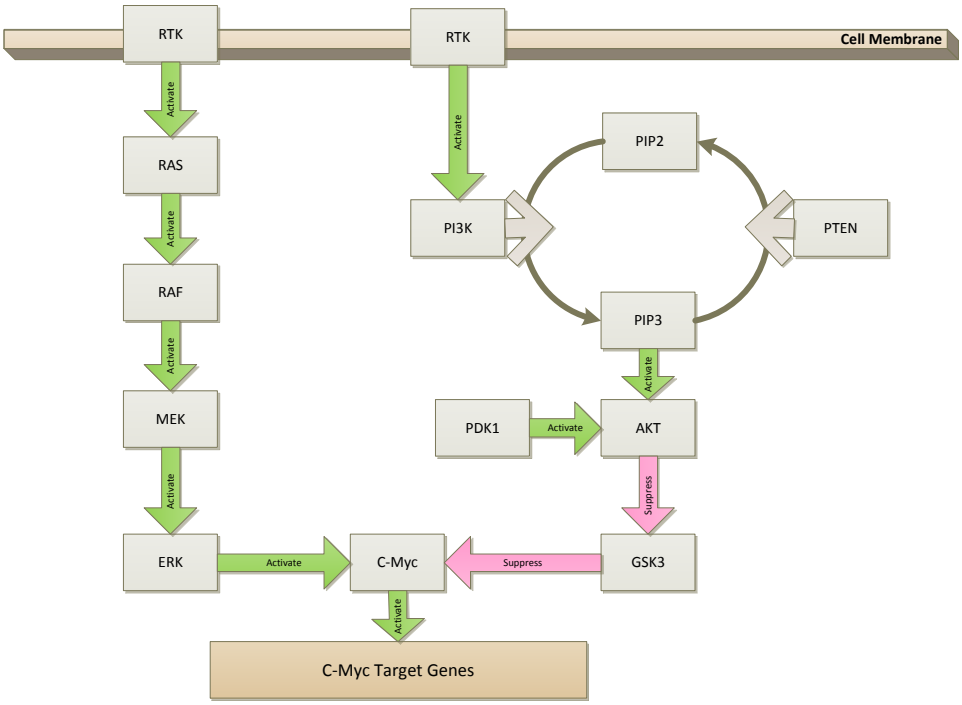
PTEN is a significant gene which controls the Akt pathway which in turn controls the replication of cells. Loss of PTEN is often seen in metastatic prostate cancer. In many ways it is the hallmark of this change. As stated in NCBI[24]:

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases,

[24] http://www.ncbi.nlm.nih.gov/gene/5728

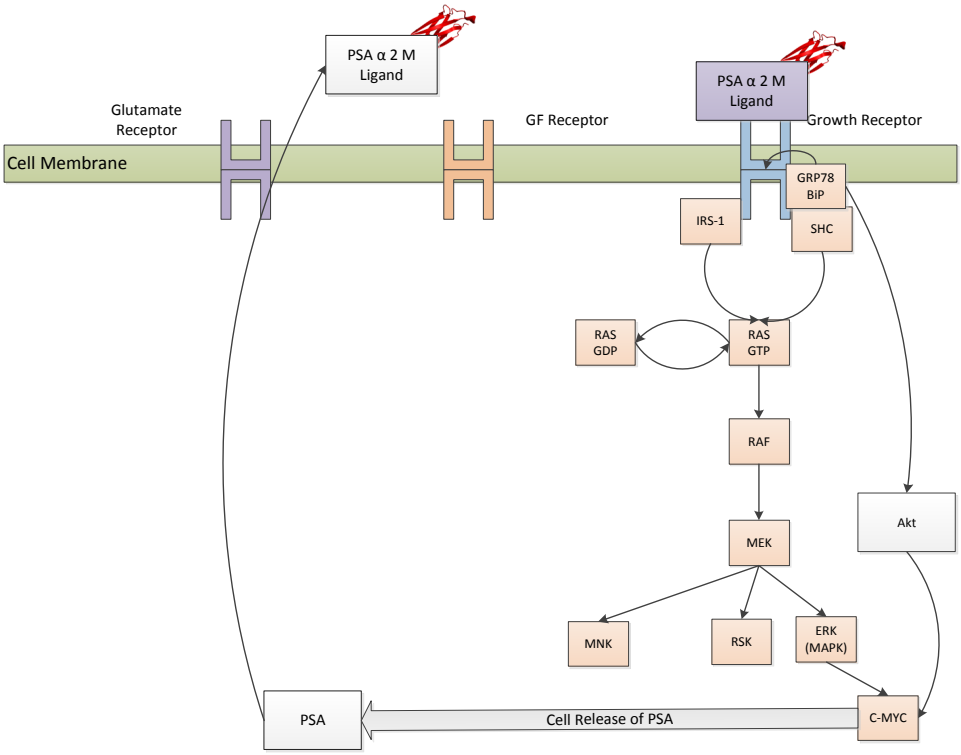
this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway.

First the PTEN pathway as shown below:



Note PTEN modulates the production of Akt which in turn modulates c-Myc which in turn controls cell reproduction. Any effect which causes PTEN to not be expressed will in turn result in unfettered cell growth.

We can amend this with the details on the Ras pathway as shown below:



9.3.3 Cyclin D

Cyclin D is one of the key regulators of the cell cycle. As Bunz states (Bunz, pp 218-221) the cell cycle goes through several well-known phases. There are phase specific kinases which are cyclins which are called that because they were found to increase or decrease in a cyclical manner as the cell cycle phase progressed.

In the cycles the cyclin binds with a cyclin-dependent kinas or CDK. The activated cyclin-CDK complex phosphoralates phase specific substrates. Cyclin D along with CDK4 and CDK6 facilitate the transition through G1 to the start of S for example. Cyclin E with CDK2 facilitates the transition from G1 to S. Cyclin A with CDK2 moves through S. Cyclin A/B with CDK1 moves through G2. Thus activation of Cyclin D is a sign that cell replication has commenced.

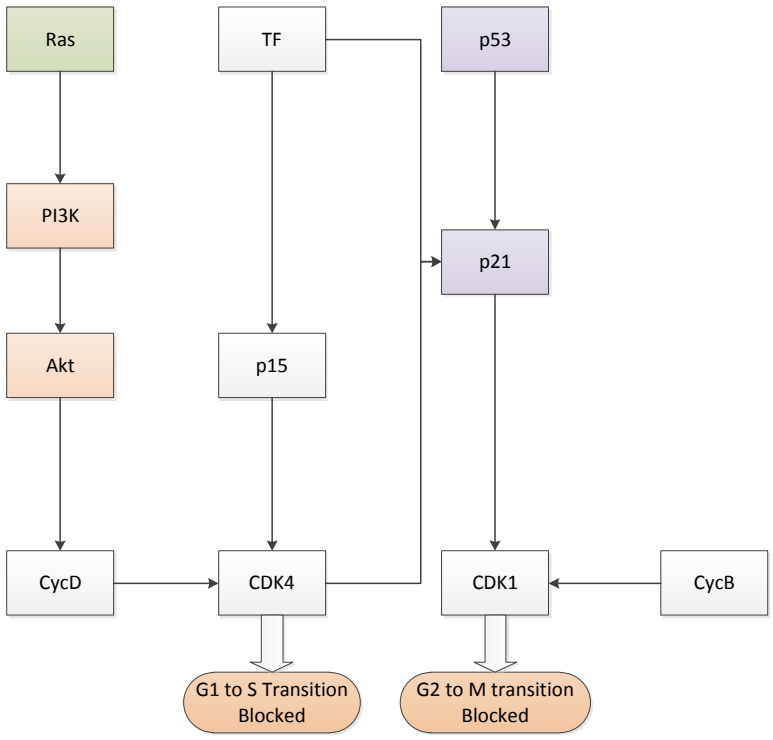
As stated in NCBI[25]:

The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb.

[25] http://www.ncbi.nlm.nih.gov/gene/595

Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis

Now we can look more closely at Cyclin D, CycD, as we show below. This we show as follows:



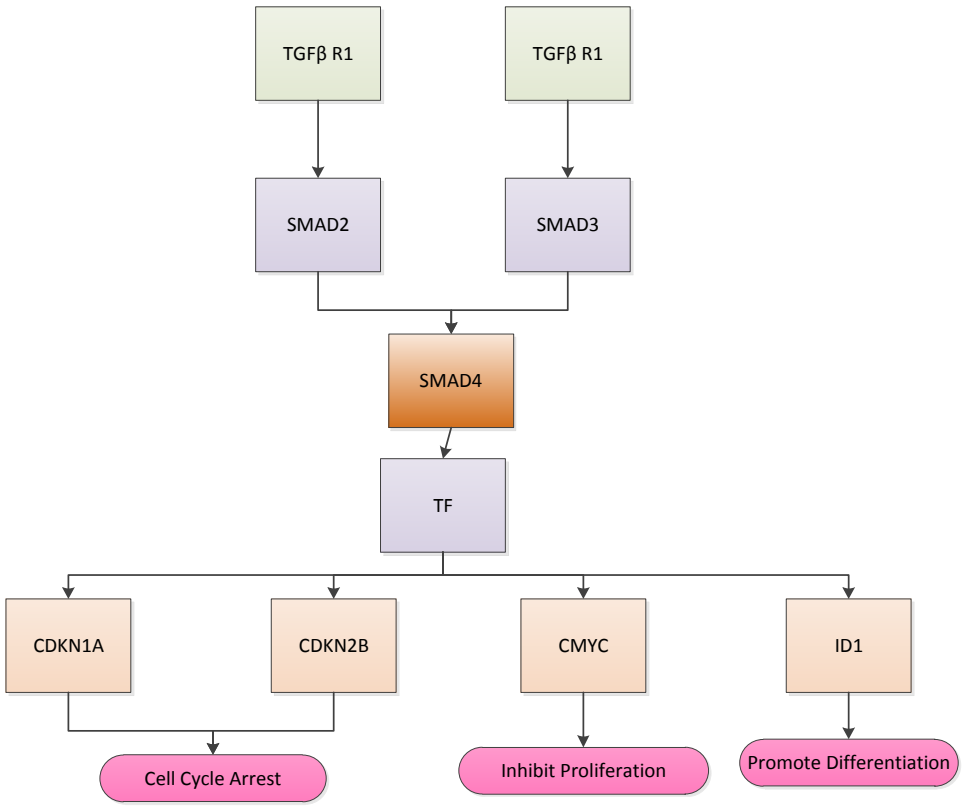
Note that Cyc D if not regulated will in turn fail to regulate the blocking of the G1 to S transition.

9.3.4 SMAD4

SMAD4 is an element in the TGF-β signalling chain. TGF is a cytokine, specifically a transforming growth factor cytokine. Like the Wnt-Apc pathway, the TGF pathway links defective development to cancer. The pathway is shown in part below (from Bunz p 199). Normal TGF signalling down-regulates the growth of most normal cells. Several of the genes in the TGF/SMAD pathway activation suppress growth. Specifically the genes CDKN1A and CDKN2B encode the cyclin dependent kinase inhibitors which suppress growth. Activated SMAD pathways also appear to suppress the transcription of other genes including c-Myc.

Gene	***Function***	***Disease***	***Pathway***
EWSR1	Translocation	Ewing's sarcomas, lymphomas, leukemias	SMAD
RUNX1	Translocation	Leukemias	SMAD
SMAD2	Inactivating codon change	Colon, breast	SMAD
TGFBR1, TGFBR2	Inactivating codon change	Colon, stomach, ovarian	SMAD

We show some of the TGF SMAD signalling below. We will elaborate this later.



SMAD4 controls the G1 to S transition. As stated in NCBI[26]:

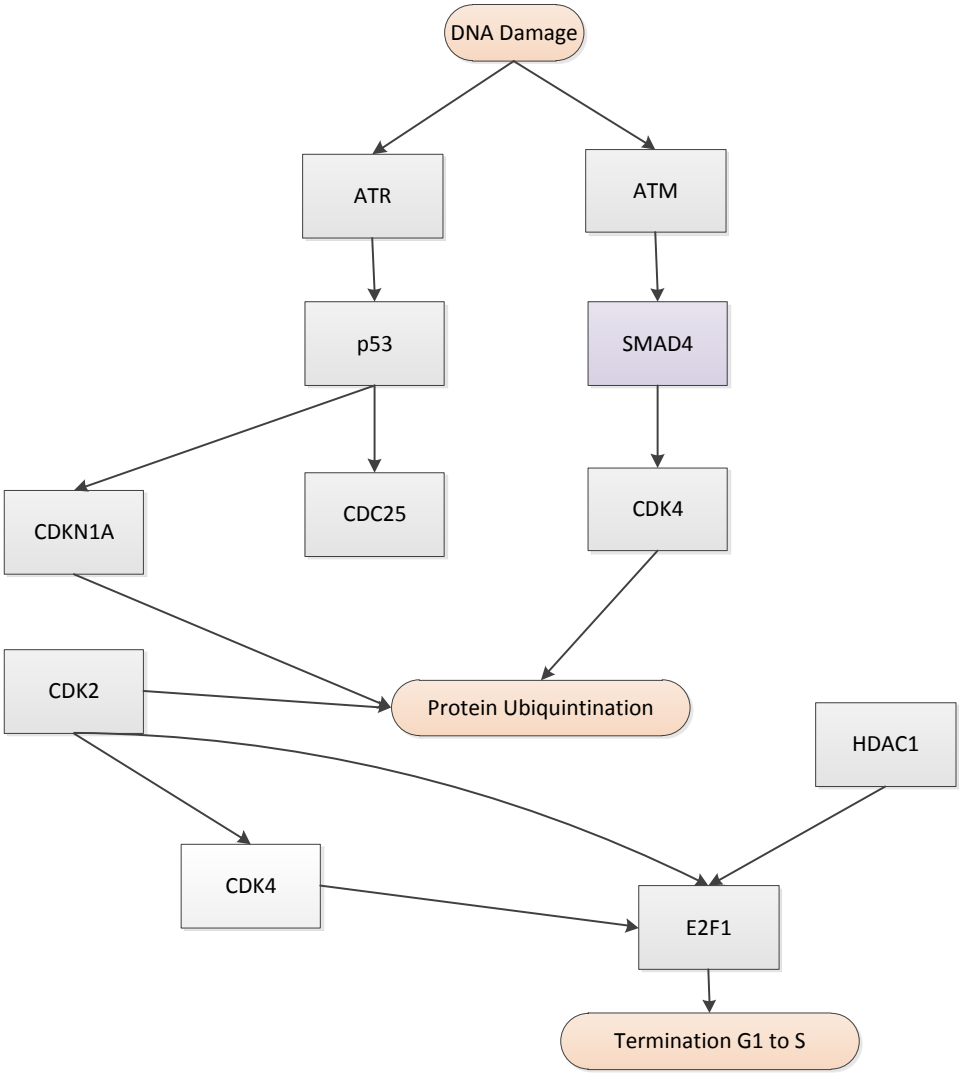
This gene encodes a member of the Smad family of signal transduction proteins. Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes.

This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome.

We use the NCI data set for its pathway[27]:

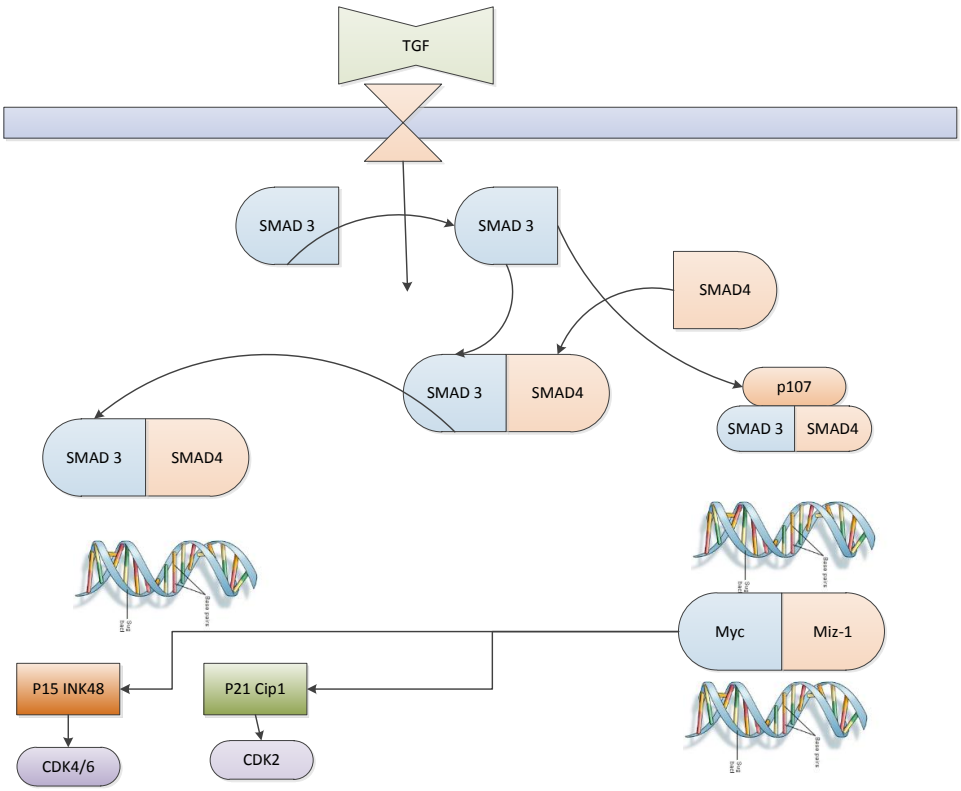
[26] http://www.ncbi.nlm.nih.gov/gene/4089

[27] http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=100160&source=BioCarta&genes_a=4089&genes_b=&what=graphic&jpg=on&ppage=1



The SMAD pathway is also detailed by NCI and one is referred to that source for further detail.

From Weinberg (p 291) we also have the SMAD4 pathway showing its immediate control of the DNA transcription.



As Weinberg states (p 292):

"… Half of all pancreatic carcinomas and more than a quarter of all colon carcinomas carry mutant inactivated Smad4 proteins. Without the presence of Smad4 neither Smad2-Smad4 nor Smad3-Smad4 complexes can form. These two complexes are the chief agents dispatched by the TGF-β receptor to the nucleus with the important assignment to shut down proliferation."

This control mechanism is shown above.

9.3.5 SPP1

SSPI is secreted phosphoprotein 1, also commonly known as Osteopontin (OPN), also known as bone sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), 2ar and Rickettsia resistance (Ric), is a human gene product which is also conserved in other species[28].

From Hendig et al, they state that SPP1 is a secreted, highly acidic phosphoprotein that is involved in immune cell activation, wound healing, and bone morphogenesis and plays a major role in regulating mineralization processes in various tissues. Increased SPP1 expression is often associated with pathological calcification. Furthermore, SPP1 is a constitutive component of human skin and aorta, where it is localized to the elastic fiber and hypothesized to prevent calcification in the fibers.

[28] Also see http://www.ncbi.nlm.nih.gov/gene/6696 also see http://www.wikigenes.org/e/gene/e/6696.html

SPP1 is a predominantly transcriptional regulated gene, and the *SPP1* promoter is highly conserved among different species *(22)*. Several polymorphisms in the *SPP1* gene affect SPP1 expression and have been associated with various disorders, e.g., systemic lupus erythematosus and arteriosclerosis.

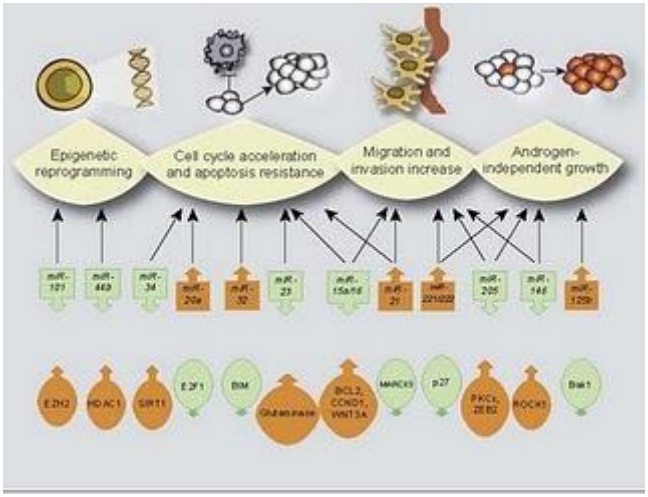
SPP1 is a SIBLING glycoprotein that was first identified in osteoblasts. OPN is an important anti-apoptotic factor in many circumstances. OPN blocks the activation-induced cell death of macrophages and T cells as well as fibroblasts and endothelial cells exposed to harmful stimuli. OPN prevents non-programmed cell death in inflammatory colitis. It has been shown that OPN drives IL-17 production; OPN is overexpressed in a variety of cancers, including lung cancer, breast cancer, colorectal cancer, stomach cancer, ovarian cancer, melanoma and mesothelioma; OPN contributes both glomerulonephritis and tubulointerstitial nephritis; and OPN is found in atheromatous plaques within arteries. Thus, manipulation of plasma OPN levels may be useful in the treatment of autoimmune diseases, cancer metastasis, osteoporosis and some forms of stress. Research has implicated osteopontin in excessive scar-forming and a gel has been developed to inhibit its effect.

9.3.6 Analysis

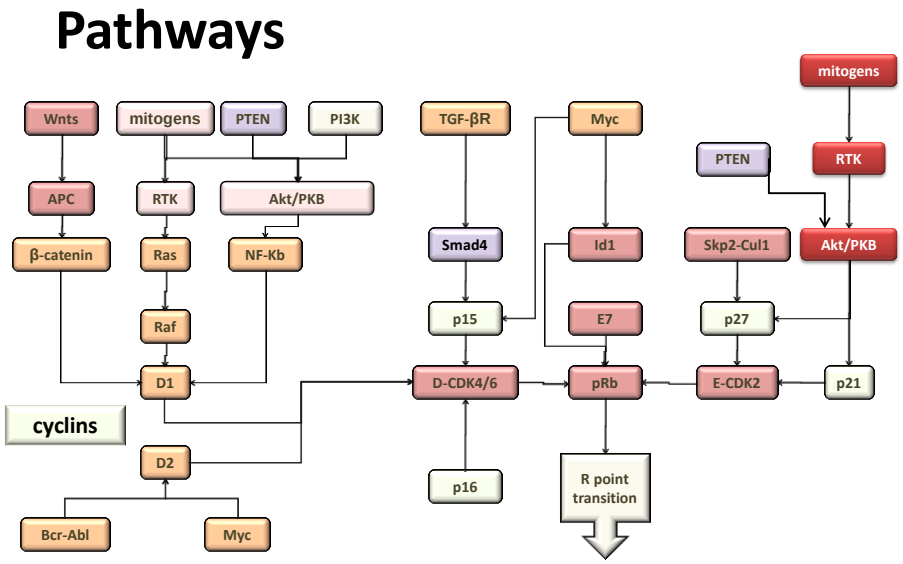
In a recent announcement from Dana Farber in Boston, a paper has been prepared that indicates that testing for four gene products significantly improves the ability to determine an indolent Prostate Cancer from an aggressive form. The results also hit the news including a WSJ release.

The release from DF states:

The four-gene signature — Pten, Smad4, SPP1, and CyclinD1 — showed its effectiveness as a predictive tool for survival when researchers drew on data from the Physicians' Health Study, which has been tracking the health of thousands of U.S. physicians for nearly 30 years. When the investigators screened prostate cancer samples from study participants for the four-gene/protein signature, it was more accurate in predicting the ultimate course of the illness than conventional methods were.



We show some of the pathway elements above. A more detailed version is below.



In the above we show the PTEN control, the Smad4 control and the presence of the cyclins. The loss of PTEN has been known for years to be a sign that metastasis may very well already started. SMAD4, SPP1 and the Cyclin D1 are in pathways that also control the growth of the cell. PTEN is most often the one pathway element of most concern.

The driving factor in the result is:

The standard measure of prostate cancer's aggressiveness, known as the Gleason score (which is based on cancer cells' appearance under a microscope), is accurate about 60 to 70 percent of the time depending on the skill of the pathologist. The four-gene signature method alone was accurate 83 percent of the time. Combining the markers and Gleason methods produced an accuracy of approximately 90 percent.

Now the above result need some clarification.

