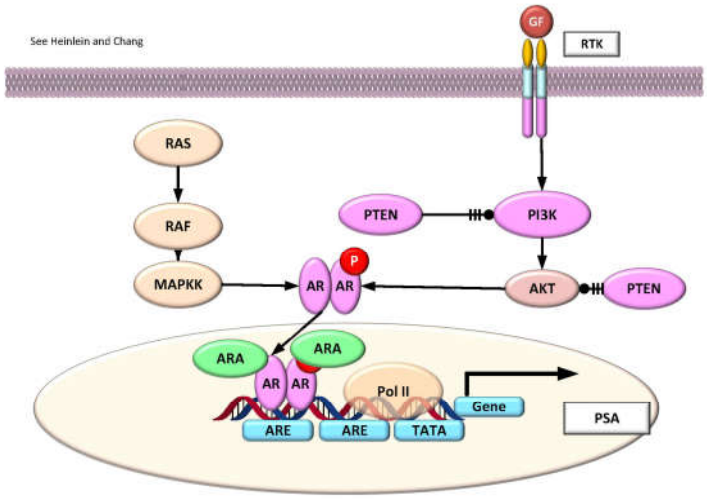

ANDROGEN RECEPTOR: WHITHER GOEST THOU?

May 2023

ABSTRACT
The Androgen Receptor, AR, is a key element in the normal maintenance of the prostate as well as a driver in malignant transformation. We examine the AR from a systems perspective and attempt to unravel the dynamics of its life cycle.

Terrence McGarty
TGL 199

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Our approach herein is to take elements of what is recent in the literature focused on a specific topic and attempt to develop a tapestry image of these connectable elements. We do not necessarily provide any new or fundamental results but merely attempt to assemble elements in a systematic and holistic manner.

Communications relating to these documents and these should be sent to:
mcgarty@alum.mit.edu.

Contents

1 WHY AR

The androgen receptor, AR, is a transcription factor which plays a pivotal role in prostate cancer, PCa. Prostate cancer management and understanding is considered by many to be twenty to thirty years behind breast cancer[1]. The tools to perform non-invasive testing do not exist and the treatment options are opportunistic at best. The AR plays a pivotal role in proliferation and in stability. Understanding the systemic interactions of AR is critical to ultimately understanding how to develop and deploy therapeutics.

Our current tools to detect the diseases in limited to PSA testing. Many believe that such tests have little value but we have argued that the logic behind many of these dicta are found seriously wanting. Frankly, it may very well be better than nothing. We do not address the issues of PSA per se but we focus on the AR and its many functions. Our approach is to look at the existing research and to attempt to assemble a broad system understanding of the multiplicity of functions of the AR.

1.1 OBJECTIVE

It has been argued that PCa may be an amalgam of multiple genetic alterations and that there is just not one PCa but many. AR plays a key role in PCa. Our intent herein is to examine AR from a systems perspective. Namely its role as regards to inputs and outputs and the system links in actions wherein AR plays a key role. Unlike the classic approach of a bench biologist, who deeps into the nooks and corners of a targeted protein, finding out more and more, we take the approach of accepting what is currently known and then try to horizontally make connections and from these inferences.

Our approach is not a systems biology approach, one where dynamics of reactions can be considered, but one more akin to an engineering approach, taking what is currently known and connecting to pieces which express actions. Our goal is to understand the system for the purpose of controlling and predicting.

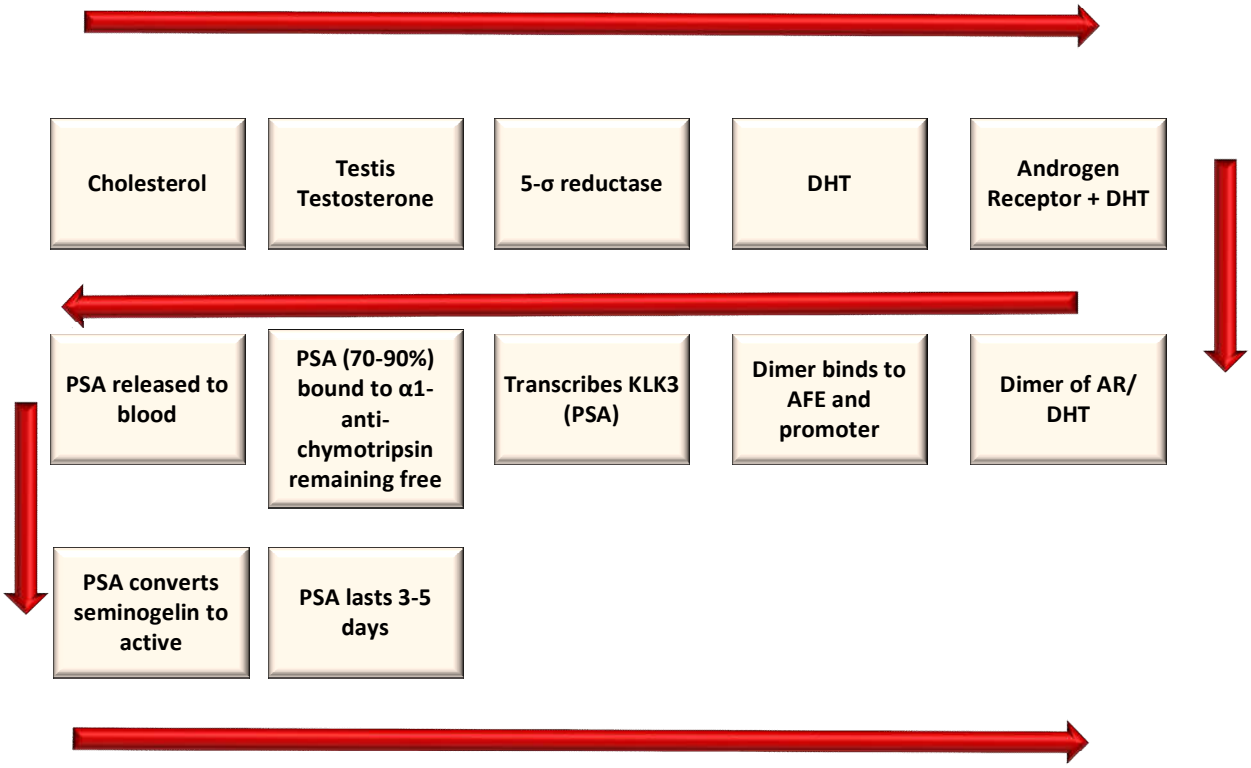
1.2 PARADIGM

Our analysis follows a structure that is similar to the paradigm of the flow of PSA or KLK3, from cholesterol to the final breakdown of KLK3 in the circulation. We show this construct below. One starts with cholesterol and ends with a decaying PSA after it has functioned in releasing certain other proteins. The driver is testosterone[2]. The result is PSA activation and

[1] https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach We wrote this concept more than a decade ago. It was a first attempt to examine PCa horizontally in a system manner. Since that time we have added almost 200 different Technical Notes examining various issues and expanding on PCa. This Technical Note is in a simple way just an extension of the 2012 book.

[2] See Melmed et al, Endocrinology, Elsevier, 2020 *Testosterone is a nuclear receptor ligand that is produced by the Leydig cells of the testis; it can circulate as a hormone and act on muscle, bone, and other tissues, but it also acts as a paracrine agent on neighboring seminiferous tubules.*

conversion of seminogelin to an active state. Thus we go from and input molecule to an output stated wherein AR acts as a transcription factor.



The above is but one of many possible cuts through what AR influences. We will attempt to do this in a broader context.

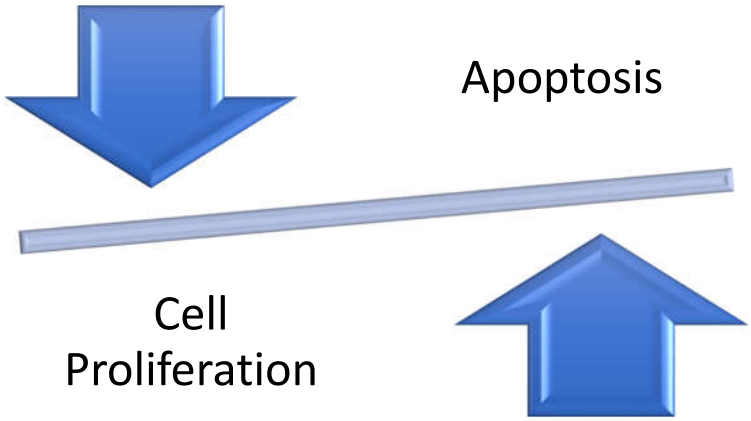
1.3 OUTLINE

The following is a brief outline of this Note:

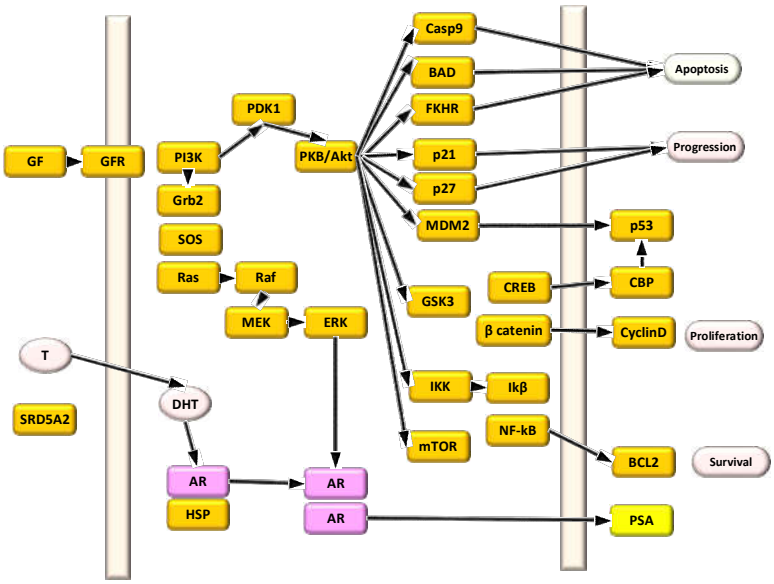
1. The Androgen Receptor: We examine the genetic details of the androgen receptor, AR. It appears to be dominant on the cells in the prostate and not elsewhere. It is principally driven by the introduction of testosterone into the cell and the conversion into DHT.
2. Transcription Factors: AE is a transcription factor. We examine some of the details as to how AR functions in that role. Specifically we look at drivers, facilitators and outputs. The drivers are factors which are principal movers of AR as a transcription factor. The facilitators are secondary factors that enhance the functions. The outputs are those elements that are produced as a result of AR activity. This begins to lay out the system functions of the AR. Our goal I not to dive deeply in each but to assemble the pieces so as to expose an integrated landscape.
3. CRPC: Castration resistant prostate cancer, CRPC, is an evolution of the initial PCa growth and its now independence of AR activation by testosterone.
4. All Drivers and Facilitators: We now details all drivers and facilitators.

5. All Outputs or Products: This sections details the many products facilitated by the functioning of AR. It should be noted that the result is a highly complex network of elements that in turn drive the cell in various directions.
6. AR and Stroma: There then is an evaluation and examination of the cell stroma, the collection of support cells that enhance the malignant state.
7. AR and Cell Cycle: Proliferation is the result of an active cell cycle. We examine the role of AR in this process and there is a focus on the cell cycle, AR and proliferation.
8. AR and Apoptosis: Cell death occurs in a variety of ways but the normal homeostatic means is via apoptosis. We examine the role that AR plays here.
9. AR and the Immune System: Finally we examine the interaction between the malignant cell, AR, and the immune system.

The key question is balance. Proliferation may be balanced by apoptosis. However stress often results in cell death but also excess proliferation. For example chronic prostatitis often is a forerunner of PCa. The infection process sometimes drives the cells into an uncontrolled malignant state.

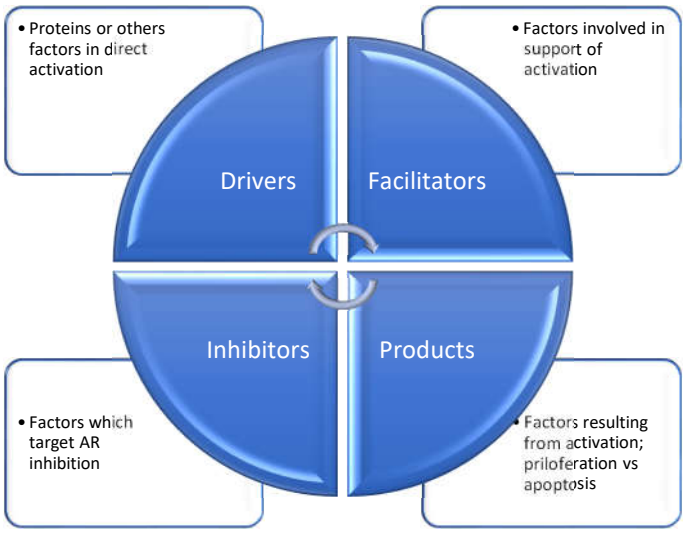


The following graphic sets out many of the pathways involved in PCa (this is a modified plot of the KEGG pathways). We have highlighted AR and in this diagram AR solely acts upon PSA. In this Note we extend this to many more interactions showing that this simplified world view severely delimits understanding AR



What we shall see herein is that AR and apoptosis has a limited linking. In contrast AR and cell cycles and thus proliferation is closely linked.

Finally as we noted above, we delve deeply into the elements as part of the AR system. As show below it consists of Drivers, Facilitators, Products or Outputs, and Inhibitors. We leave the latter for a subsequent discussion of therapeutics.



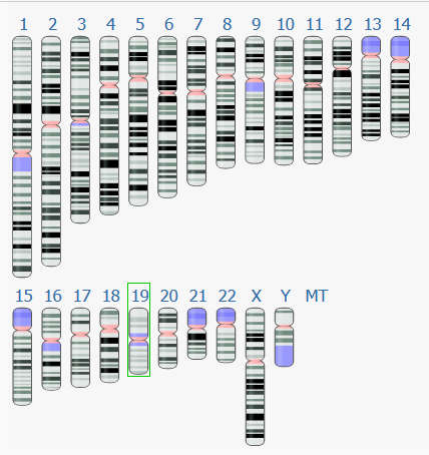
2 AR GENES

We begin with a details understanding of the AR gene and its variations.

2.1 VARIATIONS

We first examine the genetic structure of the AR. There are multiple variations and it is essential to understand which one is being operated.

The KLK3 gene is located at chromosome location 19q13.33[3]. We note that below:



KLK3 gene is approximately 5,850 nucleotides long and results in a coded protein of about 1,000 amino acids[4].

In contrast the AR gene is located at Xq12 and is approximately 186,599 nucleotides long[5].

As Fujita and Monomura note:

Androgen receptor (AR) is a steroid receptor transcriptional factor for testosterone and dihydrotestosterone consisting of four main domains, the N-terminal domain, DNA-binding domain, hinge region, and ligand-binding domain. AR plays pivotal roles in prostate cancer, especially castration-resistant prostate cancer (CRPC). Androgen deprivation therapy can suppress hormone-naïve prostate cancer, but prostate cancer changes AR and adapts to survive under castration levels of androgen. These mechanisms include AR point mutations, AR overexpression, changes of androgen biosynthesis, constitutively active AR splice variants without ligand binding, and changes of androgen cofactors.

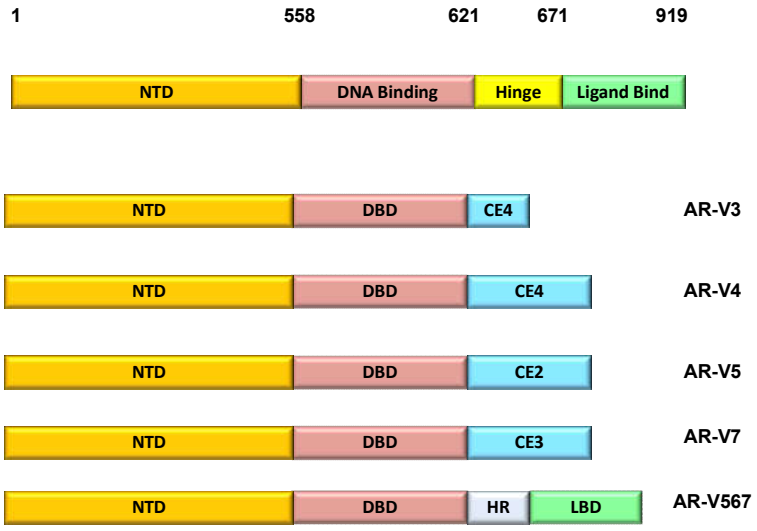
[3] https://www.ncbi.nlm.nih.gov/gene/354

[4] https://www.ncbi.nlm.nih.gov/gene/354

[5] https://www.ncbi.nlm.nih.gov/gene/367

Studies of AR in CRPC revealed that AR was still active in CRPC, and it remains as a potential target to treat CRPC. Enzalutamide is a second-generation antiandrogen effective in patients with CRPC before and after taxane-based chemotherapy. However, CRPC is still incurable and can develop drug resistance. Understanding the mechanisms of this resistance can enable new-generation therapies for CRPC. Several promising new AR-targeted therapies have been developed. Apalutamide is a new Food and Drug Administration-approved androgen agonist binding to the ligand-binding domain, and clinical trials of other new AR-targeted agents binding to the ligand-binding domain or N-terminal domain are underway. This review focuses on the functions of AR in prostate cancer and the development of CRPC and promising new agents against CRPC.

They further detail the various proteins reflecting AR protein as shown below:



As Ozturan et al note:

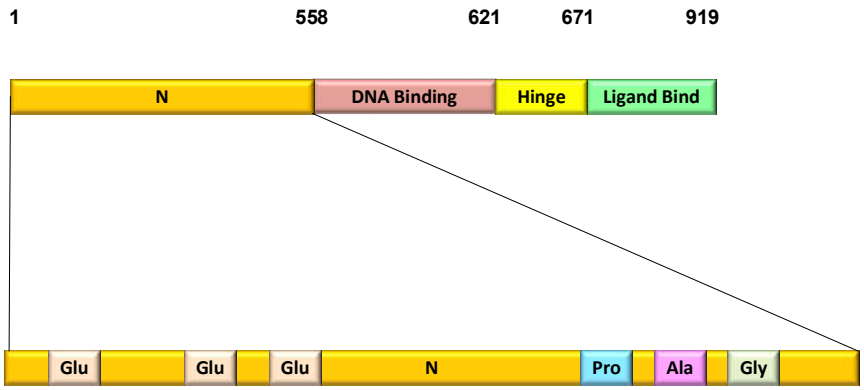
The AR is a 919 amino acids (110 kDa) protein that contains an N-terminal domain (NTD), DNA-binding domain (DBD), and C-terminal ligand-binding domain (LBD).

The inactive apo-form of AR primarily resides in the cytoplasm, where it is stabilized by chaperone proteins. When activated by androgens, most commonly testosterone or the more potent metabolite 5α-dihydrotestosterone (DHT), the AR undergoes an allosteric modification, homodimerizes, and then translocates into the nucleus where it binds to DNA at AR binding sites (ARBS) . The location of these ARBS is influenced by numerous features including the DNA primary sequence or motif, protein–protein interactions, transcription factor (TF) occupancy, and chromatin accessibility.

Once bound to enhancer cis-regulatory elements (CREs), the AR recruits coregulators , remodeling complexes , and other TFs to create a transcriptional hub that initiates an AR-dependent transcriptional program, which impacts the expression of several hundred target genes The specific genes associated with these complex cellular processes are controversial,

though several have been proposed, including c-Myc, EVT1, and EIF5A2 (eukaryotic translation initiation factor).

When looking at essentiality from published genome-wide CRISPR screens of AR-regulated genes in PCa cells (LNCaP), we find many known and novel essential genes, including coactivators such as GRHL2 (grainyhead-like transcription factor 2); metabolic genes such as DNM1L (dynamin-related protein 1), SREBP (sterol regulatory element-binding protein) cleavage activating protein SCAP and mTOR…



As Meehan and Sadar note:

The effects of androgens are mediated through the AR. The AR is a member of the nuclear receptor superfamily. The AR was first described in 1969 and then later cloned in 1988 by two independent groups. ***The AR gene is located on the X chromosome at Xq11-12 and lacks TATA and CAAT sequences in the upstream regulatory region.***

This gene contains 8 coding exons which span a length of approximately 90 kb. The AR gene has pur/pyr and GC box SP1 binding sites with two initiation sites for transcription that results in the production of two AR transcripts with differing lengths of 3' untranslated sequence that are 10 kb and 8.5 kb long.

The AR protein consists of 910-919 amino acids and has a theoretical molecular weight of 98 kDa , but runs as a 110-112 doublet in denaturing gel electrophoresis.

The AR protein consists of three functional domains:

1. ***the amino-terminal domain (NTD),***
2. ***the DNA binding domain (DBD) and***
3. ***the ligand-binding domain (LBD).***

The NTD is coded by exon 1 and comprises almost one-half of the entire AR molecule. The NTD is the most variable between nuclear receptors in terms of both length and sequence. The AR contains two discrete overlapping regions within the NTD that contribute to transactivation. Ligand-inducible transcriptional activity of the full-length receptor requires activation function 1 (AF-1) that is located between residues 141 and 338 of the NTD. A unique feature of the AR is the occurrence of several homopolymeric stretches of amino acids in the NTD. These include a polyglycine tract (24 residues), a polyproline tract (9 residues), and a polyglutamine tract. The length of the polyglutamine tract is polymorphic with the normal variation in repeat length ranging from 11 to 31 trinucleotide units, and a modal length of 20.

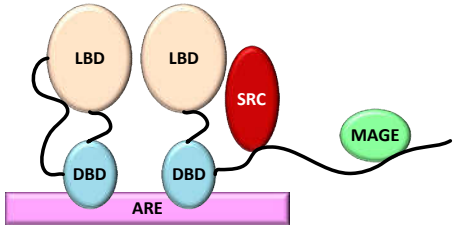
The length of the glutamine-rich region of the AR affects gene transcription. Although a number of studies have demonstrated that men with shorter CAG repeat length are at higher risk of developing prostate cancer, others present conflicting data. In most studies, however, the length of CAG repeats corresponds to racial variation in prostate cancer risk.

African Americans, with a high risk for developing prostate cancer, have a shorter CAG repeat length, while Asian men, with a lower risk for developing prostate cancer, have a longer repeat length.

Consistent with the notion that a shorter repeat length may increase the risk of developing prostatic malignancy, a shorter repeat length is also associated with early onset ovarian cancer

2.2 FUNCTIONALITY

Now Feng and He provide more detail regarding AR and its influence. They show below:



And they note as follows:

The unique molecular features of the androgen receptor and its coregulator recruitment. The primary sequence of the androgen receptor contains several functional domains: NH2-terminal Activation Function 1 (AF1), the central DBD, the carboxyl-terminal LBD, and two AR-specific FxxLF and WxxLF motifs. Schematic diagram of homodimeric androgen receptor bound to a

palindromic androgen response element (ARE). Dimerization of the androgen receptor is mediated by both DBD and LBD.