1. Gleason scores are score based upon the histological presentation of the cells. Gleason 1 for example is low grade and shows the cells as small gland like structures but lacking gland architecture. Gleason 5 is a mass of undifferentiated cells clustered about the stroma, internal part of the prostate, with no structure. Gleason scores are the sum of the most prevalent cell type plus the next most prevalent so a 7 is a 4+3 or a 3+4. Clearly a 4+3 is more severe than a 3+4. But Gleason alone tells one little about the metastatic potential.

2. Genetic pathway changes are often the sine qua non for determination. But what genes and in what cells. The problem is the existence of the cancer stem cell idea, namely that one of the many cancer like cells is pluripotent and if this is true in prostate cancer then it is that cell we want. So perhaps in a biopsy we missed the killer cell, or it may have already escaped.

3. This procedure statistically takes us a step forward, now we can test to see if we do have some very bad cells. However one may ask if we are finding out when the cow has already left the barn. Namely what we may have is a test which conforms the fact that the patient's cancer is one of those very bad types so nothing much can be done. On the other hand we may have a patient with an aggressive form which is just a threshold below the bad level. Thus one need significant clinical tests.

4. The question then is; are there other pathway constituents that are prognostic enough to have a meaningful result in mortality, namely how well do we know the PCa pathways. What are the pathway dynamics between these four, we know some, and many are available through NCI.

5. The results appear to have quite extensive, but causality and dynamics still need some filling in. What caused the change. In addition one may look at the HGPIN cases and see that we have HGPIN which all too often is considered as pre-malignant, but we know that HGPIN also regresses to a fully benign prostate. What happened.

The questions that seem to remain all relate to pathway dynamics. They are:

1. What are the pathway dynamics of each of these markers. Is there some causal relationship or are they independent events?

2. What caused the change in expression? Is there a change in the gene or are there other factors. Are there epigenetic issues such as miRNA or methylation. Are there activator or repressor genes related to the transcription of these genes which result in the change. If so how are these genes related in the overall gene network.

3. What are the temporal issues of this gene network? Which changes are causal and which are simultaneous.

4. What are the spatial issues, namely do the modified cells communicates with others to allow for the development of PCa clusters and if so how do these genes function in such an environment.

5. What of the stem cell issue? If there are PCa stem cells and these cells are drivers of the overall metastatic process, does sampling for these markers reflect the stem cell or the tumor mass in general? The CSC is a significant question and if one must select that cell how does one identify the cell? Are there CSC markers for CSC PCa cells.

These are but a few of the questions. The lingering one however is when these markers are detected is it really one where the cow is out of the barn or is it just time to bar the door?

As the WSJ states:

Dr. DePinho said the new study suggests a test based on the four genes, when added to current prediction protocols could improve the accuracy to about 90%....Charles Sawyers, a cancer expert and Howard Hughes Medical Institute Investigator at Memorial Sloan Kettering Cancer

Center in New York, said if it turns out that such a test is that effective, "it would have a huge impact." He wasn't involved in this research.

Dr. Sawyers cautioned that such gene signatures have been proposed to predict cancer progression in the past and haven't panned out. He said the genes the new research focuses on appear to have a functional role in regulating cancer development, but that more research will be necessary to validate whether a test would have value for doctors and patients..."We'll have to wait and see," he said.

Indeed one must wait and see. However this will be a challenge to Medicare, since so many of the patients are Medicare patients. How effective is this test? If the genes are all showing aggressive growth then we act? Or is it too late already. If the genes do not show aggressive growth do we deny the patient care even if the patient has strong family history of aggressive PCa? The issue is that all too often the family history dominates. Perhaps this creates more issues than it solves, however it is a step in a good direction, it may or may not be the right one.

9.3.7 Gene Tables

The following Tables are taken from the paper by Chandran et al and represent a significant amount of detail on all genes which have been identified as either over or under expressed in PCa. Causality is not evident.

Table 3: Transcripts with median values with at least 3 fold difference between metastatic and primary tumor samples

Gene Symbol	**Probe_ID**	P1	P2	P3	P4
HBB	32052_at	22.37	5.78	13.25	56.28
SPP1	34342_s_at	24.16	26.78	4.75	5.39
HBA1///HBA2	31525_s_at	15.14	4.47	13.65	108.11
LGR4	43585_at	7.39	7.43	20.89	24.82
AR	1577_at	14.35	12.97	12.24	14.78
PRO1073	49666_s_at	4.56	13.25	10.01	13.50
UTRN	42646_at	10.11	6.02	12.11	16.31
HNT	59070_at	5.37	9.69	12.08	13.67
SDCCAG3	43014_at	7.99	8.57	11.24	17.32
LOC64744	42739_at	7.30	9.64	9.57	14.51
---	1089_i_at	5.06	4.14	12.12	22.01
SPP1	2092_s_at	14.05	12.94	3.35	4.07
UBE2H	58777_at	9.50	7.45	6.55	15.13
SRPK1	63687_at	6.06	4.36	10.61	12.82
NCK2	33003_at	5.00	9.14	8.50	7.34
HIST1H3H	36757_at	7.26	17.07	8.47	5.61
PPP4R2	48663_at	5.09	6.97	8.59	16.16
C8orf16	47339_at	6.54	8.15	9.53	7.39
---	55943_at	3.41	7.47	15.31	8.01
---	64642_s_at	8.25	6.42	7.04	10.60
EP400	47518_at	5.94	9.27	4.51	9.32
GOLT1A	45144_at	3.90	6.17	8.37	12.32
---	52853_g_at	9.83	7.10	6.25	7.03
LOC284058	44791_at	8.25	10.17	4.55	5.86
DAPK1	51580_at	3.42	6.04	8.03	11.32
NFATC2IP	38864_at	3.26	4.83	9.58	9.19
SEL1L	40689_at	4.71	7.84	6.13	10.94
TM4SF9	47746_at	3.43	6.26	8.92	7.52
MLLT2	65205_at	3.43	7.13	6.57	13.01
SC4MOL	46802_at	22.91	7.35	5.62	6.17
---	62671_at	6.38	7.13	5.74	11.03
BIRC6	46558_at	5.67	8.59	7.50	5.92
MAP4K4	51474_at	4.86	4.32	8.70	8.52
MLLT2	53300_at	4.65	3.99	9.39	8.10
---	52851_at	8.71	5.94	6.35	6.25
MRRF	51635_at	4.23	4.87	7.39	8.23
ACAS2	62783_at	4.29	6.14	7.02	5.90
---	60658_at	3.40	6.84	5.19	9.10
SUMO1	49551_at	4.05	7.20	4.77	7.75
AR	1578_g_at	7.56	4.86	5.32	6.37
GALNT7	59101_at	8.41	4.12	5.11	6.54
GPR75	44203_at	5.14	8.32	3.90	6.31
TBL1XR1	65001_r_at	3.53	12.30	4.06	7.19

HSD17B12	43292_at	4.74	8.88	3.64	6.28
MRPS28	43095_at	5.79	5.39	5.58	5.14
FN1	64719_at	27.07	6.02	4.05	4.93
GPR158	44214_at	7.21	3.33	4.32	6.62
---	48069_at	6.27	9.88	3.38	4.55
FLJ21657	58778_at	4.34	5.60	6.17	5.18
MLL5	43301_at	4.76	3.61	5.87	10.34
---	55761_at	3.78	4.88	5.65	6.93
DLG1	47231_at	3.40	4.77	6.22	5.70
MYO5B	63281_r_at	3.29	6.17	4.29	6.84
---	49268_at	3.55	19.86	3.61	6.75
FUS	43501_at	3.93	3.78	6.42	8.97
CCDC35	54684_at	4.90	8.14	3.55	5.43
---	43435_at	6.85	4.83	4.82	5.49
SMA4	32921_at	4.68	5.53	5.74	4.26
NCOA1	45953_at	6.53	4.13	3.58	6.06
S100A8	41096_at	4.22	5.89	3.80	22.58
PRKCBP1	53493_at	4.65	7.37	4.50	5.35
RNPC2	65083_at	3.18	3.96	6.01	9.19
CAMSAP1	62630_at	4.45	5.80	3.36	5.36
EEF1G	41903_at	5.19	4.58	4.31	5.34
EIF5	51379_at	3.44	4.08	5.62	11.07
MAML3	49879_at	3.39	3.22	10.27	5.87
C21orf106	59651_at	3.19	4.02	5.23	6.44
VCIP135	42715_at	3.37	3.61	5.52	8.55
FOXO3A	55502_at	3.48	4.37	6.97	4.74
C7orf20	49143_s_at	4.23	4.62	4.41	5.78
GNMT	46482_at	3.59	4.84	4.24	4.64
DONSON	48549_at	4.10	3.58	4.66	5.28
---	43436_g_at	4.98	3.75	3.58	5.09
PKP4	66327_at	3.31	3.88	4.56	6.20
PCBP2	55393_at	3.73	3.19	4.36	6.29
CPEB4	57169_at	3.70	3.92	4.14	4.48
CUGBP1	34683_at	4.26	3.76	3.13	4.78
FALZ	47458_at	4.21	3.65	3.82	4.09
---	51586_at	3.51	4.00	4.99	3.89
RALA	39253_s_at	3.92	4.30	3.29	3.85
MLL5	45092_at	4.36	3.21	4.48	3.39
PABPC1	44806_at	3.74	3.98	4.20	3.07
EIF1AX	34278_at	3.99	3.47	3.84	3.19
C7orf2	42173_at	3.15	3.27	5.07	4.04
---	63147_at	3.25	5.40	3.12	4.04
RAD23B	41157_at	3.20	3.46	3.64	4.45
---	61037_at	3.44	3.56	3.47	3.73
NFATC1	39143_at	3.13	3.21	9.06	3.78
JARID1A	50532_at	3.22	3.32	3.54	4.12
PDLIM5	37366_at	3.02	3.58	3.42	3.16

Table 3: Transcripts with median values with at least 3 fold difference between metastatic and primary tumor samples

Gene Symbol	**Probe_ID**	P1	P2	P3	P4
NEFH	33767_at	(117.15)	(147.36)	(9.90)	(17.18)
C10orf116	32527_at	(35.49)	(29.63)	(46.85)	(66.50)
KLK11	40035_at	(23.65)	(19.24)	(39.73)	(62.15)
FAM3B	59657_at	(15.81)	(27.92)	(26.09)	(25.97)
PGM5	52140_at	(23.87)	(26.50)	(44.27)	(17.72)
MRGPRF	52946_at	(15.61)	(18.57)	(30.59)	(70.95)
KRT15	37582_at	(21.85)	(20.74)	(19.22)	(33.68)
PTN	34820_at	(11.62)	(31.95)	(10.24)	(27.11)
SELM	64449_at	(6.36)	(8.40)	(29.23)	(39.36)
MYLK	46276_at	(5.87)	(15.22)	(22.57)	(20.86)
SYNPO2	50361_at	(15.14)	(15.77)	(20.15)	(84.14)
KRT5	613_at	(13.21)	(11.12)	(22.66)	(32.96)
FOS	2094_s_at	(10.72)	(25.75)	(13.72)	(16.45)
PKP1	51214_at	(11.57)	(16.34)	(11.83)	(17.85)
---	42921_at	(9.96)	(11.67)	(15.61)	(16.50)
RAB34	45269_at	(14.36)	(11.54)	(17.49)	(10.35)
---	48927_at	(10.61)	(14.93)	(8.77)	(21.91)
ALOX15B	37430_at	(12.47)	(12.41)	(14.17)	(9.10)
FOS	1915_s_at	(7.59)	(26.38)	(11.03)	(12.11)
TMEM16G	62387_at	(9.63)	(13.32)	(12.59)	(9.93)
---	64676_at	(17.30)	(9.39)	(6.32)	(13.05)
SFRP1	32521_at	(13.10)	(5.73)	(8.29)	(16.73)
NDFIP2	60510_at	(7.20)	(9.23)	(11.72)	(15.15)
FHOD3	50298_at	(9.96)	(12.84)	(5.59)	(10.96)
WNT5B	61292_s_at	(8.72)	(11.85)	(5.42)	(13.92)
SYNPO2	48039_at	(11.04)	(8.80)	(12.64)	(9.34)
BOC	64423_s_at	(3.63)	(8.16)	(11.80)	(54.66)
SLC20A2	1137_at	(9.27)	(5.08)	(10.51)	(12.61)
COL8A2	52652_g_at	(7.95)	(9.99)	(11.56)	(9.75)
---	52678_at	(9.69)	(9.99)	(3.76)	(17.93)
FOS	1916_s_at	(7.58)	(21.81)	(6.93)	(11.58)
ARGBP2	51939_at	(7.77)	(13.86)	(10.40)	(8.71)
CTGF	64342_at	(4.21)	(4.15)	(20.44)	(14.87)
EPHB6	39930_at	(8.61)	(9.66)	(8.32)	(19.41)
SYNPO2	60532_at	(9.77)	(5.54)	(8.77)	(9.03)
NR4A1	280_g_at	(8.68)	(13.49)	(5.82)	(8.58)
DKFZP564O0823	54033_at	(4.67)	(3.72)	(11.83)	(20.00)
GSTO2	45609_at	(4.73)	(6.81)	(9.60)	(16.18)
---	49321_at	(7.91)	(8.41)	(9.24)	(3.88)
EGR3	40375_at	(9.89)	(7.71)	(8.49)	(6.44)
SYNPO2	61681_at	(7.85)	(8.33)	(4.56)	(18.57)

PI15	58361_at	(3.59)	(4.26)	(12.77)	(11.74)
FOSB	36669_at	(8.81)	(6.27)	(7.60)	(8.39)
OGN	43507_g_at	(3.56)	(8.26)	(7.19)	(25.54)
MOXD1	36834_at	(5.40)	(11.70)	(10.00)	(3.85)
LSAMP	43930_at	(3.05)	(7.62)	(9.76)	(7.67)
EGR2	37863_at	(7.70)	(5.52)	(7.23)	(15.41)
DKFZp686D0853	49770_at	(10.18)	(7.66)	(7.16)	(4.39)
LGP1	52826_at	(13.75)	(5.94)	(3.83)	(8.11)
ME3	35216_at	(7.45)	(9.26)	(6.54)	(5.32)
PPP1R14A	58774_at	(6.68)	(6.14)	(7.31)	(7.87)
FLJ22386	50198_at	(6.80)	(3.64)	(6.98)	(6.65)
NR4A1	279_at	(5.31)	(8.04)	(5.11)	(8.48)
WFDC1	64111_at	(3.79)	(11.21)	(6.64)	(6.66)
ZFP36	40448_at	(6.39)	(6.86)	(7.25)	(3.61)
CACHD1	43554_at	(6.68)	(3.34)	(17.46)	(6.57)
RLN1	35070_at	(6.78)	(11.78)	(5.14)	(6.39)
---	49975_at	(6.43)	(6.16)	(6.74)	(10.11)
CYBRD1	65852_at	(6.43)	(4.79)	(6.70)	(7.23)
PER3	53766_at	(15.43)	(6.79)	(5.56)	(6.29)
MN1	37283_at	(4.47)	(7.36)	(5.55)	(7.48)
DNCI2	35788_at	(4.20)	(8.68)	(3.02)	(10.64)
MRVI1	43966_at	(6.76)	(5.28)	(12.19)	(6.09)
AZGP1	35834_at	(6.32)	(3.86)	(38.18)	(6.18)
MGC14839	48949_at	(8.96)	(4.19)	(8.25)	(3.61)
SMTN	64499_s_at	(5.20)	(15.22)	(7.18)	(4.42)
HSPC157	50179_at	(5.66)	(3.18)	(6.63)	(8.09)
WFDC2	33933_at	(5.30)	(6.50)	(5.73)	(6.81)
BTG2	36634_at	(6.99)	(3.13)	(9.25)	(5.22)
AXIN2	64129_at	(4.97)	(6.97)	(7.18)	(4.20)
PDGFC	45217_at	(4.32)	(7.53)	(8.81)	(3.97)
MLLT10	63345_at	(7.20)	(5.85)	(5.90)	(3.84)
BMP7	49273_g_at	(4.58)	(4.89)	(6.82)	(13.13)
MCC	49504_r_at	(5.90)	(5.71)	(5.08)	(5.84)
HEXA	39340_at	(8.15)	(5.65)	(4.18)	(5.88)
GSTT2	1099_s_at	(6.47)	(5.05)	(6.80)	(4.66)
SSPN	65647_at	(5.40)	(5.88)	(3.12)	(17.61)
UPK3A	36379_at	(5.37)	(4.71)	(5.81)	(6.91)
PDE5A	54668_at	(4.44)	(5.17)	(5.87)	(9.56)
PSD3	63832_at	(3.19)	(6.04)	(4.98)	(6.58)
ALDH7A1	61965_at	(5.85)	(5.14)	(5.88)	(3.13)
FMOD	33431_at	(7.62)	(4.30)	(4.90)	(6.04)
TSPAN2	53693_at	(6.38)	(4.49)	(4.54)	(6.77)
DKFZP586H2123	40017_at	(6.52)	(6.49)	(4.32)	(3.91)
EFS	33883_at	(5.43)	(3.58)	(6.35)	(5.18)
PODN	63953_at	(4.16)	(5.30)	(4.84)	(4.98)
DUSP1	1005_at	(6.53)	(16.66)	(3.02)	(3.19)
SLC22A17	58898_s_at	(4.93)	(5.81)	(4.66)	(4.44)

CDH10	47535_at	(4.87)	(3.19)	(8.27)	(4.65)
---	64163_at	(3.66)	(5.03)	(4.79)	(4.70)
---	42587_at	(4.68)	(4.62)	(4.90)	(3.45)
TSPAN2	57331_at	(4.44)	(8.06)	(3.42)	(4.71)
SORBS1	56409_at	(5.45)	(5.70)	(3.17)	(3.53)
C21orf63	50658_s_at	(4.54)	(3.36)	(4.15)	(5.31)
NBL1	37005_at	(3.34)	(4.27)	(4.31)	(6.36)
CIRBP	39864_at	(4.38)	(3.53)	(4.19)	(6.80)
KLF4	48587_at	(3.77)	(3.62)	(4.57)	(12.50)
ZCSL2	45320_at	(3.10)	(3.19)	(5.88)	(5.13)
C12orf10	53911_at	(3.62)	(4.44)	(3.86)	(6.46)
CERKL	60314_at	(4.68)	(3.03)	(7.37)	(3.62)
NOV	39250_at	(3.20)	(3.90)	(4.38)	(7.37)
EPB41L5	60293_at	(4.33)	(4.97)	(3.06)	(3.92)
WNT5B	66142_s_at	(3.94)	(3.87)	(4.49)	(4.16)
ACYP2	64090_s_at	(3.36)	(4.33)	(3.68)	(5.82)
C9orf103	56186_at	(3.14)	(4.62)	(4.03)	(3.73)
FBXO2	57811_at	(3.51)	(3.37)	(4.16)	(5.33)
CD38	40323_at	(3.25)	(3.37)	(4.27)	(4.27)
BCAS1	37821_at	(4.96)	(3.19)	(4.26)	(3.34)
TMSL8	36491_at	(3.03)	(4.11)	(3.45)	(7.67)
ISL1	39990_at	(3.12)	(3.78)	(3.61)	(3.91)
HSPB8	56474_at	(3.45)	(3.87)	(3.04)	(7.50)
B3GALT3	53879_at	(3.04)	(4.02)	(3.77)	(3.48)
CYBRD1	50955_at	(3.70)	(3.51)	(3.21)	(5.60)
EFEMP2	63644_at	(3.25)	(3.91)	(3.28)	(3.97)
TU3A	45260_at	(3.14)	(3.94)	(3.22)	(4.82)
LOC57228	34176_at	(3.68)	(5.30)	(3.41)	(3.16)
IER2	36097_at	(4.79)	(3.20)	(3.11)	(3.88)
DKFZP564K1964	65860_at	(3.53)	(3.11)	(3.52)	(4.62)

10 PATHWAY MODELS

We now consider in more formalistic terms how to develop models. There are three approaches under consideration:

Boolean Networks: The model assumes discrete time on or off nets controlled by the dynamics of the pathway. The Boolean model consists of two elements: first, an assumption that a gene or protein is on or off, second, that the control of that constituent is a Boolean control consisting of AND, OR and NOT Boolean type functions dependent upon precursor constituents. We discuss this in some detail related to PTEN. The Boolean approach is interesting in that it works well at times identifying non-dynamic pathways using massive micro-array data.

Bayesian Networks: The Bayesian model assumes that the value may be analog not binary and that there exists a graph which is what is the desired output. It uses a Bayes model which yields the best fitting graph given data from a microarray and assuming that there are linkages consisting of probabilities in a directed manner. The Bayesian may become time varying as well. As with the Boolean approach it works well with ascertaining linkages and graphs using microarray data and in this case the microarray data may be of an analog nature not just binary.

State Models: These are continuous time and continuous state models of specific pathway concentrations where we employ enzymatic reaction rate models that we have discussed previously. We have used these models in other circumstances and they have proven their worth. Also these models are often more amenable to stochastic effects as well as examination for observability and controllability.

10.1 BOOLEAN NETWORKS

The simplest model for pathways is the Boolean model. This has been described in Klipp et al in simple terms. A classification of Boolean Networks has been done in Gershenson. It is based upon three simple assumptions.

First, genes and their products are either on or off, expressed or not expressed. This denies concentration effects.

Thus as an example we may have PTEN and Akt, and they are either 1 or 0. Thus we have the four states, {PTEN, Akt} as 00, 10, 01, 11. Not all may exist, we will show that next.

Second, the effects on a set of gene products on other gene products can be expressed by a Boolean expression.

Thus, for example we have the Binary or Boolean expression:

Akt = NOT PTEN.

Thus if PTEN is 0 then Akt is 1 and likewise if PTEN is 1 then Akt is 0. The states 11 and 00 are not allowed.

Third, this is a discrete time state system where we have the kth instance influencing the k+1 th state.

Thus as above we should state:

Akt(k+1)=NOT PTEN(k)

We may add asynchrony and probabilistic transitions as well to the model.

Now let us formalize the model a bit. We assume that there are N genes or gene products that are of interest. Then the state of the system at any time is:

$$x(k)=\begin{bmatrix} x_1(k) \\ \dots \\ x_N(k) \end{bmatrix}$$

where the xs can take on only 0 or 1 in value, on or off.

Now we assume that for each x(k) we have some Boolean equation of the following type:

$$x_j(k+1)=(x_1(k)ANDx_3(k))NOTx_4(k)ORx_6(k)$$

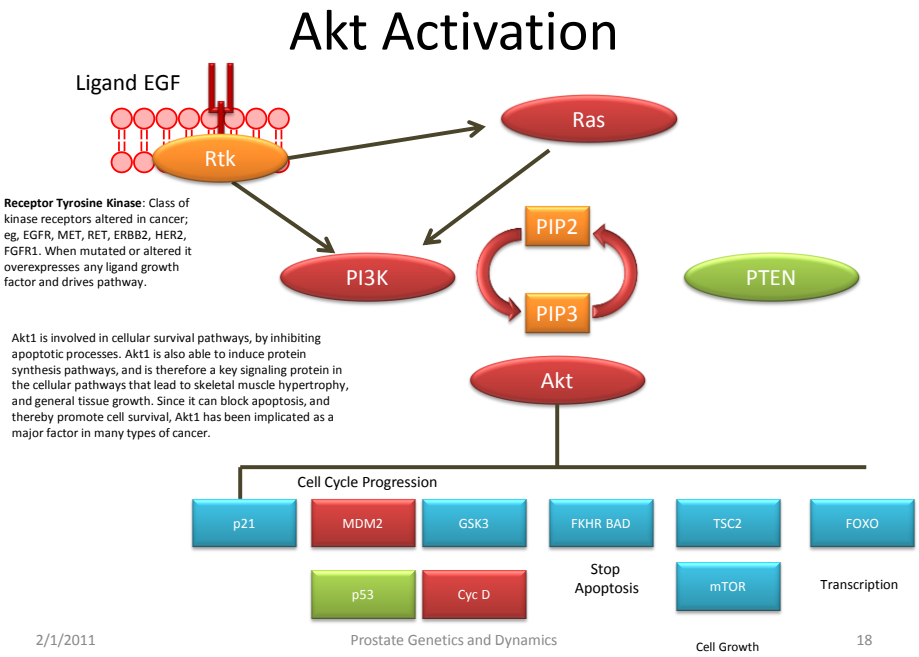
We now ask where did this equation come from. We defer that for the moment. But let us assume that we can find that for every one of the x values. The we have:

$$x(k+1)=F(k+1,k)$$

where

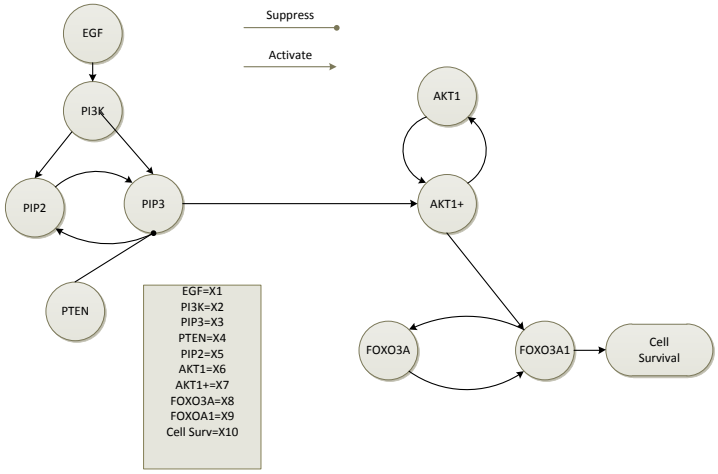
$$F(k+1,k)=\begin{bmatrix} f_1(k+1,k;x_1(k)\dots x_N(k)) \\ \dots \\ f_N(k+1,k;x_1(k)\dots x_N(k)) \end{bmatrix}$$

Now let us return to our PCa model and look at a simplified set of it as shown below. First, from Lin et al we have for the simple model showing that "*PTEN Decreases AR Protein Levels via Promotion of AR Degradation"* which we show below;



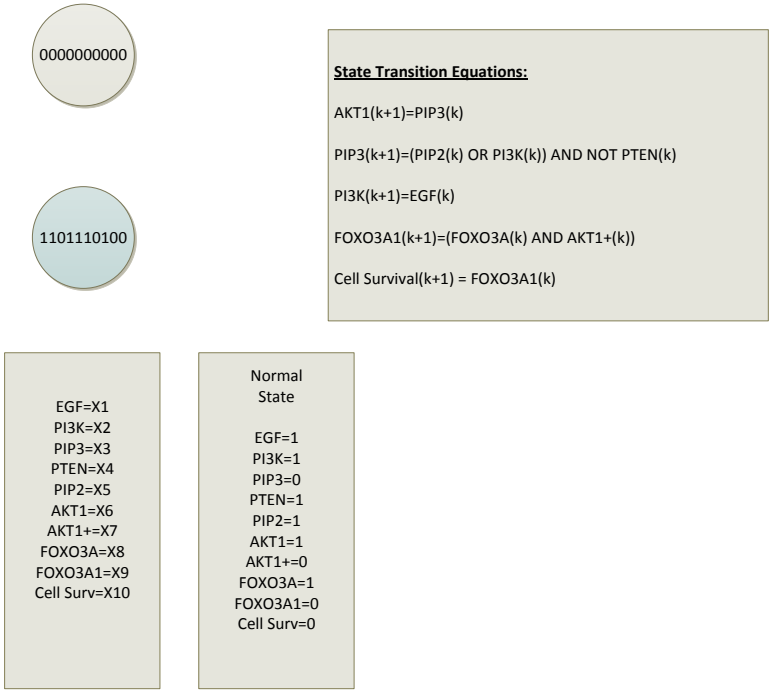
:

Then from the above we can generate a simple model of pathways with some detail as below:



Now in this model we assume that an EGF ligand drives PI3K which moves PIP3 to activate AKT to AKT+1 the activated form which drives FOXO to enhance cell survival. We see 10 states all of which are binary. Ideally we would have 2^{10} possible states, roughly a million. But the state transitions will prohibit this.

The following depict the transition equations, the Boolean f functions from above, as we have somewhat arbitrarily created them, and the stable state.



Note that we can assume that EGF is one and PTEN is 1 and thus PTEN blocks everything. Note also that only 5 state equations are specified. The others are just stable states. We can now take any states, namely ones where EGF and PTEN go from 0 to 1 or 1 to 0 and see what happens. Note that we have in this model the assumption that PI3K needs EGF. Also if PTEN is on then it suppresses cell growth via FOXO.

One can use microarray technology to validate this model. However it has significant drawbacks.

First it is binary. Real world models are not that way.

Second it is discrete time, again not a reflection of reality.

Third, it assumes binary reactions, and the real reactions are a bit more subtle.

The use of the pathway data is oftentimes effective at the gross level (see Kim et al). Also the ability of correlating pathway models with microarray data is also of significant value. Oftentimes the microarray data itself is binary and thus it maps fairly well onto this model (see Driscoll and Gardner).

Expansion of this model to non-discrete times and random transitions has been shown in the wok of Shmulevich et al (three papers in 2002, 2002, 2002)

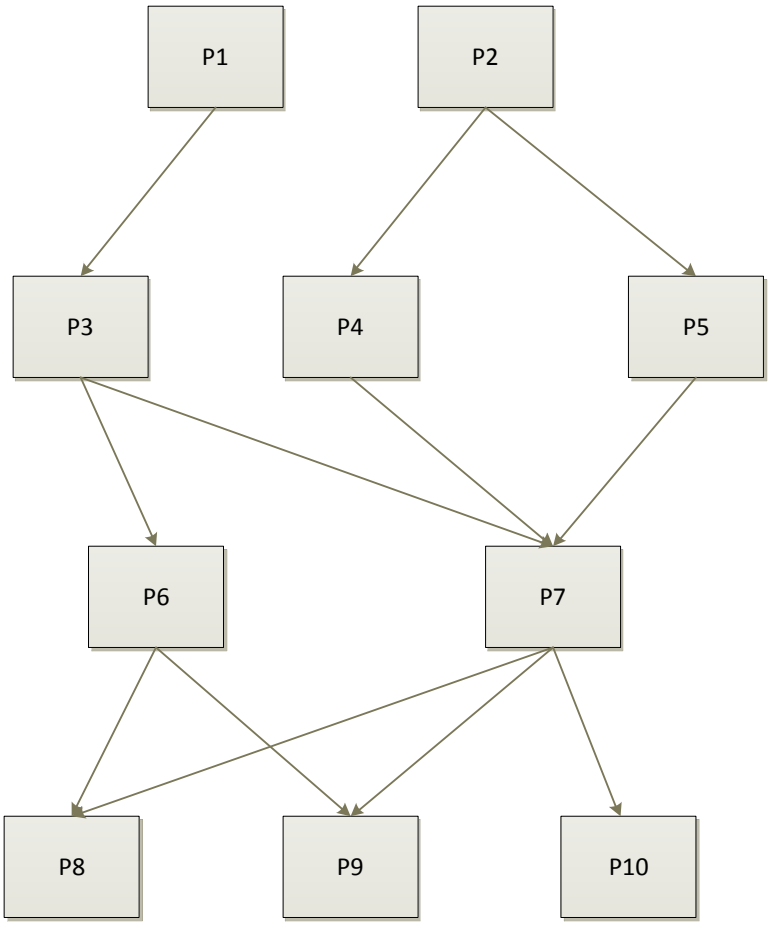
10.2 BAYESIAN NETWORKS

In the development of a Boolean Network we assumed two things; first that the proteins or other relative constituent was either on or off and second that the interaction between constituents was

controlled via some Boolean function of AND, OR, NOT type combinations. Now in the Bayesian world we assume Bayes type relationships and then try to infer dependency thereupon.

10.2.1 Bayesian Analysis

Let us begin by looking at the following network. We assume that the Ps are influencing the Ps they are connected to in some manner. For simplicity we shall assume they are all positive influences.



Now in a Bayes world we generally have some data and we then try to infer from the data the structure as we see it above.

Let us look at the above flow. Here we have assumed a priori that there are well defined influencing factors. But what if all we had was 10 products and we just thought that they somehow interact. What we want to do is use some logical method to arrive at the chart above. How could we approach this?

Let us assume we have some microarray data. Let us further assume that it is simple microarray data showing genes on or off.

We could write in general the following (see Theodoridis and Koutroumbas, pp 64-68):

$$p(P10,P9,P8,...P1) = p(P10|P9,...P1)p(P9|P8,...P1)...p(P2|P1)p(P1)$$

Now that does not reflect the graph, G, which we had shown at the top. One may look at the set of all possible graphs, say:

$$\Gamma = \{G_i\}$$

where we have any possible G as a set. We are really interested in the "best" G give say a data set D.

So let us go to the data set, D. What can we use to assist in determining the best possible G. Let us assume we have a microarray. The microarray presents data in one of two forms; active or no effect. Namely the array elements lights up if active and does not light up is not active. Let us assume we have 20 samples and we test for the ten products. We get an array as below. Here red is active.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
P1																				
P2																				
P3																				
P4																				
P5																				
P6																				
P7																				
P8																				
P9																				
P10																				

From this we have a data set given by;

$$d_i = \begin{bmatrix} d_{i,1} \\ ... \\ d_{i,10} \end{bmatrix}$$

and

$$D = \{d_1,...d_N : N = 1,...,20\}$$

We can now look at the problem in a Bayesian sense. Let G be the graph and D the data as we demonstrated above. Now as a Bayes approach we can seek:

$$max\left[log\, P\left[G|D \right] \right] =$$
$$max\left[log\, P\left[D|G \right] + log\, P\left[G \right] + c \right]$$

We thus seek to maximize the Bayes score. We seek the G which given the D gives the best result.

Now we can model the system as follows:

$$P_i = \sum_{k=1,\neq i}^{10} a_{i,k} P_k$$
and
$$d_i = b_i P_i$$

The a values are determined by the graph G. We can add noise to the measurement and even to the system. We can then find the set of a that given the D set minimize the measure we stated above. Since we can assume that we have:

$$P_i = \sum_{k=1,\neq i}^{10} a_{i,k} P_k + n_i$$
and
$$d_i = b_i P_i + w_i$$

where they are zero mean Gaussian, then the metric used to attain a minimum on the data set D to determine the graph G is simple, yet a complex calculation.

Namely we have (see Shmulevich and Dougherty):

$$max\left[log\, P\left[G|D \right] \right] =$$
$$max\left[log\, P\left[D|G \right] + log\, P\left[G \right] + c \right]$$
or
$$max\left[-\sum_{k=1}^{10} \sum_{i=1}^{20} \left(d_{i,k} - \hat{d}_{i,k} \right)^2 \right] + log\, P\left[G \right]$$

where we have used the estimated d for the specific graph G being tested. We do this for every possible graph, G. That is a very computationally complex calculation. Indeed it can become non-calculateable.

We demonstrate in the next section that we can measure analog values for the measurements as well as for the desired connections.

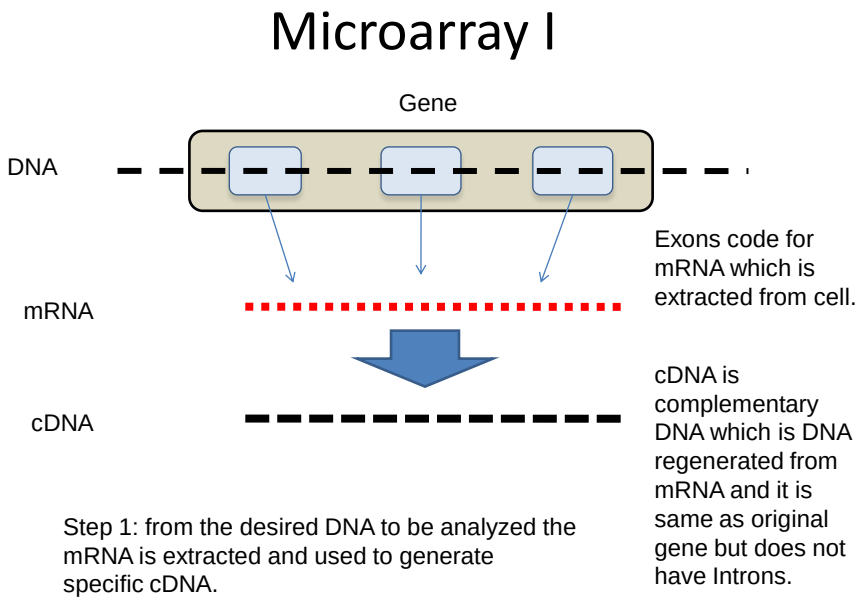
10.2.2 Microarrays

We briefly look at microarrays in some detail since we will use them in the Bayesian case as well in the full system modeling case.

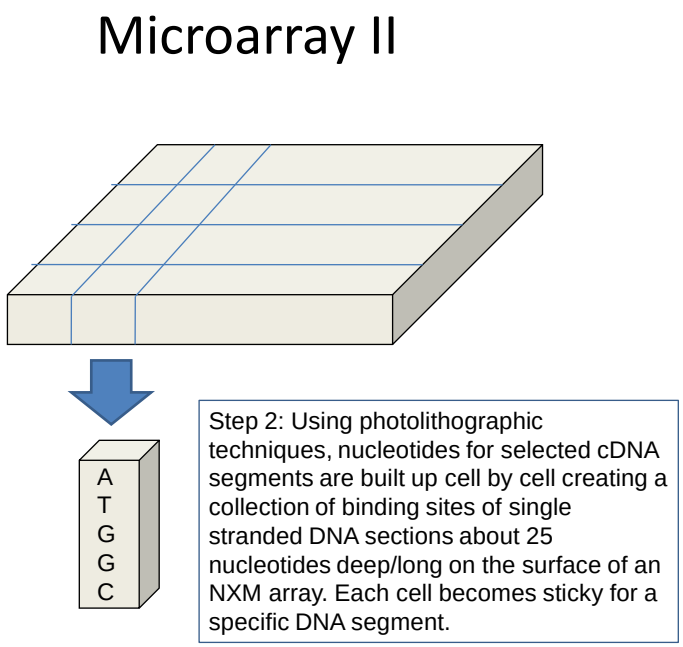
Microarrays are simply a set of matrices where we take samples from many cells and place them in an N by M matrix, say using rows for genes and columns for cell samples. By selecting the rows such that we can place in each row a gene specific binding site, which we can even tag with some visible marker, we can then determine if on a sample by sample basis we have genes being expressed, namely that they bind at the binding site. It is also possible to attempt to ascertain the concentration of the gene or gene product by having the amount of binding in each sample be reflected by a linear or similar color change. We can say have red for no binding, yellow for some and green for a great deal, or whatever color combination we so desire. Indeed it may then be possible to calibrate for relative concentrations in each cell entry via a colorimetric measurement.

Microarrays is a unique approach which allows for the analysis of millions of samples, it is a marriage of high tech solid state chip technology with DNA bonding. We describe it in the following four steps, each step accompanied by a Figure.

Step 1: The first step in a micro array is the production of cDNA, or complementary DNA. cDNA is that set of nucleotides which account for the encoding of mRNA. It does not include the non-coding regions which are the introns.

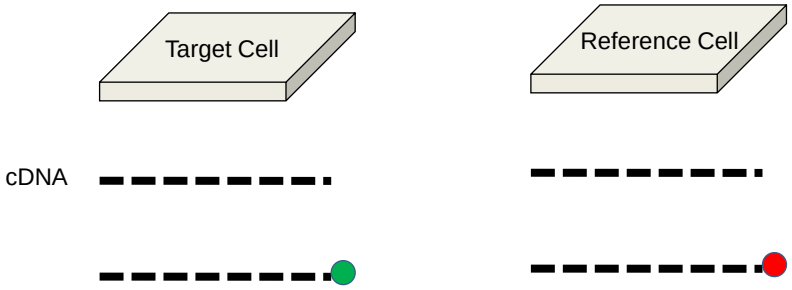


Step 2: In a separate environment we make the microcell. This is created in a manner identical to the making of integrated circuits which entails photo-masking techniques. Instead of silicon we used nucleotides. The array has millions of small holes in an array like manner. Each hole we fill with nucleotide, one nucleotide at a time.



Step 3: Now we take two DNA samples, one from what we call the Target, the plane we wish to categorize. We then take the segments we collected in step one and tag then with green or red tags, green say for the Target and Red for the Reference.

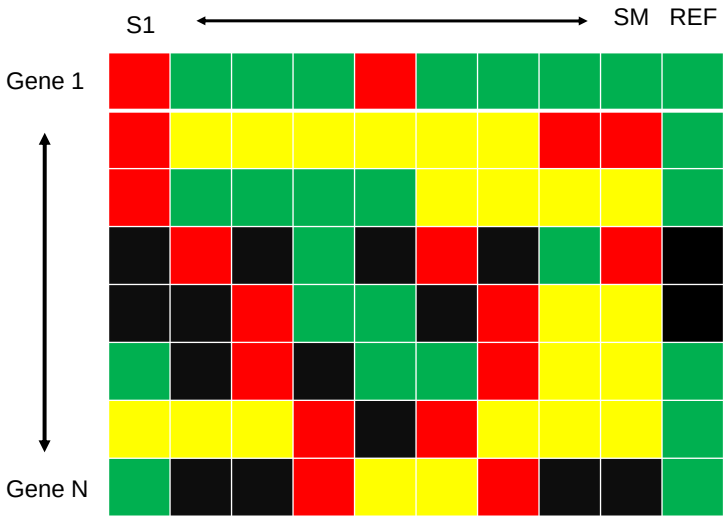
Microarray III



Step 3: For the DNA to be analyzed and a "Reference" target DNA, the mRNA is extracted from each and the cDNA is produced for every gene in the cells to be analyzed, and then it is tagged with a dye which is red for one and green for the other. Typically we tag the target red and Reference green.

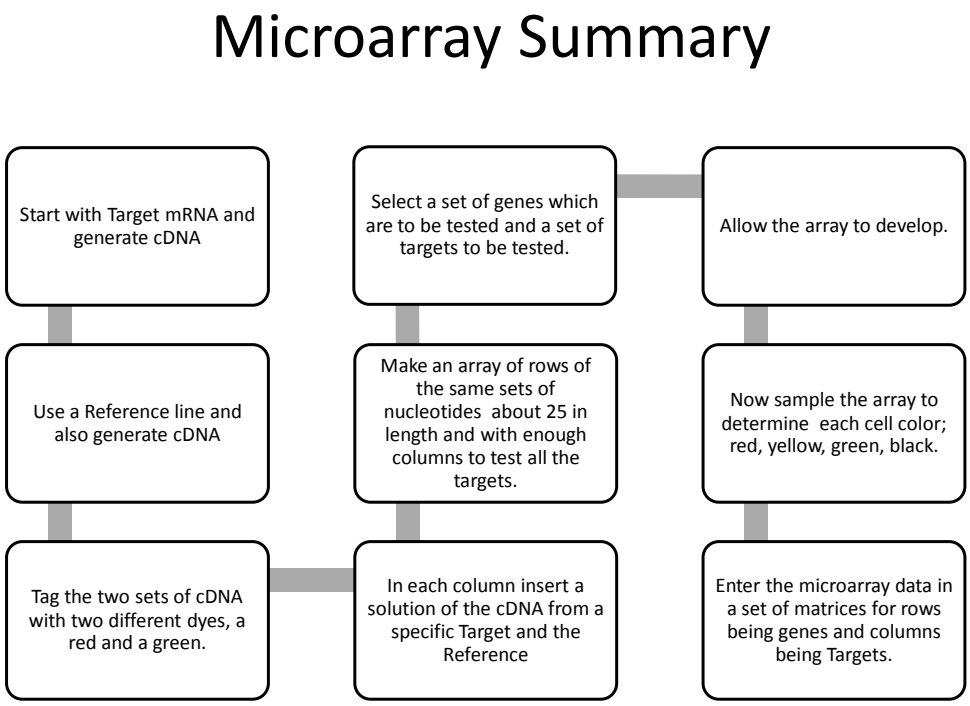
Step 4: We then take the samples from the differing plants, one in each column, and look at the array. If the microarray cell has the gene sequence we are seeking to march, and the Target has that sequence, it will bond and stick. If the Reference has it, it too will bond. If we just get the Target the cell will be green, if we just get the Reference the cell is red, if we get both the cell turns yellow, and if we have neither the cell is black. The result of a sample scan is shown below.

Microarray IV



Now, we even get to try and look at the intensity of the red, green, or yellow. This we can try to see how much is expressed not just whether it is or is not. We will not discuss that here. In the above matrix we can see that many genes are expressed in one or both or none. If we have enough genes than we can argue we have the basis for an exceptionally good means to develop a classification.

In the following Figure we summarize the microarray process.



This is a brief summary of microarray technology. We refer the reader to the volume of literature available and referenced to herein.

10.2.3 Bayesian Network Summary

The Bayesian network approach can be posed as a general problem. Yet it is often delimited by assuming a priori that certain graphs, G, and no possible, and the minimization or maximization process then is performed over the subset of graphs. There is often still quite a great deal of calculation required.

On the negative side the Bayesian approach as is the case for the Boolean approach does not take into account the underlying chemical dynamics that we have been observing. In addition for most of the pathways we are looking at we already know the key pathway structure and it is the reaction kinetics that we are looking for.

Thus Boolean and Bayesian are useful for identifying pathways whereas the full system model works when the pathway is somewhat well understood and we are looking more for the dynamics and details of the reactions. In a sense they are useful at different stages of the process.

10.3 STATE DYNAMIC NETWORKS

The state dynamic network model for pathways is the heart of what we have been developing herein. It consists of the following:

1. Intracellular Pathways: The description of pathways within the cell and a description which focuses on concentrations of products and exogenously extracellular products that affect the pathways.

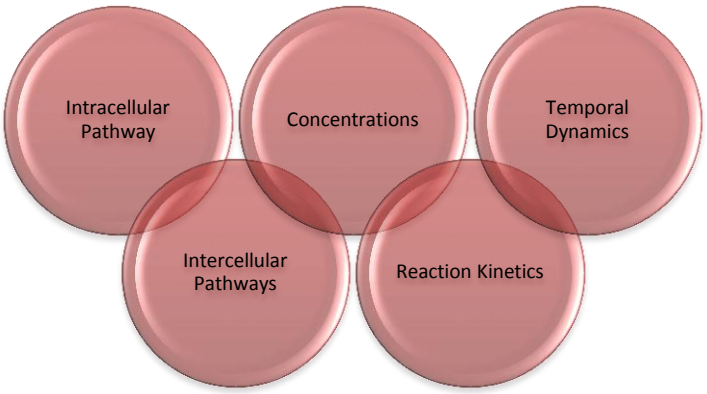
2. Intercellular Pathways: This is the modeling of pathways between and amongst the cells. It is intercellular signalling pathways where receptors and their associated ligands are studied. Again this may generally be focused on concentrations of ligands and similar extracellular and intercellular communicators.

3. Concentrations: The focus is on concentrations of the gene products. The issue here is that it is concentrations which reflect reality and although binding and pathway control may be seen as promoted or inhibited by other proteins, the reality of reaction kinetics is that it is concentrations which are reflective not just single molecule presence or absence. This element is dramatically different from what we see in Boolean or Bayesian models. Here with the modeling with concentrations we are modeling closer to reality.

4. Reactions: Reaction kinetics describes how one substance can control the conversion of another substance into a third. The reaction kinetics are essential to the system dynamics model. The challenge is to know and understand which of the reaction models apply. For example are there rate limiting factors due to concentration limitations of the reaction.

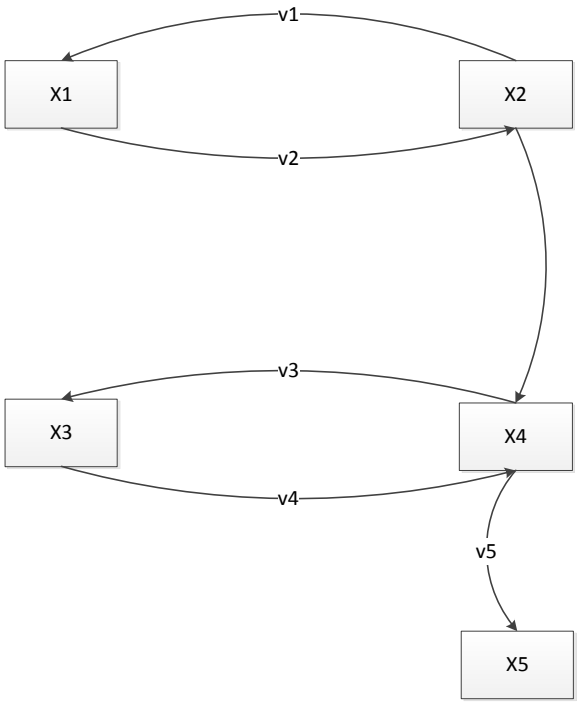
5. Time Variations: The models are all time varying. Thus understanding reaction kinetics from a temporal perspective is essential. This may take our knowledge to the limit and perhaps beyond at the current time.