Shown in the diagram are FxxLF motif-mediated N/C interaction, recruitment of the SRC/p160 by AF1 and AF2, recruitment of FxxLF motif-containing ARA proteins by AF2, and recruitment of MAGE-A11 through the AR NH2-terminal extended FxxLF motif. Competition likely exists among different FxxLF, WxxLF, and LxxLF motifs for binding to the same AF2 site on AR LBD (1). SRC, steroid receptor coactivator; ARA, AR-associated protein; AF1, activation function 1; AF2, activation function 2, a hydrophobic cleft in the LBD; ARE, androgen response element; DBD, DNA binding domain; LBD, ligand binding domain.

As Heinlein and Chang note:

After the development of the prostate, androgens continue to function in promoting the survival of the secretory epithelia, the primary cell type thought to be transformed in prostate adenocarcinoma . In the normal prostate, the rate of cell death is 1–2% per day, which is balanced by a 1–2% rate of proliferation . The reduction of serum and prostatic DHT levels by castration results in a loss of 70% of the prostate secretory epithelial cells due to apoptosis in adult male rats, but the basal epithelia and stromal cell populations are relatively unaffected .

In the intact rat prostate, the secretory epithelial cells show strong AR immunoreactivity, whereas the majority of basal epithelial cells are AR negative, suggesting an explanation for their different sensitivity to androgen. However, AR is also expressed in the prostatic stroma, although castration results in the loss of stromal AR expression . The prostatic stroma therefore has the capacity to respond to androgen, but androgen is not required for its survival. Physiological testosterone levels prevent secretory rat prostate epithelial apoptosis. However, normal epithelial function is dependent on prostatic DHT levels . Superphysiological levels of serum androgen in dogs and in human habitual anabolic steroid users result in an increase in cellular proliferation in the prostate .

In humans, the proliferation occurs predominantly in the transitional zone of the prostate, the region that is primarily affected in benign prostatic hypertrophy but is seldom the initial site of prostate carcinoma formation *.*

Although individual cases of prostate cancer have been reported in anabolic steroid users , epidemiological studies have failed to establish a link between elevated serum testosterone, DHT, or adrenal androgens and prostate cancer risk, ***suggesting that elevated testicular and adrenal androgens alone do not significantly promote prostate carcinogenesis.***

In addition to apoptosis of secretory epithelial cells, castration also results in apoptosis and degeneration of prostatic capillaries and constriction of larger blood vessels, which precedes the appearance of epithelial apoptosis . These observations suggest that the reduction of blood flow to the prostate may contribute to epithelial apoptosis.

However, castration does not induce necrosis or apoptosis in all prostatic cell types, suggesting that if secretory epithelial cell loss is influenced by the alteration in blood flow, these cells are

more sensitive to this change than other prostate cell types. Administration of testosterone to castrated rats results in vascular regrowth followed by reconstitution of the secretory epithelia . However, the vascular endothelial cells of the rat prostate do not express AR .

In the normal prostate, cellular homeostasis is modulated in part by paracrine growth factor regulation between epithelial and stromal cells .

A subset of these growth factors, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor, can be regulated by androgens and can influence vascular survival. It is possible that castration initially alters prostatic growth factor production in the stroma, which contributes to a decrease in vascular function. The resulting reduction in blood flow, combined with an altered growth factor environment and decreased expression of other androgen regulated proteins, may contribute to apoptosis of the secretory epithelia

2.3 AR-V7 AND METASTASIS

We have examined AR-V7 and its impact on PCa[6]. As Sobhani et al have noted:

Metastatic prostate cancer is the most common cancer in males and the fifth cause of cancer mortality worldwide. Despite the major progress in this field, leading to the approval of novel anti-androgens, the prognosis is still poor.

A significant number of patients acquire an androgen receptor splice variant 7 (AR-V7), which is constitutively activated and lacks the ligand-binding domain (LBD) while maintaining the nuclear localization signal and DNA-binding domain (DBD).

This conformational change, even in the absence of the ligand, allows its retention within the nucleus, where it acts as a transcription factor repressing crucial tumor suppressor genes. AR-V7 is an important oncogenic driver and plays a role as an early diagnostic and prognostic marker, as well as a therapeutic target for antagonists such as niclosamide and TAS3681.

Anti-AR-V7 drugs have shown promise in recent clinical investigations on this subset of patients.

This mini-review focuses on the relevance of AR-V7 in the clinical manifestations of castration-resistant prostate cancer (CRPC) and summarizes redemptive therapeutic strategies … Several androgen receptor splice variants have been identified in PCa cell lines and xenograft tumors at the mRNA level [25,26]. In 2010, Sun et al. developed a novel human AR splice variant in which exons 5, 6, and 7 were deleted (ARv567es) and demonstrated that this variant could contribute to cancer progression in human prostate cancer xenograft models [27].

Due to the availability of a specific antibody validated for immunohistochemistry on tumor tissue samples, androgen receptor variant 7 (AR-V7) is the best-characterized variant [28]. Since its

[6] https://www.researchgate.net/publication/343669352_AR-V7_A_Driver_of_Prostate_Metastasis

detection in circulating tumor cells (CTCs), it has been associated with resistance to enzalutamide and abiraterone in preclinical studies [29]. AR-V7 may induce a distinctive set of gene expression compared to full-length receptor (DOI: 10.1158/0008- 5472.CAN-11-3892).

The ARV-7 preferentially leads to the expression of cell cycle regulatory genes, while the full-length AR represses that program and favors instead genes related to metabolism, differentiation, and macromolecular synthesis. Additionally, the resulting truncated AR variant is constitutively active and can promote AR signaling without ligand interaction. The mechanisms of cross-resistance of current anti-androgen drugs could be therefore influenced by the presence of AR-V7 …

In recent years, AR-V7 genomic alterations have been associated with progression to mCRPC, patients with detectable AR-V7 in liquid biopsies usually manifesting more aggressive disease and shorter survival [48]. Emerging evidence suggests that AR-V7 status can act as a prognostic marker in mCRPC [49]. More aggressive features (e.g., worse performance status, higher PSA serum levels, and higher disease burden) and poorer clinical outcomes have been observed in AR-V7-positive patients.

Zhang et al have noted:

Many therapeutic options are now available for men with metastatic castration-resistant prostate cancer (mCRPC), including next-generation androgen receptor axis-targeted therapies (AATTs), immunotherapy, chemotherapy, and radioisotope therapies. No clear consensus has been reached for the optimal sequencing of treatments for patients with mCRPC, and few well-validated molecular markers exist to guide the treatment decisions for individual patients. The androgen receptor splice variant 7 (AR-V7), a splice variant of the androgen receptor mRNA resulting in the truncation of the ligand-binding domain, has emerged as a biomarker for resistance to AATT. AR-V7 expression in circulating tumor cells has been associated with poor outcomes in patients treated with second- and third-line AATTs.

Clinically validated assays are now commercially available for the AR-V7 biomarker. In the present review of the current literature, we have summarized the biology of resistance to AATT, with a focus on the AR-V7; and the clinical studies that have validated AR-V7 expression as a strong independent predictor of a lack of clinical benefit from AATTs. Existing evidence has indicated that patients with AR-V7epositive mCRPC will have better outcomes if treated with taxane chemotherapy regimens rather than additional AATTs. …

Given the increasing number of treatment options and the limited survival of patients with mCRPC, biomarkers that can guide clinical decision-making will have clear clinical utility. The expression of the AR-V7 splice variant in prostate cancer cells appears to be the first well-established (negative) predictive factor to guide patient treatment for men with mCRPC. The detection of AR-V7 in the CTCs of patients with mCRPC has been clinically validated as a predictor of resistance to AR-directed therapies in sequential lines of treatment.

Multiple reported studies have shown that ARV7epositive patients will have improved outcomes if treated with taxanes instead of AATT. AR-V7 testing represents an important actionable advance toward personalizing the treatment selection for men with high-risk mCRPC.

3 AR AS TRANSCRIPTION FACTOR

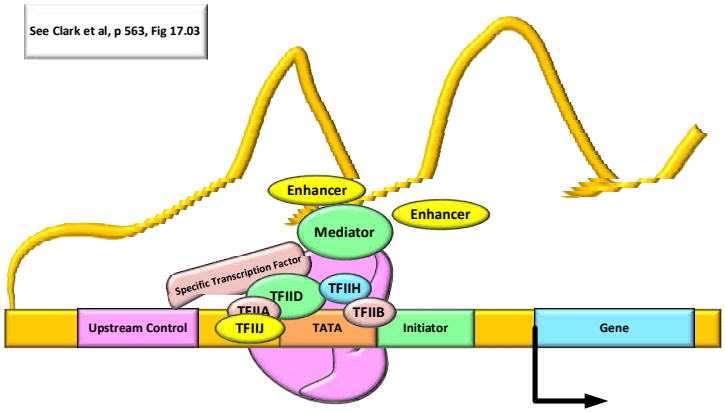
AR is a transcription factor. In that role it facilitate the transcription of a multiplicity of genes that are central to the development and evolution of PCa. We now discuss transcription factors and AR specifically.

3.1 TRANSCRIPTION FACTORS

There are a multiplicity of transcription factors in the human gene set. AR is one of them. As Lambert et al note:

Transcription factors (TFs) recognize specific DNA sequences to control chromatin and transcription, forming a complex system that guides expression of the genome. Despite keen interest in understanding how TFs control gene expression, it remains challenging to determine how the precise genomic binding sites of TFs are specified and how TF binding ultimately relates to regulation of transcription. This review considers how TFs are identified and functionally characterized, principally through the lens of a catalog of over 1,600 likely human TFs and binding motifs for two-thirds of them. Major classes of human TFs differ markedly in their evolutionary trajectories and expression patterns, underscoring distinct functions. TFs likewise underlie many different aspects of human physiology, disease, and variation, highlighting the importance of continued effort to understand TF-mediated gene regulation.

We show a simple example below.



The TF are elements necessary for the expression of certain genes. Lambert et al continue:

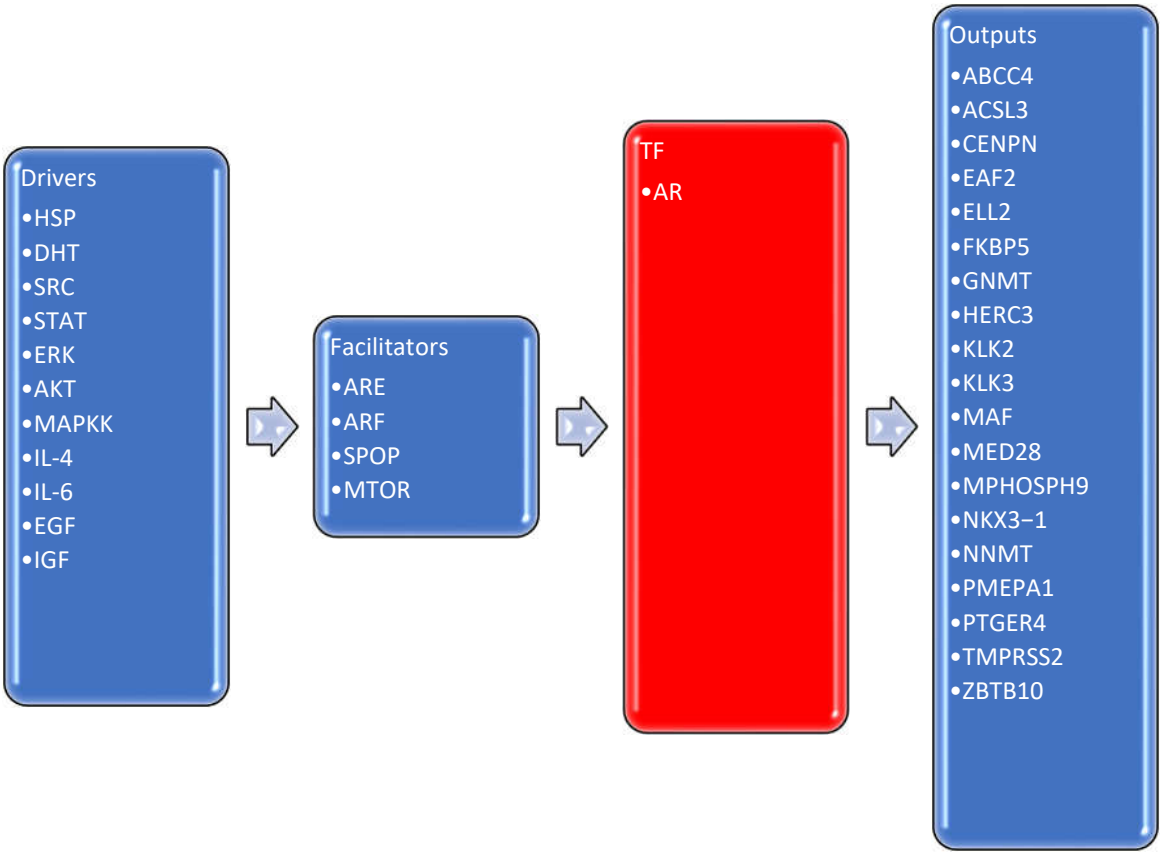
Transcription factors (TFs) directly interpret the genome, performing the first step in decoding the DNA sequence. Many function as ‘‘master regulators’’ and ‘‘selector genes’’, exerting control over processes that specify cell types and developmental patterning and controlling specific pathways such as immune responses. In the laboratory, TFs can drive cell differentiation and even de-differentiation and trans-differentiation. Mutations in TFs and TF-binding sites

underlie many human diseases. Their protein sequences, regulatory regions, and physiological roles are often deeply conserved among metazoans, suggesting that global gene regulatory ‘‘networks’’ may be similarly conserved. And yet, there is high turnover in individual regulatory sequences, and over longer timescales, TFs duplicate and diverge.

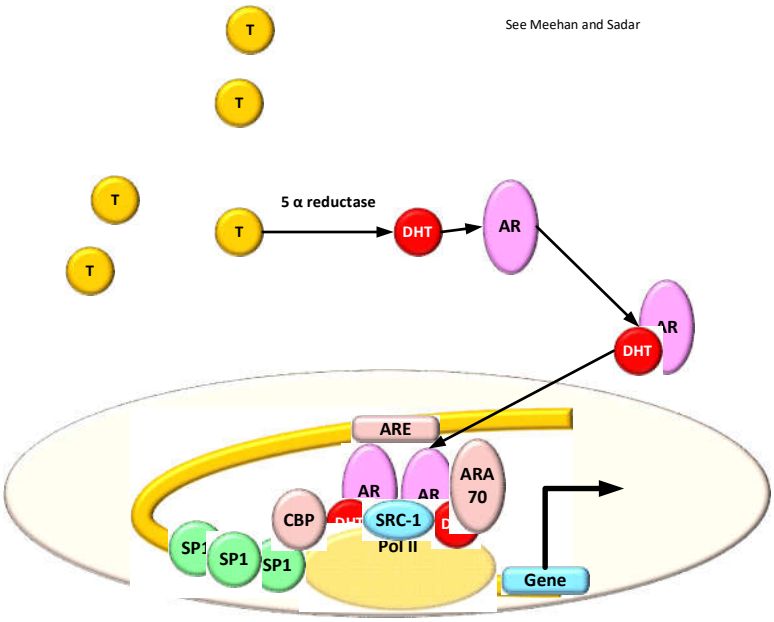
The same TF can regulate different genes in different cell types (e.g., ESR1 in breast and endometrial cell lines), indicating that regulatory networks are dynamic even within the same organism. Determining how TFs are assembled in different ways to recognize binding sites and control transcription is daunting yet paramount to understanding their physiological roles, decoding specific functional properties of genomes, and mapping how highly specific expression programs are orchestrated in complex organisms. … today, most known and putative TFs have instead been identified by sequence homology to a previously characterized DNA-binding domain (DBD), which is also used to classify the TF. With the possible exception of the very simple AT-hook (Aravind and Landsman, 1998), all extant examples of DBDs are assumed to be derived from a small set of common ancestors representing the major DBD folds, with the families arising by duplication…

3.2 Drivers, Facilitators and Outputs

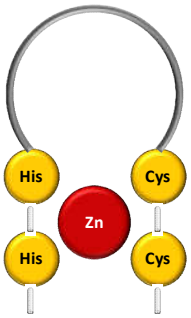
Transcription factors can relate to three other factors. First, Drivers are factors that force the process of the transcription factor. They initiate the process. Second, Facilitators are factors that assist in the process such as DBD. The are necessary but not sufficient. They facilitate the Drivers in having the TF function. Third, Outputs are the resulting gene that is read and translated such as PSA in the case of AR. There may not be a one to one relationship between TF and Outputs. Namely a TF may produce or facilitate several outputs.



We now show this for AR as below.



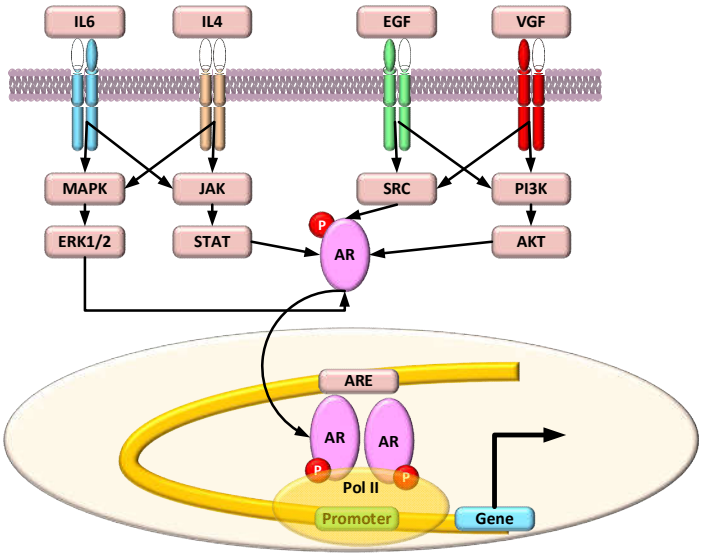
The zinc finger structure below is a common in the connection of the proteins to the selected gene sequences.



3.3 DRIVERS

We begin by examining what are drivers to the AR protein to act as a transcription factor. One of the first drivers is the Heat Shock protein which is initially bound to AR and replaced by DHT which initiates the process. Then there are other pathways that begin the AR chain in a variety of gene expressions.

From Tang et al we start with the graphic example below:



See Tang et al

The above depicts the canonical elements we will examine as AR effects transcription.

As Pincik et al note:

Furthermore, there is need for novel targeted therapies of metastatic PCa based on a better molecular understanding of the disease4. The lack of markers to stratify PCa cases into low- and high-risk groups results in overtreatment of 20–42% of patients5. STAT3, the major downstream

mediator of IL-6 signalling, was shown to be related to advanced tumour growth, by tumour-autonomous mechanisms and by modulating tumour-associated stroma6. Although STAT3 activation is observed in B50% of PCa7 its functional role in tumorigenesis and metastasis has not been elucidated. Data from the majority of human PCa cancer cell lines support an oncogenic and growth promoting role of IL-6 and STAT3 in vitro8. However, metastatic LNCaP cells were growth inhibited in vitro and in vivo in response to IL-6 treatment8. Moreover, treatment of patients with an IL-6 blocking antibody did not result in a survival advantage in patients with advanced PCa.

Thus, addressing the precise in vivo role of IL-6/STAT3 in PCa is of utmost importance to reassess diagnostic and therapeutic approaches. PTEN is one of the most frequently deleted or mutated tumour suppressors in PCa, with an estimated incidence of 70% in metastatic PCa, causing aberrant activation of the PI3K– AKT–mTOR signalling pathway. Loss of Pten leads to senescence, which is critically regulated by the ARF–p53 pathway. While the tumour suppressor ARF (p14ARF in humans; p19ARF in mice) is readily degraded in normal cells, it is stabilized to increase p53 function on loss of Pten. ARF was shown to augment p53 stability by promoting the degradation of Mdm2, a negative regulator of p53.

Concomitant inactivation of Pten and p53 leads to bypass of senescence and as a consequence to a malignant PCa phenotype. Previous studies report PTEN–STAT3 signalling crosstalk in malignant glioblastoma, but the detailed molecular mechanisms in cancer progression and metastasis remain unresolved. In this study, we show that loss of IL-6/Stat3 signalling in a Pten-deficient PCa model accelerates cancer progression leading to metastasis. Loss of IL-6/Stat3 signalling in PCa bypasses senescence via disrupting the ARF–Mdm2–p53 tumour suppressor axis.

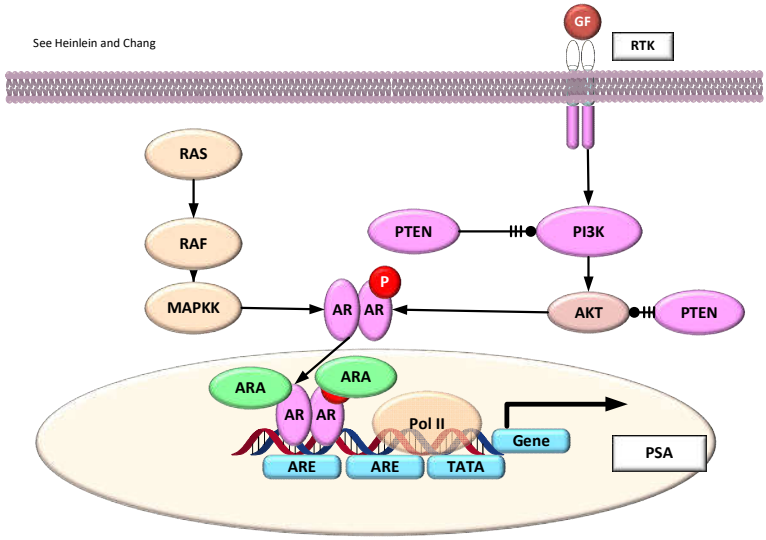
We identify ARF as a novel direct Stat3 target.

Notably, loss of STAT3 and p14ARF expression correlates with increased risk of recurrence in PCa patients. In addition, STAT3 and p14ARF expression was lost in metastasis compared with the primary tumours. We identified STAT3 and CDKN2A mutations in primary PCa patients. Furthermore, PCa metastases show a high frequency of STAT3 and CDKN2A deletions. We propose STAT3 and ARF as prognostic markers for high versus low risk PCa patient stratification

As Heinlein and Chang have noted:

Crosstalk of MAPK and PI3K/Akt pathways with A/AR. Both MAPK and PI3K/Akt may influence the phosphorylation of AR and AR coregulators, resulting in modulation of AR activity. The tumor suppressor PTEN can modulate AR activity via PI3K/Akt pathways or by interacting directly with AR. MAPKK, MAPK kinase; A/AR, androgen/androgen receptor; RTK, receptor tyrosine kinase; APPL, adapter protein containing PH domain, PTB domain, and leucine zipper motif; P, protein phosphorylation.