We graphically demonstrate these factors below:



In order to best understand the system dynamic model we again briefly return to the reaction rate model and its part in defining the reaction rate equations.

Let us consider a simple model as below:



Recall that if we have a pathway with say N=5 constituents then we have the following formula where the rates v are yet to be determined:

$$\frac{d[x_1(t)]}{dt} = v_1 - v_2$$
$$\frac{d[x_2(t)]}{dt} = v_2 - v_1$$
$$\frac{d[x_3(t)]}{dt} = v_4 - v_3$$
$$\frac{d[x_4(t)]}{dt} = v_3 - v_4$$
$$\frac{d[x_5(t)]}{dt} = v_5$$

Now let us return to the rate analysis. We will use the paper by Segel and Slemrod as the basis. This paper presents an excellent analysis of the quasi steady state assumptions for rate and reaction with enzymatic issues as we will encounter.

Assume we have the following reaction:

$$E + S \rightleftarrows C \rightarrow E + P$$

We further assume that the forward rate from E+S to C us k_1 and the reverse is k_{-1} and the forward rate from S to E+P is k_2. Then we have the following:

$$\frac{dE}{dt} = -k_1 ES + k_{-1} C + k_2 C$$
$$\frac{dS}{dt} = -k_1 ES + k_{-1} C$$
$$\frac{dC}{dt} = k_1 ES - k_{-1} C - k_2 C$$
$$\frac{dP}{dt} = k_2 C$$

Note that as we have discussed before the equations are nonlinear due to the product terms. We could linearize them by assuming that we are dealing with small changes or we can use the quasi steady state assumptions which reduces the equations yet keeps the nonlinearity. We shall do the later. Also note the mapping of the above specific reactions to the generalized equations we have used above. Also note that when we have two constituents using an enzyme we see the enzyme as a single combine entity and the initial components as two separate ones. Also we have reaction rates thus dependent to the product of the concentrations on each side of the reaction.

Now let us proceed with the enzymatic reaction. We can restrict the analysis to the following conditions:

$$E(0) = E_0$$
$$S(0) = S_0$$
$$C(0) = 0$$
$$P(0) = 0$$

This is a simple defined initial state when we have just the initial entities which we desire to react. But we also can readily show:

$$E(t) + C(t) = E_0$$

Thus we can simplify the result to what we have below by elimination:

$$\frac{dS}{dt} = -k_1(E_0 - C0S + k_{-1}C$$
$$\frac{dC}{dt} = k_1(E_0 - C)S - k_{-1}C$$
$$S(0) = S_0$$
$$C(0) = 0$$

These are still rather complex differential equations and we will now assume that the rates of each are dramatically different. Namely one proceeds quite quickly and the other slowly. That being the case we can thus assume that one is steady state and the other dominates the observable time variation. This is in essence the quasi steady state assumption. This assumption is:

$$\frac{dC}{dt} \approx 0$$

Then we obtain:

$$C = \frac{E_0 S}{K_m + S}$$

where

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

and finally we have the single differential equation for S, which is:

$$\frac{dS}{dt} = \frac{k_2 E_0 S}{K_m + S}$$

This can be applied to the above initial generic form depending upon what the reaction is. Simple reactions do not involve an enzymatic catalyst as we have shown here but in general we will always be dealing with some nonlinearities.

The above deals with four of the five elements we discussed previously: intracellular pathway which was given, rate reactions, concentrations and temporal dynamics.

Thus for any of the pathways we have described above we can readily apply the model we have developed. The issue will be one of simplification. In the previous quasi steady state analysis we eliminate one reaction as not being significant to the "scale" of the reaction or reactions of interest. We assume it has reached a steady state by the time we enter the second reaction. There are two other approaches; first neglect scale and just deal with the complex nonlinearities and compute results or at the other extreme linearize the system.

Let us apply linearization to the above:

$$\frac{dS}{dt} = \frac{k_2 E_0 S}{K_m + S}$$

let

$$S = S_0 + s$$

where

s = *small variation*

then

$$\frac{ds}{dt} = \frac{k_2 E_0 (S_0 + s)}{K_m + S_0 + s} \approx a + bs$$

where

$$a = \frac{k_2 E_0 S_0}{K_m + S_0}$$

$$b = \frac{k_2 E_0}{K_m + S_0}$$

Thus we can look at small variations in such a linear manner.

11 IDENTIFICATION, REGRESSION AND ITS DRIVERS

The issue of determining the reaction constants in the system model is of significant importance. In this section we address the issues regarding such estimation, often called system identification. In simplest terms we may have determined the following:

1. Pathway: We can ascertain what the pathway and related constituents are with some certainty.

2. Reaction Models: We may for each set of related elements in the pathways be able to determine what the reaction dynamics can be.

3. Dynamic Model: Having the first two we may then be able to posit a dynamic model for all of the material concentrations of constituents, and yet now have specific values for the reaction rates.

4. Unknown Reactants: There may be unknown reactants or even noise in the system. These may be modeled by an extended identification process or noise. We will demonstrate both herein.

5. Spatial Dynamics: We have referred to the spatial dynamics from time to time but as we have stated, albeit critical, it lacks adequate experimental data to make any progress at this time.

6. Stem Cell Dynamics: We believe that there is substantial evidence of a stem cell model with a CSC in PCa. However, at this time we cannot differentiate these. Yet using the methodology as we develop it, we believe that understanding the dynamics of CSC PCa cells can be determined.

11.1 IDENTIFICATION

This section addresses the ability to determine the detailed concentrations of each of the colorants in a cell if one knows the cell effective optical length and the extinction coefficients for each of the constituents. The models for performing these tasks also show what the maximum resolution that can be achieved as well and the maximum number of constituents. The results in the maximum bounding resemble the same results that are found in such areas as ascertaining the accuracy in ambiguity functions for phased arrays. The latter problem was solved by the author in the mid-1970s.

11.1.1 Network Model

As we have discussed earlier, the network may be characterized by a matrix differential equation of the form:

$$\frac{dx(t)}{dt} = f(x,t) + w(t)$$

where

$$x = \begin{bmatrix} x_1 \\ \dots \\ x_n \end{bmatrix}$$

and

$$f(x,t) = \begin{bmatrix} f_1(x,t) \\ \dots \\ f_n(x,t) \end{bmatrix}$$

and

$$w = \begin{bmatrix} w_1 \\ \dots \\ w_n \end{bmatrix}$$

We assume that w is a white noise zero mean process and that the f have reasonable mathematical properties. This generalization can be specified for any know gene or gene product network.

We will assume that we can linearize this model. Namely we can do what we had done earlier when looking at the ability to linearize an enzymatic reaction network. This will assume that we have small variations in the constituents. This yields:

$$\frac{dx(t)}{dt} = Ax(t) + w(t)$$

where, as above, x is an n by 1 vector and A is an n by n matrix evaluated about the stability point. That is:

$$A = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} \dots \frac{\partial f_1}{\partial x_n} \\ \dots\dots\dots\dots \\ \frac{\partial f_n}{\partial x_1} \dots \frac{\partial f_n}{\partial x_n} \end{bmatrix}$$

Now the steady state profile would be:

$0 = Ax$

and

x_0

such that

$0=Ax_0$

where we have defined the steady state concentrations. Arguably if we know the steady state concentrations then A must yield the zero vector resulting therefrom.

11.1.2 Measurement Model

Now we use the microarray method to measure. The issue however is to measure amongst groups of common cells. Consider the following microarray form. We have n sample across the columns and m gene or gene related samples across the columns.

The microarray can be constructed so that the color spectrum of the reflected light is a function of the density of the targeted gene or gene counterpart. Thus the microarray must be aligned to deal with samples from large, organized and segmented samples. Below we show 15 gene products and 19 samples. The relative concentrations are also shown.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
G1	1	3	1	2	3	1	2	3	2	1	3	1	1	3	2	2	3	2	1
G2	2	2	2	3	2	2	3	2	3	2	2	2	2	2	3	3	2	3	2
G3	3	2	2	3	1	3	3	1	3	3	2	2	3	1	3	3	2	3	3
G4	3	2	2	3	1	3	3	1	3	3	2	2	3	1	3	3	2	3	3
G5	4	2	4	2	1	4	2	1	2	4	2	4	4	1	2	2	2	2	4
G6	6	1	5	1	2	6	1	2	1	6	1	5	6	2	1	1	1	1	6
G7	3	2	5	2	3	3	2	3	2	3	2	5	3	3	2	2	2	2	3
G8	2	4	5	5	4	2	5	4	5	2	4	5	2	4	5	5	4	5	2
G9	3	5	6	6	5	3	6	5	6	3	5	6	3	5	6	6	5	6	3
G10	1	7	6	6	6	1	6	6	6	1	7	6	1	6	6	3	7	6	1
G11	2	9	2	7	7	2	7	7	7	2	9	2	2	7	7	3	9	7	2
G12	5	7	1	7	4	5	7	4	7	5	7	1	5	4	7	2	7	7	5
G13	6	4	2	8	4	6	8	4	8	6	4	2	6	4	8	2	4	8	6
G14	7	3	3	8	4	7	8	4	8	7	3	3	7	4	8	8	3	8	7
G15	2	2	4	9	3	2	9	3	9	2	2	4	2	3	9	9	2	9	2

Now the color of the cell will reflect the concentration of what the cell has been targeted for, a gene or gene product. Thus by measuring the cell color we can infer the cell concentration and thus the concentration of that specific product in a cell.

The first simplistic identification model will be developed. We thus make the following assumptions:

1. We assume we have n gene products and m samples.

2. We assume we have for each (n,m) tuple a sample which is a color and that we can calibrate color to concentration. We will discuss this in some detail in the next section.

3. We assume that the samples are either from a steady state model or are from a dynamic model and labeled accordingly. We may have samples from multiple cell conditions, that is we may have benign samples, HGPIN samples and PCa samples, and even divided by grade.

4. We assume that the samples are an (n,1) tuple and we then have k of them.

5. We may also have knowledge of the A matrix whereby we know that certain linkages exist, are positive or negative and that certain linkages do not exist. Thus from Boolean or Bayesian analyses we may have a priori knowledge.

6. We then pose the following problem:

Given :

$$x(i) = \begin{bmatrix} x_1(i) \\ ... \\ x_n(i) \end{bmatrix}$$

$$i = 1, M$$

Find

$$\hat{A} = \begin{bmatrix} \hat{a}_{1,1} ... \hat{a}_{1,n} \\ ..... \\ \hat{a}_{n,1} ... \hat{a}_{n,n} \end{bmatrix}$$

such that A yields the best fit to the data and subject to the known constraints.

Now what do we mean by best fit to the data? We mean that at steady state the product of any data set and A is Ax=0. But we have k data sets and from these data sets we want to obtain an estimate of A, namely its element entries, subject to whatever constraints we may know a priori.

We know the following:

$$Ax = 0$$

or

$$\sum_{i=1}^{M} a_{j,i} \tilde{x}_i(k)$$

for j=1,M and all k measurements;

where $\tilde{x}$ is a measured value

Then again the above is subject to whatever a priori constraints we also have. In effect we are looking for a entries which can minimize a metric of the form:

$$min \sum_{k=1}^{m} \left[\sum_{i=1}^{n} a_{j,i} \tilde{x}_i(k) \right]^2 ; \forall j$$

That is for each j entry we want to find the a which minimize the metric above, subject to the constraints. Thus for any j we can say:

$$min \sum_{k=1}^{M} \left[\sum_{i=1}^{n} a_{j,i} \tilde{x}_i(k) \right]^2$$

implies

$$g(a_{j,i}) = \sum_{k=1}^{M} \left[\sum_{i=1}^{n} a_{j,i} \tilde{x}_i(k) \right]^2$$

and

$$\frac{\partial g(a_{j,i}; i = 1,n)}{\partial a_{j,i}} = 0; \forall j$$

Thus simplistically; if we have enough measurements we can estimate the a values by performing the above analysis. Let us look at a simple example.

$$min \sum_{k=1}^{m} \left[a_{j,1} \tilde{x}_1(k) + a_{j,2} \tilde{x}_2(k) + a_{j,3} \tilde{x}_3(k) \right]^2$$

implies

$$g(a_{j,i}) = \sum_{k=1}^{m} \left[a_{j,1} \tilde{x}_1(k) + a_{j,2} \tilde{x}_2(k) + a_{j,3} \tilde{x}_3(k) \right]^2$$

and

$$\frac{\partial g(a_{j,i}; i = 1,n)}{\partial a_{j,i}} = 0, \forall j$$

We can look at the details as follows:

$$g(a) = \sum_{k=1}^{N} \left(a_{11}x_1 + a_{12}x_2 + a_{13}x_3 \right)^2$$

thus

$$\frac{\partial g}{\partial a_{11}} = \sum_{k=1}^{N} 2a_{11}x_1 \left(a_{11}x_1 + a_{12}x_2 + a_{13}x_3 \right) = 0$$

or

$$\sum_{k=1}^{N} x_1 \left(a_{11}x_1 + a_{12}x_2 + a_{13}x_3 \right) = 0$$

or

$$a_{11}R_{11} + a_{12}R_{12} + a_{13}R_{13} = 0$$

where

$$R_{ij} = \sum_{k=1}^{N} x_i x_j$$

Now we will use a heuristic approach to this solution. It will be a modified Newton method, discussed later. It should be remembered that we have here a simple dynamic system at steady state. We have assumed:

1. Linear relationships for reaction rates.
2. Steady state.
3. An assumed set of initial network maps with some minimal guidance as to the entries, even at the 0,1 level.
4. A wealth of microarray data adequate to assist a convergence.

Now let us assume the following:

Let

$$a_j(k) = \begin{bmatrix} a_{j1} \\ \dots \\ a_{jn} \end{bmatrix}$$

Thus we will develop an estimator for each of these vector elements which comprise the matrix A. The above are the rows of A.

Now we posit as per Newton's method:

$$\hat{a}_j(k+1) = \hat{a}_j(k) + \kappa_j\left[\hat{A}(k)x(k) - \hat{A}(k-1)x(k-1)\right]$$

where

$$\hat{A}(k) = \begin{bmatrix} \hat{a}_1(k) \\ ... \\ \hat{a}_n(k) \end{bmatrix}$$

we choose the weighting constant on a trial and error basis. This works as follows:

1. We start with an initial set of estimates for the a values. These may be from a Boolean or Bayesian estimator. This yields the existing links and we would expect that A would be a somewhat sparsely populated matrix. One can see from our previous analysis how this may be the case.

2. Then we calculate estimates for the zeroth state and we enter an estimate for the zeroth concentration matrix.

3. Then we make a measurement and this measurement is used to adjust the initial guess, as well as subsequent guesses.

4. Then we change the guesses accordingly as we see whether it is greater or less than the previous guess. If we can drive the answer to zero, namely the difference of the Ax product, which is the goal of a Newton procedure, then we can achieve convergence. The constant is chosen heuristically to time the convergence.

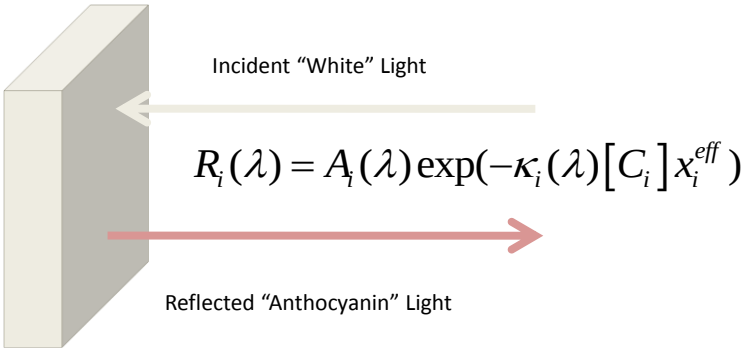
11.2 MEASUREMENTS

To understand the use of microarray data we look briefly at the issue of reflectance from the surface and relate it to concentrations. It is essential to understand this process since it becomes an integral part of the overall calibration method.

Let us begin with a simple model of reflectance. We look at the Figure below and see a white light impinging on a cell and the light reflected back is seeing at one specific wavelength, frequency, as an attenuated version of what was transmitted at the wavelength. A is the amplitude of the transmission and the exponentially reduced A value is what is reflected. Thus if absorption is in the red and blue as we saw with chlorophyll then we reflect green and that is what we see. This is an application of Beer's Law[29]. Beer's law is a statistical approach to absorption. It reflects what experimentally is obtained and does not provide a detailed analysis as we had been developing in prior sections.

[29] See Cantor and Schimmel, pp. 60-68.

Example



2/1/2011 41

We define the reflect light at a specific frequency, wavelength, as follows:

$$R_i(\lambda) = A_i(\lambda)\exp(-\kappa_i(\lambda)[C_i]x_i^{eff})$$

where [C] is a concentration and x is the effective thickness of the cell.

Here R is the reflected light we see at the wavelength specified and at the ith anthocyanin.
A is the incident light amplitude at the wavelength specified. The exponent is Beer's law where C is the concentration of anthocyanin I and x the effective depth of that anthocyanin.

Now we can write Beer's law for one or two or even more absorbents. We show the case for one and two absorbents as follows:

$$\frac{dR}{R} = -C_k\kappa_k dx$$

or

$$\frac{dR}{R} = -C_k\kappa_k dx - C_{k+1}\kappa_{k+1}dx$$

Note that the reduction in reflected light or in transmitted light is reduced by a result of the additive reduction of separate collisions with separate molecules.

$$R_{Total}(\lambda) = A(\lambda)\exp(-\sum_{i=1}^{N}\kappa_i(\lambda)[C_i]x_i(\lambda))$$

The log of the ratio of intensities is the sum of the weighted concentrations. We assume we know the κ values for each absorbing element at each wavelength. Then we can use the above to estimate the separate concentrations

$$I(\lambda) = \ln \frac{R_{Total}(\lambda)}{A(\lambda)} = -\sum_{i=1}^{N} \kappa_i(\lambda)[C_i]x_i(\lambda)$$

The problem is simply stated. We measure the intensity at say M values of wavelength and this gives us M samples. We then must find values of the [C] which give the best fit to the measurements obtained using the model assumed. That is for every wavelength, we define an error as the difference between the measurement and what the measurement would have been using the estimates of the [C] values and the best [C] values are those which minimize the sum of the squares of these errors. There are M measurements and N concentrations and M is much larger than N. That is:

Choose $[C_n]$ *such that they minimize*

$$\min(\sum_{m=1}^{M}\left(I(m) - \hat{I}(m)\right)^2$$

where

$I(m)$ *is the mth measurement*

and

$$\hat{I}(m) = \sum_{i=1}^{N} \kappa_i(\lambda)[C_i]x_i(\lambda)$$

This is an optimization problem which can be solved in many ways. We address some of them in the next section.

11.3 INVERSION APPROACHES

We will now look at several general methods of inversion, mostly applied to dynamic system inversions, where we may have dynamics in space and/or time. In many ways this is an example of the Inverse Problem already solved by McGarty (1971):

1. CIE approach: This assumes that one can unravel the exponents of the x,y,z model. The problem is that we will not have an adequate number of degrees of freedom.

2. Splines: This assumes we can generate curves and then separate them and then focus on their coefficients[30].

3. Steepest Descent: This is the incremental approach of best fit. It assumes we are trying to solve an optimization problem.

[30] See Hildebrand pp. 478-494. The use of splines is an approach which tries to match coefficients of polynomials.

4. Least Squares Fit: A statistical best fit method.

5. Kalman Filter: This is the statistical solution using steepest descent but with correlation matrices.

6. Matched Filter: This approach assumes we know the waveforms of each absorption curve for each colorant and that we receive a resulting absorption curve which is the sum of all of them, and that we then try to estimate the "amplitudes" of each curve, in effect the concentrations.

11.3.1 CIE Approach

We briefly look at using the color data directly. This we call the CE approach since it employs the CIE color structure. We may define the problem as follows:

Let $R(\lambda)$ *be determinable for a given set of* $[C_i]$ *and let*

$R(\lambda)$ *be the measured received spectrum power and*

$I(\lambda)$ *be the log of the received to incident power*

at the wavelength

Find the set of $[C_i]$, $i=1...N$, *such that*

$\left(\widetilde{R(\lambda)} - \widehat{R(\lambda)}\right)^2$ *is minimized where* $\widehat{R(\lambda)}$*is the*

estimated received spectral element

We may also characterize the variables as follows:

Let

$$x(k) = \begin{bmatrix} C_1 \\ \cdot \\ \cdot \\ C_n \end{bmatrix} = x(k+1)$$

and

$$z(k) = c^T(k)x(k) + n(k)$$

where

$$c(k) = \begin{bmatrix} -\kappa_1(k)x_1 \\ \cdot \\ \cdot \\ -\kappa_n(k)x_n \end{bmatrix}$$

and for this case k and λ *are identical increments*

We now consider three possible approaches.

11.3.2 Newton Steepest Descent

The Newton Steepest descent approach is one where we define an optimization and this optimization results in solving a polynomial equation. We then employ an iterative method to solve that equation. We now seek the following:

Find the a such that:

$$a = \begin{bmatrix} \hat{a}_1 \\ .. \\ \hat{a}_n \end{bmatrix} = \begin{bmatrix} [C_1] \\ . \\ . \\ [C_N] \end{bmatrix}$$

such that

$$\min\left[\sum_{i=1}^{M}(I_i - \hat{I}_i)^2\right]$$

Let us recall the simple optimization result:

$$h(a) = \left[\sum_{i=1}^{M}(I_i - \hat{I}_i)^2\right]$$

and

$$\frac{\partial h(a)}{\partial a_n} = g_n(a) = 0$$

is the optimal point, so we seek to solve the vector equation:

g(a)=0

We can now state the general solution in terms of Newton's Method[31]:

[31] See Athans et al, Systems, Networks and Computation, Multivariable Methods, McGraw Hill (New York) 1974, pp-115-122.

$g(a) = 0$

is the desired result. Define:

$$A(a) = -\left[\frac{\partial g(a)}{\partial a}\right]^{-1}$$

where we define:

$$\left[\frac{\partial g(a)}{\partial a}\right] = \begin{bmatrix} \frac{\partial g_1}{\partial a_1} ..... \frac{\partial g_1}{\partial a_n} \\ \frac{\partial g_n}{\partial a_1} ..... \frac{\partial g_n}{\partial a_n} \end{bmatrix}$$

and the estimate at sample k+1 is:

$\hat{a}(k+1) = \hat{a}(k) + A(\hat{a}(k))g(\hat{a}(k))$

Note that we use this iterative scheme as one of several means to achieve the result. For each tuple of data we do the following:

$\hat{a}(0) = a^0$, an n x 1 vector guess. Then we use the first data tuple:

$\hat{a}(1) = \hat{a}(0) + A(\hat{a}(0))g((\hat{a}(0))$

where we use the difference:

$a_{k,measured}(0) - \hat{a}(0)$

as the data entry element for each of the elements of a.

The Newton algorithm is but one of many possible algorithms. We know the conditions for Newton convergence. We can also estimate the accuracy of this algorithm as well.

11.3.3 Kalman Filter

The method of estimating the structural elements of the gene expression can be structured using a standard set of methodologies. In particular we use the two approaches. The approach was applied to estimating the constituent chemical concentrations of the upper atmosphere, namely the inversion problem, using transmitted light as the probe mechanism. In this case we seek to estimate the gene expression matrix using the concentrations of secondary chemicals as expressed in color concentrations. This is in many ways a similar problem.

Let us consider a six gene model, two color modifying genes and four control genes, two each. The model is as follows. First is a general linear model for the gene production:

$$\frac{dx(t)}{dt} = Ax(t) + u(t) + n(t)$$

Then the entries are as follows:

$$A = \begin{bmatrix} a_{11}..a_{12}..a_{13}..0..0..0 \\ 0...a_{22}..0...0...0...0 \\ 0...0...a_{33}..0...0...0 \\ 0...0...0...a_{44}..a_{45}..a_{46} \\ 0...0...0...0...a_{55}..0 \\ 0...0...0...0...0...a_{66} \end{bmatrix}$$

and

$$u(t) = \begin{bmatrix} u_1 \\ ... \\ u_6 \end{bmatrix}$$

And we assume a system noise which is white with the following characteristic:

$$E\left[n(t)\right] = 0$$

and

$$E\left[n(t)n(s)\right] = N_0 I\delta(t-s)$$

Now we can define:

$$A = \begin{bmatrix} A_1...0 \\ 0...A_2 \end{bmatrix}$$

Where we have partitioned the matrix into four submatrices. This shows that each gene and its controller are separate. Now we can determine the concentrations of each protein in steady state as follows, neglecting the Gaussian noise element for the time being:

$$\begin{bmatrix} x_1 \\ x_2 \\ x_3 \end{bmatrix} = -A_1^{-1} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \end{bmatrix}$$

and

$$\begin{bmatrix} x_4 \\ x_5 \\ x_6 \end{bmatrix} = -A_2^{-1} \begin{bmatrix} u_4 \\ u_5 \\ u_6 \end{bmatrix}$$

We argue that finding either the matrix elements or their inverse relatives is identical. Thus we focus on the inverse elements. Now the concentrations of the anthocyanins are given by the 2 x 2 vector as follows:

$$\begin{bmatrix} z_1 \\ z_2 \end{bmatrix} = \begin{bmatrix} c_{11}...0...0...0...0...0 \\ 0...0...0...c_{24}...0...0 \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{bmatrix} = Cx$$

The color model remains the same.

The system model is as follows. Let us begin with a model for the vector a that we seek:

$$\frac{da(t)}{dt} = 0 : where$$
$$a(t) = \begin{bmatrix} a_1 \\ ... \\ a_5 \end{bmatrix}$$

In this case we have assumed a is a 5 x 1 vector but it can be any vector. The measurement system equation is given by:

$$z(t) = g(a,t) + w(t)$$

Where z is an m x 1 vector. In this case however we have for the measurement the following:

$$z(t) = \begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ x_1 \\ ... \\ x_6 \end{bmatrix} = g(a,t) + w(t)$$

We now expand in a Taylor series the above g function:

$$g(a,t) = g(a_0,t) + C(a_0,t)\left[a(t) - a_0(t)\right] +$$
$$\frac{1}{2}\sum_{i=1}^{N} \gamma_i \left[a - a_0\right]^T F_i \left[a - a_0\right] + ...$$

Where we have:

$$C = \begin{bmatrix} \frac{\partial g_1}{\partial a_1} & \cdots & \frac{\partial g_1}{\partial a_n} \\ .. & .. & .. \\ \frac{\partial g_m}{\partial a_1} & \cdots & \frac{\partial g_m}{\partial a_n} \end{bmatrix}$$

Thus we have for the measurement:

$$z(t) = C(t)a(t) + \left[g(a_0) - C(a_0)a_0(t) \right]$$

We now use standard Kalman theory to determine the mean square estimate;

$$\frac{d\hat{a}(t)}{dt} = P(t)C^T(t)K^{-1}(z - C(t)\hat{a}(t))$$

where

$$\frac{dP(t)}{dt} = -P(t)C^T(t)K^{-1}C(t)P(t) +$$

$$\sum_{i=1}^{N} PF_i P\gamma_i^T K^{-1}(z - g(a_0)$$

where

$$K\gamma(t-s) = E\left[w(t)w^T(s) \right]$$

In discrete time we have the equation:

$$\hat{a}(k+1) = \hat{a}(k) + PCK^{-1}\left[z(k) - \hat{z}(k) \right]$$

This is identical to the equation we derived from the Newton method.

11.3.4 The Matched Filter Approach

This is a different approach and it is an application of signal detection taken from classic communication theory. It assumes we have N signals and each signal shape is known but the amplitude of the individual signals is not known. Then we ask how we can estimate the amplitude of each signal if what we have is a received signal which is the sum of the N plus noise. We begin this approach as follows:

Let us assume there are two waveforms bounded on an interval [0, T]

Let

$$s_1(t) = s_1^{orthog}(t) + s_1^{remain}(t)$$

$$s_2(t) = s_2^{orthog}(t) + s_2^{remain}(t)$$

such that

$$\int_0^T s_1^{orthog}(t) s_2^{orthog}(t) dt = 0$$

Now there are three questions which we may pose:

1. Does such a decomposition exist, if so under what terms?

2. What is a constructive way to perform the decomposition?

3. Is there an optimum decomposition such that the "distance between the two orthogonal signals is maximized"?

Namely:

$\exists$ *a set* $\{s_1, s_2\}$
such that

$$\int_0^T s^2{}_1(t) dt = E_1$$

$$\int_0^T s^2{}_2(t) dt = E_2$$

and

$\exists \max$

$$\int_0^T s^{2,orth}{}_1(t) dt = E_1$$

$$\int_0^T s^{2,orth}{}_2(t) dt = E_2$$

Let us approach the solution using the theory of orthogonal functions[32]. Now we can specifically use a Fourier series approach. We do the following:

[32] See Sansone, Orthogonal Functions.

Let

$$s_1(t) = \sum_{n=1}^{\infty} s_1^n \cos(\frac{2\pi}{T} nt) + r_1(t)$$

where

$$r_1(t) = s_1(t) - FS\cos$$

and

$$FS\cos = \sum_{n=1}^{\infty} s_1^n \cos(\frac{2\pi}{T} nt)$$

Likewise

Let

$$s_2(t) = \sum_{n=1}^{\infty} s_2^n \sin(\frac{2\pi}{T} nt) + r_2(t)$$

where

$$r_2(t) = s_2(t) - FS\sin$$

and

$$FS\sin = \sum_{n=1}^{\infty} s_2^n \sin(\frac{2\pi}{T} nt)$$

Clearly FS_{cos} and FS_{sin} are orthogonal. The residual functions r are the sin and cos elements respectively of the expansions. We could have just as easily transposed the sin and cos allocations between the two s functions. As to answering the third question we are effectively asking if the r residual functions can be minimized. The answer is not with a Fourier Transform. Then the question would be; is there another set of orthogonal functions which would minimize the residuals, namely:

$$\int_0^T r_1^2(t)dt = R_1$$

and

$$\int_0^T r_2^2(t)dt = R_2$$

are to be minimized. For a Fourier Transform as the orthogonal base we are left with residuals, R, at whatever they may be. However using the Fourier Transform approach we can extract the two signals as follows:

$$P(\lambda) = [C_1]\tilde{s}_1(\lambda) + [C_2]\tilde{s}_2(\lambda) + r_{TOT}(\lambda)$$

Note we can interchange t and λ since they represent the same variable. We now have a "signal" with amplitudes to be determined and a bias which is known. Using standard "signal detection theory" we can readily solve this problem as well. This becomes the "matched filter problem"[33].

What we have sought to accomplish in this paper is to describe color and it generation in plants and to present a set of methods and means to determine the constituents which give rise to those colors. In effect we have created a world view of color, apart from the classic colorimetry approach, and used this and the physical measurements related thereto to affect a method and means to determine concentrations of colorants in flowers.

The simple application of Beer's law and the use of the known spectra of anthocyanins and other colorants allow us to use data from FTS to determine the concentrations of each colorant on literally a cell by cell basis. Beer's law is a simplistic but fairly accurate and consistent method. It would be interesting to explore the details of the transmission of light to a deeper level but the complexity of the cell structure prohibits that at this time.

Having a methodology of the type developed herein we can now more readily examine the genetic pathways and expression systems in the genus Hemerocallis. This paper details multiple ways to ascertain concentrations on a cell by cell basis.

[33] See VanTrees, Detection, Estimation and Modulation Theory. He presents details on this solution.

12 CONCLUSION

We have examined HGPIN and the genetic defects observed in PCa. The answers to progression from benign to HGPIN to PCa are not at all well understood. There is a correlative relationship between gene loss or enhancement and the stage of PCa but there clearly is no definable and replicateable path. PTEN is lost in some but not all, and the same holds for all of the genes and their aberrations. There is not a common and predictable pathway at this time. The pathway to understanding the forward progression is filled with many paths. Perhaps that is just the very nature of this cancer. It lacks the consistency of a Vogelstein model that colon cancer has, but in many ways it is a much more typical cancer.

One of the most obvious conclusions is that an effective model of prostate genetic dynamics is still a work in progress. There are several factors which limit what we can accomplish:

1. The details regarding the effects of the genes that have been targeted are still qualitative and not adequately detailed.

2. The complete epigenetic networks are not fully complete. For example with regard to PTEN or the AR genes, we do not know if they are eliminated or epigenetically suppressed and if the later by what specific mechanism, methylation, miRNA, or other gene products yet to be determined.

3. The mechanism for regression is not well understood. The presence of p27 or GSTP1 and other genes is suggestive at best and not necessarily causal. It will be necessary in this case to work through full details. In addition, there is the issue of immune response and ligan control.

We have focused on pathways and the dynamics of the reaction kinetics. The primary focus has been within cells. There are reasons for understanding the detailed concentration dynamics. As we have seen, many researchers have developed models which are understandings of pathways at the highest levels, namely what products relate to what other products. Other researchers as they delve into the dynamics of the pathways have employed the Boolean and Bayesian methods. We have argued that these methods are tuned for ascertaining pathway structure and not necessarily pathway dynamics. We have argued that detailed dynamic concentration models must be used.

To understand why the concentrations and their dynamics are useful we look at a recent paper by Carracedo et al where they are discussing PTEN and the authors state:

The importance of PTEN (phosphatase and tensin homolog located on chromosome 10) in cancer has surpassed all predictions and expectations from the time it was discovered and has qualified this gene as one of the most commonly mutated and deleted tumor suppressors in human cancer. PTEN levels are frequently found downregulated in cancer, even in the absence of genetic loss or mutation. PTEN *is heavily regulated by transcription factors, microRNAs, competitive endogenous RNAs (such as the PTEN pseudogene), and methylation, whereas the tumor suppressive activity of the PTEN protein can be altered at multiple levels through aberrant phosphorylation, ubiquitination, and acetylation. These regulatory cues are presumed*

to play a key role in tumorigenesis through the alteration of the appropriate levels, localization, and activity of PTEN. The identification of all these levels of PTEN regulation raises, in turn, a key corollary question: How low should PTEN level(s) or activity drop in order to confer cancer susceptibility at the organismal level? Our laboratory and others have approached this question through the genetic manipulation of Pten *in the mouse. This work has highlighted the exquisite and tissue-specific sensitivity to subtle reductions in Pten levels toward tumor initiation and progression with important implications for cancer prevention and therapy.*

The very title of their paper, namely looking at what amount of PTEN is too little frames the question well.

Now looking at a single cell is but one step. We also believe that one must look at the inter-cell signalling as well. Thus the spatial dynamics of inter-cell signalling we believe will also play a significant role in cancer development. We have examined the issue of inter-cell communications when looking at the control of secondary pathways elsewhere. However in the development of cancer and its propagation we have little to begin with other than recognizing its importance.

Having described the multiple genes and their products who presence or absence is identified with PCa and having further identified the pathways and then their dynamics in terms of measurable results, we now seek to ask two additions questions.

First, how can we identify the many constants in the models which we have developed. namely can we observe the model in adequate detail so as to have confidence in what has been speculated.

Second, if we can observe the models to a reasonable degree of accuracy and predictability, can we then develop means and methods to control the actual system, and be capable of driving it from one state to another? Can we control PCa or even more so can we regress PCa?

Apoptosis is the normal progression and death of cells for a variety of reasons and predominantly because they are no longer functional, no longer do what they should be doing. Apoptosis is self-recognized in the cell and the cell takes it upon itself to die. The loss of this capability is one of the major factors in the development of a cancer. The existence of this factor in HGPIN remission may be a natural path to returning to normality. But the question still remains; what causes the remission, and if it is apoptotic then what makes it so?

The immune system responds when it perceives some antigen, a non-self-indicator on a cell, which initiates a set of cascades within the complexity of the immune system to recognize the invader and take actions to cleanse the body of this invader.

13 APPENDIX A GRADING

In this sections we provides more detail on grading of PCa. The emphasis here is upon histological change and does not reflect any changes in pathways.

13.1 CANCER

Prostate Cancer is simply the growth of abnormal glandular like structures outside of the normal prostate glands the resulting continued growth of the cells making up those structures both within and without the prostate. The PCa cells take over the stroma, pushing aside the normal stromal cells and then migrate in a metastatic fashion throughout the body.

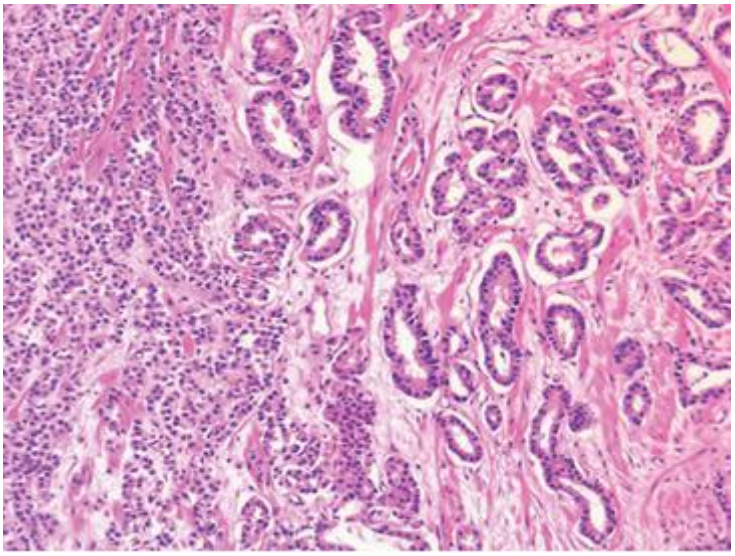
We will use the Gleason grading score as a means to characterize the level of cancer progression within the prostate.

13.2 GRADING

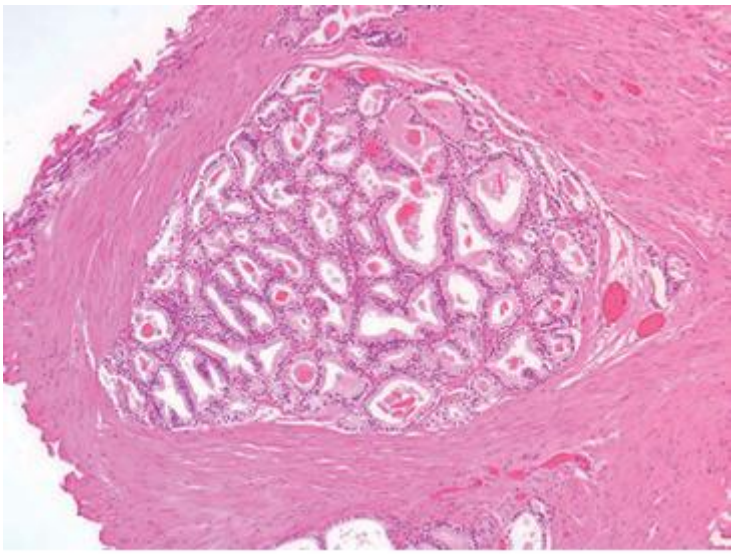
We present the grading system developed by Gleason. On the one hand this has been used as a gold standard for ascertaining future progress and yet it is still just a morphological tool. It fails to determine the pathways and regulators in a cell by cell basis.

13.2.1 Gleason 1

The following is a Gleason 1 grade tumor. Note that there are a proliferation of small glandular like clusters with dark basophillic stains and they are separate and have clear luminal areas. Gleason 1 is generally composed of many single and separate and closely packed glands of well circumscribed uniforms glands. One rarely sees Gleason 1 grade tumors, and they are often found as incidental findings when examining for other issues.



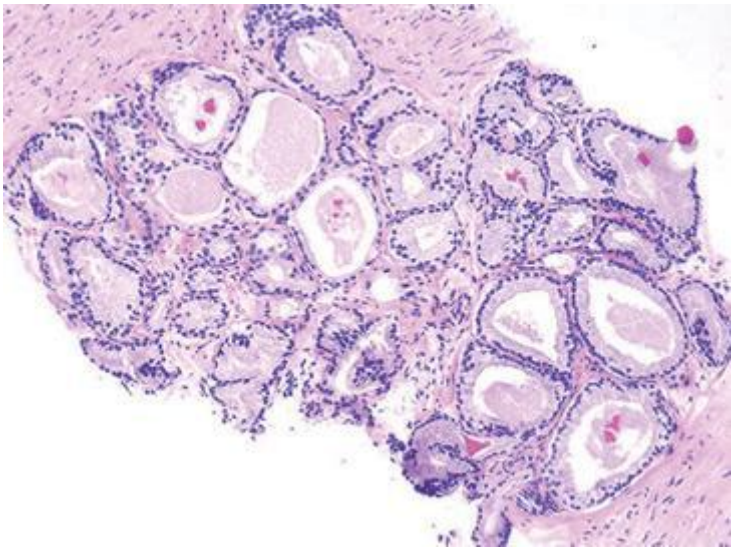
We show another view of a Gleason 1 below. This is especially descriptive of such a form because it appears almost as a single and isolated structure. The interesting question will be if this is PCa then if PCa is clonal is this cluster an aberrant outgrowth of a normal cells, if so which one, and if so is this just one cell growing. It appears that at this stage the intercellular signaling is still trying to function. However the clarity of cell form is being degraded.



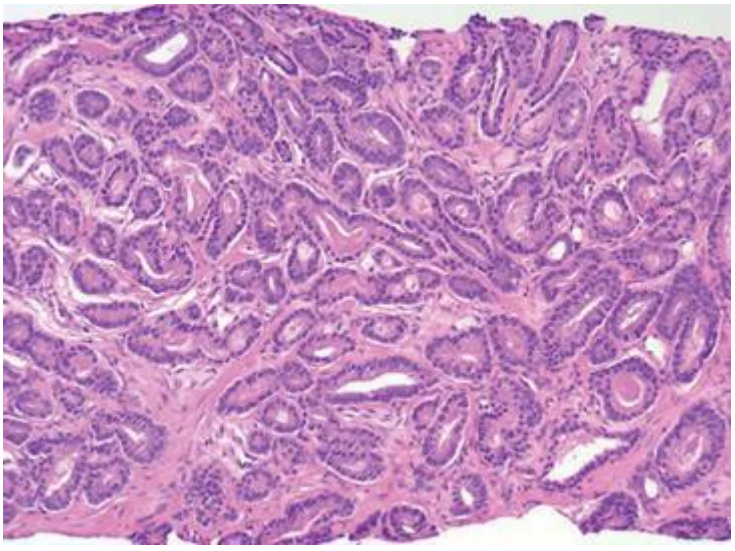
13.2.2 Gleason 2 and 3

Gleason 2 shows many more new glandular like cells but now of varying larger sizes. As Epstein notes: "*Grade 2 … is still fairly circumscribed, at the edge of the tumor nodule there can be minimal extension by neoplastic glands into the surrounding non-neoplastic prostate. The glands are more loosely arranged and not as uniform as Gleason 1*." We see those in the figure below which combines Gleason 2 and 3.

Gleason 3 is often composed of single glands. The Gleason 3 infiltrates in and amongst the non-neoplastic glands. Gleason 3 still can be seen as a separate gland and there are no single cells starting to proliferate. In Gleason 3 we still have some semblance of intercellular communications and coordination, albeit with uncontrolled intracellular growth. Again in the figure below we see both the smaller 2 and the larger 3 with gland structure being preserved and no separate cells proliferating.

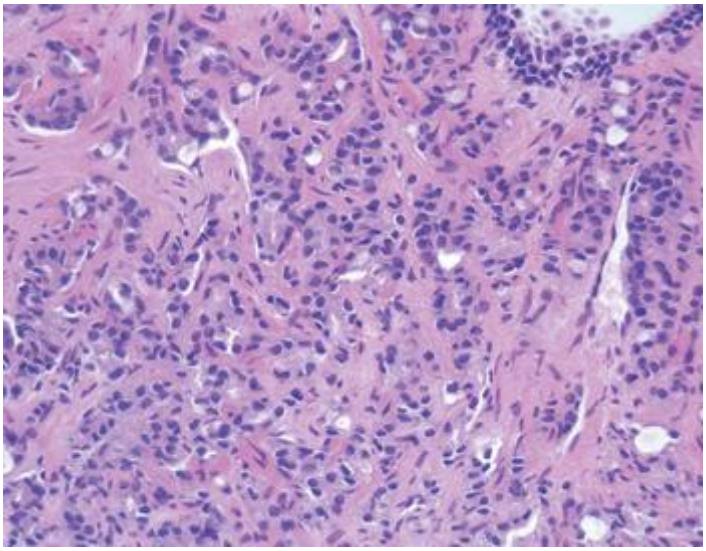


A Gleason 3 throughout is shown below.

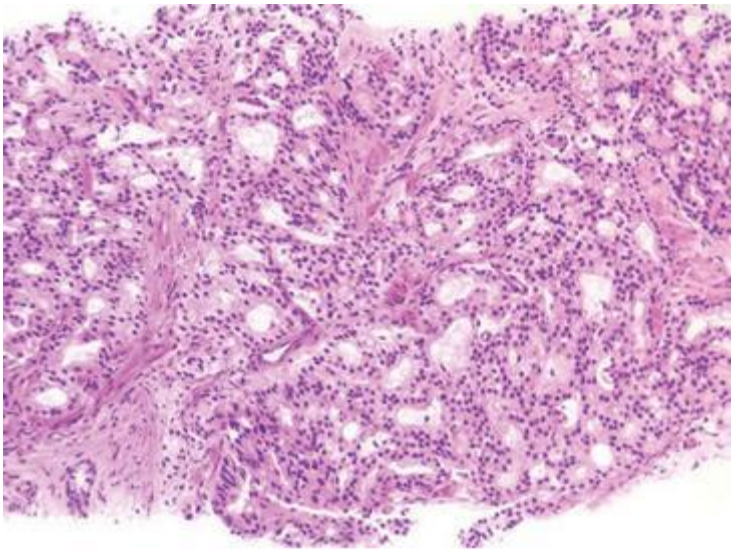


13.2.3 Gleason 4

Gleason 4 consists mostly of cribiform cells (perforated like a sieve) or fused and ill-defined glands with poorly formed glandular lumina. The glands appear to start to "stick" together. A Gleason 4 with a Gleason 3 is shown below. Note the sieve like structure and the closing of the glands.

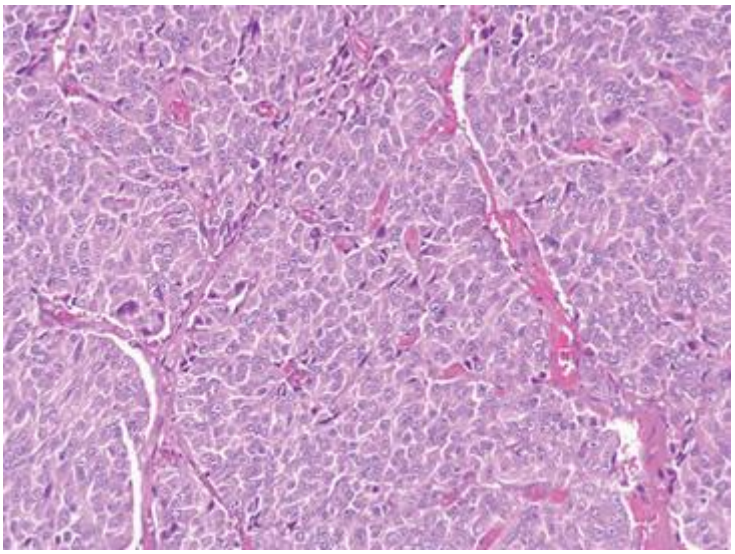


A Gleason all 4 is shown below. Note that the cells are sticking closed and the entire mass appears as a sieve like mass.