And as shown below



3.3.1 Heat Shock

Heat Shock proteins are bound to AR before the AR become activated by DHT, They drive the AR to a state whereby the activation can occur.

As Dubey et al note:

Heat shock proteins (HSPs) are the molecular chaperones, that are not only expressed during the normal growth process of cell cycle consecutively, but also get induced in cells during various stress conditions produced by cellular insult, environmental changes, temperature, infections, tumors etc.

According to their molecular weight and functions, HSPs are divided into five major families. HSP90, HSP70, HSP60 and HSP100 are the most studied members of the family. Experimental studies have proved that overexpression and/or inhibition of HSPs play an important role in maintaining the tolerance and cell viability under above-described stress conditions. HSP90 is found to be a promising candidate for the diagnosis, prognosis and treatment of cancer. Similarly, HSP70, HSP60 and small HSPs experimentally and clinically have potential for the treatment of neurodegenerative disease, ischemia, cell death, autoimmunity, graft rejection, etc….

The heat shock response was first described in 1962 by Ritossa and is named as heat shock proteins (HSPs) based on their increased synthesis after heat shock in house fly . Later it has been noted that HSPs exist in all the organisms from bacteria to humans, and they are among the most conserved proteins known . HSPs, are multimolecular complexes expressed constitutively (up 5-10% of the total protein) under normal growth condition in cells and act as molecular chaperones, which play a regulatory role in the folding of proteins, intracellular transport of

proteins in cytosol, endoplasmic reticulum and mitochondria; repair or degradation of proteins and refolding of misfolded proteins .

In addition to being constitutively expressed, these proteins are markedly induced (up to 15%) by a range of environmental, pathological, or physiological stimuli . These proteins are also modulated by nutrient deprivation, oxidative stress, hypoxia-ischemia, apoptotic stimuli and neuronal injury in the brain, etc..

HSPs are divided into five major families, HSP100, 90, 70, 60, and the small HSP (sHSP)/α-crystallins, according to their molecular weight, structure and function. HSP synthesis results in tolerance to insult, such as thermotolerance or stress tolerance in various organisms .

In various acute and chronic cell injuries, pathogenic conditions such as malignancies, and infectious diseases overexpression of HSPs were found to be playing cytoprotective and immunoregulatory roles . Recently, HSP reactivity in autoimmune diseases and transplantation have been proven to be down-regulated in the disease process. Togetherness, induction or inhibition of HSPs provides vast area of therapeutic target for combating various diseases. Considering, the regulatory role of HSPs in physiological and pathological conditions, HSPs have emerged as potential drug candidates for drug development and can be a breakthrough in the near future. Regulation of HSPs Stress condition causes protein unfolding, misfolding or aggregation, which triggers the stress response that leads to the induction of gene transcription of proteins.

HSP gene transcription is mediated by the interaction of the heat shock factor (HSF1) with heat shock elements (HSEs) in the HSP gene promoter regions. In unstressed state, HSF1 is present in the cytoplasm as a latent monomeric molecule.

Under stress, HSF1 is hyperphosphorylated in a ras-dependent manner by members of the mitogen-activated protein kinase (MAPK) subfamilies (e.g. ERK1, JNK/SAPK, p38 protein kinase). HSF1 is then converted to phosphorylated trimers with the capacity to bind DNA and translocates from the cytoplasm to the nucleus. The generation of HSPs is transient, and the presence of HSPs negatively influences the protein homeostasis. The activity of HSF trimers is downregulated by HSPs (e.g. HSP70) and the heat shock binding protein 1 which is found in the nucleus.

As Ciocca et al have noted:

The heat shock proteins (HSP) constitute a superfamily of chaperone proteins present in all cells and in all cell compartments, operating in a complex interplay with synergistic/overlapping multiplicity of functions, even though the common effect is cell protection.

Several reasons explain the need for investigating HSP in prostate cancer:

(1) these molecules function as chaperones of tumorigenesis accompanying the emergence of prostate cancer cells,

(2) they appear as useful molecular markers associated with disease aggressiveness and with resistance to anticancer therapies including hormone therapy, radiotherapy, chemotherapy and hyperthermia, and

(3) they can be used as targets for therapies.

The latter can be accomplished by:
(i) interrupting the interaction of HSP (mainly HSPC1) with various client proteins that are protected from degradation when chaperoned by the HSP;

(ii) using the chaperone and adjuvant capabilities of certain HSP to present antigenic peptides to the immune system, so this system can recognise the prostate tumour cells as foreign to mount an effective antitumoral response; and

(iii) using treatment planning models taking into account the HSP expression levels to obtain more effective therapies.

In summary, the study of the HSP during tumorigenesis as well as during cancer progression, and the inclusion of treatment designs targeting HSP combined with other treatment modalities, should improve prostate cancer survival in the near future…

Carcinogenesis involves a cascade of molecular events that mediate the transformation of normal cells into cancer cells. Although prostate cancer is a malignancy with a high incidence, the events associated with its initiation remain poorly understood and there are still many enigmas about the pathophysiology of prostate cancer.

Early prostate tumorigenesis appears to be associated with a dysplasia that initiates with proliferative inflammatory atrophy (PIA), and progresses to prostatic intraepithelial neoplasia (PIN), which in some cases leads to carcinoma.

Existing evidence suggests that these early lesions may be initiated by inflammation that occurs with exposure to different infectious agents and/or ingestion of carcinogens.

When a premalignant lesion progresses to primary cancer, to metastatic cancer, and to androgenindependent cancer, genetic alterations continue to accumulate within the tumour cells. Moreover, normal prostate and early-stage prostate cancers cells depend on androgens for growth and survival. As the cancer advances and metastasizes, it becomes dominated by cells that proliferate and survive independently of androgens.

With a practical/ didactic purpose we can identify the following entities during prostate cancer progression:

(1) normal prostate epithelium,

(2) PIA,

(3) PIN,

(4) localised prostate cancer,

(5) metastatic prostate cancer (all of them androgen-dependent), and

(6) androgen-independent prostate cancer.

Owing to their role as molecular chaperones, HSP participate in many events related to cancer, starting from the beginning of carcinogenesis . During this process, the transformed cells begin to express abnormal/elevated levels of HSP, and in some cases this induction continues during tumour progression. At present there exists an important body of evidence to support the participation of this family of proteins in the initiation and progression of prostate carcinogenesis. In accordance with the above, an interesting paper of Byun et al. has demonstrated that during prostate tumorigenesis the expression of several sets of housekeeping genes (including HSP) are differentially expressed, suggesting that the process is driven by modulation of the expression of these genes.

The expression of HSP was up-regulated during the transition of localised prostate cancer to metastatic prostate cancer, indicating that in advanced stages prostate tumour cells could be under cellular stress. Therefore, the authors suggest that during this period of cellular stress the prostate tumour may be more vulnerable and responsive to treatment….

The identification and assessment of level of these genes/proteins in the prostate tumour progression will allow the best management of prostate cancer patients and to improve the treatments that have HSP as potential targets for the therapy.

As Jin et al noted:

The androgen receptor (AR) is a member of hormonal transcription factors. The expression of AR protein and its activation by male hormone androgen are fundamental to prostate development during pubertal and malignant transformation during later ages.

These biological/ pathological processes are determined by critical regulation of downstream molecules/pathways by the AR. AR is a DNA-binding protein that regulates a wide-range of target genes through directly binding to cis-regulatory elements. In the absence of androgen, the AR is sequestered in the cytoplasm by the ***chaperone super-complex including heat shock proteins (Hsp) 90, 70 and 56.***

Once bound by androgen, AR undergoes conformational changes to dissociate from Hsp complex, becomes phosphorylated and translocates into the nucleus.

Albany and Hahn note:

Heat shock proteins HSPs are highly conserved stress-induced factors that play an essential role as molecular chaperones by regulating protein folding, stability transport and aggregation. HSPs have cytoprotective roles and are essential for cancer cell survival. HSPs are often upregulated in cancer and this constitutive expression is necessary for cancer cells' survival.7

Several of these proteins have demonstrated a direct interaction with components of the cell signaling pathways. For example, the androgen receptor (AR) is a major player in PCa growth and progression and is a well-known interacting factor of HSPs.

Since AR function is very dependent on HSP activity, many emerging compounds address AR-associated HSPs as novel drug targets.

HSPs have been classified into four families according to their molecular weight: HSP90, HSP70, HSP60 and small HSPs (15–30kDa) that include HSP27. HSPs are powerful regulators of apoptosis through an ability to interact with key components of the apoptotic signaling pathway, in particular, those involved in caspase activation. HSP90 is a molecular chaperone involved in the conformational maturation and function of a large number of 'client' proteins that have been implicated in oncogenesis.

The AR, a key driver of PCa growth and treatment resistance, is an HSP90 client and its function is dependent on HSP90 chaperone activity.

HSP27 and HSP70 are the most strongly induced chaperones during cellular stress. HSP27 is an ATP-independent, small HSP that, once phosphorylated, forms a chaperoning oligomer that regulates multiple cell survival and signaling pathways.

At the post-mitochondrial level, HSP27 binds to cytochrome C and inhibits caspase activation and apoptotic cell death. HSP27 and CLU act together to stabilize the cell against apoptotic stressors.

From Ratajczak et al we have:

Two out of three diseases of the prostate gland affect aging men worldwide. Benign prostatic hyperplasia (BPH) is a noncancerous enlargement affecting millions of men. Prostate cancer (PCa) in turn is the second leading cause of cancer death. The factors influencing the occurrence of BPH and PCa are different; however, in the course of these two diseases, the overexpression of heat shock proteins is observed.

Heat shock proteins (HSPs), chaperone proteins, are known to be one of the main proteins playing a role in maintaining cell homeostasis. HSPs take part in the process of the proper folding of newly formed proteins, and participate in the renaturation of damaged proteins. In addition, they are involved in the transport of specific proteins to the appropriate cell organelles and directing damaged proteins to proteasomes or lysosomes.

Their function is to protect the proteins against degradation factors that are produced during cellular stress. HSPs are also involved in modulating the immune response and the process of apoptosis.

One well-known factor affecting HSPs is the androgen receptor (AR)—a main player involved in the development of BPH and the progression of prostate cancer. HSPs play a cytoprotective role

and determine the survival of cancer cells. These chaperones are often upregulated in malignancies and play an indispensable role in tumor progression.

Therefore, HSPs are considered as one of the therapeutic targets in anti-cancer therapies.

In this review article, we discuss the role of different HSPs in prostate diseases, and their potential as therapeutic targets…. In normal cells under physiological conditions, in a state of undisturbed homeostasis, cytoprotective mechanisms operate, thanks to which they are able to survive the stressful conditions. Cells that are not exposed to stress factors show enough HSP expression to protect their proteome and ensure cellular homeostasis (proteostasis).

A number of significant changes take place in neoplastic cells, including, at the level of activity of the transcription factors and metabolic activity, glycolysis levels, lipid metabolism or amino acid metabolism.

Cancer cells are exposed to high levels of proteotoxic stress.

They enter stress response pathways for survival and proliferation and become dependent on stress-induced HSPs. Moreover, the intracellular homeostasis of neoplastic cells is regulated by the increased expression of HSPs. In this case, the HSP-mediated cytoprotection of cancer cells takes place by inhibiting apoptosis, which is important for the proliferation, invasiveness and metastasis of tumor cells . In addition, the high level of HSP expression promotes the folding of oncoproteins, which ensures their stability and reduces the likelihood of their proteolytic degradation.

The expression of HSPs is induced in response to a variety of physiological and environmental factors, including anti-cancer chemotherapy.

Such a strategy allows the cells to survive even under lethal conditions. Importantly, in neoplastic diseases, HSP expression is usually increased, which has been confirmed in gastric cancer , breast cancer , endometrial cancer, ovarian cancer, gastrointestinal cancers , lung cancer and in prostate cancer .

Many signaling pathways play an important role in the pathogenesis of neoplastic diseases, and their incorrect regulation leads to changes in the cell phenotype and disturbances of such important processes, such as the regulation of the cell cycle, growth, death, differentiation and cell adhesion .

In eukaryotic cells, two complementary processes aimed at the degradation of native intracellular proteins can be distinguished: lysosomal degradation, including macroautophagy, and proteasomal degradation. Lysosomes mainly break down extracellular proteins that enter the cell through endocytosis, or, in the case of macroautophagy, also the intracellular proteins under strong cellular stress.

Proteasomes, in turn, are responsible for the controlled degradation of proteins with lower molecular weights, including signaling proteins with a short half-life and misfolded proteins .

Current therapeutic strategies for neoplastic diseases mainly aim to induce apoptosis in these cells by genotoxic action or the inhibition of their proliferation.

Proteasome inhibitors lead to an increase in the transcription of genes encoding proteins from the HSP90, HSP70, HSP40, HSP28, HSP APG-1 and mitochondrial HSP75 families. These proteins play a significant role in the development of mechanisms of resistance to therapeutic compounds.

Cancer cells treated with proteasome inhibitors aim to compensate for the decreased activity of this protease by increasing its synthesis and the synthesis of chaperone molecules

3.3.2 SRC

From Kim et al:

Src family kinases (SFKs) have a critical role in cell adhesion, invasion, proliferation, survival, and angiogenesis during tumor development.

SFKs comprise nine family members that share similar structure and function. Overexpression or high activation of SFKs occurs frequently in tumor tissues and they are central mediators in multiple signaling pathways that are important in oncogenesis. SFKs can interact with tyrosine kinase receptors, such as EGFR and the VEGF receptor.

SFKs can affect cell proliferation via the Ras/ERK/MAPK pathway and can regulate gene expression via transcription factors such as STAT molecules.

SFKs can also affect cell adhesion and migration via interaction with integrins, actins, GTPase-activating proteins, scaffold proteins, such as p130CAS and paxillin, and kinases such as focal adhesion kinases.

Furthermore, SFKs can regulate angiogenesis via gene expression of angiogenic growth factors, such as fibroblast growth factor, VEGF, and interleukin 8. On the basis of these important findings, small-molecule SFK inhibitors have been developed and are undergoing early phase clinical testing. In preclinical studies these agents can suppress tumor growth and metastases. The agents seem to be safe in humans and could add to the therapeutic arsenal against subsets of cancers.

3.3.3 STAT

Signal transducer and activator of transcription (STAT) proteins are powerful controllers of gene expression. Recent work has involved them in Prostate Cancer along with the many other targets which have been identified. We examine this specific gene and its recently identified significance. The specific STAT is STAT3. Previously it has been linked to aggressive cancers. In fact attempts have been made to therapeutically target this pathway. The authors in a recent paper however contend that it is just the opposite. Namely STAT3 actually prevent metastatic behavior.

This discussion is a critical one as we examine further the targeting of genes and their behavior. The STAT3 issue seems to state that on one hand over-expression is bad, yet then on the other hand over-expression is good. This highlights the issue of cross talk between paths as well as the yet to be fully understood dynamics of pathways. Add to this is the fact that STAT3 is driven by IL-6 and this links in the immune system as well.

We begin the discussion with information in Science Daily which reports[7]:

A gene that is responsible for cancer growth plays a totally unexpected role in prostate cancer. The gene Stat3 is controlled by the immune modulator interleukin 6 and normally supports the growth of cancer cells. The international research team led by Prof. Lukas Kenner from the Medical University of Vienna, the Veterinary University of Vienna, and the Ludwig Boltzmann Institute for Cancer Research (LBI-CR) discovered a missing link for an essential role of Stat3 and IL-6 signalling in prostate cancer progression.

Interleukin 6 (IL-6) is an important cytokine that controls the cell survival and tumor growth. Hyperactive IL-6 may support cancer growth, particularly as it controls STAT3, which was shown to have an oncogenic role in most tumours. Many therapies are therefore designed to suppress IL-6 or STAT3. But the situation is different in prostate cancer. Lukas Kenner's research group has shown that, contrary to expectations; active STAT3 suppresses cell growth in prostate tumours. It activates the gene $p14^{ARF}$, which blocks cell division and thus inhibits tumour growth.

IL-6 is one of many interleukin cytokines, activating immune cells and leading to their proliferation. In a classic model for STAT3, it is activated by IL-6 and then it progresses via phosphorlyation to act as a promoter or enhancer for a multiplicity of genes whose expression leads to cancerous growth. However there is an alternative pathway, the ARF-MDM2-p53 pathway the controls and may mitigate some of these processes. This paper focuses on this crossover effect.

The article continues:

For this reason, STAT3 and $p14^{ARF}$ are ideally suited to act as biomarkers for the prognosis of this disease. If these two factors are missing in tissue samples, the risk is massively increased that the tumour grows and forms metastases.

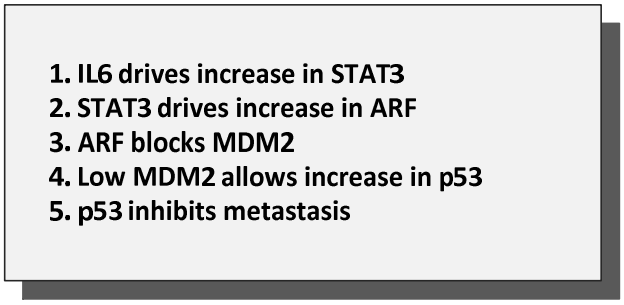
According to Lukas Kenner, this is important, as the predictive power of these proteins as biomarkers is twice as good as the previous gold standard. As only about 10 % of patients with prostate cancer die from the disease, this can help to prevent unnecessary therapeutic interventions with severe side effects such as incontinence and impotence. A non-invasive nuclear medical test based on these findings might soon be able to replace the painful removal of tissue samples to be examined.

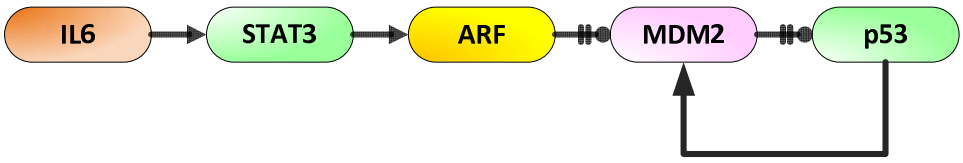
[7] http://www.sciencedaily.com/releases/2015/07/150722081410.htm

The reversed role of interleukin 6 as an inhibitor of prostate cancer has an additional significance. Blockade of interleukin 6 is used to treat other diseases, such as rheumatoid arthritis. According to Kenner, this means that therapies that block the IL-6 pathway may enhance the growth of prostate cancer.

Thus, the drug that is used to treat inflammatory disease may exacerbate malignancies. "Applying IL-6/Stat3 blockers to clinical practice might be dangerous for patients with cancerous lesions, further studies are mandatory to assess the possibility of increased cancer risk right now," says coauthor of this study, Helmut Dolznig, also from the Medical University of Vienna. The study was financed mainly by the LBI-CR and the FWF..

The following is a generalized paradigmatic summary of Pencik et al. Namely; they observed that IL6 controls STAT3 which in turn controls the ARF-MDM2-p53 pathway, which is critical in the overall control of PCa metastasis.





Now it should also be noted that the above is not the complete presentation. For example in this pathway p53 actually drives MDM2. There are other linkages that should be considered as well. We shall discuss some of these later.

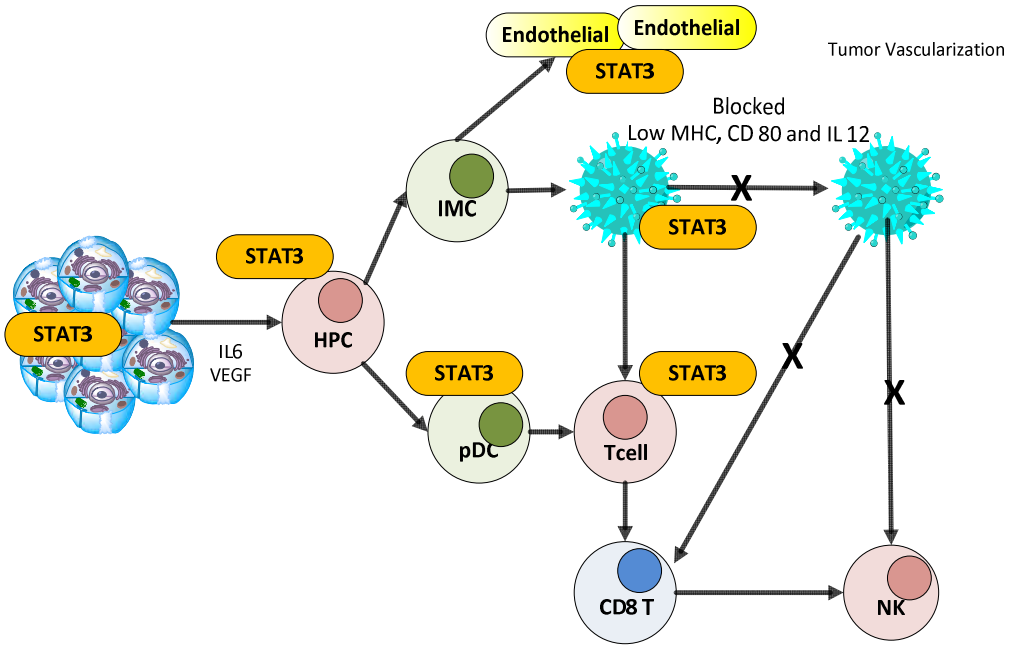
Now from the paper in question, namely Pencik et al, they conclude:

We have uncovered a paradigm shift in understanding the key function of STAT3 in tumorigenicity and metastatic progression in PCa. Therefore, our results call for cautious use of anti-IL-6- STAT3 signalling blockers in the treatment of PCa as this may turn low-grade tumours into highly malignant cancers by loss of senescence controlled by the STAT3–ARF axis. As IL-6/STAT3 signalling blockers are successful in the treatment of chronic inflammatory or autoimmune diseases, their influence on PCa development needs to be carefully evaluated in future studies.

Reactivating the IL-6/STAT3/ARF-dependent senescence pathway57 might be a promising strategy for PCa therapy via downregulation of Mdm2 (ref. 58) or p53 induction59. Alternatively, triggering ARF–p53-independent cellular senescence by a small molecule inhibitor could be beneficial for PCa patients in whom other therapies have failed.

Namely, they argue that the STAT3 control of the ARF-MDM2-p53 pathway should not be interfered with. That pathway actually enables control over metastatic behavior. We will discuss each element in some detail in what follows.