13.2.4 Gleason 5

Gleason 5 is a complete conversion to independent malignant cells. They have lost all intercellular coordination. As shown below it is a mass or mat or sheet of independent cancer cells and it has lost any of the sieve like structures. There may also appear to be some necrosis



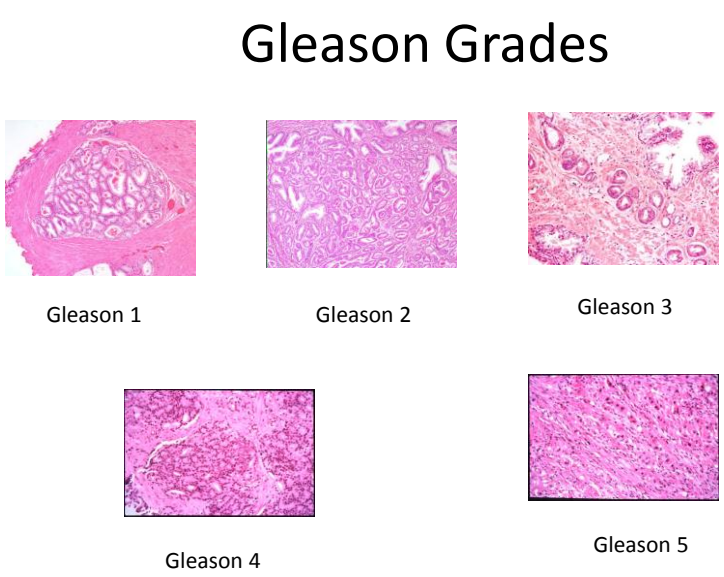
13.2.5 Gleason Summary

The Gleason scores are then determined by taking the predominant type and adding it to the secondary type. Thus a 4+3 yields a Gleason combined 7 but it is 4+3 and that is more aggressive than say a 3+4 with the same total score.

We repeat the grading commentary below.

Gleason 1	Gleason 2	Gleason 3	Gleason 4	Gleason 5
Many acini with no basal layers and large nucleoli. Closely packed clumps of acini.	Many very small single separate glands (acini) with no basal layer and large nucleoli . Glands, acini, are more loosely arranged and not close packed.	Many small microglands extending throughout the stroma and out of the normal gland structure	Glands are now spread out and fused to one another throughout the stroma.	No gland structure seen, all luminal cells throughout the stroma with large nucleoli.

The following chart is a summary of the progression.



13.3 MODELS FROM GRADING

In looking at the grading one may also hypothesize a possible path of progression. The steps appear to be:

1. Movement from existing benign gland to a separate but glandular like proliferation. Cells which would normally remain dormant go through a replication cycle, apoptosis and cell proliferation control seems lost. New glands appear clustered but appear separate.

2. Growth of the new glands makes them expand but remain morphologically glandular. They close packing begins to disappear and glands start to stand on their own. It is as if they are expanding and growing and the basal layer begins to disappear. Luminal like cancer cells start to be predominant.

3. Many small micro-glands start expanding and cell growth accelerates and the cells appear more cancer like but there is still some morphological glandular structure left.

4. The many glands have dramatically different shaped and start closing in one another and appear sieve like with small openings. They look as if they are losing any intercellular communications resulting is a common mat of cells.

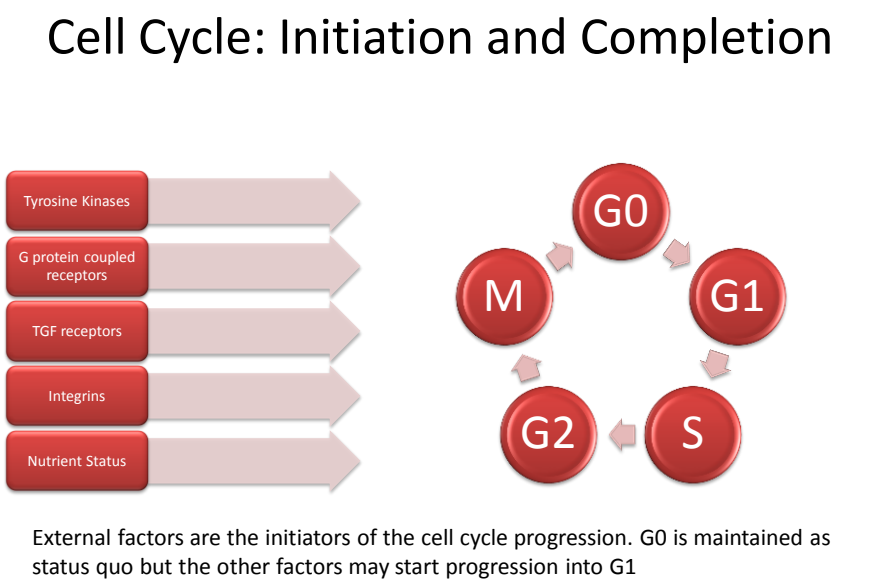
5. Cells have lost any morphological form related to glands and appear as a mat of cancer cells replacing the stroma totally. No intercellular communications is left and cellular growth control has been eliminated totally.

These five steps are consistent with the Gleason grading but they also parallel the way the intracellular and intercellular controls are lost. We will look at these mechanism later.

14 APPENDIX B CELL CYCLE

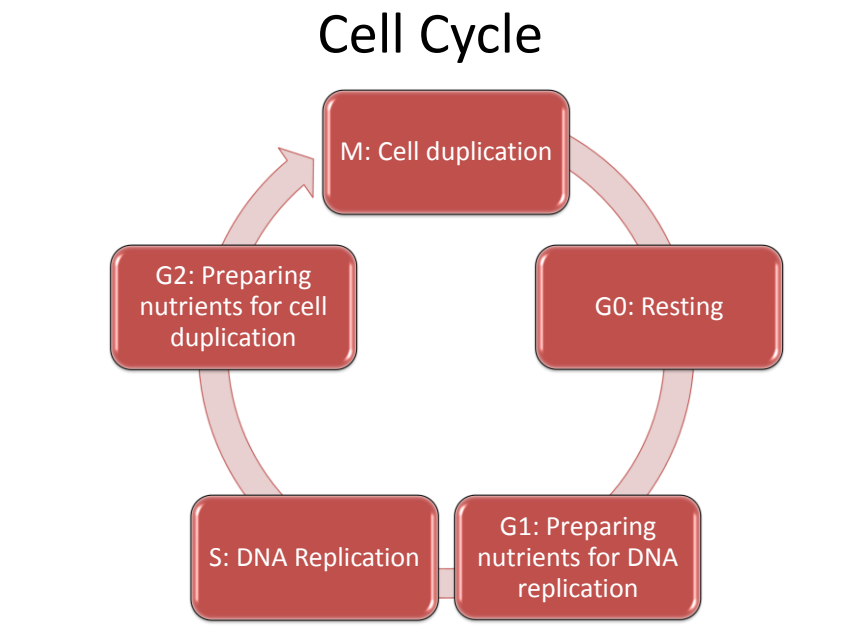
Before detailing the full set of pathways we often examine a hypothetical cancer which is characterized by uncontrolled growth, and that means abnormal controls on the cell cycle. As we have also discussed, such may exist only on the cancer stem cell and not on all the malignant cells.

We find it useful to look at the cell cycle and then introduce some simplified pathways We then model the disturbances to the genes controlling pathway proteins and see what the impact is. We then follow the dynamics of the cell cycle. Mitosis is the process of cell reproduction. Cells can: Replicate, Die, Stasis. Cell Cycle is a controlled process, key to understanding cancer



The external factors influence the instigation and control of the cell cycle. The cell goes from the stable ground state G0 into the first step of reproducing the G1 state and then through S, G2 and M and then back to a resting state. Again we must emphasize that this may apply to the stem cell only.

We duplicate the above no detailing the specific steps in between.



14

Virtually all cells require external stimuli to begin G1. There are 10 cells in body
Consulting the external environment occurs from onset G1 to about 1-2 hours before S phase begins, this is the R point or the Restriction Point; if cell goes past r point it is committed to replicate. The R point is one of several that are check points in cell replication that in benign cells act as a point at which any imperfect replication can be stopped. That is not the case in a malignant cell.

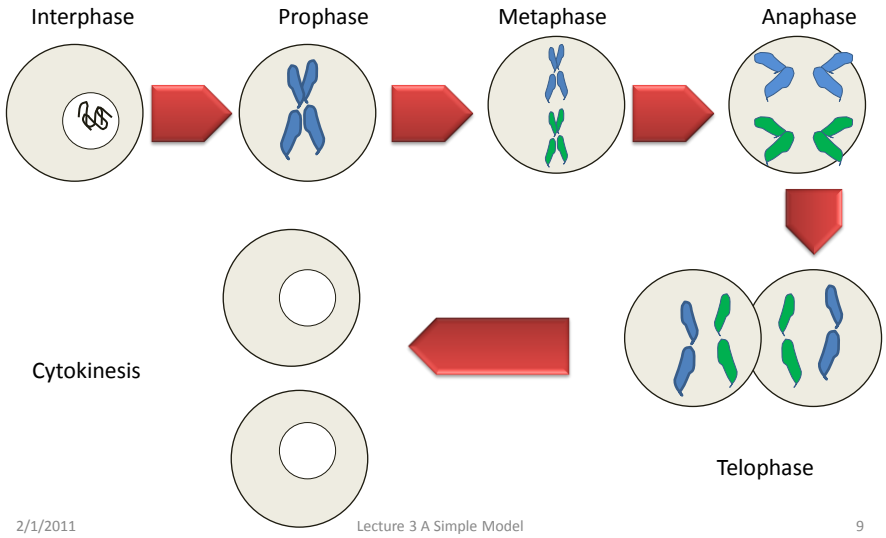
G1 and R Point

The deregulation of the R point is the major critical determinant of whether cells will duplicate and grow or not. Weinberg and others contend that this R point decision is key to cancer growth.

2/1/2011 Lecture 3 A Simple Model 7

Mitosis in the M phase is the detailed breaking apart of the chromosomes and their replication. This is often a critical step because it is here that we may see translocations as we have discussed internally.

M Phase: Mitosis and Subphases



2/1/2011 Lecture 3 A Simple Model 9

This is a brief summary of the cell cycle as regards to the growth of cancer cells.

15 APPENDIX C: OTHER KEY PATHWAYS

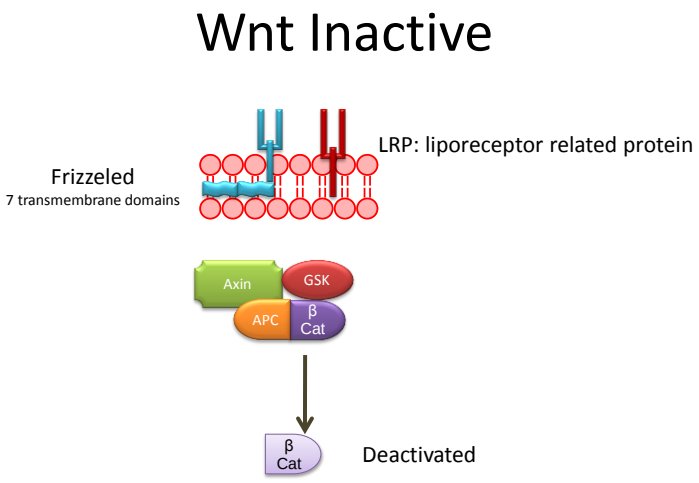
There are several other pathway elements that are worth commenting on. We do three here; WNT, Hedgehog, and Notched.

15.1 WNT

Wnt is characterized as follows:

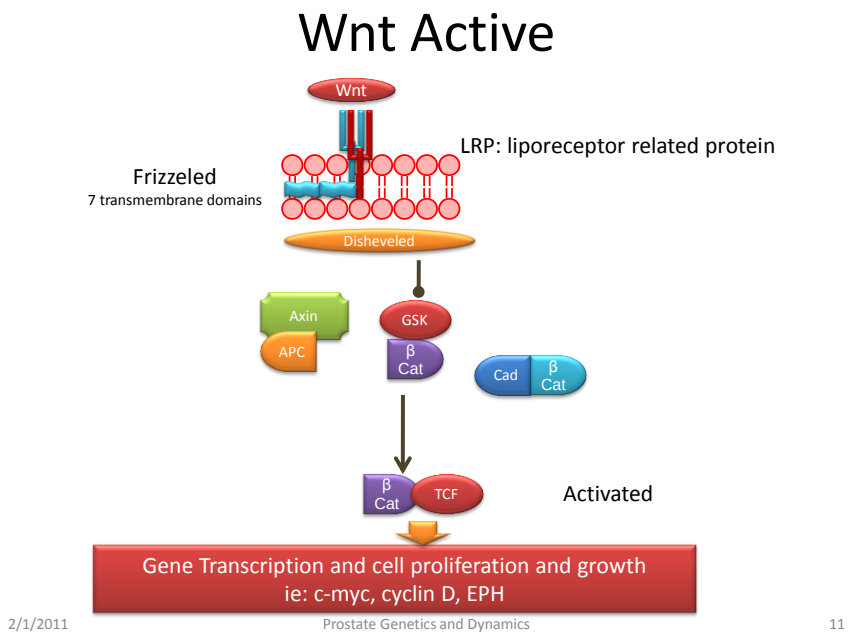
- This comes from the “wingless” gene and thus the Wn prefex. The was related to discovery on fruitflies.
- The **canonical Wnt pathway** describes a series of events that occur when Wnt proteins bind to cell-surface receptors of the Frizzled family, causing the receptors to activate Dishevelled family proteins and ultimately resulting in a change in the amount of β-catenin that reaches the nucleus
- Dishevelled (DSH) is a key component of a membrane-associated Wnt receptor complex which, when activated by Wnt binding, inhibits a second complex of proteins that includes axin, GSK-3, and the protein APC
- The axin/GSK-3/APC complex normally promotes the proteolytic degradation of the β-catenin intracellular signaling molecule.
- After this "β-catenin destruction complex" is inhibited, a pool of cytoplasmic β-catenin stabilizes, and some β-catenin is able to enter the nucleus and interact with TCF/LEF family transcription factors to promote specific gene expression

We depict this below, first the inactive state:



2/1/2011 Prostate Genetics and Dynamics 10

Then we depict the activated state with Wnt attached to the receptor:

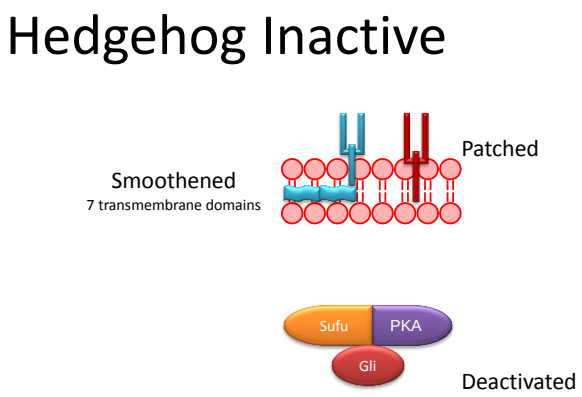


15.2 HEDGEHOG

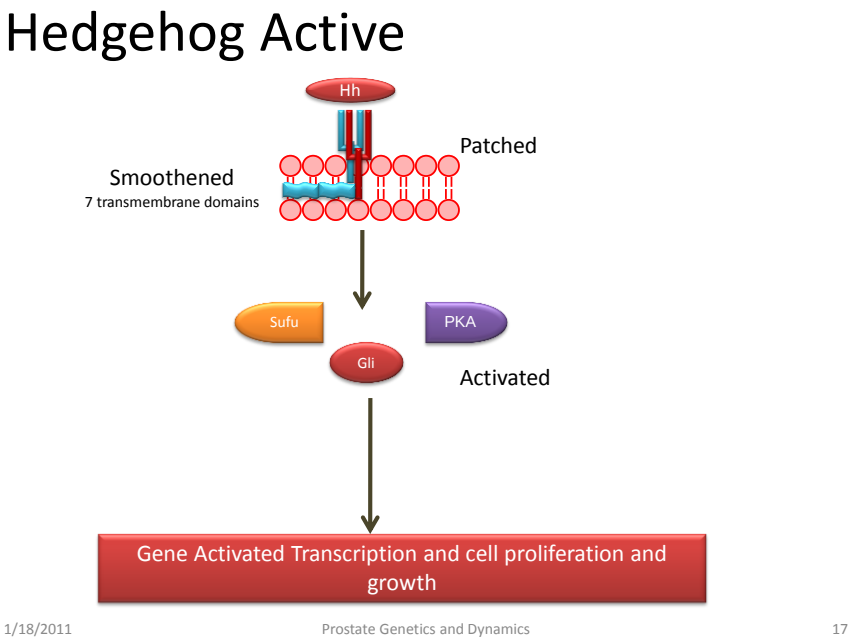
The Hedgehog pathway is also a key element characterized as follows:

- In the absence of Hh a cell-surface transmembrane protein called Patched (PTCH) acts to prevent high expression and activity of a 7 membrane spanning receptor called Smoothened (SMO).
- Patched has sequence similarity to known membrane transport proteins. When extracellular Hh is present , it binds to and inhibits Patched, allowing Smoothened to accumulate and inhibit the proteolytic cleavage of the Ci protein.
- In cells with Hh-activated Patched, the intact Ci protein accumulates in the cell cytoplasm and levels of CiR decrease, allowing transcription of some genes such as decapentaplegic (dpp, a member of the BMP growth factor family).
- For other Hh-regulated genes, expression requires not only loss of CiR but also the positive action of uncleaved Ci acting as a transcriptional activator.

First we show it inactivated as below:



Then the activated pathway as follows:



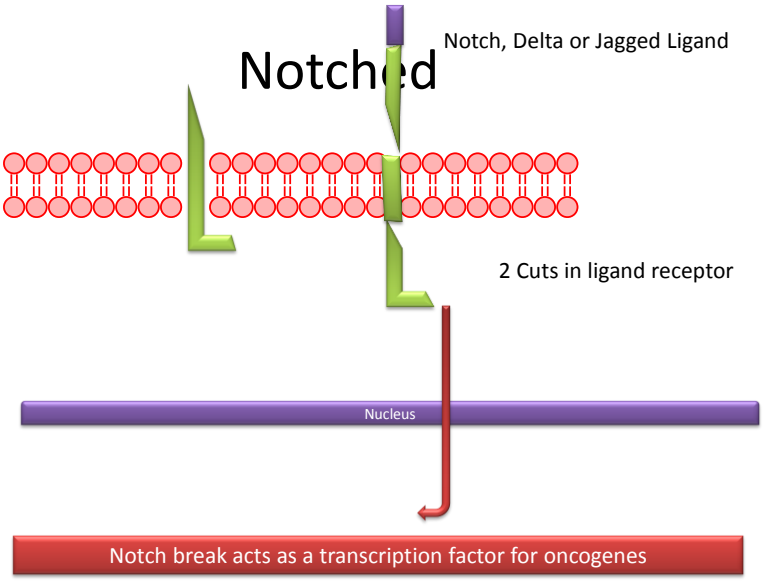
15.3 NOTCHED

The notched pathway is characterized as follows:

The notch protein sits like a trigger spanning the cell membrane, with part of it inside and part outside. Ligand proteins binding to the extracellular domain induce proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to alter gene expression. The notch signaling pathway is important for cell-cell communication, which involves gene regulation mechanisms that control multiple cell differentiation processes during embryonic and adult life. Notch signaling also has a role in the following processes:

1. neuronal function and development
2. stabilization of arterial endothelial fate and angiogenesis
3. regulation of crucial cell communication events between endocardium and myocardium during both the formation of the valve primordial and ventricular development and differentiation
4. cardiac valve homeostasis, as well as implications in other human disorders involving the cardiovascular system
5. timely cell lineage specification of both endocrine and exocrine pancreas
6. influencing of binary fate decisions of cells that must choose between the secretory and absorptive lineages in the gut
7. expansion of the hematopoietic stem cell compartment during bone development and participation in commitment to the osteoblastic lineage, suggesting a potential therapeutic role for notch in bone regeneration and osteoporosis
8. T cell lineage commitment from common lymphoid precursor
9. regulation of cell-fate decision in mammary glands at several distinct development stages
10. possibly some non-nuclear mechanisms, such as control of the actin cytoskeleton through the tyrosine kinase Ab

Notch signaling is dysregulated in many cancers


Notched
Notch, Delta or Jagged Ligand
2 Cuts in ligand receptor
Nucleus
Notch break acts as a transcription factor for oncogenes

16 REFERENCES

1. Abrahamsson, P., D. Bostwick, The Biological Nature of Prostate Cancer, to be published 2010 (Univ Malmo Sweden).
2. Ahn, K., Transcription Factor NF-kB, Ann NY Aca Sci, V 1056, 2005, pp 218-233.
3. Ailles, L., I. Weissman, Cancer Stem Cells in Solid Tumors, Curr Opp in Biotech, 2007, pp 460-466.
4. Allis, C., et al, Epigenetics, CSH (Cold Spring Harbor) 2007.
5. Alonzo, T., Verification Bias corrected estimators of relative true and false positives of two binary screening tests, Stat in Med 2005 pp 403-417.
6. Ando, S., H. Iba, Quantitative Modeling of Gene Regulatory Network, Univ Tokyo.
7. Ando, S., H. Iba, Quantitative Modeling of Gene Regulatory Network, Univ Tokyo.
8. Andriole, G., et al, Mortality Results from Randomized Prostate Cancer Screening Trial, NEJM, 2009 V 360 pp 1310-1319.
9. Appasani, K., MicroRNAs, Cambridge (New York) 2008.
10. Araud, T., Overview of the miRNA Pathways, Thesis, U Geneva, 2008.
11. Asadi, F., R. Sharifi, Effects of Sex Steroids on Cell Growth and C myc Oncogene Expression in LN-CaP and DU-145 Prostatic Carcinoma, Int Uro and Neph, V 27, 1995, pp 67-80.
12. Athans, M. et al., System, Networks, & Computation; Multivariable Methods, McGraw Hill (New York) 1974
13. Atkins, P. Physical Chemistry, Freeman (New York) 1990.
14. Augustin, H., et al, Prostate Cancers in the Transition Zone Part II, BJU Inter V 94 2004 pp 1226-1229.
15. Austin, R., et al, Role of Hyperhomocysteinemia in Endothelial Dysfunction, Cell Death and Diff 2004 pp 556-564.
16. Ayala, G., et al, High Levels of Phosphorylated Form of Akt-1 in Prostate Cancer and No Neoplastic Prostate Tissues are Strong Predictors of Biochemical Recurrence, Clin Can Res, V 10, 2004, pp 6572-6578.
17. Baici, A., *Enzyme Kinetics*, The Velocity of Reactions, Biochem Journal, 2006, pp. 1-3.
18. Baker, C.A.H., et al, GeneVis: Visualization Tools for Genetic Regulatory Network Dynamics, Univ Calgary.
19. Baker, C.A.H., et al, GeneVis: Visualization Tools for Genetic Regulatory Network Dynamics, Univ Calgary.
20. Baldo, P., et al, mTOR Pathway and mTOR inhibitors as Agents for Cancer Therapy, 2008, Curr Can Drug Targets, pp 647-668.
21. Barry, M., Screening for Prostate Cancer among Men 75 Years of Age and Older, NEJM, 2008, V 359, pp 2515-2516.
22. Barry, M., Screening for Prostate Cancer, NEJM, 2009 V 360 pp 1351-1354.
23. Bartel, D., MicroRNAs: Genomics, Biogenesis, Mechanism, and Function, Cell 2004 pp 281-297.
24. Begg, C., R. Greenes, Assessment of Diagnostic Tests when Disease Verification is Subject to Selection Bias, Biometrics 1983 V 39 pp 207-215.
25. Bellomo, N., et al, Selected Topics in Cancer Modelling, Birkhauser (Boston) 2008.

26. Berns, R. S., Principles of Color Technology, Wiley (New York) 2000.

27. Bolouri, H., Computational Modeling of Gene Regulatory Networks, Imp Coll Lon Press (London) 2008.

28. Born, M., E. Wolf, Principles of Optics, 4th Ed, Pergamum (New York) 1970.

29. Brooks, J., et al, CG Island Methylation Changes Near the GSTP1 Gene in Prostatic Intraepithelial Neoplasia, Can Epid 1998, pp 531-536.Donkena, K., et al, Oxidative Stress and DNA Methylation in Prostate Cancer, Ob & Gyn Internat 2010, pp .

30. Buhmeida, A., et al, Prognostic Factors in Prostate Cancer, Diag Path 2006 pp 1-15.

31. Bunz, F., Principles of Cancer Genetics, Springer(New York) 2008.

32. Calin, G., C. Croce, Chromosomal Rearrangements and Micro RNAs, Jrl Clin Inv 2007 pp 2059-2066.

33. Calin, G., C. Croce, MicroRNA Signatures in Human Cancers, Nat Rev 2006 pp 857-866.Calin, G., C. Croce, Chromosomal Rearrangements and microRNAs, Jrl Clin Inves 2007 pp 2059-2066.

34. Campbell, A., L. Heyer, **Genomics, Proteomics, and Bioinformatics**, Benjamin Cummings (New York) 2003.

35. Campbell, A., L. Heyer, Genomics, Proteomics, and Bioinformatics, Benjamin Cummings (New York) 2003.

36. Carrecedo, A., et al, PTEN Level in Tumor Suppression, How Much is Too Little?, *Cancer Res; 71(3); pp. 629–33. 2011.*

37. Carter, H., et al, Detection of Life Threatening Prostate Cancer With Prostate Specific Antigen Velocity During a Window of Curability, Jrl Nat Canc Inst, V 98, 2006, pp 1521-1527.

38. Carter, H., et al, Recommended Prostate Specific Antigen Testing Intervals, JAMA 1997, V 277, pp 1456-1460.

39. Catalona, W., et al, Prostate Cancer Detection in Men, JAMA, 1997 V 277, pp 1452-1455.

40. Causton, H. et al, **Microarray Gene Expression and Analysis**, Blackwell (Malden, MA) 2003.

41. Causton, H. et al, Microarray Gene Expression and Analysis, Blackwell (Malden, MA) 2003.

42. Chandran, U., et al Gene Expression Profiles of Prostate Cancer Reveal Involvement of Multiple Molecular Pathways in Metastatic Process, BMC Cancer, 2007 1-21.

43. Chen, H., et al, NKX3.1 Interacts with Prostate Derived ETS Factor and Regulates the Activity of PSA Promoter, Can Res, V 63, 2002, pp 330-340.

44. Chen, P., J. Chen, A Markovian Approach to the Control of Genetic Regulatory Networks, Bio Sys, 2007 PP 535-545.

45. Chen, T., et al, Modeling Gene Expression with Differential Equations, Pacific Symposium on Biocomputing, 1999 pp. 29-40.

46. Chen, T., et al, Modeling Gene Expression with Differential Equations, Pacific Symposium on Biocomputing, 1999 pp. 29-40.

47. Chow, L. et al, PTEN Function in Normal and Neoplastic Growth, Cancer Ltr, V 241, 2006, pp 184-196.

48. Cohen, R., et al, A Proposal on the Identification, Histologic Reporting, and Implications of Intraductal Prostatic Carcinoma, Arch Path Lab Med, V 131, 2007. pp 1103-1109.

49. Coppola, V. et al, MicroRNAs and Prostate Cancer, End Related Cancer V 17, 2010, pp 1-17.

50. Costa, F., et al, Concise Review: Cancer Testis Antigens, Stem Cells and Cancer, Stem Cells 2007 pp 707-711.

51. Crampin, E., et al, Extracting Biochemical Reaction Kinetics from Time Series Data, LNAI 3214 pp 329-336 2004 Springer Verlag.

52. Croce, C., Causes and Consequences of microRNA Dysregulation in Cancer, Nature 2009 pp 704-714.

53. Croce, C., Oncogenes and Cancer, NEJM, V 358, 2008, pp 502-511.

54. Cunningham, W., Nonlinear Analysis, McGraw Hill (New York) 1958.

55. Dagvodorj, A., et al, Transcription Factor Signal Transducer and Activator of Transcription 5, Clin Can Res, V 14, 2008, pp 1317-1324.

56. Dahia, P., PTEN, a Unique Tumor Suppressor Gene, Endo Rel Can, V 7 2000, pp 115-129.

57. Dahlman, A., et al, Effect of Androgen Deprivation Therapy on the Expression of Prostate Cancer Biomarkers, Pros Can and Prost Dis 2010 pp 1-7.

58. Dalerba, P., et al, Cancer Stem Cells: Models and Concepts, Stem Cells, 2008 pp 267-284.

59. D'Amico AV; Schnall M; Whittington R; Malkowicz SB; Schultz D; Tomaszewski JE; Wein A. Endorectal Coil Magnetic Resonance Imaging Identifies Locally Advanced Prostate Cancer In Select Patients With Clinically Localized Disease. *Urology* 1998 Mar;51(3):449-54.

60. D'Amico, A., et al, Preoperative PSA Velocity and the Risk of Death from Prostate Cancer after Radical Prostatectomy, NEJM, V 351, 2004, pp 125-135.

61. DeMarzo, A., et al, Inflammation in Prostate Carcinogenesis, Nature Rev Cancer 2007 pp 256-269.

62. DeMarzo, A., et al, Pathological and Molecular Aspects of Prostate Cancer, Lancet, V 361, 2003, pp 955-964.

63. Demichelis, F., et al, Distinct Genomic Aberrations Associated with ERG Rearranged Prostate Cancer, Gene, Chromo, Cancer, V 48, 1999, pp 366-380.

64. Demichelis, F., et al, TMPRSS2:ERG Gene Fusion Associated with Lethal Prostate Cancer in a Watchful Waiting Cohort, Onco 2007 pp. 1-4.

65. Deutsch, E., et al, Environmental. Genetic, and Molecular Features of Prostate Cancer, Lancet, 2004, pp 303-313.

66. DeVita, V., et al, Cancer, Lippincott (Philadelphia) 2008.

67. Dey, P. M., J. B. Harborne, Plant Biochemistry, Academic Press (New York) 1997.

68. di Bernardo, D., et al, Chemogeneomic Profiling on a Genome Wide Scale Using Reverse Engineered Gene Networks, Nature Biotech, V 23, 2005, pp 377-383.

69. Diamond, J., et al, The Use of Morphological Characteristics and Texture Analysis in the Identification of Tissue Composition in Prostatic Neoplasia, Human Path, V 35, 2004, pp 1121-1131.

70. Djebbari, A., J. Quackenbush, Seeded Bayesian Networks, BioMed Cent Sys Bio 2008 pp 1-13.

71. Dobi, A., et al, ERG Expression Levels in Prostate Tumors Reflect Functional Status of the Androgen Receptor, Open Cancer Jrl, V 3, 2010, pp 101-108.

72. Donkena K., et al, Oxidative Stress and DNA Methylation in Prostate Cancer, Obs & Gyn Int 2010, pp.

73. Dougherty, E., Epistemology and the Role of Mathematics in Translational Science, To Be Published, Texas A&M.

74. Dougherty, E., et al, Epistemology of Computational Biology, Jour Bio Sys, V 14, 2006, pp 65-90.

75. Dougherty, E., et al, Validation of Computational Methods in Genomics, Curr Gen, V 8, 2007, pp 1-19.

76. Dougherty, E., On The Epistemological Crisis in Genomics, Current Genomics, V 9, 2008, pp 69-79.

77. Dougherty, E., Translational Science: Epistemology and the Investigative Process, Curr Gen, V 10, 2009, pp 102-109.

78. Dressler, D., H. Potter, Discovering Enzymes, Scientific American Press (New York) 1991.

79. Driscoll, M., T. Gardner, Identification and Control of Gene Networks in Living Organisms, Jrl Proc Contrl 2006 pp 303-311.

80. Durrett, H., Color, Academic Press (New York) 1987.

81. Easton, J., P. Houghton, mTOR and Cancer Therapy, Oncogene, 2006, pp 6436-6446.

82. Ebersdobler, A., et al, Prostate Cancers in the Transition Zone Part I, BJU Inter V 94 2004 pp 1221-1225.

83. Epstein, J., G. Netto, Biopsy Interpretation of the Prostate, Lippincott (New York) 2008.

84. Ergun, A., et al, A Network Biology Approach to Prostate Cancer, Mole Sys Bio, V 3, 2007, pp 1-6.

85. Ernst, T., et al, Decrease and Gain in Gene Expression are Equally Discriminatory Markers for Prostate Cancer, Am Jrl Path, V 160, 2002, pp 2169-2180.

86. Esgueva, R., et al, Prevalanece of TMPRSS2-ERG and SLC45A3-ERG Gene Fusions in a Large Prostatectomy Cohort, Mod Path, V 2010, pp 1-8.

87. Essaghir, A., et al, The Transcription of FOXO Genes is Stimulated by FOXO3 and Repressed by Growth Factors, Jrl Bio Chem 2009, pp 10334-10342.

88. Esserman, L., et al, Rethinking Screening for Breast Cancer and Prostate Cancer, JAMA 2009 V 302 pp 1685-1692.

89. Esteller, M., Epigenetics in Cancer, NEJM, 2008 V 358 pp 1148-1159.

90. Feero, W. G., et al, Genomic Medicine – An Updated Primer, NEJM, 2010, pp 2001-2011.

91. Feldman, B., D., Feldman, The Development of Androgen Independent Prostate Cancer, Nature Reviews Cancer, Vol 1, 2001, pp 34-45.

92. Figueiredo, M., et al, Advances in Preclinical Investigation of Prostate Cancer Gene Therapy, Am Soc Gene Therapy 2007 V 15 pp 1053-1064.

93. Finn, O., Cancer Immunology, NEJM V 358, 2008 pp 2704-2715.

94. Foster, C., D. Bostwick, Pathology of the Prostate, Saunders (Philadelphia) 1998.

95. Freytag, S., Prostate Cancer Gene Therapy Clinical Trials, Am Soc Gene Therapy V 15 2007 pp 1042-1052.

96. Frohling, S., Chromosomal Abnormalities in Cancer, NEJM 2008, pp 722-734.

97. Fukuhara, H., et al, Maximum Tumor Diameter, Pros Canc and Prost Dis 2010 V 13 pp 244-247.

98. Gardenes, J., et al, On the robustness of complex heterogeneous gene expression networks, Biophys Chem, 118 2005 pp. 225-228.

99. Gardenes, J., et al, On the robustness of complex heterogeneous gene expression networks, Biophys Chem, 118 2005 pp. 225-228.

100. Garnick MB. Prostate Cancer Screening: Part I: The Screening Dilemma, Screening Tests and the Role of Prostate Specific Antigen Testing. Primary Care Reports. 2006; 12:1-12

101. Garnick MB. Prostate Cancer Screening: Part II: Treatment and Outcomes. Primary Care Reports. 2006; 12: 13-28

102. Gershenson, C., Classification of Random Boolean Networks, in Artificial Life, MIT Press 2002., pp 1-8.

103. Goentoro, L., et al, The Incoherent Feedforward Loop Can Provide Fold Change Detection in Gene Regulation, Mol Cell 2009 pp 894-899.

104. Goldstein, A. et al, Identification of a Cell of Origen for Human Prostate Cancer, Science, 2010 V 329, pp 568-571.

105. Gonzalgo, M., et al, Prostate Cancer Detection by GSTP1 Methylation Analysis of Post biopsy Urine Specimen, Clin Can Res, 2003, pp 2673-2677.

106. Gottwein, E., B. Cullen, Viral and Cellular MicroRNAs as Determinants of Viral Pathogenesis and Immunity, Cell Host 2008, pp 375-387.

107. Gregg, J., EGF Mediated Regulation of EGR1 In Prostate Cancer Cells, PhD Thesis Kent State, Dec 2010.

108. Grochow, J., M.. Kellis, Network Motif Discovery Using Subgraph Enumeration and Symmetry Breaking, Speed and Huang Ed, RECOMB Springer 2007 pp 92-106.

109. Grunkemeier, M, R. Vollmer, Predicting Prostate Biopsies, Ana Path 2006 V 126 pp 110-112.

110. Gurel, B., et al, Molecular Alterations in Prostate Cancer as Diagnostic Prognostic, and Theraputic Targets, Adv Anal Path, V 15, 2008, pp 319-331.

111. Harmans, K., Unravelling of the Major Genetic Defects in Prostate Cancer, Doctoral Thesis, Erasmus Univ, Rotterdam, 2009.

112. Hatzimanikatis, V., Dynamical Analysis of Gene Networks Requires Both mRNA and Protein Expression Information, Metabolic Engr, Vol 1, 1999, pp. 275-281.

113. Hatzimanikatis, V., *Dynamical Analysis of Gene Networks Requires Both mRNA and Protein Expression Information*, Metabolic Engr, Vol 1, 1999, pp. 275-281.

114. Hatzimanikatis, V., Dynamical Analysis of Gene Networks Requires Both mRNA and Protein Expression Information, Metabolic Engr, Vol 1, 1999, pp. 275-281.

115. Hay, N., The Akt-mTOR tango and its relevance to Cancer, Cancer Cell, V 8, 2005, pp 179-183.

116. He, L., G. Hannon, MicroRNAs: Small RNAs with a Big Role in Gene Regulation, Nature, 2004 pp 522-532.

117. Hendig, D., et al, SPP1 Promoter Polymorphism, Mol Diag and Gen Clin Chem 2007 pp 829-835.

118. Herman, J., S. Baylin, Gene Silencing in Cancer in Association with Promoter Hypermethylation, NEJM 2003, pp 2042-2034.

119. Ho, J., et al, Comparative Effectiveness of Minimally Invasive vs Open Radical Prostatectomy, JAMA 2009 V 302 pp 1557-1564.

120. Holmes, S., Graphs and Networks in Bioinformatics, Stanford Univ Notes.

121. Houston, P., Chemical Kinetics and Reaction Dynamics, Dover (Mineola) 2001.

122. Hsu, P., D., Sabatini, Cancer Cell Metabolism, Warburg and Beyond, Cell 2008 pp 703-707.

123. Huang, C., et al, A Study of Cancer Related MicroRNAs through Expression Data and Literature Search, World Acad Sci & Engr, 2009 254-256.

124. Huang, H., PTEN Induces Chemosensitivity in PTEN-mutated Prostate Cancer, Jrl Bio Chem, V 276, 2001, pp 38830-38836.

125. Huang, S., et al, Blockade of NF-kB Activity in Human Prostate Cancer Cells is Associated with Suppression of Angiogenesis, Invasion, and Metastasis, Oncogene, V 20, 2001, pp 4118-4197.

126. Hurt, E., et al, CD44+CD44- Prostate Cells are Early Cancer Progenitor Stem Cells that Provide a Model for Patients with Poor Prognosis, Brit Jrl Can 2008 pp 756-765.

127. Iljin, K., et al, TMPRSS2 Fusions with Oncogenes ETS Factors in Prostate Cancer, Cancer Res, V 66, 2006, pp 10242-10246.

128. Imoto, S., et al, Combining Microarrays and Biological Knowledge for Estimating Gene Networks via Bayesian Networks, IEEE Com Sys Bio 2000 pp .

129. Ivanov, I., E. Dougherty, Reduction Mappings Between Probabilistic Boolean Networks, Jrl App Sig Pro 2004 pp 125-131.

130. Iwata, T., et al, Myc Overexpression Induces Prostatic Intraepithelial Neoplasia and Loss of Nkx3.1 in Mouse Luminal Epithelial Cells, Open Access, PLOSon,V 5, 2010, pp e9427

131. Jaakola, L. et al, Expression of Genes Involved in Anthocyanin Biosynthesis, Plant Physiology, Vol 130 Oct 2002, pp 729-739.

132. Jacobsen, S., Gene Silencing: Maintaining Methylation Patterns, Curr Bio 1999, pp 617-619.

133. Jansen, R., et al, A Bayesian Networks Approach for Predicting Protein-Protein Interactions from Genomic Data, Science, 2003 pp 449-453.

134. Jaqmaspishvili, T., et al, Urine Markers in Monitoring Prostate Cancers, Pros Canc and Prost Dis, V 13, 2010, pp 12-19.

135. Jeet, V., et al, Modelling Prostate Cancer, Canc Met Rev, V 29, 2010, pp 123-142.

136. Jelovac, D., B. Park, *PTEN* Promoter Silencing and Cowden Syndrome, JAMA, 2010, pp 2744-2745.

137. Jenkins, F. A., H. E. White, Fundamentals of Optics, McGraw Hill (New York) 1957.

138. Jeong, J., et al, BRAF Activation Initiates but does not Maintain Invasive Prostate Adenocarcinoma, Plus One, 2008 pp 3949-.

139. Jones, J., Prostate Biopsy, Humana (Totowa, NJ) 2008.

140. Jones, P., S. Baylin, The Epigenomics of Cancer, Cell, 2007 pp 683-692.

141. Jordan, C., et al, Cancer Stem Cells, NEJM 2006, pp 1253-1262.

142. Judd, D. B. et al, Color 2ND Edition, Wiley (New York) 1963.

143. Kanamori, Y., et al, Correlation Between Loss of PTEN Expression and Akt Phosphorylation in Endometrial Carcinoma, Clin Can Res, V 7, 2001, pp 892-895.

144. Kang T et al, Functional heterogeneity of prostatic intraepithelial neoplasia: the duration of hormonal therapy influences the response, BJU Int 2007 May;99(5):1024-7 Epub 2007 Jan 22.

145. Kang, T et al, Functional heterogeneity of prostatic intra-epithelial neoplasia: Length of hormonal therapy influences response, Journal of Clinical Oncology, 2006 ASCO Annual Meeting Proceedings (Post-Meeting Edition).Vol 24, No 18S (June 20 Supplement), 2006: 4648

146. Kantoff, P. et al, Prostate Cancer, Lippincott (Philadelphia) 2002.

147. Kilkov, V., Identifying Gene Regulatory Networks from Gene Expression Data, .

148. Kim, H., X. Yang, Prevalence of High Grade Prostatic Intraepithelial Neoplasia and its Relationship to Serum Prostate Specific Antigen, Clin Uro, V 28, 2002, pp 413-417.

149. Kim, S., et al Can Markov Chain Models Mimic Biological Regulation, Jrl Bio Sys 2002, pp 337-357.

150. Kim, S., et al, Inferring Gene Networks from Time Series Microarray Data Using Dynamic Bayesian Networks, Brief on Bioinfo 2003, pp 228-235.

151. King, J., Cooperativity of TMPRSS2-ERG with PI3 kinase pathway Activation in Prostate Oncogenesis, Nature Gen, V 41, 2009, pp 524-526.

152. Kinkaide, C., et al, Targeting Akt/mTOR and ERK MAPK Signalling Inhibits Hormone Refractory Prostate Cancer in Preclinical Mouse Model, Jrl Clin Inv 2008, 99 3051-3064.

153. Klemm, K., S. Bornholdt, Topology of Biological Networks and Reliability of Information Processing, PNAS 2005 pp 18414-18419.

154. Klipp, E., et al, Systems Biology, Wiley (Weinheim, Germany) 2009.

155. Kohane, I., et al, **Microarrays for an Integrative Genomics**, MIT Press (Cambridge) 2003.

156. Kohane, I., et al, Microarrays for an Integrative Genomics, MIT Press (Cambridge) 2003.

157. Kokontis, J., et al, Increased Androgen Receptor Activity and Altered c-myc Expression in Prostate Cancer Cells after Long Term Androgen Deprivation, J Can Res, V 54, 1994, pp 1566-1573.

158. Krane, D., M. Raymer, Bioinformatics, Benjamin Cummings (New York) 2003.

159. Krause, D., R. VanEtten, Tyrosine Kinases as Targets for Cancer Therapy, NEJM 2005 pp 172-187.

160. Lam, E., et al, FOXO Transcription Factors, Biochem Soc Trans 2006, pp 722-726.

161. Lawson, D., et al, Isolation and Functional Characterization of Murine Prostate Stem Cells, PNAS, 2007, V 104 pp 181-186.

162. Lawson, D., O. Witte, Stem Cells in Prostate Cancer Initiation and Progression, Jrl Clin Inv, 2007 pp 2044-2050.

163. Lee, A., GRP78 Induction in Cancer, Can Res 2007 pp 3946-3499.

164. Leite, K., et al, Repeat Prostate Biopsies Following Diagnoses of Prostate Intraepithelial Neoplasia and Atypical Small Gland Proliferation, Inter Brazil Jrl Uro, V 31, 2005, pp 131-136.

165. Lepor, H., R. Lawson, Prostate Diseases, Saunders (Philadelphia) 1993.

166. Lesk, A., **Bioinformatics**, Oxford (New York) 2002.

167. Lesk, A., Bioinformatics, Oxford (New York) 2002.

168. Levi, L., Applied Optics, Wiley (New York) 1968.

169. Levine, A., A. Puzio-Kuter, The Control of the Metabolic Switch in Cancers by Oncogenes and Tumor Suppressor Genes, Science, 2010, pp 1340-1344.

170. Li, G., et al, Selective Anticancer Strategies via Intervention of the Death Pathways Relevant to Cell Transformation, Cell Death and Diff 2008 pp 1197-1210.

171. Li, J., et al, PTEN, a Putative Protein Tyrosine Phosphatase Gene Mutated in Human Brain, Breast, and Prostate Cancer, Science, V 275, 1997, pp 1943-1947.

172. Li, X., et al, NKX3.1 is Regulated by Protein Kinase CK2 in Prostate Tumor Cells, Mol Cell Bio, V 26, 2006, pp 3008-3017.

173. Lin, H., et al, Regulation of Androgen Receptor Signalling by PTEN, Mol Endo, V 18, 2004, pp 2409-2425.

174. Liu, A., et al, Correlated Alterations in Prostate Basal Cell Lauer and Basement Membrane, Int Jrl Bio Sci, V 5, 2009, pp 276-285.

175. Ljung, L. System Identification, Prentice Hall (Englewood Cliffs) 1987.

176. Lobo, N., et al, The Biology of Cancer Stem Cells, Ann Rev Cell Dev 2007 pp 675-699.

177. LoPiccolo, J., et al, Targeting the PI3K/Akt/mTOR Pathway, Drug Res Up, 2008, pp 32-50.

178. Luo, S., et al, Introduction of Grp78/BiP by Translational Block, Jrl Bio Chem, 2003 pp 37375-37385.

179. Lu-Yao, G., et al, Survival Following Primary Androgen Deprivation Therapy Among Men With Localized Prostate Cancer, JAMA, V 300 2008 pp 173-181.

180. Maira, S., et al, PI3K Inhibitors for Cancer Treatment, Biochem Soc Trans, V 37, 2009, pp 265-272.

181. Majumder, P., et al, A prostatic intraepithelial neoplasia-dependent p27 Kip1 checkpoint induces senescence and inhibits cell proliferation and cancer progression. Cancer 2008 146-155.

182. Majumder, P., et al, mTOR inhibition reverses Akt-dependent Prostate Intraepithelial Neoplasia Through Regulation of Apoptotic and HIF-1-denendent Pathways, Nature Med 2004 pp 594-501.

183. Marcucci, G., et al, High Expression Levels of the ETS Related Gene ERG Predict Adverse Outcome, Jrl Clin Onc, V 25, 2007, pp 3337-3343.

184. McGarty, T Gene Expression in Plants: Use of System Identification for Control of Color, submitted to IEEE JSAC for 2008.

185. McGarty, T. Achieving the "Blue Daylily": Genetic Capabilities for Color in the Genus Hemerocallis, Draft, MIT, August 2010

186. McGarty, T. Backcrossing: A Mathematical Analysis of Gene Insertion in Existing Hybrids and a Statistical Validation, Draft, MIT August 2008

187. McGarty, T. P, The Structure of the Upper Atmosphere, IEEE Automatic Control 1971.

188. McGarty, T. P., Bayesian Outlier Rejection and State Estimation, IEEE AC 1975, pp. 682-687

189. McGarty, T. PSA Evaluation Methodologies: A Look at Multiple Alternatives and Maximum Likelihood Techniques, Draft, MIT, December 2010.

190. McGarty, T. The PSA Controversy: Details, Models, Analysis and Recommendations, Draft, MIT, December 2010.

191. McGarty, T., Flower Color and Patterning in the Genus Hemerocallis and its Hybrids: A Mathematical Model and Experimental Analysis , MIT, Draft, August 2009.

192. McGarty, T., Stochastic Systems and State Estimation, Wiley (New York) 1974.

193. McMenamin, M., et al, Loss of PTEN Expression in Paraffin-embedded Primary Prostate Cancer, Can Res, V 59, 1999, pp 4291-4296.