The classic understanding of STAT3 is that is acts to promote cancers. The figure below is a modification from Yu et al:



STAT3 signalling allows crosstalk between tumour cells and dendritic cells, forming an immunosuppressive network. *Tumour-associated factors such as vascular endothelial growth factor (VEGF), IL-10 and IL-6 can not only be upregulated by signal transducer and activator of transcription 3 (STAT3), but are also STAT3 activators. Increased STAT3 activity in haematopoietic progenitor cells (HPCs) promotes the generation of immature myeloid cells (iMCs) and increases the numbers of both immature dendritic cells and plasmacytoid dendritic cells (pDCs), each of which promotes the accumulation of regulatory T (TReg) cells in the tumour microenvironment. ...preventing their maturation and compromising their ability to stimulate the anti-tumour effects of CD8+ T cells and natural killer (NK) cells.*

As Yu et al state:

Immune cells in the tumour microenvironment not only fail to mount an effective anti-tumour immune response, but also interact intimately with the transformed cells to promote oncogenesis actively. Signal transducer and activator of transcription 3 (STAT3), which is a point of convergence for numerous oncogenic signalling pathways, is constitutively activated both in tumour cells and in immune cells in the tumour microenvironment.

Constitutively activated STAT3 inhibits the expression of mediators necessary for immune activation against tumour cells. Furthermore, STAT3 activity promotes the production of immunosuppressive factors that activate STAT3 in diverse immune-cell subsets, altering gene-expression programmes and, thereby, restraining anti-tumour immune responses. As such, STAT3 propagates several levels of crosstalk between tumour cells and their immunological microenvironment, leading to tumour-induced immunosuppression. Consequently, STAT3 has emerged as a promising target for cancer immunotherapy.

Thus the classic view is that STAT3 is an essential element in the pathology of tumorogenesis which as we indicated earlier is in contrast to the recent results. Thus do we block it or allow it? That is the question. Yu et al conclude:

The ability of STAT3 to broadly and profoundly affect tumour immunity strongly indicates that constitutively activated STAT3 both in tumour cells and in tumour stromal immune cells is an attractive target for cancer immunotherapy. Another unique and appealing aspect of targeting STAT3 for cancer immunotherapy is due to the crucial role of STAT3 in tumour-cell survival and tumour angiogenesis. Many experiments have shown that tumour rejection mediated by CD8+ T cells is always preceded by the inhibition of tumour-induced angiogenesis.

Because targeting STAT3 is expected to decrease the survival and angiogenic potential both of tumour cells and of the tumour stroma, targeting STAT3 could facilitate immune-cell-mediated anti-tumour effects at several levels. Although STAT3 is the first oncogenic target for cancer immunotherapy, other important onco proteins, such as MAPKs, might have similar roles. With the emergence of targeted delivery systems, and small molecule inhibitors or RNAi technology to block STAT3 and other relevant oncogenic pathways, a new era of molecular targeting for cancer immunotherapy is on the horizon.

Yu et al are focusing on hematopoietic cells not prostate cells. There is no reason why one should expect the same effect in different cells. Yet from a therapeutic perspective if such a drastically different model is functioning, the results would be problematic at best.

As Niu et al have stated:

Loss of p53 function by mutation is common in cancer. However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations.

It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53.

We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates

expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

Thus, Niu et al also present a model for Stat3 inhibiting p53, again in contrast to the paper in question. Niu et al conclude:

1. Stat3 protein interacts with the p53 promoter.
2. Stat3 inhibits p53 expression at the transcription level.
3. Stat3 binds to the p53 promoter in vitro as determined by EMSA.
4. Interaction between Stat3 protein and the p53 promoter contributes to Stat3-mediated inhibition.
5. Stat3 activity inhibits the p53-responsive element and UV-induced p53-mediated growth arrest.
6. Blocking Stat3 activates p53 expression in human cancer cells.
7. Blocking Stat3 induces p53-mediated tumor cell apoptosis and facilitates UV-induced tumor cell growth inhibition.

The results of these two studies seem fairly conclusive regarding Stat3. Namely it is oncogenic. But despite the study in question here seems to reverse that position. We will examine that in some detail.

Let us now review what is understood about the ARF-MDM2-p53 pathway. This will be necessary before linking this pathway to STAT3 and its functions.

Now this is a classic pathway whose ultimate control mechanism is p53 expression. p53 is generally understood to be a control gene, keeping the cell in some homeostasis and preventing malignancy. As we will not later this may not always be the case but that will not apply to the current discussion.

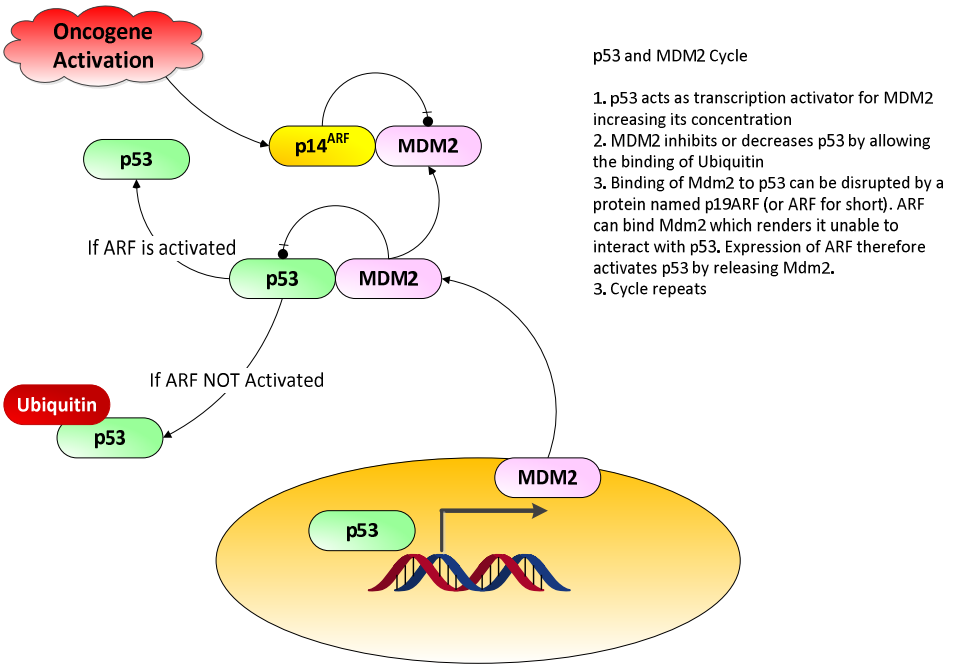
The following Figure depicts the process of the three gene control mechanism. Simply:

1. p53 activates the production of MDM2

2. MDM2 can bind to p53 and result in its dissolution via an Ubiquination

3. ARF can bind to MDM2 and allow the p53 to survive.

4. The process, albeit a bit complex, reaches a steady state for all three proteins.

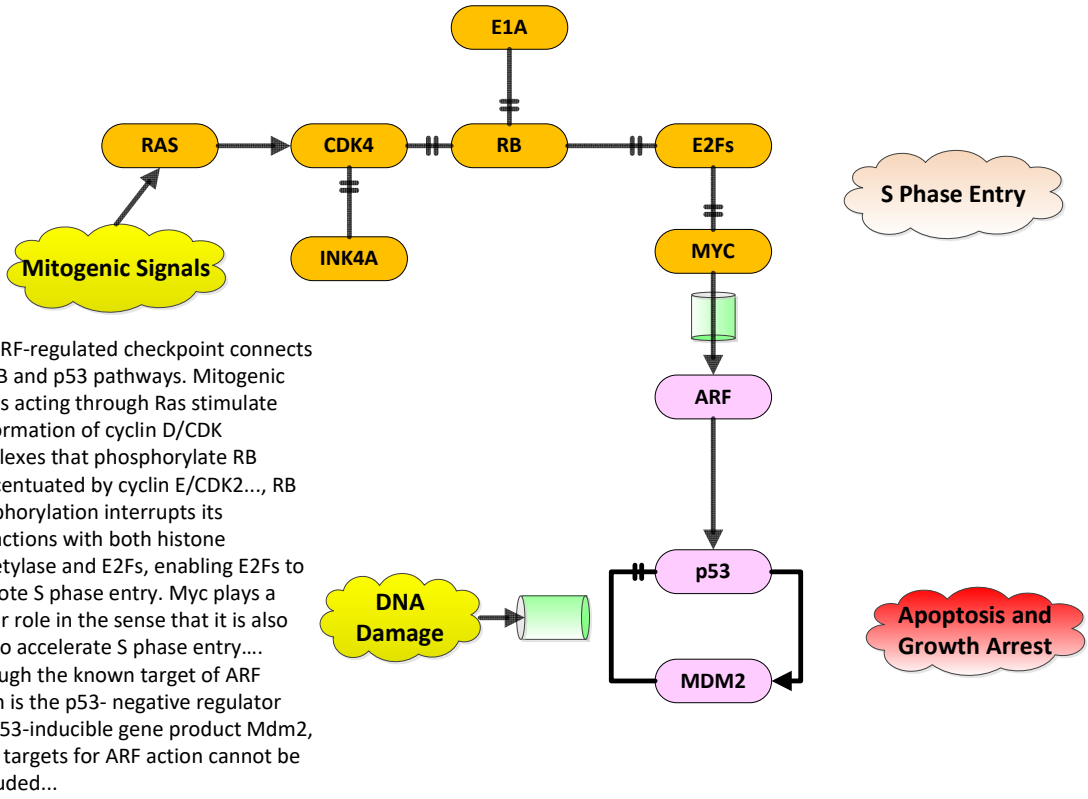


p53 and MDM2 Cycle

1. p53 acts as transcription activator for MDM2 increasing its concentration
2. MDM2 inhibits or decreases p53 by allowing the binding of Ubiquitin
3. Binding of Mdm2 to p53 can be disrupted by a protein named p19ARF (or ARF for short). ARF can bind Mdm2 which renders it unable to interact with p53. Expression of ARF therefore activates p53 by releasing Mdm2.
3. Cycle repeats

From Sherr and Weber (as modified) we have the following details as well shown graphically:

From Sherr and Weber



The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB ….Accentuated by cyclin E/CDK2..., RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry…. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded...

Note in the above we have the cyclic MDM2 and p53 control as well as the cell instigators.

Now Van Maerken, T., et al notes the following regarding the details of this feedback loop:

The p53-MDM2 autoregulatory feedback loop.

(a) The p53 protein induces expression of MDM2, which negatively regulates the stability and activity of p53, providing a means to keep p53 levels and activity low in unstressed cells and to switch off p53 at the end of a stress response.

(b) The p53-mediated expression of MDM2 results from binding of p53 to response elements in the MDM2 gene and subsequent transactivation of MDM2. The domain structure of p53 is shown schematically:

i. *TAD, transactivation domain, amino acids;*
ii. *PRD, proline-rich domain, amino acids; DBD, DNA-binding domain, amino acids;*
iii. *TD, tetramerization domain, amino acids;*
iv. *CTD, C-terminal regulatory domain, amino acids.*

(c) The p53-inhibitory activity of MDM2 relies on multiple mechanisms. Binding of MDM2 to p53 conceals the TAD and consequently blocks the transcriptional activity of p53. MDM2 also recruits several corepressor proteins to p53, including HDAC1, CTBP2, YY1, and KAP1.

The E3 ubiquitin ligase activity of MDM2 results in ubiquitination of lysine residues in the CTD of p53, preventing acetylation of p53, favoring nuclear export, and promoting proteasomal degradation (see text for details). Some of these lysine residues can also be neddylated by MDM2, resulting in inhibition of the transcriptional activity of p53. Finally, MDM2 may also serve as a p53-specific transcriptional silencer by binding and monoubiquitinating histone proteins in the proximity of p53-responsive promoters. Nd, NEDD8; Ub, ubiquitin. …

They continue the discussion as follows:

The $p14^{ARF}$ protein is predominantly localized to the nucleolus, in which it is stabilized by binding to nucleophosmin within maturing pre-ribosomal particles, pointing to a function in the regulation of ribosome biogenesis.

Nucleophosmin promotes the processing of ribosomal RNA precursors and the nuclear export of ribosomal subunits, whereas overexpression of $p14^{ARF}$ or its murine homolog $p19^{ARF}$ interferes with transcription and processing of ribosomal RNA, impedes nucleocytoplasmic shuttling of nucleophosmin, and inhibits ribosome nuclear export. However, the precise biological function of the nucleophosmin– $p14^{ARF}$ complexes remains a subject of debate. Stress signals trigger the disruption of the interaction between $p14^{ARF}$ and nucleophosmin, and induce translocation of p14ARF to the nucleoplasm.

This redistribution enables $p14^{ARF}$ to interact with p53-bound MDM2 and to antagonize MDM2 function by inhibiting its E3 ubiquitin ligase activity and by blocking nucleocytoplasmic shuttling of MDM2 and p53, resulting in p53 stabilization. The p53-inhibitory activity of MDM2 may also be neutralized by $p14^{ARF}$ -mediated mobilization of MDM2 into the nucleolus, although this mechanism is not strictly required for the p53-dependent functions of $p14^{ARF}$.

This is clearly a highly complex mechanism. They continue:

Furthermore, the $p14^{ARF}$ protein is capable of inhibiting the activity of another E3 ubiquitin ligase that targets p53 for degradation, ARF-BP1/Mule, and of counteracting the p53-antagonizing NF-kappaB pathway. It should be noted that p14ARF also exerts a potent tumor suppressor activity independently of p53.

Various researchers have tried to model these systems using different techniques. One technique is the use of Petri Nets[8]. From CSML we have a Petri Net models describing the details of such a network and they state[9]:

Proteins p53, MDM2, and $p19^{ARF}$ are proteins closely related to cancer. The protein p53 is a protein which suppresses the formation of tumors, and the protein MDM2 promotes the formation of tumors by decreasing the activity of the protein p53.

Understanding of control mechanism of these proteins connects to development of an effective medicine for suppressing the tumor. It is known that protein p53 works as a transcription factor for many genes and its transcriptional activity is controlled by a complex formed with proteins MDM2 and $p19^{ARF}$.

However, it is still unclear whether protein p53 keeps its transcriptional activity in the form of the trimer with proteins p53, MDM2 and $p19^{ARF}$. …

a hybrid functional Petri net (HFPN) model which has been constructed by compiling and interpreting the information of p53-MDM2 interactions... With our HFPN model, we have simulated mutual behaviors between genes p53, MDM2, $p19^{ARF}$, and their products. Through simulation, we discussed whether the complex p53-MDM2-$p19^{ARF}$ has transcriptional activity for genes Bax and MDM2 or not.

It is worth examining these structures, namely the Petri Nets. We leave the examination to the reference. From Moll and Petrenko we have the following result:

Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53- specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growthsuppressive function in

[8] See Reisig

[9] http://www.csml.org/models/csml-models/p53-arf-dependent-stabilization-pathway/

unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes.

The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

Let us go back and re-examine the functions of STAT3 and this time in the context of the paper in study. As NCBI states[10]:

The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators.

This protein is activated through phosphorylation in response to various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein.

As Niu et al have noted:

Loss of p53 function by mutation is common in cancer.

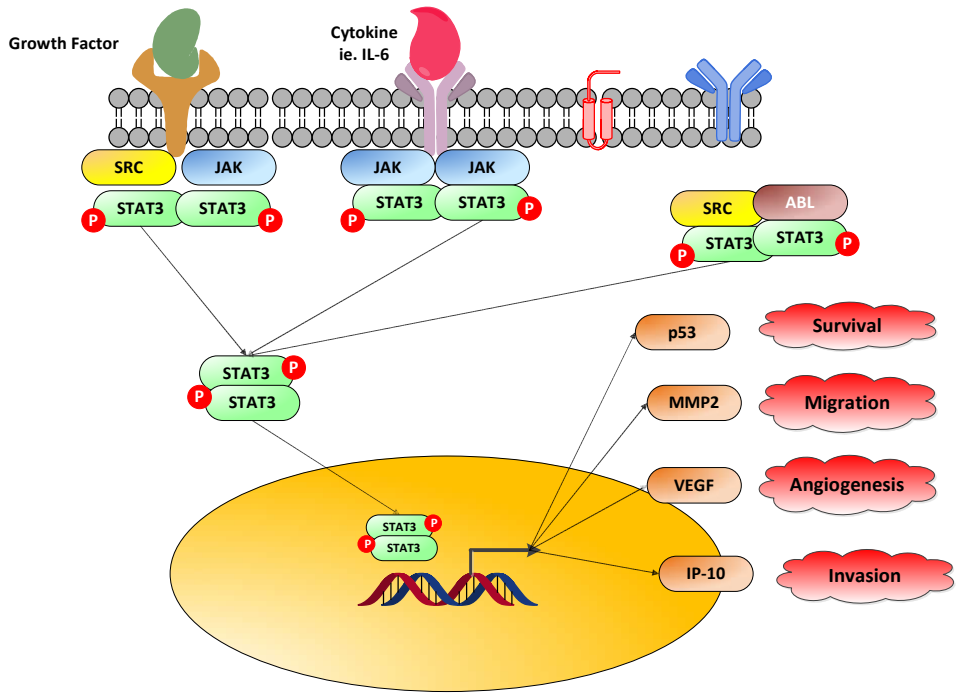
However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations. It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53. We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

[10] http://www.ncbi.nlm.nih.gov/gene/6774

Namely in many cancers the excess expression of STAT3 leads to an inactivation of p53 and thus an oncogenic state. The figure below is a depiction of this process.



However, Pencik et al have recently noted the following as regards to PCa.

Prostate cancer (PCa) is the most prevalent cancer in men. Hyperactive STAT3 is thought to be oncogenic in PCa. However, targeting of the IL-6/STAT3 axis in PCa patients has failed to provide therapeutic benefit. Here we show that genetic inactivation of Stat3 or IL-6 signalling in a Pten-deficient PCa mouse model accelerates cancer progression leading to metastasis. Mechanistically, we identify p19ARF as a direct Stat3 target.

Loss of Stat3 signalling disrupts the ARF–Mdm2–p53 tumour suppressor axis bypassing senescence. Strikingly, we also identify STAT3 and CDKN2A mutations in primary human PCa. STAT3 and CDKN2A deletions co-occurred with high frequency in PCa metastases. In accordance, loss of STAT3 and p14ARF expression in patient tumours correlates with increased risk of disease recurrence and metastatic PCa. Thus, STAT3 and ARF may be prognostic markers to stratify high from low risk PCa patients. Our findings challenge the current discussion on therapeutic benefit or risk of IL-6/STAT3 inhibition.

But Pencik et al further note:

PTEN is one of the most frequently deleted or mutated tumour suppressors in PCa, with an estimated incidence of 70% in metastatic PCa, causing aberrant activation of the PI3K– AKT–mTOR signalling pathway

We have examined this extensively in our analyses of PCa.

Loss of Pten leads to senescence, which is critically regulated by the ARF–p53 pathway.

PTEN is a major controller of PI3K and its pathway. Loss of PTEN is common in most PCa. On the other hand we have the ARF-MDM2-p53 dynamic which we shall discuss later.

While the tumour suppressor ARF ($p14^{ARF}$ in humans; $p19^{ARF}$ in mice) is readily degraded in normal cells, it is stabilized to increase p53 function on loss of Pten. ARF was shown to augment p53 stability by promoting the degradation of Mdm2, a negative regulator of p53.

Concomitant inactivation of Pten and p53 leads to bypass of senescence and as a consequence to a malignant PCa phenotype.

Loss of PTEN and of p53 is potentially a universally catastrophic event. It is a loss of two of the most significant stabilization elements in any cell, especially the prostate.

Previous studies report PTEN–STAT3 signalling crosstalk in malignant glioblastoma, but the detailed molecular mechanisms in cancer progression and metastasis remain unresolved.

In this study, we show that loss of IL-6/Stat3 signalling in a Pten-deficient PCa model accelerates cancer progression leading to metastasis. Loss of IL-6/Stat3 signalling in PCa bypasses senescence via disrupting the ARF–Mdm2–p53 tumour suppressor axis.

We identify ARF as a novel direct Stat3 target. Notably, loss of STAT3 and p14ARF expression correlates with increased risk of recurrence in PCa patients. In addition, STAT3 and p14ARF expression was lost in metastasis compared with the primary tumours.

This is the nexus between the STAT3 pathway and the ARF-MDM2-p53 pathways. Namely the authors seem to argue that STAT3 targets ARF and it is through this "targeting" that the latter pathway becomes defective.

We identified STAT3 and CDKN2A mutations in primary PCa patients. Furthermore, PCa metastases show a high frequency of STAT3 and CDKN2A deletions.

We propose STAT3 and ARF as prognostic markers for high versus low risk PCa patient stratification.

Pencik et al also note the following inference:

Stat3 regulates the ARF–Mdm2–p53pathway. Since loss of Pten triggers senescence thereby restricting cancer progression and metastasis, we next tested whether Stat3 exerts a tumour suppressive function by activating senescence-inducing programmes in Ptenpc-/-PCa cells at an early stage of PCa development.

Senescence is generally characterized by upregulation of p53, cyclin-dependent kinase inhibitor 1 (Cdkn1, p21), promyelocytic leukaemia protein (PML) and elevated senescence-associated-b-galactosidase activity. Of note, Ptenpc-/-Stat3-/- tumours lacked p21 expression, displayed reduced numbers of PML nuclear bodies and decreased SA-b-Gal activity compared with Ptenpc-/- tumours, suggesting Stat3 as a novel mediator of senescence in response to loss of Pten.

Again the statement is "suggesting" and there is no definitive well defined mechanism.

Senescence associated with loss of Pten was shown to be bypassed by deletion of p53 leading to early lethality. We show here that loss of Stat3 and Pten revealed a phenotype strikingly similar to that of p53 and Pten loss. Intriguingly, Stat3 and Pten deletion resulted in downregulation of p53 expression in the prostate epithelium, which was accompanied by the loss of p19ARF

The authors make the following statement:

The p53 expression in the tumour stromal cells remained unchanged. Since $p19^{ARF}$ is a critical regulator of Mdm2 degradation, our results suggest that the tumour suppressive capacity of Stat3 in senescent tumour cells may rely on the p19ARF–Mdm2–p53 tumour suppressor axis.

The conclusion is still a bit tentative. Just what the mechanism is may not be well understood.

Now Yu et al state:

The Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) proteins, particularly STAT3, are among the most promising new targets for cancer therapy. In addition to interleukin-6 (IL-6) and its family members, multiple pathways, including G-protein-coupled receptors (GPCRs), Toll-like receptors (TLRs) and microRNAs were recently identified to regulate JAK–STAT signalling in cancer.

Well known for its role in tumour cell proliferation, survival, invasion and immunosuppression, JAK–STAT3 signalling also promotes cancer through inflammation, obesity, stem cells and the pre-metastatic niche. In addition to its established role as a transcription factor in cancer, STAT3 regulates mitochondrion functions, as well as gene expression through epigenetic mechanisms. Newly identified regulators and functions of JAK–STAT3 in tumours are important targets for potential therapeutic strategies in the treatment of cancer.

Huang, et al state that STAT3 is a preferred target for cancer therapy. Specifically:

Numerous cytokines, growth factors, and oncogenic proteins activate signal transducer and activator of transcription 3 (Stat3), which has been recognized as one of the common pathways in cancer cells. Stat3 signaling affects the expression and function of a variety of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and immune evasion.

Evidently, the Stat3 signaling pathway regulates cancer metastasis and constitutes a potential preventive and therapeutic target for cancer metastasis. .

Furthermore Huang et al outline the reasons for this:

Contribution of Stat3 signaling pathway to cancer metastasis.

Stat3 in the cytoplasm of unstimulated cells becomes activated by recruitment to phosphotyrosine motifs within complexes of growth factor receptors (e.g., epidermal growth factor receptor), cytokine receptors (e.g., IL-6 receptor), or non-receptor tyrosine kinases (e.g., Src and BCR-ABL) through their SH2 domain. Stat3 is then phosphorylated on a tyrosine residue by activated tyrosine kinases in receptor complexes.

Phosphorylated Stat3 forms homodimers and heterodimers and translocates to the nucleus. In the nucleus, Stat3 dimers bind to specific promoter elements of target genes and regulate gene expression. The Stat3 signaling pathway regulates cancer metastasis by regulating the expression of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and tumor immune evasion.

It would be useful if somehow these conflicting views could be brought into alignment. In addition we have the work Marcias et al, who state:

Pathways associated with Stat3 activation. Stat3 is activated downstream of receptor tyrosine kinases (e.g., EGFR), cytokine receptors via associated Janus family kinases (JAKs) (e.g., IL-6 receptor), and nonreceptor-associated tyrosine kinases (e.g., c-src). Tumor promoters such as TPA and UVB activate Stat3 in keratinocytes primarily via the EGFR.

Activation of PKCs by tumor promoters leads to the processing of membrane-bound preforms of EGFR ligands such as heparin-binding EGF (HB-EGF) by matrix metalloproteinases (MMPs). In addition, PKCs associate with and phosphorylate Stat3 at Ser727, which is necessary for maximal Stat3 transcriptional activity. Furthermore, transcriptional induction of cytokines and EGF ligands can lead to autocrine stimulation and sustained Stat3 phosphorylation.

After phosphorylation, STAT3 dimerizes and translocates to the nucleus, where Stat3 dimers directly regulate gene expression of transcriptional targets including Bcl-xL, cyclin D1, c-myc, Twist and Survivin. STAT3-mediated regulation of target gene expression is involved in various cellular functions including cell differentiation, proliferation, survival, and oncogenesis. Stat3 can also act through noncanonical signaling pathways. In this regard, unphosphorylated Stat3 (U-Stat3) can drive gene expression of a subset of genes that are different from p-Stat3 dimers in an NF-κB-dependent and independent manner.

In addition, p-Stat3 Ser727 can translocate into the mitochondria and influence mitochondrial respiratory chain activity. These noncanonical Stat3 signaling pathways have protumorigenic roles in certain cell/tissue types; however their role in epithelial carcinogenesis has not been evaluated.

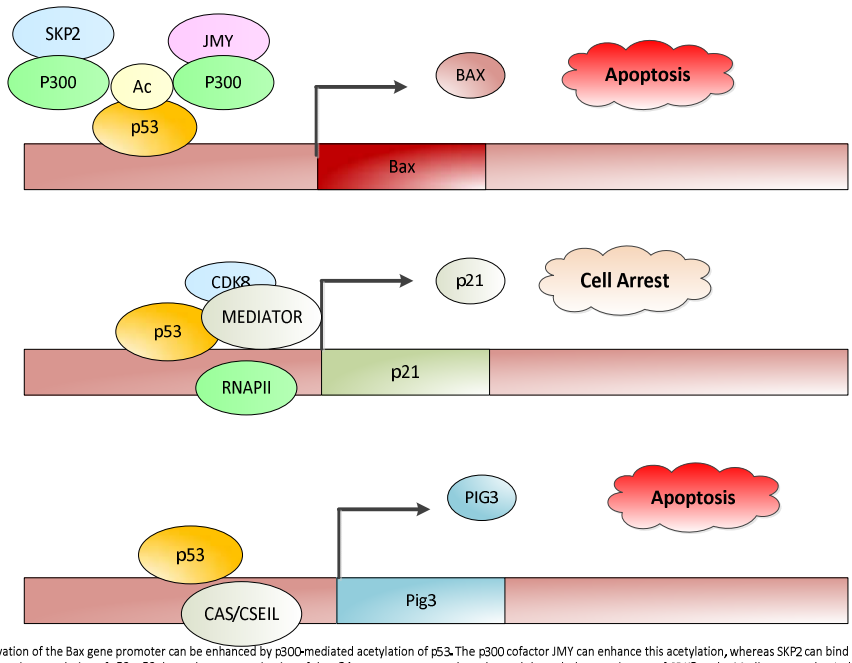
Thus the nature of STAT3 and its importance must be better investigated.

This paper by Pencik et al presents an interesting challenge to the ability to identify genetic markers for various cancers. What may at one time seem to be a problem may later be understood in a more complete fashion to be a necessary control element. To some degree we have observed this with BRAF inhibitors in melanoma, which lead to SCC and thus require a MEK inhibitor. In some sense unless a full dynamic understanding of pathways is established one may continue to see this "whack a mole" approach to therapeutics.

To reiterate the Pencik et al observations:

1. *Co-deletion of Stat3 and Pten triggers PCa*: We know that PTEN loss is found in PCa and we also know that active Stat3 is a significant factor in many malignancies. Yet the loss of both may appear as being of significance.
2. *Stat3 regulates the ARF–Mdm2–p53pathway*: This is the key observation which they articulate and stress and the main divergence from standard thought.
3. *Loss of IL-6 and Pten leads to cancer and metastasis*: We know that IL-6 drives Stat3 and that loss of IL-6 would most likely lead to a loss of Stat3 expression. As noted above loss of both Pten and Stat3 would lead to a malignant state.
4. *Loss of STAT3 and ARF in PCa is associated with metastases*: ARF is key to the ARF-MDM2 –p53 pathway. MDM2 inhibits p53. Thus the association of Stat3 being the "driver" of the ARF process is essential.

We reiterate the p53 processes as shown below. The three lead to either apoptosis or cell arrest as one would expect. In all cases p53 plays a key role but it is also clear that other proteins are required in some cases.



Activation of the Bax gene promoter can be enhanced by p300-mediated acetylation of p53. The p300 cofactor JMY can enhance this acetylation, whereas SKP2 can bind p300 and antagonize acetylation of p53. p53-dependent transactivation of the p21 gene promoter can be enhanced through the recruitment of CDK8 to the Mediator complex. Independent binding of the long-range chromatin modifier CAS/CSE1L, which is the human orthologue of yeast Cse1, to promoters, such as the Pig3 gene promoter, can also enhance p53-dependent transcription.

Pencik et al finally note:

Interestingly, loss of PTEN expression in primary human PCa did not correlate with overall survival and could not predict PCa-specific death. Moreover, heterozygous PTEN deletions far outnumber homozygous deletions in primary human PCa and we show here that PTEN is mutated or lost only in a small subset (4.7%) of a large cohort of patients with primary PCa.

However, PTEN is lost in >50% of human PCa metastases suggesting an important role for PTEN in this process. Finally, we show in our study that STAT3 is co-deleted with PTEN in 66% of human PCa metastases in two independent data sets.

Since PTEN is mutated or lost in only a minor fraction of primary PCa, other aberrations must occur (oncogene induction or loss of tumour suppressor function) to activate STAT3 and ARF to induce senescence in human cancers. Indeed, several studies indicate that different aberrations can lead to induction of senescence in human cancers

From Soissi and Wiman:

The standard classification used to define the various cancer genes confines tumor protein p53 (TP53) to the role of a tumor suppressor gene. However, it is now an indisputable fact that many p53 mutants act as oncogenic proteins.

This statement is based on multiple arguments including the mutation signature of the TP53 gene in human cancer, the various gains-of-function (GOFs) of the different p53 mutants and the heterogeneous phenotypes developed by knock-in mouse strains modeling several human TP53 mutations.

In this review, we will shatter the classical and traditional image of tumor protein p53 (TP53) as a tumor suppressor gene by emphasizing its multiple oncogenic properties that make it a potential therapeutic target that should not be underestimated.

Analysis of the data generated by the various cancer genome projects highlights the high frequency of TP53 mutations and reveals that several p53 hotspot mutants are the most common oncoprotein variants expressed in several types of tumors.

The use of Muller's classical definition of mutations based on quantitative and qualitative consequences on the protein product, such as 'amorph', 'hypomorph', 'hypermorph' 'neomorph' or 'antimorph', allows a more meaningful assessment of the consequences of cancer gene modifications, their potential clinical significance, and clearly demonstrates that the TP53 gene is an atypical cancer gene.

There is an interesting paper from CSHL on progress on cancer classification. Linnaeus some 300 years ago came up with a classification system for various species. Aristotle was driven by his desire to classify, and ever since we have people trying their best to do that task. Patients always want to know what they have, and that is a form of classification.

We classify cancers based upon organs. We may modify it based on cell types or based on cell markers such as immunological markers. I remember back in the 60s that Leukemias were simple; acute or chronic, you died now or later. Now we have a plethora of subtypes and a multiplicity of therapeutics.

But we also know genomic data. Perhaps then we should classify cancers based upon genes, not upon organs, binding proteins, or the like,

As the authors state:

Classification is an everyday instinct as well as a full-fledged scientific discipline. Throughout the history of medicine, disease classification is central to how we organize knowledge, obtain diagnosis, and assign treatment. Here we discuss the classification of cancer, the process of categorizing cancers based on their observed clinical and biological features. Traditionally, cancer nomenclature is primarily based on organ location, e.g., "lung cancer" designates a tumor originating in lung structures. Within each organ-specific major type, further subgroups can be defined based on patient age, cell type, histological grades, and sometimes molecular markers, e.g., hormonal receptor status in breast cancer, or microsatellite instability in colorectal cancer. In the past 15+ years, high-throughput technologies have generated rich new data for somatic variations in DNA, RNA, protein, or epigenomic features for many cancers. These data, representing increasingly large tumor collections, have provided not only new insights into the biological diversity of human cancers, but also exciting opportunities for discovery of new cancer subtypes.

They continue:

An ever finer classification system has many potential benefits. It is needed to capture the full spectrum of biological diversity—the "endless forms" that Darwin spoke of. It could lead to a better recognition of patient-specific disease mechanisms, and importantly, could suggest treatment options that are more accurately matched to the patient's tumor. Precision medicine, at its very foundation, relies on valid and continuously optimized disease classification that reflects the underlying mechanisms. However, a fine-grained classification system also has many potential drawbacks. The newly proposed splits may not be technically robust. Even when the finer categories are robustly supported by statistical significance and by replication, they may still lack a clear biological meaning, or have little impact on treatment options (#3 below) if it turns out that some subtypes share the same clinical endpoint, or if treatment options are limited.

Indeed, we may find it much more powerful to have a new Linnaeus type look at classification. Classifying genomically, via genes, RNA, and epigenetic factors, may help stratify and focus on therapeutics. This article raises an interesting dialog.

Overall we can make some summary observations:

1. Perhaps one should be cautious as regards to murine and human models. All too often what we see in mouse models does not pan out in human. The reasons may very well be the complexity of the signally paths.

2. Signalling paths are complex and dynamic. What may work at one instant may not at another? The question then is: how critical are realistic repeatable and predictive models in assisting in both prognostic evaluation and therapeutic approaches?

3. Cells are not the same everywhere. Thus when we perform a prostate biopsy we may get one profile but when that cell metastasizes to other organs we get dramatically different cells. As we have discussed before the paper by Gundem et al presets a compelling picture of the complexity of gene expression in PCa. Namely each cell cluster may have complex and disparate genes expressed. If that is the case then we would also be concerned that we look at similar expression when performing biopsies.

3.3.4 ERK

As Torrealba et al note:

Prostate cancer may emerge as result of dysregulated balance between cell proliferation and death rates, increased angiogenesis and chronic.

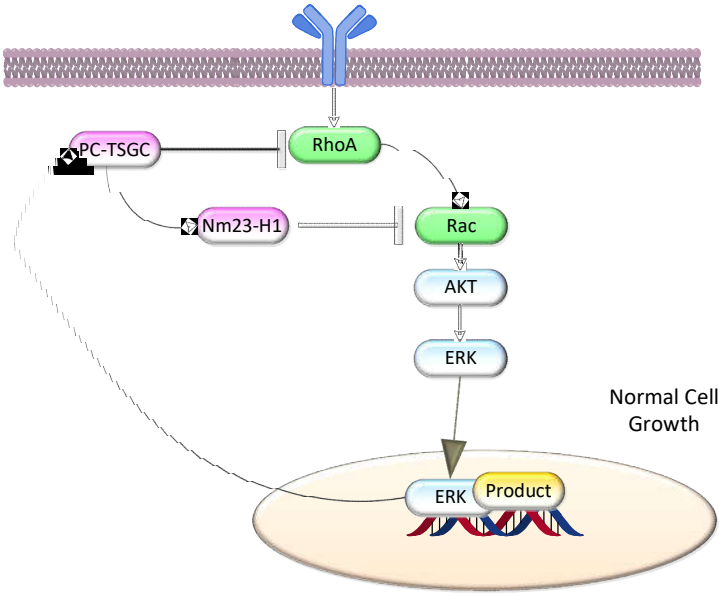
These processes are regulated by numerous signaling proteins, including the mitogen-activated protein kinases (MAPKs). JNK, p38 and extracellular signal-regulated kinase (ERK) are the three major sub-families of MAPKs. The pro-oncogenic effects of ERK isoforms (ERK1 and ERK2) lie in their aberrant activation through phosphorylation by any mutation along the pathway of receptor tyrosine kinase (RTK)-Ras-Raf-MEK-ERK1/2. Once activated, ERKs phosphorylate cytoskeletal proteins, kinases, and transcription factors.

Active ERK proteins induce strong proliferative and anti-apoptotic effects.

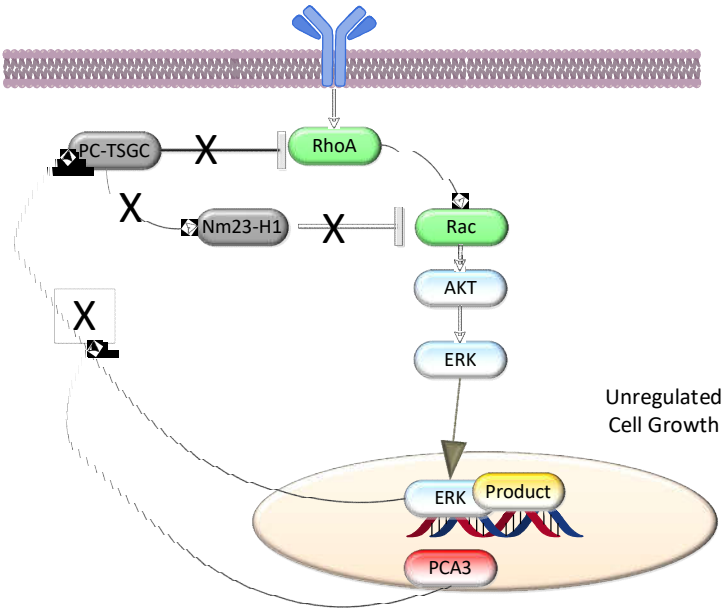
Our group has tested variations in expression, activation and localization of ERKs in human prostate. Differential ERK1/2 expression and phosphorylation status may be linked to the progression of prostate cancer. The major striking observation is that ERKs are expressed in tumors with higher proportion than normal prostate.

We believe that this is an important notion because the status (expression, localization, phosphorylation and the ERK1/ERK2 ratio) of ERK in the prostate may be developed into an important prognostic marker that predicts patient responce to the anti-cancer treatment.

We show the key paths below:



The Figure below depicts the results of path blockage resulting in unregulated growth.



3.3.5 AKT

As Shorning et al note:

AKT isoforms 1, 2, and 3 (encoded by AKT1, AKT2, and AKT3 respectively) form a subfamily of serine/threonine protein kinases that possess both overlapping and distinct cellular functions to regulate a variety of cellular processes during normal tissue homeostasis and cell transformation.

PI3K activity elevates PIP3 levels to recruit AKT to the plasma membrane where is it activated (Figure 1). AKT is activated by multiple kinases, including PDK1 and mTORC2 that phosphorylate AKT at residues Thr308 and Ser473 respectively, triggering a wave of phosphorylation through multiple downstream targets that stimulate cell survival, proliferation, metabolism and differentiation to promote tumor growth.

AKT downstream targets include PRAS40 (a component of mTORC1), BAD, FOXOs, and MDM2 (reviewed in). AKT signaling is negatively regulated by several protein phosphatases that dephosphorylate and inactivate AKT, including protein phosphatase 2 (PP2A), and PH domain and leucine-rich repeat protein phosphatase-1 and -2 (PHLPP1 and PHLPP2).

…we outline the various genetic alterations within the AKT isoforms and their regulators that have been detected in prostate cancer, and discuss their potential to activate AKT signaling and promote prostate tumor growth.

AKT Mutation and Amplification AKT genetic aberrations that increase AKT activity have been detected in multiple malignancies and are especially common in breast cancer, where AKT3 amplification and AKT1 E17K oncogenic mutation have been reported in up to 24% and 1–8% of cases respectively.

AKT1, AKT2, and AKT3 activating mutations are rare in prostate cancer (≤0.9%, predominantly in AKT1 at E17K), whereas AKT1, AKT2, and AKT3 high-level gene amplification that can increase AKT activity is more common, particularly in advanced disease.

Moreover, AKT activation in prostate cancer has been shown to positively correlate with Gleason score and invasive progression , and over-expression of myristoylated AKT (which causes constitutive AKT activation) causes prostate neoplasia in mice . In support of an oncogenic role in prostate cancer and therapeutic resistance, conditional activation of AKT in either the LNCaP human prostate cancer cells or a transgenic mouse results in increased cell proliferation and inhibits cell

3.3.6 MAPKK

As Burotto et al note:

There are four independent MAPK pathways composed of four signaling families: the MAPK/ERK family or classical pathway, and Big MAP kinase-1 (BMK-1), c-Jun Nterminal kinase (JNK), and p38 signaling families. These families share a basic organization composed of two serine/threonine kinases and one double specificity threonine/ tyrosine kinase.

Generically, these kinases are designated from upstream to downstream, closer to the nucleus, as MAPK kinase-kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. The canonical MAPK/ERK pathway is composed of three types of MAPKKK: A-RAF, B-RAF and RAF-1 or C-RAF kinases. BRAF is the gene most commonly mutated at this level in human cancer.

One level below are the MAPKKs, which are composed of MEK1 and MEK2. Finally, further downstream are ERK1 and ERK2, which are the final effectors of the MAPK pathway.

From Burotto et al,

The MAPK/ERK pathway is activated by upstream genomic events and/or activation of multiple signaling events where information coalesces at this important nodal pathway point. This pathway is tightly regulated under normal conditions by phosphatases and bidirectional communication with other pathways, such as the AKT/m-TOR pathway. Recent evidence indicates that the MAPK/ERK signaling node can function as a tumor suppressor as well as the more common prooncogenic signal.

The effect that predominates depends on the intensity of the signal and the context or tissue in which the signal is aberrantly activated. Genomic profiling of tumors has revealed common mutations in MAPK/ERK pathway components, such as BRAF. Currently approved for the

treatment of melanoma, inhibitors of B-RAF kinase (BRAFi) are being studied alone and in combination with inhibitors of the MAPK and other pathways to optimize treatment of many tumor types.

Therapies targeted toward MAPK/ERK components have variable response rates when used in different solid tumors, such as colorectal cancer and ovarian cancer.

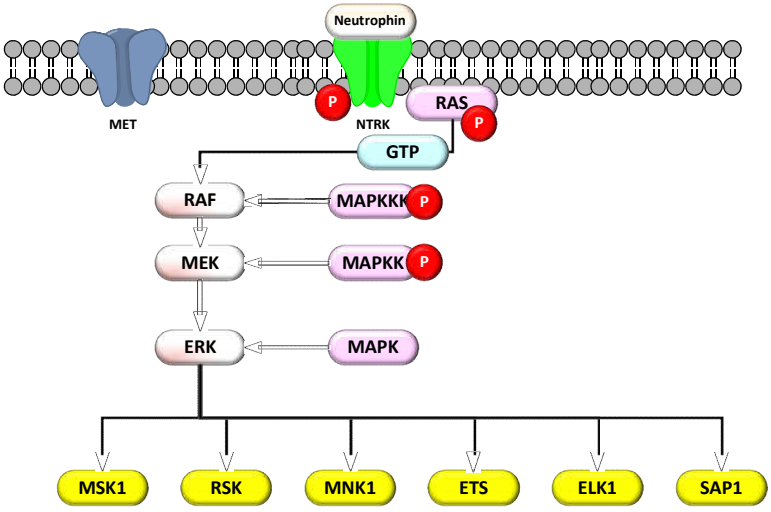
Understanding the differential nature of activation of the MAPK/ERK pathway in each tumor type is critical in developing single and combination regimens, as different tumors have unique mechanisms of primary and secondary signaling and subsequent sensitivity to drugs. …

There are four independent MAPK pathways composed of four signaling families: the MAPK/ERK family or classical pathway, and Big MAP kinase-1 (BMK-1), c-Jun Nterminal kinase (JNK), and p38 signaling families.

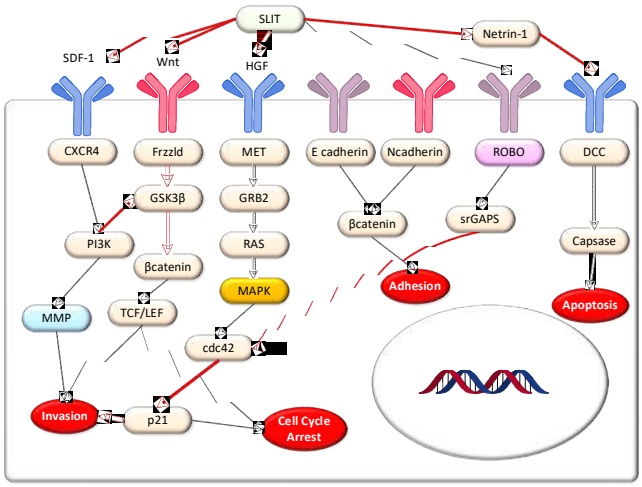
These families share a basic organization composed of two serine/threonine kinases and one double specificity threonine/ tyrosine kinase. Generically, these kinases are designated from upstream to downstream, closer to the nucleus, as MAPK kinase-kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK. The canonical MAPK/ERK pathway is composed of three types of MAPKKK: A-RAF, B-RAF and RAF-1 or C-RAF kinases.