194. McMurry, J., Begley, T., The **Organic Chemistry of Biological Pathways**, Roberts & Company Publishers, 2005.

195. McMurry, J., Begley, T., The Organic Chemistry of Biological Pathways, Roberts & Company Publishers, 2005.

196. McNeil, B., J., Hanley, Statistical Approaches to the Analysis of Receiver Operating Curves, Med Dec Making V 4 1984 pp137-149.

197. Mello, C., Return of the RNAi World: Rethinking Gene Expression and Evolution, Nobel Laureate Lecture 2006.

198. Milo, R., et al, Network Motifs: Simple Building Blocks of Complex Networks, Science, 2002, pp 824-827.

199. Miranda, T., P. Jones. DNA Methylation: The Nuts and Bolts of Repression, Cll Phys 2007 pp 384-390.

200. Misra, U., Ligation of Prostate Cancer Cell Surface GRP78 Activates a Proproliferative and Antiapoptotic Feedback Loop: A Role for Secreted Prostate-Specific Antigen, Jrl Bio Chem 2011, pp 1248-1259.

201. Mjolsness, E., et al Gene Regulation Networks with Signaling and Cell Division in Development Satiations, IEEE Biomed Engr Houston TX Oct 2002.

202. Mjolsness, E., et al Gene Regulation Networks with Signaling and Cell Division in Development Satiations, IEEE Biomed Engr Houston TX Oct 2002.

203. Mooi, W., D. Peeper, Oncogene Induced Cell Senescence, NEJM, 2006 pp 1037-1045.

204. Mora, L., et al, Prostate Adenocarcinoma: Cellular and Molecular Abnormalities, Can Contrl, V 8, 2001, pp 551-561.

205. Morgan, D., The Cell Cycle, Sinauer (London) 2007.

206. Mosquera, J., et al, Characterization of TMPRSS2-ERG Fusion in High Grade Prostatic Intraepithelial Neoplasia and Potential Clinical Implications, Clin Can Res, V 14, 2008, pp 3380-3385.

207. Mosquera, J., et al, Morphological Features of TMPRSS2-ERG Gene Fusion Prostate Cancer, Jrl Path 2007 pp 91-101.

208. Mosquera, J., et al, Prevalence of TMPRSS2-ERG Fusion Prostate Cancer among Men Undergoing Prostate Biopsy in the United States, Clin Can Res, V 15, 2009, 4706-4711.

209. Murphy, K., S. Mian, Modelling Gene Expression Data Using Dynamic Bayesian Networks, .

210. Murray, J., **Mathematical Biology**, Springer (New York) 1989.

211. Murray, J., Mathematical Biology, Springer (New York) 1989.

212. Murrell, J., Understanding Rate of Chemical Reactions, University of Sussex.

213. Murrell, J., *Understanding Rate of Chemical Reactions*, University of Sussex.

214. Murrell, J., Understanding Rate of Chemical Reactions, University of Sussex.

215. Nantermet, P., et al, Identification of Genetic Pathways Activated in Androgen Receptor during the Induction of Proliferation in the Ventral Prostate Gland, Jrl Bio Chem, V 279, 2004. pp 1310-1322.

216. Narayanan, B., et al, Regression of Mouse Prostatic Intraepithelial Neoplasia by Nonsteroidal Anti Inflammatory Drugs, Clin Can Res 2004 pp 7727-7737.

217. Nelson, E., et al, Inhibition of Akt Pathways in the Treatment of Prostate Cancer, 2007, Pros Can and Pros Dis, pp 331-339.

218. Nelson, W. G., et al, Prostate Cancer, NEJM, V 349 N 4 July 2003, pp 366-381.

219. Nelson, W., et al, The Role of Inflammation in the Pathogenesis of Prostate Cancer, Jrl Uro 2004 pp 1-7.

220. Neuwirt, H., at al, Suppressor of Cytokine Signaling (SOCS)-1 Is Expressed in Human Prostate Cancer, Am Jrl Path, 2009, pp 1921-1930.

221. Niu, Y., et al, Androgen Receptor is a Tumor Suppressor and Proliferator in Prostate Cancer, PNAS 2008 pp 12182-12187.

222. Nusse, R., Relays at the Membrane, Nature, 2005, V 438, pp 747-748.

223. Odom, D., et al, Core Transcriptional Regulatory Circuits in Human Hepatocytes, Mol Sys Bio, 2006, pp 1744-1754.

224. Oesterling JE; Martin SK; Bergstralh EJ; Lowe FC. The use of prostate-specific antigen in staging patients with newly diagnosed prostate cancer. *JAMA* 1993 Jan 6;269(1):57-60.

225. O'Shaughnessy, J., et al, Treatment and Prevention of Intraepithelial Neoplasia, Clin Cancer Res, V 8, 2002, pp 314-346.

226. Palapattu, G., et al, The Case for the Role of Chronic Inflammation in the Development of Prostate Cancer, US Onc Rev, 2006, pp 112-113.

227. Pali, S., K. Robertson, Epigenetic Control of Tumor Suppression, Clin Rev in Euk Gene Exp 2007, pp 295-316.

228. Papatsoris, A., et al, The Power and Promise of "Rewiring" the Mitogen Activated Protein Kinase Network in Prostate Cancer Theraputics, Mol Can Ther, V 6, 2007, pp 811-819.

229. Partin, A., et al, Combination of Prostate Specific Antigen, Clinical Stage and Gleason Score, JAMA, 1997 V 277 pp 1445-1451.

230. Payne, S., C. Kemp, Tumor Suppressor Genetics, Carcinogenesis, V 26, 2005, pp 2031-2045.

231. Pecorino, L., Molecular Biology of Cancer, Oxford (New York) 2008.

232. Peng, C., et al, An Introduction to Logistic Regression Analysis and Reporting, Jrl Ed Res, V 96 2002 pp 3-9.

233. Percus, J., Mathematics of Genome Analysis, Cambridge (New York) 2004.

234. Perkins, T., et al, Inferring Models of Gene Expression Dynamics, Journal of Theoretical Biology, Vol 230, 2004, pp. 289-299.

235. Perkins, T., et al, *Inferring Models of Gene Expression Dynamics*, Journal of Theoretical Biology, Vol 230, 2004, pp. 289-299.

236. Perkins, T., et al, Inferring Models of Gene Expression Dynamics, Journal of Theoretical Biology, Vol 230, 2004, pp. 289-299.

237. Pestell, R., M. Nevalainen, Prostate Cancer, Humana (Totowa, NJ) 2008.

238. Petrocca, F., et al, Emerging Role of miR-106b/miR-17-92 Clusters in the Control of Transforming Growth Factor β Signalling, Can Res 2008, pp 8191-8194.

239. Petrocca, F., et al, Emerging Role of miR-106b-25/miR-17-92 Clusters in the Control of Transforming Growth Factor β Signalling, Can Res 2008, pp 8191-8194.

240. Phe, V., et al, Methylated Genes as Potential Biomarkers in Prostate Cancer, BJUI, 2010 pp 1364-1370.

241. Planet, P. et al, Systematic Analysis of DNA Microarray Data: Ordering and Interpreting Patterns of Gene Expression, Cold Spring Harbor, Genome Research, 2001 pp 1149-1155.

242. Poliseno, L., et al, Identification of the miR-106b MicroRNA Cluster as a Proto Oncogenic PTEN Targeting Intron That Cooperates with the Host Gene MCM7 in Transformation, Sci Sig 2010, pp 29- .

243. Poliseno, L., et al, Identification of the miR-106b-25 MicroRNA Cluster as a Proto-oncogenic PTEN-targeting Intron That Cooperates with Host Gene MCM7 in Transformation, Sci Sig 2010 .

244. Pollack A, Zagars GK, et al. Preliminary results of a randomized radiotherapy dose-escalation study comparing 70 Gy with 78 Gy for prostate cancer. *J Clin Oncol*. 2000 Dec 1;18(23):3904-11.

245. Pootrakul, L., et al, Expression of Stress Response Protein Grp78 is Associated with the Development of Castration Resistant Prostate Cancer. Hum Can Bio 2006 pp 5987-5993.

246. Porkka, K., Differentially Expressed Genes in Prostate Cancer, Doctoral Dissertation, Univ Tampere, Finland, 2003.

247. Punglia, R., et al, Effect of Verification Bias on Screening for Prostate Cancer, NEJM, 2003 V 349, pp 335-342.

248. Putzi, M., A. De Marzo, Morphologic Transitions Between Proliferative Inflammatory Atrophy and High Grade Prostatic Intraepithelial Neoplasia, Adult Uro 2000 pp 828-832.

249. Radu, A., et al, PTEN Induces Cell Cycle Arrest by Decreasing the Level and Nuclear Localization of Cyclin D1, Mol and Cell Bio, V 23, 203, pp 6139-6149.

250. Rakhlin, O. et al, MicroRNA-34 Mediates AR-dependent p53-indiced Apoptosis in Prostate Cancer, Can Bio & Therapy 2008 pp 1288-1296.

251. Ramoutar, R., J. Brumaghim, Antioxidant and Anticancer Properties and Mechanisms of Inorganic Selenium, Oxo Sulfur, and Oxo Selenium Compounds, Cell Biochem 2010, pp 1-23.

252. Reya, T., et al, Stem Cells, Cancer, and Cancer Stem Cells, Nature, 2001 pp 105-111.

253. Risbridger, G., R. Taylor, Minireview: Regulation of Prostatic Stem Cells by Stromal Niche in Health and Disease, Endo 2008 pp 4303-4306.

254. Robertson, K., A. Wolffe, DNA Methylation in Health and Disease, Nat Rev Gen 2000 pp 11-19.

255. Robertson, K., DNA Methylation, methyltransferase and cancer, Oncogene 2001 pp 3139-3155.

256. Rosen, J., C. Jordan, The Increasing Complexity of the Cancer Stem Cell Paradigm, Science, 2009, pp 1670-1672.

257. Rosenberg, S., Gene Therapy for Cancer, JAMA 1992 pp 2416-2419.

258. Rosenberg, S., The Transformed Cell, Putnam (New York) 1992.

259. Rossi, B., Optics, Addison Wesley (Reading, MA) 1957.

260. Roy, S., et al, Inhibition of PI3K/AKT and MAPK/ERK pathways causes Activation of FOXO Transcription Factor, Jrl Mol Sig 2010, pp 1-13.

261. Rubin, M., A. Chinnaiyan, Bioinformatics Approach Leads to the Discovery of the TMPRSS2:ETS gene Fusion in Prostate Cancer, Lab Inv 2006, pp. 1099-1102.

262. Rubin, M., et al, Overexpression Amplification and Androgen Regulation of TPD52 in Prostate Cancer, Can Res 2004 pp 3814-3822.

263. Rubinow, S., Mathematical Biology, Dover (New York) 2002.

264. Sachs, K., et al, Causal Protein Signalling Networks Derived from Multiparamemeter Single Cell Data, Science, 2005, pp 523-529.

265. Sage A. P., J. Melsa, System Identification, Academic Press (New York) 1971.

266. Saitou, N., M. Nei, *The Neighbor Joining Method: A New Method for Reconstructing Phylogenetic Trees*, Molecular Biological Evolution, Vol 4 1987 pp 406-425.

267. Samaratunga, H., J. Epstein, What is the Molecular Pathology of Low Risk Prostate Cancer, World J Uro, V 26, 2008, pp 431-436.

268. Sanda, M., I. Kaplan, A 64 Year Old Man with Low Risk Prostate Cancer, JAMA, V 301, 2009, pp 2141-2151.

269. Savli, H., et al, Gene Network and Canonical Pathway Analysis in Prostate Cancer, Exp Mol Med, V 40, 2008, pp 176-185.

270. Scherl, A., et al, Prostatic Intraepithelial Neoplasia and Intestinal Metaplasia in Prostates of Probasin-RAS Transgenic Mice, The Prostate 2004 pp 448-459

271. Schnell. S, T. Turner, Reaction Kinetics in Intracellular Environments with Macromolecular Crowding, Biophys and Molec Bio vol 85 2004 pp. 235-260.

272. Schroder, F., et al, Screening and Prostate Cancer Mortality in a Randomized European Study, NEJM, 2009 V 360 pp 1320-1328.

273. Schroder, F., Progress in Understanding Androgen Independent Prostate Cancer, Euro Uro, V 53, 2008, pp 1129-1137.

274. Schroder, F., R., Kranse, Verification Bias and the Prostate Specific Antigen Test, NEJM, 2003, V 349, pp 393-395.

275. Seoane, J., et al, Integration of Smad and Forkhead Pathways in the Control of Neuroepithelial and Glioblastoma Cell Proliferation, Cell 2004, pp. 211-223.

276. Sears, F. W., Optics, Addison Wesley (Reading, MA) 1949.

277. Segel, L., M. Slemrod, The Quasi Steady State Assumption: A Case Study in Perturbation, SIAM, 1989, pp 446-477.

278. Sevigani, C., et al, Mammalian microRNAs: A Small World for Fine-tuning Gene Expression, Mam Gene V 17 2006, pp 189-201.

279. Sevli, S., et al, The Function of microRNAs, small but potent molecules, in Human Prostate Cancer, Pros Can and Pros Dis, V 13, 2010, pp 208-217.

280. Sharifi, N., et al, Androgen Deprivation Therapy for Prostate Cancer, JAMA, V 294, 2005, pp 238-244.

281. Shen, M., C. Abate-Shen, Roles of the Nkx3.1 Homeobox Gene in Prostate Organogenesis and Carcinogenesis, Dev Dynam, V 228, 2003, pp 767-778.

282. Shen-Orr, S., et al, Network Motifs in the Transcriptional Regulation Network of E Coli, Nat Gen, 2002, pp 64-68.

283. Shmulevich, I, et al, Gene Perturbation and Intervention in Probabilistic Boolean Networks, Bioinfo 2002 pp 1319-1331.

284. Shmulevich, I. et al, Control of Stationary Behavior in Probabilistic Boolean Networks, J Bio Sci 2002, pp 431-445.

285. Shmulevich, I., E., Dougherty, Genomic Signal Processing, Princeton (Princeton) 2007.

286. Shmulevich, I., et al, From Boolean to Probabilistic Networks as Models of Genetic Regulatory Networks, Proc IEEE 2002 pp 1778-1792.

287. Shyam, E. et al, The ERG Gene: A Human Gene Related to the ETS Oncogene, PNAS 1987, pp 6131-6135.

288. Simon, W., Mathematical Techniques for Biology and Medicine, Dover (New York) 1986.

289. Slingerland, J., M. Pagano, Regulation of Cdk Inhibitor p27 and Its Deregulation in Cancer, Jrl Cell Phys 2000 pp 10-17.

290. Smith MR, Finkelstein JS, et al. Changes in body composition during androgen deprivation therapy for prostate cancer. *J Clin Endocrinol Metab*. 2002 Feb;87(2):599-603.

291. Spirtes, P., et al, Constructing Bayesian Network Models of Gene Expression Networks from Microarray Data, .

292. Stein, G., et al, The Molecular Basis of Cell Cycle and Growth Control, Wiley (New York) 1999.

293. Strathdee, G., R., Brown, Aberrant DNA Methylation in Cancer; Potential Clinical Interventions, Exp Rev Mol Med, 2002, pp 1-17.

294. Strong, J., Concepts of Classical Optics, Freeman (San Francisco) 1958.

295. Studier, J., K. Kappler, *A Note on the Neighbor Joining Algorithm*, Molecular Biological Evolution, Vol 5 1988 pp 729-731.

296. Styerberg, E., et al, Prediction of Indolent Prostate Cancer, Jrl Uro, 2007, V 177, pp 107-112.

297. Su, L., Early Diagnosis and Treatment of Cancer: Prostate Cancer, Saunders(New York) 2010.

298. Swanson, K, et al, Prostate Specific Antigen, Am Jrl Clin Path, 2006, V 125 pp 331-333.

299. Szallasi, Z. **System Modeling in Cellular Biology: From Concepts to Nuts and Bolts**. MIT Press (Cambridge) 2006.

300. Szallasi, Z. System Modeling in Cellular Biology: From Concepts to Nuts and Bolts. MIT Press (Cambridge) 2006.

301. Szallasi, Z., et al, System Modelling of Cellular Biology, MIT Press (Cambridge) 2006.

302. Taby, R., J-P Issa, Cancer Epigenetics, CA 2010 pp376-392.

303. Taichman, R., et al, The Evolving Biology and Treatment of Prostate Cancer, Jrl Clin Invest 2007, pp 2351-2360.

304. Tang, D., et al, Prostate Cancer Stem/Progenitor Cells, Mol Car 2007 pp. 1-14.

305. Taylor, B., Integrative Genomic Profiling of Human Prostate Cancer, Cancer Cell, V 18, 2010, pp 1-12.

306. Taylor, W., et al, p27 Deficiency Promotes Prostate Carcinogenesis But Does Not Affect the Efficacy of Retinoids in Suppressing the Neoplastic Process, BMC Cancer, 2010 10:541.

307. Thompson, I. et al, Operating Characteristics of Prostate Specific Antigen, JAMA 2005 V 294 pp 66-70.

308. Thompson, I., et al, Assessing Prostate Cancer Risk, Jrl Nat Cancer Inst V 98 2006 pp 529-534.

309. Tinoco, I. et al, Physical Chemistry, Prentice Hall (Englewood Cliffs, NJ) 1995.

310. Tobias, A., Directed Evolution of Biosynthetic Pathways to Carotenoids with Unnatural Carbon Bonds, PhD Thesis, Cal Tech, 2006.

311. Tomlins, A., ETS Rearrangements and Prostate Cancer Initiation, Nature, V 448, 2007, pp 595-599.

312. Tomlins, S., et al, ETS Gene Fusion in Prostate Cancer, Eur Jrl Uro 2009 pp 1-12.

313. Tomlins, S., et al, Recurrent Fusion of TMPRSS2 and ETS Transcription Factor Genes in Prostate Cancer, Science, V 310, 2005, pp 644-648.

314. Turing, A., The Chemical Basis of Morphogenesis, Phil Trans Royal Soc London B337 pp 37-72, 19459.

315. Turner, D., The Role of ETS Transcriptional Regulation in Hormone Sensitive and Refractory Prostate Cancer, Open Can Jrl, 2010, V 3, pp 40-48.

316. Tuxhorn, J., et al, Reactive Stroma in Human Prostate Cancer, Clin Cancer Res V 8 2002 pp 2912-2923.

317. van der Heide, L., et al, The Ins and Outs of FOXO Shuttling, Biochem Jrl 2004, pp 297-309.

318. Van Trees, H. L., Detection, Estimation and Modulation Theory, Wiley (New York) 1968.

319. Vander Heiden, M., et al, Understanding the Warburg Effect, Science 2009 pp 1029-1033.

320. Vazquez, F., et al, The PTEN Tumor Suppressor Protein, Biochimica et Biophysica, 2000 pp 21-25.

321. Vicini FA, Abner A, Baglan KL, Kestin LL, Martinez AA. Defining a dose-response relationship with radiotherapy for prostate cancer: is more really better? *Int J Radiat Oncol Biol Phys*. 2001 Dec 1;51(5):1200-8.

322. Vis, A., F. Schroder, Key Targets of hormonal treatment of prostate cancer; Part 2 the androgen receptor and 5α reductase, BJUI 2009 pp 1191-1197.

323. Vliestra, R., et al, Frequent Inactivation of PTEN in Prostate Cancer Cell Lines and Xenografts, Can Res, V 58, 1998, pp 2720-2723.

324. Vogelstein, B., K., Kinzler, Cancer Genes and the Pathways They Control, Nature Med, V 10, 2004, pp 789-799.

325. Vohradsky, J., Neural Network Model of Gene Expression, FASEB Journal, Vol 15, March 2001, pp. 846-854.

326. Vohradsky, J., *Neural Network Model of Gene Expression*, FASEB Journal, Vol 15, March 2001, pp. 846-854.

327. Vohradsky, J., Neural Network Model of Gene Expression, FASEB Journal, Vol 15, March 2001, pp. 846-854.

328. Vollmer, R., Predictive Probability of Serum Prostate Specific Antigen for Prostate Cancer, Am Jrl Clin Path, 2006 pp 336-342.

329. Vollmer, R., Predictive Probability of Serum Prostate Specific Antigen for Prostate Cancer, Am Jrl Clin Path 2006 V 125, pp 336-342.

330. von Knobloch, R., et al, Genetic Pathways and New Progression Markers for Prostate Cancer Suggested by Microsatellite Allelotyping, Clin Can Res, V 10, 2004, pp 1064-1073.

331. Walsh, P., et al, Localized Prostate Cancer, NEJM, V 357, 2007, pp 2696-2705.

332. Wang, E., Cancer Systems Biology, CRC (New York) 2010.

333. Wang, X, et al, Notch-1 Expressing Cells are Indispensible for Prostatic Branching Morphogenesis, Jrl Bio Chem, V 279, 2004, pp 24733-24744.

334. Wang, X., et al, A Luminal Epithelial Stem Cell That is a Cell of Origen for Prostate Cancer, Nature 2009 V 461 pp 495-500.

335. Warburg, O., On the Origin of Cancer Cells, Science 1956 pp …

336. Watson, D. et al, ETS Transcription Factor Expression and Conversion During Prostate and Breast Cancer Progression, Open Access Cancer Journal, 2010, pp 24-39.

337. Watson, J. et al, Molecular Biology of the Gene, Pearson (San Francisco) 2004.

338. Watson, J., et al, Molecular Biology of the Gene, Benjamin Cummings (San Francisco) 2004.

339. Weber DC; Bieri S; Kurtz JM; Miralbell R. Prospective Pilot Study of Sildenafil For Treatment of Postradiotherapy Erectile Dysfunction In Patients With Prostate Cancer. *J Clin Oncol* 1999 Nov;17(11):3444-9.

340. Weinberg, R., The Biology of Cancer, Garland (New York) 2007.

341. Werhli, A., D. Husmeier, Reconstructing Gene Regulatory Networks with Bayesian Networks, 2007 Bioinfo and Stat pp 1-45.

342. Wolf, D., et al, Transcriptional Down Regulation of c-myc in Human Prostate Carcinoma Cells by the Synthetic Mibolerone, Br Jrl Cancer, V 65, 1992, pp 376-382.

343. Wu., F.X. et al, Modeling Gene Expression From Microarray Expression Data with State Space Equations.

344. Wu., F.X. et al, Modeling Gene Expression From Microarray Expression Data with State Space Equations.

345. Xia, H., Great Potential of MicroRNA in Cancer Stem Cell, Jrl Can Mol 2008 pp 79-89.

346. Yang, G., et al, Elevated Skp2 Protein Expression in Human Prostate Cancer, Clin Can Res, 2002 pp 3419-3426.

347. Yang, J., et al, Activation of FOXO3 is Sufficient to Reverse Mitogen Activated Protein/Extracellular Signal, Can Res, 2010 pp 4709-4718.

348. Yemelyanov, A., et al, Tumor Suppressor Activity of Glucocortoid Receptor in the Prostate, Oncogene, 2006, pp 1-12.

349. Yen, K., et al, Myc Driven Murine Prostate Cancer Shares Molecular Features with Human Cancer, Cancer Cell, 2003, pp 223-238.

350. Yu, J., et al, An Integrated Network of Androgen Receptor and TMPRSS2-ERG Fusiona in Prostate Cancer Progression, Cancer Cell 2010 pp. 443-454.

351. Zak, D., Continuous Time Identification of Gene Expression Models, Journal of Integrative Biology, Vol 7 No 4 2003 pp 373-386.

352. Zak, D., Continuous Time Identification of Gene Expression Models, Journal of Integrative Biology, Vol 7 No 4 2003 pp 373-386.

353. Zhang, L. et al, NF-kB Regulates Androgen Receptor Expression and Prostate Cancer Growth, Am Soc Invest Path, V 175, 2009, pp 489-499.

354. Zilberman, D., The Evolving Functions of DNA Methylation, Curr Opin in Plt Bio, 2008, pp 554-559.

355. Zong, Y., et al, ETS Family Transcription Factors Collaborate with Alternative Signalling Pathways to Induce Carcinomas from Adult Murine Prostate Cells, PNAS, V 106, 209, pp 12465-12470.

356.