BRAF is the gene most commonly mutated at this level in human cancer. One level below are the MAPKKs, which are composed of MEK1 and MEK2. Finally, further downstream are ERK1 and ERK2, which are the final effectors of the MAPK pathway….

The figure below shows the MAPKKK element in its pathway.

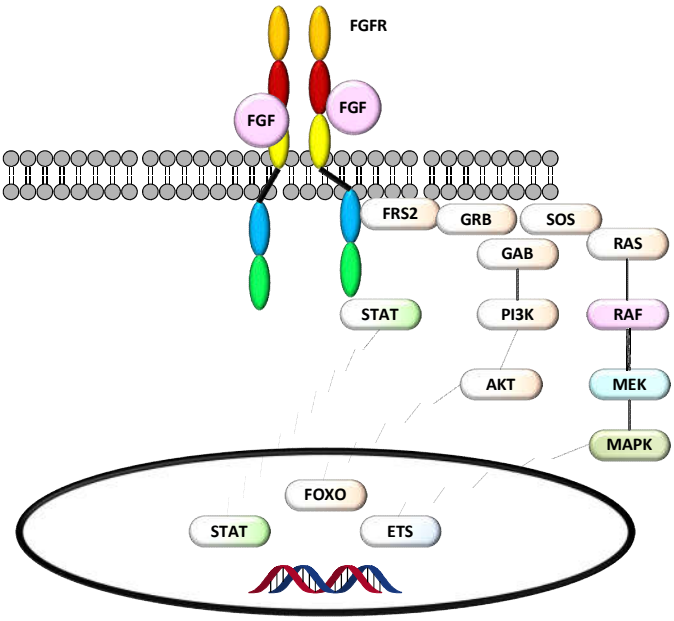


The effects of the various pathways are shown below. The insertion of MAPK and its derivatives play a significant role in invasion and cell cycle control.



From: Dickinson and Duncan

The figure below incorporates details regarding receptors, here FGFR and ligand FGF. They un turn activate MAK and derivatives.



3.3.7 IL-6

As Pencik et al note:

Interleukin-6 (IL-6) is a multifunctional cytokine that is implicated in the regulation of immune responses, inflammation and cellular processes in several cancers, including cancer of the prostate.

IL-6 is detectable in stromal cells, but preferentially localised in the epithelium of prostate tissue. The IL-6 receptor displays a highly restricted expression pattern including hepatocytes, leucocyte subsets and megakaryocytes, but is also ubiquitously expressed in prostate cancer cells . In benign prostatic tissue IL-6 expression is confined to the basal cells of the epithelium. In particular, the androgen receptor-negative human prostate cancer cell lines (DU-145 and PC3) express high levels of IL-6 . Whether this is a result of a direct mechanism involving the androgen receptor remains unknown. IL-6 expression is governed by nuclear factor kappa B, which is suppressed by treatment with androgenic hormones and may otherwise result in an aberrant activation of the androgen receptor …,

IL-6 expression levels are high in the tissue of prostate cancer patients after radical prostatectomy, as well as in sera of patients with advanced prostate cancer that is resistant to therapy. IL-6 levels are upregulated by transforming growth factor-beta (TGF-β) as well, which is an important determinant of metastatic transformation .

Thus, IL-6 signal transduction is important for regulating cellular processes in prostate cancer. Studies with primary cells also demonstrated that there is a positive growth effect of IL-6 in such a condition….

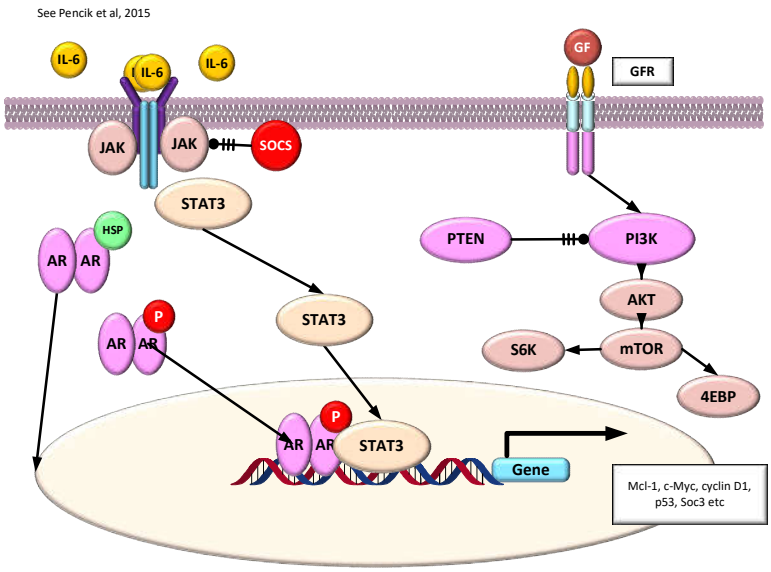
They then note the dynamics as follows:

Overview of IL-6/STAT3/ARF and PI3K/PTEN/AKT/mTOR pathways.

Activation of IL-6/STAT3 signalling leads to phosphorylation and translocation of STAT3 to the nucleus, which is associated with AR interaction regulated by HSP.

JAKs serve to phosphorylate tyrosine (Y) or serine (S) residues of STAT3 and to translocate to nucleus or mitochondrial matrix. Some of these downstream signalling events (STAT3-AR-S6K) could regulate activity or expression of prostate cancer related genes. ARF = alternative reading frame protein; IL-6 = interleukin-6; PI3K = phosphatidylinositol-3-kinase; PTEN = phosphatase and tensin homologue; STAT3 = signal transducer and activator of transcription-3…

as shown below:



As Neuwirt et al noted:

Prostate cancer initiation and progression strongly depend on activation of the AR, but chronic inflammation of the prostate may also play an important role.

Therefore, it is not surprising that the role of the proinflammatory cytokine interleukin-6 (IL-6) in prostate carcinogenesis has received a considerable interest. IL-6 is a multifunctional cytokine that acts in a cell type-specific manner through activation of signaling pathways of Janus kinases/signal transducer and activator of transcription factors (STAT), mitogen-activated protein kinases, and/or phosphotidylinositol 3-kinase.

In prostate cancer cells, either pro-differentiation or survival effects of IL-6 have been described. The mechanisms responsible for differential activation of IL-6 signaling pathways in prostate tumor cells are being investigated. It is assumed that various regulators of phosphorylation of STAT3, in particular suppressors of cytokine signaling ( ) and protein inhibitors of activated STAT, determine activation status of this transcription factor. The family comprises eight members, 1 through 7 and CIS.3 family members share the central Src homology 2 domain and box in the carboxy-terminal end, which plays a crucial role in proteasomal degradation of binding partners. -1 and -3 contain a kinase inhibitory region, which has a pivotal function in antagonizing activation of Janus kinases.

3.3.8 IL-17

As Kuen et al note:

IL-17 is produced by RAR-related orphan receptor gamma t (RORγt)-expressing cells including Th17 cells, subsets of γδT cells and innate lymphoid cells (ILCs).

The biologicalsignificance of IL-17-producing cells is well-studied in contexts of inflammation,autoimmunity and host defense against infection. While most of available studies in tumorimmunity mainly focused on the role of T-bet-expressing cells, including cytotoxic CD8+T cells and NK cells, and their exhaustion status, the role of IL-17-producing cells remainspoorly understood.

While IL-17-producing T-cells were shown to be anti-tumorigenic inadoptive T-cell therapy settings, mice deficient in type 17 genes suggest a protumorigenicpotential of IL-17-producing cells. This review discusses the features of IL-17-producingcells, of both lymphocytic and myeloid origins, as well as their suggested pro- and/or antitumorigenic functions in an organ-dependent context. Potential therapeutic approachestargeting these cells in the tumor microenvironment will also be discussed…

The biologicalsignificance of IL-17-producing cells is well-studied in contexts of inflammation,autoimmunity and host defense against infection. While most of available studies in tumorimmunity mainly focused on the role of T-bet-expressing cells, including cytotoxic CD8+T cells and NK cells, and their exhaustion status, the role of IL-17-producing cells remainspoorly understood.

Potential therapeutic approaches targeting these cells in the tumor microenvironment will also be discussed granulocytic in nature in squamous cervical cancers, and associated with poor survival.

In addition, IL-17-expressing cells were independently associated with poor survival in early stage of the disease . IL-17 producing mast cells in esophageal squamous cell carcinoma were found to be densely located in the muscularis propria, and were suggested to function in the recruitment of effector CTLs and M1 macrophages to the site of tumor, thus acting as a favorable prognostic factor…

Now Zhang et al (2012) noted:

The contributions of interleukin (IL)-17 to cancer remain unclear and somewhat controversial.

We took a genetic approach to explore its role in prostate cancers by interbreeding IL-17 receptor C (IL-17RC)–deficient mice with mice that are conditionally mutant for PTEN, one established preclinical model for prostate cancer. Mice that were IL-17RC–deficient (IL-17RC) displayed prostates that were smaller than mice that maintained IL-17RC expression (IL-17RCþ).

In addition, IL-17RC mice developed a reduced number of invasive prostate adenocarcinomas with lower rates of cellular proliferation and higher apoptosis than IL-17RCþ mice. Moreover, the fibromuscular stroma surrounding prostatic glands was relatively thicker in IL-17RC mice and was associated with decreased matrix metalloproteinase (Mmp)7 expression and increased

Timp1, 2, and 4 expression, whereas administration of recombinant mouse IL-17 induced prostatic expression of Mmp7.

Taken together, our results suggested that IL-17 promotes the formation and growth of prostate adenocarcinoma, and that an IL-17–MMP7 signaling axis is required for the transition of prostatic intraepithelial neoplasia to frank adenocarcinoma.

Zhang et al (2017) then noted:

Chronic inflammation has been associated with a variety of human cancers. Approximately 15% of all human cancers have been suggested to result from infection and chronic inflammation.1 Almost all surgical prostate specimens contain evidence of inflammation.

Chronic inflammation invokes proliferative inflammatory atrophy of prostate – a potential precursor lesion to prostatic intraepithelial neoplasia (PIN) and carcinoma. The cause of prostatic inflammation includes infection, urine reflux, diet, estrogen, and physical trauma. Inflammation is a complex response involving many immune cells, chemokines, and cytokines as well as matrix-degrading enzymes.

Interleukin-17 (IL-17, also named IL-17A) is a key pro-inflammatory cytokine that plays critical roles in many inflammatory and autoimmune diseases. IL-17 has been demonstrated to promote development of colon cancer, skin cancer, breast cancer, prostate cancer, lung cancer, and pancreas cancer. IL-17 is secreted by T helper 17 (TH17) cells, γδ T cells, natural killer cells, and other immune cells.

IL-17 acts on IL-17RA/ IL-17RC receptor complex to recruit nuclear factor-κB (NF-κB) activator 1 (Act1). Act1 activates tumor necrosis factor receptor-associated factor 6 (TRAF6),2and subsequently activates transforming growth factor-β-activated kinase 1 (TAK1) and IκB kinase (IKK) complex, resulting in activation of NF-κB pathway that initiates transcription of a variety of chemokines and cytokines, such as C-X-C motif ligand 1 (CXCL1), C-C motif ligand 20 (CCL20), IL-1β, and IL-6.

These IL-17-downstream factors promote cancer formation through increased cellular proliferation, attenuated apoptosis, and sustained angiogenesis, as well as creation of an immunotolerant microenvironment. …

We have previously generated an IL-17 receptor C (Il-17rc) and prostate-specific conditional phosphatase and tensin homolog (Pten) double knockout (KO) mouse model. IL-17RCdeficient (IL17-RC− or RC−) mice display smaller prostates and develop a reduced number of invasive prostate adenocarcinomas, compared to IL-17RC-sufficient (IL17-RC+ or RC+) mice.

Further, matrix metalloproteinase 7 (MMP7) expression is increased in RC+ mice compared to RC−mice. However, whether MMP7 mediates IL-17's action and the underlying molecular mechanisms remain unknown. MMP7 (also known as putative metalloproteinase I or matrilysin) is exclusively expressed in the epithelial cells.

MMP7 is overexpressed in human prostate cancer, but not expressed in normal prostate glands. Here, we investigated the role of MMP7 in mediating IL-17's action, using an Mmp7 and Pten double KO mouse model. Our findings demonstrate that MMP7 mediates IL-17's function in promoting prostate carcinogenesis through induction of epithelial-tomesenchymal transition (EMT) …

E-cadherin interacts with a β-catenin-based complex to act on actin cytoskeleton and mediate adhesion-dependent signaling, and several proteinases including MMP7 are known to be able to cleave E-cadherin. Thus, we tested if MMP7 could cleave E-cadherin in three human prostate cancer cell lines. …

Together, these results suggested that MMP7 cleaved E-cadherin to release β-catenin from E-cadherin/β-catenin complex, leading to nuclear translocation of β-catenin and subsequently activation of downstream transcription factors Snail and Slug, hence inducing EMT

3.3.9 EGF

The epidermal growth factor, EGF, is another GF associated with malignancies. *As NCBI* notes[11]:

This gene encodes a member of the epidermal growth factor superfamily. The encoded preproprotein is proteolytically processed to generate the 53-amino acid epidermal growth factor peptide. This protein acts a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of numerous cell types. This protein acts by binding with high affinity to the cell surface receptor, epidermal growth factor receptor. Defects in this gene are the cause of hypomagnesemia type 4.

Dysregulation of this gene has been associated with the growth and progression of certain cancers. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed.

3.3.9.1 EGF Functions

We begin with a simple overview of the EGF functions. As Singh et al note:

EGF is the prototypic and founding member of the EGFR ligand family, first identified from submaxillary gland extracts during nerve growth factor studies. The EGF-EGFR ligand-receptor system has greatly enhanced our understanding of receptor tyrosine kinase signaling, as evidenced by more than 70,000 publications for EGF alone. A recent review has distilled our current understanding of EGF and its actions.

More recently, a study uncovered that EGF-induced EGFR signaling enhances production of intracellular reactive oxygen species (ROS) by dual oxidase 1 (DUOX1) This nicely complements earlier studies in which ROS were shown to enhance EGFR signaling by

[11] https://www.ncbi.nlm.nih.gov/gene/1950

modulating both positive and negative regulators of EGFR signaling (ADAMs and protein tyrosine phosphatases). In another recent study, urinary EGF has been shown to be an independent risk factor for progression of chronic kidney disease, substantiating earlier findings.

They then note its functioning:

Modes of signaling via epidermal growth factor receptor (EGFR) ligands.

Autocrine signaling occurs when a ligand is released from a cell and binds to EGFR on that same cell.

Paracrine signaling refers to the released ligand acting on a nearby cell, usually a different cell type.

Juxtacrine signaling occurs when a non-cleaved, transmembrane ligand binds to EGFR on an adjacent cell; this is best documented for heparin-binding epidermal growth factor-like growth factor (HBEGF). Amphiregulin (AREG), transforming growth factor-alpha (TGFA), and HBEGF, as well as EGFR, can be packaged into signaling competent exosomes. Uptake of exosomal AREG by recipient cells is, at least in part, dependent on EGFR, leading to the term exosomal targeted receptor activation (ExTRAcrine).

ExTRAcrine signaling has features of autocrine, paracrine, and juxtacrine signaling as well as possibly endocrine signaling since EGFR and AREG can be detected in human plasma exosome.

3.3.9.2 EGF and Cancer

Relationships between EGF and cancers are significant. From Yang et al we have the following:

EGF and its receptor (EGFR) have been associated with tumour cell invasion and metastasis initiation.

Dysregulation of EGFR signalling, including receptor over expression and/or activation has been shown to be a significant effector in the progression of human cancers including neoplasms of the brain, lung, breast, ovary, prostate, and pancreas.

A recent study investigated the relationship between EGFR and the adhesion molecule-integrin in human pancreatic carcinoma cells and demonstrated that the crosstalk between EGFR signalling and integrin in the cancer cell membrane is implicated in carcinoma cell invasion and metastasis. Integrins are a family of adhesion proteins that regulate cell migration.

The fact that EGF stimulated integrins-mediated carcinoma cell migration on vitronectin suggests that EGFR regulates cancer cell migration through the adhesion proteins, the integrins. EGFR inhibitors, such as erlotinib, provide clinical benefit in patients with advanced non-small cell lung cancer metastasis which suggests a critical role for EGF and its receptor in the initial steps of cancer metastasis. The mechanism of EGF activation of adhesion proteins in cancer cell remains to be elucidated.

Some studies indicate EGF induces tumour cell invasion and metastasis through de-phosphorylation and downregulation of focal adhesion kinase, while other studies suggest EGFR activates the Src family of kinases (SFK). The fact that activated Src kinase is involved in the rearrangement of the actin cytoskeleton, cell-matrix interactions, and cell-cell adhesion processes that promote cell invasion suggests a role for Src activity in tumour metastasis development.

Added insight is provided by Mendelsohn and Baselga who note:

Human carcinomas frequently express high levels of receptors in the EGF receptor family, and overexpression of at least two of these receptors, the EGF receptor (EGFr) and closely related ErbB2, has been associated with a more aggressive clinical behavior. Further, transfection or activation of high levels of these two receptors in nonmalignant cell lines can lead to a transformed phenotype. For these reasons therapies directed at preventing the function of these receptors have the potential to be useful anti-cancer treatments. In the last two decades monoclonal antibodies (MAbs) which block activation of the EGFr and ErbB2 have been developed.

These MAbs have shown promising preclinical activity and `chimeric' and `humanized' MAbs have been produced in order to obviate the problem of host immune reactions. Clinical activity with these antibodies has been documented: trastuzumab, a humanized anti-ErbB2 MAb, is active and was recently approved in combination with paclitaxel for the therapy of patients with metastatic ErbB2-overexpressing breast cancer; IMC- C225, a chimeric anti-EGFr MAb, has shown impressive activity when combined with radiation therapy and reverses resistance to chemotherapy. In addition to antibodies, compounds that directly inhibit receptor tyrosine kinases have shown preclinical activity and early clinical activity has been reported. A series of phase III studies with these antibodies and direct tyrosine kinase inhibitors are ongoing or planned, and will further address the role of these active anti-receptor agents in the treatment of patients with cancer.

Finally from Calderon and Prins[12]:

Epidermal growth factor (Egf), a secreted peptide, is produced by the luminal epithelial cells in the prostate, and is found at the highest concentration in human prostatic secretions compared to the rest of the body. Epidermal growth factor exerts its effects by binding to its tyrosine kinase receptor, epidermal growth factor receptor (EgfR).

Upon binding, EgfR can homo- or heterodimerize with erbB2 receptors, causing autophosphorylation of its tyrosine residues that in turn activate the phosphatidylinositol 3'-kinase (PI3K), mitogen activated protein kinase (MAPK), or phospholipase C-γ (PLC-γ) signaling cascades. In the developing murine prostate gland, Egf has been shown to mediate its actions through the PLC-γ signaling pathway.

[12] https://www.sciencedirect.com/science/article/pii/B9780128126363000055

Furthermore, rat UGS explants treated with exogenous Egf showed stimulation of prostate bud formation in the absence of androgens, thus positively regulating prostatic budding.

3.3.10 IGF

The IGF and the IGFR, and its respective sub-elements, are major factors in many malignancies. For example as noted in NCBI:

IGF1[13]: The protein encoded by this gene is similar to insulin in function and structure and is a member of a family of proteins involved in mediating growth and development. The encoded protein is processed from a precursor, bound by a specific receptor, and secreted. Defects in this gene are a cause of insulin-like growth factor I deficiency. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar processing to generate mature protein.

IGF1R[14]: This receptor binds insulin-like growth factor with a high affinity. It has tyrosine kinase activity. The insulin-like growth factor I receptor plays a critical role in transformation events. Cleavage of the precursor generates alpha and beta subunits. It is highly overexpressed in most malignant tissues where it functions as an anti-apoptotic agent by enhancing cell survival. Alternatively spliced transcript variants encoding distinct isoforms have been found for this gene.

There has been a great deal of study of the IGF and its constituents[15].

3.3.10.1 IGF Overview

The insulin growth factor is a key element in glucose control. Spravchikov et al have discussed the impact of poor glucose management on skin keratinocytes. This discussion is critical in trying to understand the role of the IGF and glucose on cancer initiation and progression. Thus it is worth a mild digression to understand their findings.

They note:

Glucose is known to affect insulin action as well by regulating the expression of several genes, including the IGF-I receptor (IGFR) and insulin receptor (IR) genes, at both the transcriptional and translational levels.

Moreover, hyperglycemia was shown to inhibit insulin action.

[13] https://www.ncbi.nlm.nih.gov/gene/3479

[14] https://www.ncbi.nlm.nih.gov/gene/3480

[15] https://www.sciencedirect.com/topics/neuroscience/insulin-like-growth-factor-1

This inhibition is thought to be a result of serine phosphorylation through a PKCmediated mechanism as well as by activation of protein tyrosine phosphatases, which deactivates the IR function. In addition to its possible involvement in the development of complications of chronic diabetes, glucose was shown to downregulate its own transport and metabolism.

As a result, high glucose levels create a vicious cycle in which even less glucose enters the cells, resulting in increased blood glucose levels, which in turn further disrupt the transport and metabolism of glucose into the cells.

It is therefore clear that glucose per se, either directly or via changes in insulin signaling, is an important factor in both the regulation of its own transport and metabolism and in the pathogenesis of chronic complications of diabetes… Glucose inhibits the phosphorylation of the IGFR.

We have shown so far that exposure of keratinocytes to high glucose concentrations, mimicking the hyperglycemic state, has effects on skin cells, resulting in inhibition of proliferation and an abnormal differentiation process. However, in diabetic patients, development of hyperglycemia also results in changes in insulin and IGF-I signaling….

As mentioned earlier, another effect of insulin and IGF-I on keratinocytes is an increase in cellular proliferation . Therefore, we evaluated the proliferation rate of keratinocytes in response to chronic insulin or IGF-I stimulation in the presence of 2 or 20 mmol/l D-glucose. … both insulin and IGF-I induced an increase in the proliferation rate of the cells (142 and 155% above control, respectively). However, in the presence of high glucose concentrations, the effects of both hormones—but mainly of IGF-I—were reduced (129 and 123% above control, respectively). Glucose effects were specific, as there was no effect on the activity of keratinocyte growth factor on glucose transport…

We have previously shown that in skin keratinocytes, IR and IGFR have different roles in skin proliferation that are mediated via distinct signaling pathways. In addition, we have shown in the present study that high glucose levels, in the absence of any additional perturbation, are associated with decreased cellular proliferation. Thus, glucose inhibits proliferation by both direct effects as well as by reducing the stimulatory effect of IGF-I on proliferation. In conclusion, the consequence of high glucose inhibition on the proliferation of skin keratinocytes and its enhancement of their differentiation is obvious.

By changing the proliferation-differentiation balance, which is one of the essential steps in the healing process, as well as by decreasing other possible local effects of IGF-I on wound healing, high glucose levels might indeed contribute to impaired wound healing in diabetes.

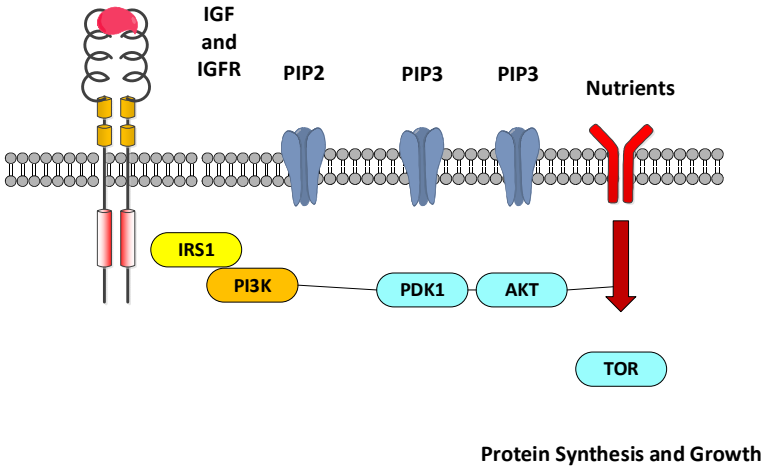
From Yang et al:

The insulin-like growth factor system consists of two ligands (IGF-I and -II), two main receptors (IGF-IR and IGFIIR), six different IGF binding proteins (IGFBP1- 6) and four IGFBP related peptides (IGFBP Rp1-4). The IGF ligands have a short life-span unless they are bound to a

binding protein which transports them in the circulation and delivers them to specific tissues. Components of the IGF system are found throughout the body in various fluids and tissues . IGFs act on a variety of mammalian cells in an endocrine, paracrine and autocrine manner to regulate cell proliferation, apoptosis, transformation and differentiation . They influence the growth of normal tissue as well as that of several cancers.

Converging data from clinical and laboratory studies clearly indicate that IGF-I is implicated in cancer cell migration and invasion. IGF-I receptor (IGF-IR) expression is correlated with colorectal cancer venous invasion and liver metastasis, and has been proposed as a predictor of liver metastasis from colorectal cancer . Blockade of the paracrine action of IGF-I can suppress liver metastases from colorectal cancer . It has been established that IGF-IR and the integrins interact together to form a complex at the colon cell-cell contact sites, whilst addition of IGF-I to this complex causes integrin redistribution within the cell-cell contact site and is associated with an increase in the migration of colorectal cancer cells.

From Morgan we have the putative interaction of IGF with the IGFR and the resulting cell reaction as shown below[16]:



3.3.10.2 IGF Cancers

Excess activation of IGF has been linked to a variety of cancers. As Murekatete et al have noted regarding melanomas:

Insulin-like growth factor (IGF)-I binds to the ECM protein vitronectin (VN) through IGF binding proteins (IGFBPs) to enhance proliferation and migration of skin keratinocytes and fibroblasts. Although evidence exists for the role of individual components of the complex (IGF-I, IGFBP-3 and VN), the cellular functions stimulated by these proteins together as a complex remains un-investigated in melanoma cells. We report here that the IGF-I:IGFBP-3:VN trimeric

[16] See Morgan, p 216

complex stimulates a dose dependent increase in the proliferation and migration of WM35 and Sk-MEL28 melanoma cells.

In 3D Matrigel™ and hydrogel cultures, both cell lines formed primary tumor-like spheroids, which increased in size in a dose-dependent manner in response to the trimeric complex. Furthermore, we reveal IGFBP- 3:VN protein complexes in malignant melanoma and squamous cell carcinoma patient tissues, where the IGFBP-3:VN complex was seen to be predominantly tumor cell-associated. Peptide antagonists designed to target the binding of IGF-I:IGFBP-3 to VN were demonstrated to inhibit IGF-I:IGFBP- 3:VN-stimulated cell migration, invasion and 3D tumor cell growth of melanoma cells. Overall, this study provides new data on IGF:ECM interactions in skin malignancies and demonstrates the potential usefulness of a growth factor:ECM-disrupting strategy for abrogating tumor progression.

They continue:

The high mortality rate of melanoma is associated with the metastasis of malignant melanoma cells to critical organs of the body1. Insulin-like growth factor-I (IGF-I), amongst others, is known to enhance tumor growth and invasion. IGF-I can act as a paracrine factor that drives malignant cell transformation through the activation of the IGF type-I receptor (IGF-IR). All melanocytic cells express the IGF-IR, with increased expression correlated with disease progression.

In addition, growth factor interactions with the extracellular matrix (ECM) play important roles in tumor biology, facilitating tumor cell attachment, proliferation and invasion, and resistance against chemotherapeutic drugs. Proteins in the IGF system have been shown to interact with ECM proteins such as fibronectin (FN), vitronectin (VN), laminins, as well as integrins, which in turn, modulate the function of IGF-I9. Previous studies have demonstrated that IGF-I interacts with VN through IGFBPs to form IGF-I:IGFBP:VN trimeric (TRI) complexes11. Further, IGFBP:VN complexes have been observed in tumor biopsies from breast cancer patients, associating with the invasive front of tumor clusters and around tumor blood vessels.

This is aligned with the concept that VN is a matricellular protein that functions as a scaffold onto which growth factors, such as IGF-I, are captured, exposing cells to concentrated foci of growth factors available for receptor stimulation. Indeed, complexes of TRI have been shown to promote enhanced cell attachment and migration, as well as protein synthesis, in human keratinocytes14 and breast cancer cell lines

3.3.11 ERG

ERG has been considered a master transcription factor[17]. As Kish et al note:

The ETS-related gene (ERG) is proto-oncogene that is classified as a member of the ETS transcription factor family, which has been found to be consistently overexpressed in about half of the patients with clinically significant prostate cancer (PCa). The overexpression of ERG can

[17] https://www.researchgate.net/publication/340539918_ERG_A_Master_Transcription_Factor

mostly be attributed to the fusion of the ERG and transmembrane serine protease 2 (TMPRSS2) genes, and this fusion is estimated to represent about 85% of all gene fusions observed in prostate cancer. Clinically, individuals with ERG gene fusion are mostly documented to have advanced tumor stages, increased mortality, and higher rates of metastasis in non-surgical cohorts.

In the current review, we elucidate ERG's molecular interaction with downstream genes and the pathways associated with PCa. Studies have documented that ERG plays a central role in PCa progression due to its ability to enhance tumor growth by promoting inflammatory and angiogenic responses.

ERG has also been implicated in the epithelial–mesenchymal transition (EMT) in PCa cells, which increases the ability of cancer cells to metastasize. In vivo, research has demonstrated that higher levels of ERG expression are involved with nuclear pleomorphism that prompts hyperplasia and the loss of cell polarity …

In prostate cancer cells, a surprisingly common occurrence involves the fusion of ERG to TMPRSS2, which forms the fusion product of TMPRSS2-ERG. The most common mechanism by which these two genes fuse involves the deletion of intronic sequences on the long arm of chromosome 21 via an intron deletion between TMPRSS2 and ERG on chromosome 21q22.2-3 (Figure 1). This fusion mechanism has been identified as being prevalent in approximately 50% of prostate cancer patients .

The frequent occurrence of this fusion protein can be attributed to the presence of a homogenous deletion site that is present between ERG and TMPRSS2 . Moreover, this deletion site is separated into two different classifications according to various start sites. In both of the deletion products, the 50 end of the TMPRSS2 gene has been ligated to the 30 end of ERG. TMPRSS2-ERG fusion results in ERG overexpression due to the androgen responsive promoter of the TMPSS2 gene allowing for the constitutive transcription of ERG, which has been shown to be correlated with increased cell proliferation, cell invasion, angiogenesis, and invasiveness in PCa cells.

In addition, this TMPRSS2-ERG fusion enhances the transcription and activates downstream oncogenes

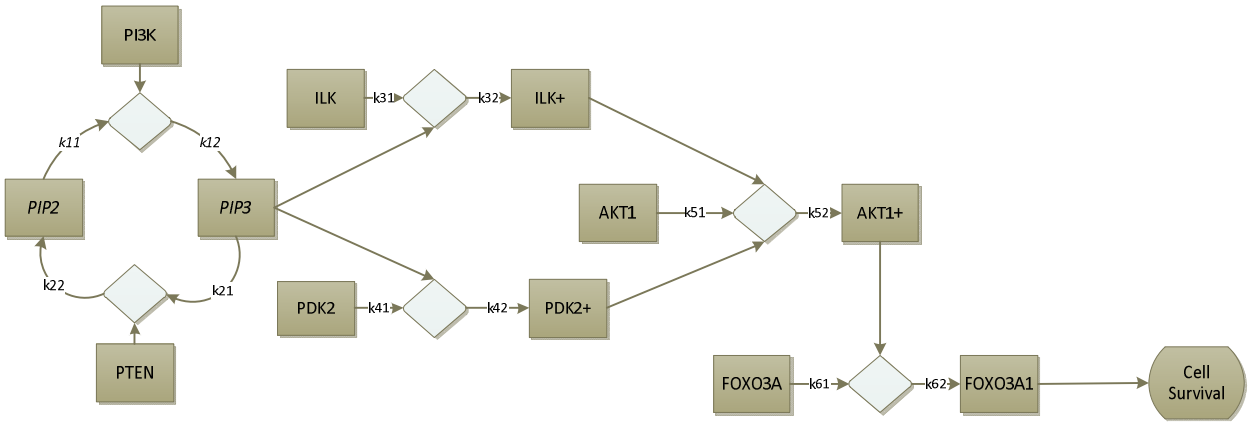
3.3.12 FOXO

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.

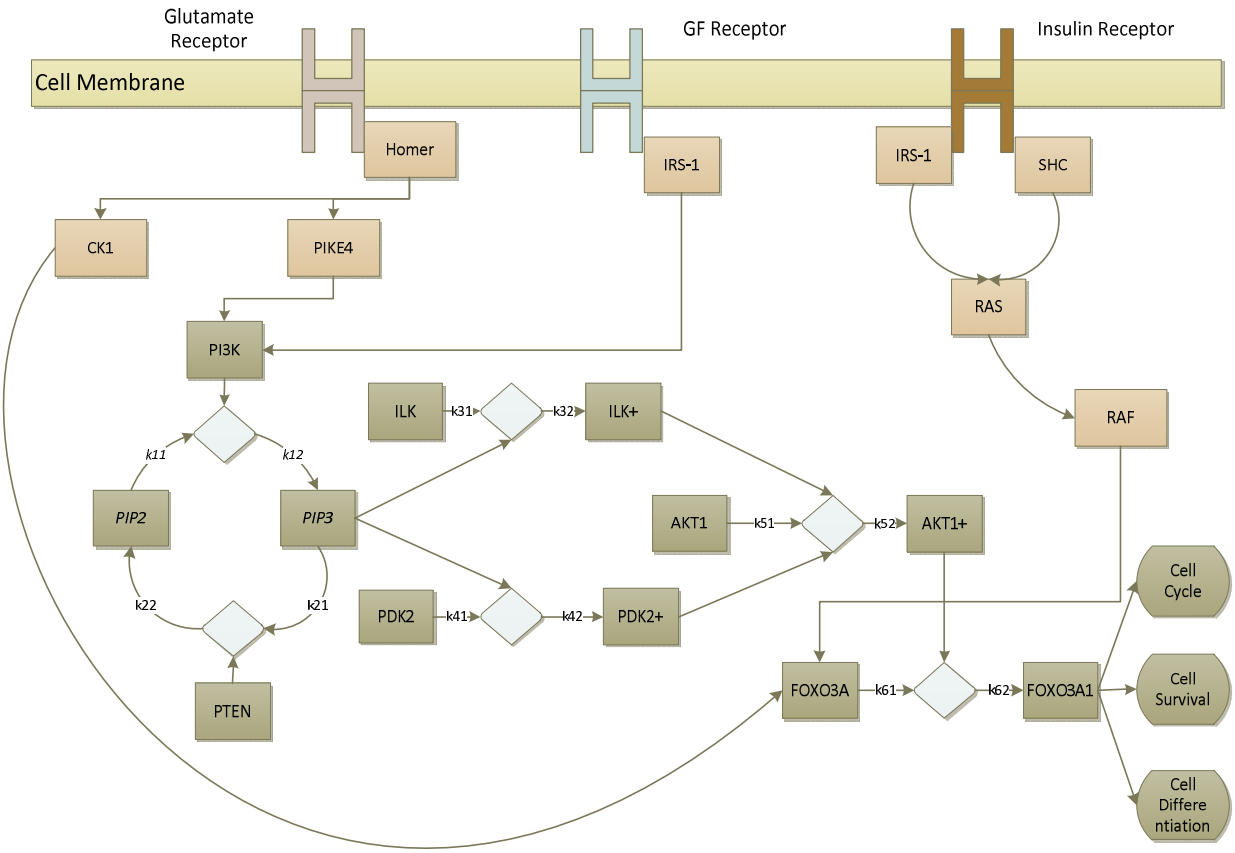
As noted by van der Heide et al, FOXO is a major player in pathways activated by Glutamate and insulin. We will depict that detail later. However the nexus to the insulin activator may also provide a connection to the role that inflammation may have in PCa and especially Type 2 Diabetes and its related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

3.3.13 SOCS1

As Neuwirt et al note:

Suppressor of cytokine signaling (SOCS) proteins play a pivotal role in the development and progression of various cancers.

We have previously shown that SOCS-3 is expressed in prostate cancer, and its expression is inversely correlated with activation of signal transducer and activator of transcription factor 3.

We hypothesized that SOCS-1, if expressed in prostate cancer cells, has a growth-regulatory role in this malignancy.

The presence of both SOCS-1 mRNA and protein was detected in all tested cell lines.

To assess SOCS-1 expression levels in vivo, we analyzed tissue microarrays and found a high percentage of positive cells in both prostate intraepithelial neoplasias and cancers. SOCS-1 expression levels decreased in samples taken from patients undergoing hormonal therapy but increased in specimens from patients who failed therapy. In LNCaP-interleukin-6 prostate cancer cells, SOCS-1 was up-regulated by interleukin-6 and in PC3-AR cells by androgens; such up-regulation was also found to significantly impair cell proliferation.

To corroborate these findings, we used a specific small interfering RNA against SOCS-1 and blocked expression of the protein. Down-regulation of SOCS-1 expression caused a potent growth stimulation of PC3, DU-145, and LNCaP-interleukin-6 cells that was associated with the

increased expression levels of cyclins D1 and E as well as cyclin-dependent kinases 2 and 4. In summary, we show that SOCS-1 is expressed in prostate cancer both in vitro and in vivo and acts as a negative growth regulator. (Am J Pathol 2009, 174:1921–1930; DOI: 10.2353/ajpath.2009.080751) Prostate cancer is the second most common cause of tumor-related deaths in the Western world. Although localized tumors can be successfully treated with surgery or radiotherapy, clinically approved therapy for advanced prostate cancer is limited to androgen ablation, blockade of the androgen receptor (AR) or chemotherapy.

Recent modest improvements in chemotherapy have been achieved with the anti-microtubule agent docetaxel …

The role of SOCS-1 and -3 in carcinogenesis is of interest since it was shown by several groups that their expression may be altered in head and neck cancer, gastric carcinoma, chronic myeloid leukemia, melanoma, or prostate cancer.

There is an increasing evidence showing that SOCS have different functions depending on the origin of the tumor. Tannapfel and colleagues have shown that methylation-dependent silencing of the SOCS-1/3 genes in head and neck squamous cell and Barretts adenocarcinoma is associated with tumor growth in vitro and in vivo. On the other hand, it was demonstrated that SOCS-1 is constitutively expressed in patients with chronic myeloid leukemia or in human melanoma.8 Our previous studies revealed that SOCS-3 is increasingly expressed in prostate cancer and can exert inhibitory effects on induction of apoptosis by cAMP.

Other researchers have reported that SOCS-1 can also act as an inhibitor of phosphorylation of STAT In particular, IL-4 and IL-13 stimulate expression of SOCS-1 in keratinocytes, which in turn inhibits phosphorylation of STAT3. The two cytokines receptors were detected in prostate cells. Furthermore, in breast cancer a N-Myc downstream-regulated gene can induce SOCS-1, which negatively regulates STAT3 activation. Thus, we have asked whether SOCS-1 is expressed in prostate cancer cell lines and patient samples and what impact it has on tumor cell proliferation

3.4 FACILITATORS

There are a wide variety of facilitators. We start with the ARF-MDM2-p53 axis.

3.4.1 ARF-MDM2-p53 Axis

Let us now review what is understood about the ARF-MDM2-p53 pathway. This will be necessary before linking this pathway to STAT3 and its functions.

Now this is a classic pathway whose ultimate control mechanism is p53 expression. p53 is generally understood to be a control gene, keeping the cell in some homeostasis and preventing malignancy. As we will not later this may not always be the case but that will not apply to the current discussion.

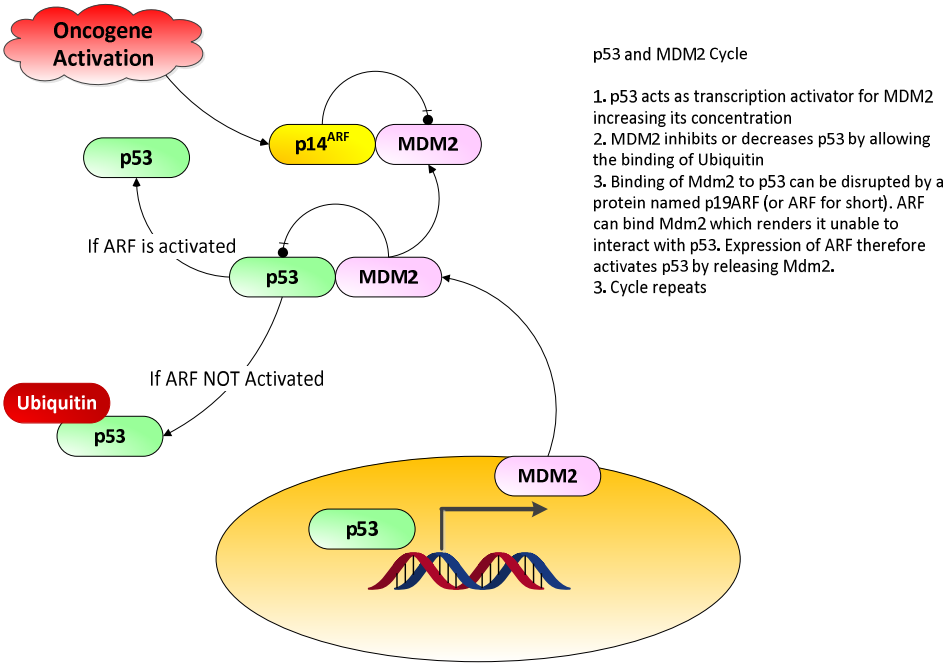
The following Figure depicts the process of the three gene control mechanism. Simply:

1. p53 activates the production of MDM2

2. MDM2 can bind to p53 and result in its dissolution via an Ubiquination

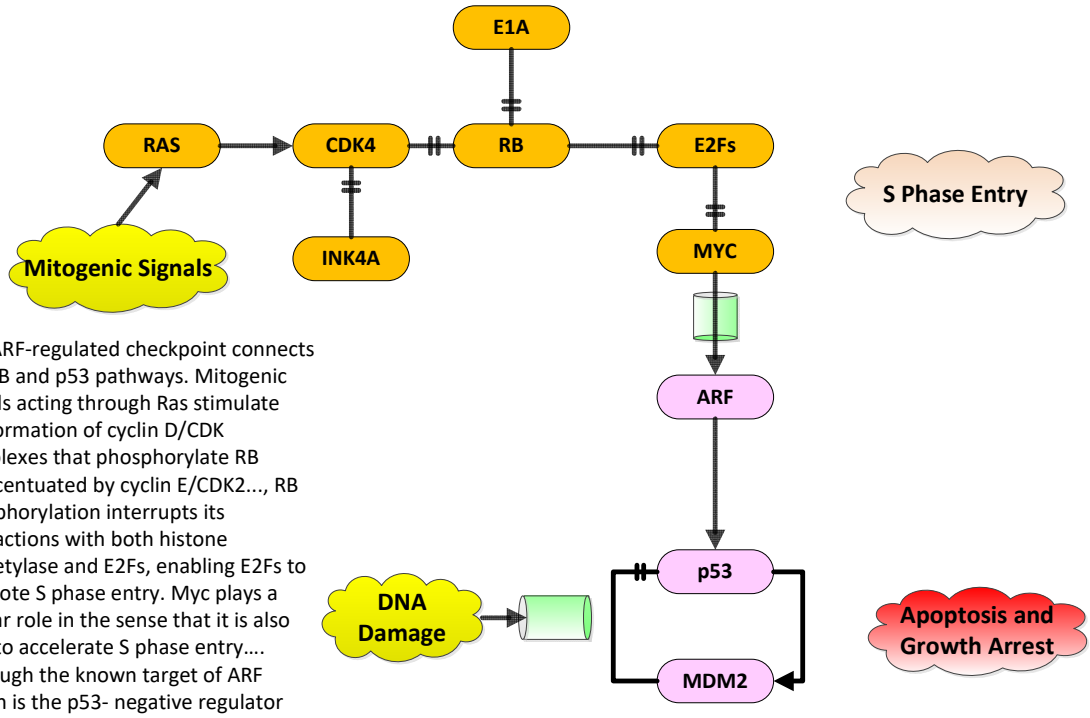
3. ARF can bind to MDM2 and allow the p53 to survive.

4. The process, albeit a bit complex, reaches a steady state for all three proteins.



From Sherr and Weber (as modified) we have the following details as well shown graphically:

From Sherr and Weber



The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB ….Accentuated by cyclin E/CDK2..., RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry…. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded...

Note in the above we have the cyclic MDM2 and p53 control as well as the cell instigators.

Now Van Maerken, T., et al notes the following regarding the details of this feedback loop:

The p53-MDM2 autoregulatory feedback loop.

(a) The p53 protein induces expression of MDM2, which negatively regulates the stability and activity of p53, providing a means to keep p53 levels and activity low in unstressed cells and to switch off p53 at the end of a stress response.

(b) The p53-mediated expression of MDM2 results from binding of p53 to response elements in the MDM2 gene and subsequent transactivation of MDM2. The domain structure of p53 is shown schematically:

v. *TAD, transactivation domain, amino acids;*
vi. *PRD, proline-rich domain, amino acids; DBD, DNA-binding domain, amino acids;*
vii. *TD, tetramerization domain, amino acids;*
viii. *CTD, C-terminal regulatory domain, amino acids.*

(c) The p53-inhibitory activity of MDM2 relies on multiple mechanisms. Binding of MDM2 to p53 conceals the TAD and consequently blocks the transcriptional activity of p53. MDM2 also recruits several corepressor proteins to p53, including HDAC1, CTBP2, YY1, and KAP1.

The E3 ubiquitin ligase activity of MDM2 results in ubiquitination of lysine residues in the CTD of p53, preventing acetylation of p53, favoring nuclear export, and promoting proteasomal degradation (see text for details). Some of these lysine residues can also be neddylated by MDM2, resulting in inhibition of the transcriptional activity of p53. Finally, MDM2 may also serve as a p53-specific transcriptional silencer by binding and monoubiquitinating histone proteins in the proximity of p53-responsive promoters. Nd, NEDD8; Ub, ubiquitin. …

They continue the discussion as follows:

The $p14^{ARF}$ protein is predominantly localized to the nucleolus, in which it is stabilized by binding to nucleophosmin within maturing pre-ribosomal particles, pointing to a function in the regulation of ribosome biogenesis.

Nucleophosmin promotes the processing of ribosomal RNA precursors and the nuclear export of ribosomal subunits, whereas overexpression of $p14^{ARF}$ or its murine homolog $p19^{ARF}$ interferes with transcription and processing of ribosomal RNA, impedes nucleocytoplasmic shuttling of nucleophosmin, and inhibits ribosome nuclear export. However, the precise biological function of the nucleophosmin– $p14^{ARF}$ complexes remains a subject of debate. Stress signals trigger the disruption of the interaction between $p14^{ARF}$ and nucleophosmin, and induce translocation of p14ARF to the nucleoplasm.

This redistribution enables $p14^{ARF}$ to interact with p53-bound MDM2 and to antagonize MDM2 function by inhibiting its E3 ubiquitin ligase activity and by blocking nucleocytoplasmic shuttling of MDM2 and p53, resulting in p53 stabilization. The p53-inhibitory activity of MDM2 may also be neutralized by $p14^{ARF}$ -mediated mobilization of MDM2 into the nucleolus, although this mechanism is not strictly required for the p53-dependent functions of $p14^{ARF}$.

This is clearly a highly complex mechanism. They continue:

Furthermore, the $p14^{ARF}$ protein is capable of inhibiting the activity of another E3 ubiquitin ligase that targets p53 for degradation, ARF-BP1/Mule, and of counteracting the p53-antagonizing NF-kappaB pathway. It should be noted that p14ARF also exerts a potent tumor suppressor activity independently of p53.

Various researchers have tried to model these systems using different techniques. One technique is the use of Petri Nets[18]. From CSML we have a Petri Net models describing the details of such a network and they state[19]:

[18] See Reisig

[19] http://www.csml.org/models/csml-models/p53-arf-dependent-stabilization-pathway/

Proteins p53, MDM2, and $p19^{ARF}$ are proteins closely related to cancer. The protein p53 is a protein which suppresses the formation of tumors, and the protein MDM2 promotes the formation of tumors by decreasing the activity of the protein p53.

Understanding of control mechanism of these proteins connects to development of an effective medicine for suppressing the tumor. It is known that protein p53 works as a transcription factor for many genes and its transcriptional activity is controlled by a complex formed with proteins MDM2 and $p19^{ARF}$.

However, it is still unclear whether protein p53 keeps its transcriptional activity in the form of the trimer with proteins p53, MDM2 and $p19^{ARF}$. ... a hybrid functional Petri net (HFPN) model which has been constructed by compiling and interpreting the information of p53-MDM2 interactions... With our HFPN model, we have simulated mutual behaviors between genes p53, MDM2, $p19^{ARF}$, and their products. Through simulation, we discussed whether the complex p53-MDM2-$p19^{ARF}$ has transcriptional activity for genes Bax and MDM2 or not.

It is worth examining these structures, namely the Petri Nets. We leave the examination to the reference. From Moll and Petrenko we have the following result:

Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53- specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growthsuppressive function in unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes.

The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

3.4.2 ARF

As NCBI notes[20]:

This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase.

The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an ***alternate open reading frame (ARF)*** *that* ***specifies a protein which is structurally unrelated to the products of the other variants****.*

[20] https://www.ncbi.nlm.nih.gov/gene/1029

This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, the E3 ubiquitin-protein ligase MDM2, a protein responsible for the degradation of p53*.*

In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.

From Casalou et al:

The ***Adenosine diphosphate-Ribosylation Factor (ARF) family belongs to the RAS superfamily of small GTPases and is involved in a wide variety of physiological processes, such as cell proliferation, motility and differentiation by regulating membrane traffic and associating with the cytoskeleton.***

Like other members of the RAS superfamily, ARF family proteins are activated by Guanine nucleotide Exchange Factors (GEFs) and inactivated by GTPase-Activating Proteins (GAPs). When active, they bind effectors, which mediate downstream functions.

Several studies have reported that cancer cells are able to subvert membrane traffic regulators to enhance migration and invasion.

Indeed, members of the ARF family, including ARF-Like (ARL) proteins have been implicated in tumorigenesis and progression of several types of cancer.

Here, we review the role of ARF family members, their GEFs/GAPs and effectors in tumorigenesis and cancer progression, highlighting the ones that can have a pro-oncogenic behavior or function as tumor suppressors.

Moreover, we propose possible mechanisms and approaches to target these proteins, toward the development of novel therapeutic strategies to impair tumor progression…

Dysregulation of expression and/or activity of ARF family proteins and/or their effectors, GEFs and GAPs has been associated with enhanced cell migration, invasion and proliferation in several types of cancer. In this section, we review the ARF family members, as well as their activity regulators and effectors that have been found overexpressed in cancer and play essential roles in cancer progression…

ARF1 plays a central role in maintaining the structure and function of the Golgi apparatus and is highly expressed in breast, prostate and ovarian cancers

In the context of cancer, ARF1 has an important function in inter- and intracellular signaling, cell cycle regulation and DNA repair, as well as necrosis and apoptosis. Moreover, ARF1

regulates breast cancer cell adhesion and proliferation, being essential for EGF-mediated phosphorylation of Focal Adhesion Kinase (FAK) and Src.

Furthermore, ARF1 sensitizes MDA-MB-231 breast cancer cells to the anti-tumor drugs actinomycin D and vinblastine through ERK and Akt signaling.

In prostate cancer, ARF1 promotes tumorigenesis by controlling MAPK activation and cell growth.

In myeloma cells, ARF1 expression promotes cell proliferation and inhibits cell adhesion, controlling proliferation- and cell adhesion-mediated drug resistance. Finally, ARF1 is upregulated in ovarian tumors, when compared with adjacent non-cancerous tissues and its overexpression is associated with ovarian cancer cell proliferation and migration through the PhosphoInositide 3-Kinase (PI3K) pathway

Lu et al note:

Androgen receptor (AR) signaling is essential for prostate cancer (PCa) development in humans. The initiation of prostate malignancy and progression to a castration-resistant stage are largely contributed by the modulation of AR activity through its coregulatory proteins.

We and others previously reported that p14 ***alternative reading frame*** *(ARF) expression is positively correlated with the disease progression and severity of PCa. Here, we provide evidence that p14ARF physically interacts with AR and functions as an AR corespressor in both an androgen-dependent and androgen-independent manner.*

Endogenous ARF (p14ARF in human and p19ARF in mouse) and AR colocalize in both human PCa cells in vitro and PCa tissues of mouse and human in vivo.

Overexpression of p14ARF in PCa cells signifiicantly attenuates the activities of androgen response region (ARR2)-probasin and prostate-specifiic antigen (PSA) promoters.

The forced expression of p14ARF in cells resulted in a suppression of PSA and NK transcription factor locus 1 (NKX3.1) expression. Conversely, knockdown of endogenous p14ARF in human PCa cells with short hairpin RNA enhanced AR transactivation activities in a dose-dependent and p53- independent manner. Furthermore, we demonstrated that p14ARF binds to both the N-terminal domain and the ligand-binding domain of AR, and the human double minute 2 (HDM2)-binding motif of p14ARF is required for the interaction of p14ARF and AR proteins. p14ARF perturbs the androgen-induced interaction between the N terminus and C terminus of AR. Most importantly, we observed that the expression of PSA is reversely correlated with p14ARF in human prostate tissues. Taken together, our results reveal a novel function of ARF in modulation of AR transactivation in PCa.

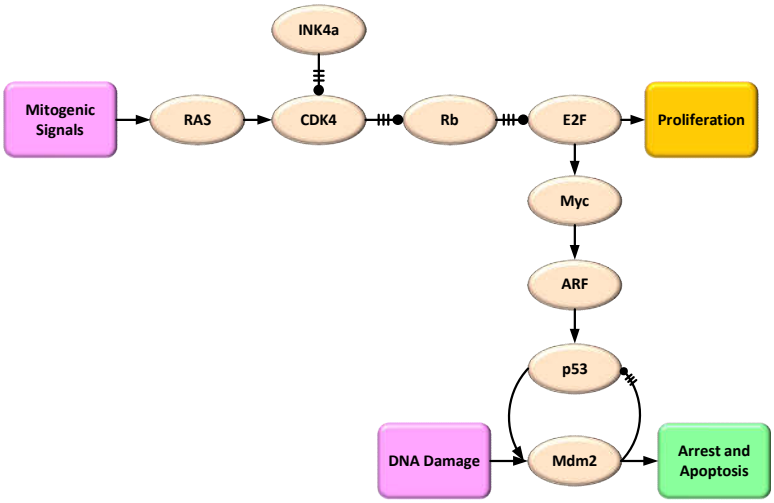
From Sherr we note:

ARF checkpoint control. ARF responds to proliferative signals that are normally required for cell proliferation. When these signals exceed a critical threshold, the ARF-dependent checkpoint (gray vertical barrel) is activated, and ARF triggers a p53-dependent response that induces growth arrest and/ or apoptosis.

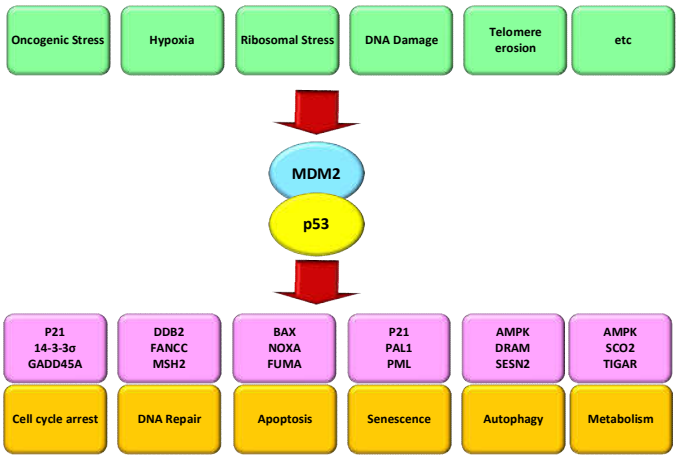
Signals now known to induce signaling via the ARF–p53 pathway include Myc, E1A, and E2F-1. In principle, 'upstream' oncoproteins, such as products of mutated Ras alleles, constitutively activated receptors, or cytoplasmic signal transducing oncoproteins, might also trigger ARF activity via the cyclin D– cdk4–Rb–E2F or Myc-dependent pathways, both of which are normally necessary for Sphase entry. In inhibiting cyclin D-dependent kinases, p16INK4a can dampen the activity of mitogenic signals.

E1A is shown to work, at least in part, by canceling Rb function, although its ability to inhibit p300 contributes to the response by interfering with mdm2 expression. Again for simplicity, Myc and E2F-1 are only shown to activate p53 via ARF. However, highly overexpressed levels of these proteins can activate p53 in ARF-negative cells, albeit with an attenuated efficiency. ARF activation of p53 likely depends on inactivation of some Mdm2-specific function (implied by the unfilled box bracketing the latter two proteins). DNA damage signals (ionizing and UV radiation, hypoxic stress, genotoxic drugs, etc.) access p53 through multiple signaling pathways shown, again for simplicity, as a single DNA damage checkpoint (gray horizontal barrel). Signals through the ARF and DNA damage pathways can synergize in activating p53.

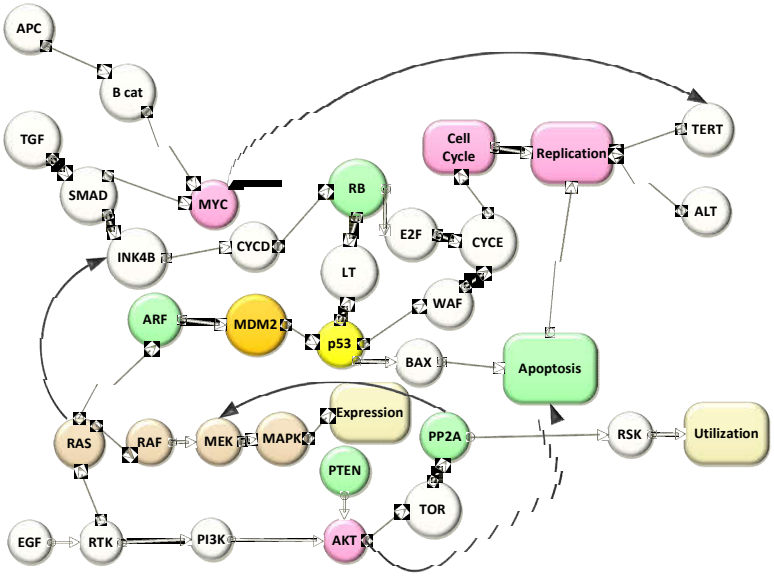
The flow shown below is a critical path involving ARF. Mitogenic signals drive RAS and in turn activates CDK4. RB plays a key role as we show herein as well for cell cycle activation. E2F drives proliferation also MYC and ultimately the MDM2-p53 loop.



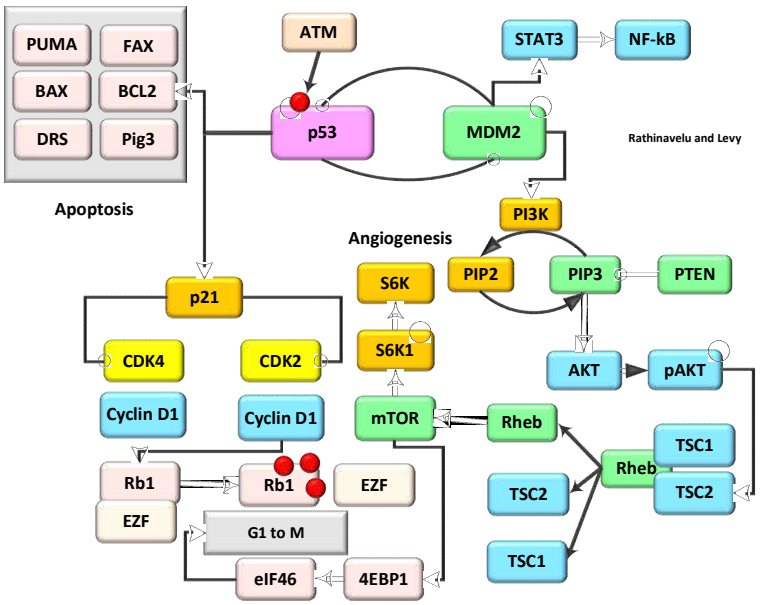
We summarize these factors in the graphic below.



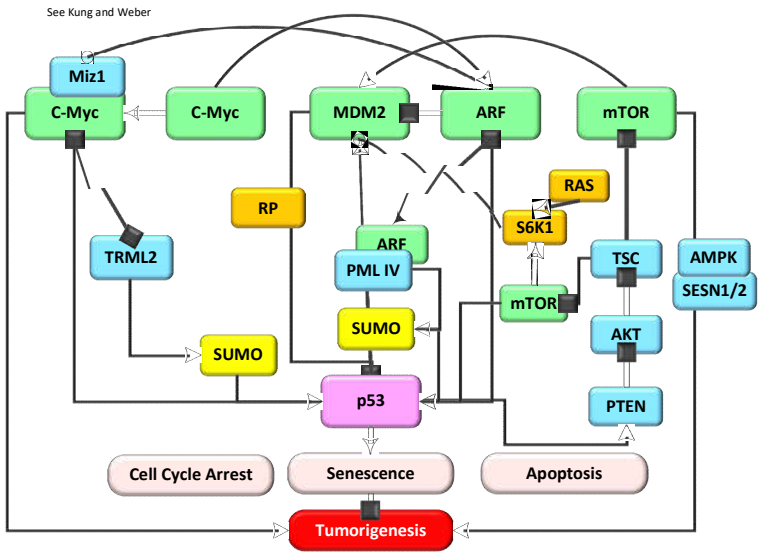
Finally the graphic below is an attempt to details all of the gene flow and control efforts in this process.



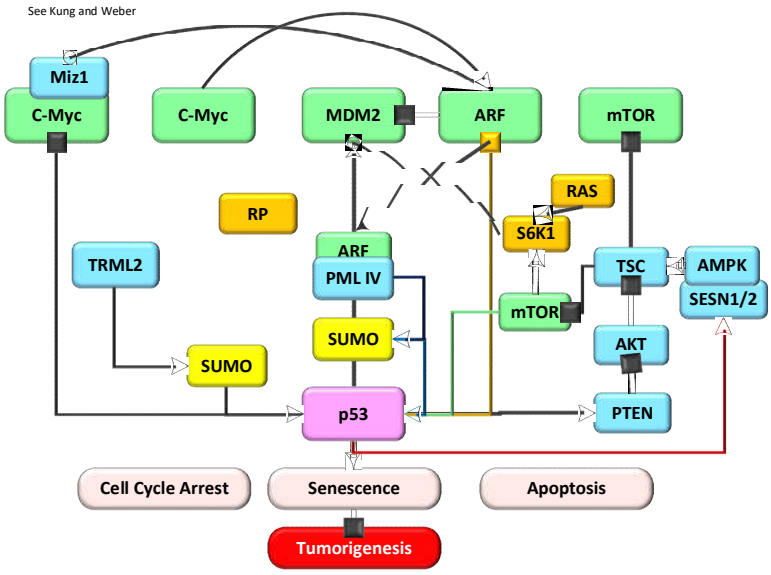
Cell apoptosis is shown below controlled by the MDM2-p53 control loop.



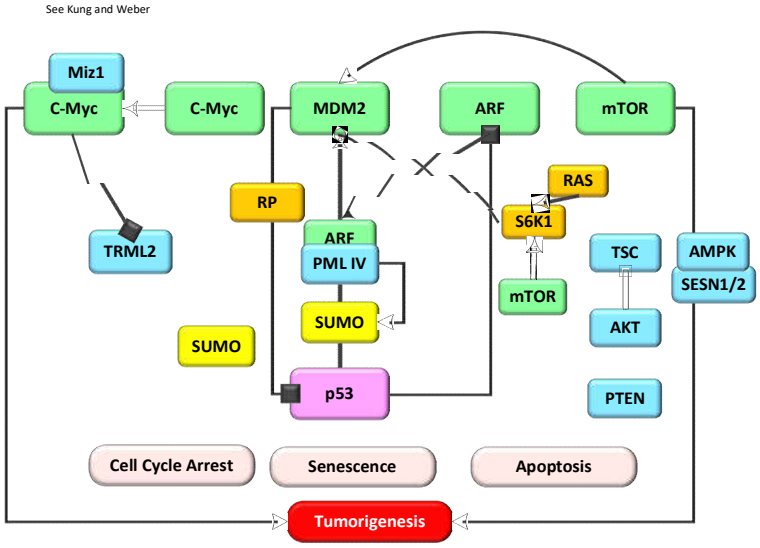
We then show in the following three graphics the flow resulting in tumor generation. The function of ARF is detailed in each of these flows.



Below we have p53 activation and tumor growth blocked.



Finally we show p53 blocked and tumor growth proceeds.

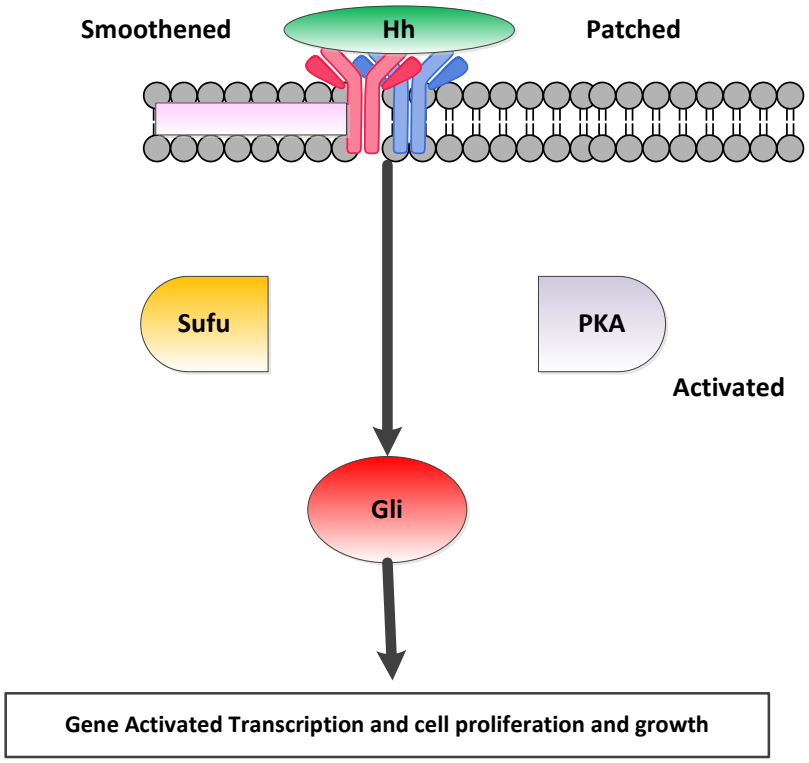


3.4.3 SPOP

SPOP is part of the Hedgehog signalling pathway[21]. The Hedgehog signalling pathway controls amongst other factors the formation of body segments in insects and in vertebrates the

[21] http://pid.nci.nih.gov/search/MoleculePage?molid=203488 and http://pid.nci.nih.gov/search/search_landing.shtml?atom_id=208460,208462&what=graphic&jpg=on and pathway at http://pid.nci.nih.gov/search/advanced_landing.shtml?what=graphic&svg=&jpg=true&xml=&biopax=&complex_uses=on&family_uses=on&degree=1&molecule=&pathway=hedgehog&macro_process=&source_id=5&evidence_co

development of the neural tube, limbs and left-right asymmetry. In adult tissues Hedgehog is responsible for homeostasis, equilibrium between cells loss and gain while maintaining total mass and function. With an overactive Hedgehog pathway one sees excess cell proliferation and tumor growth[22]. Thus SPOP has a controlling mechanism for cell replication. Here Hedgehog attaches to Patched and the Patched inhibition of Smothered is eliminated allowing Smothered to start a transcription process enabling replication.



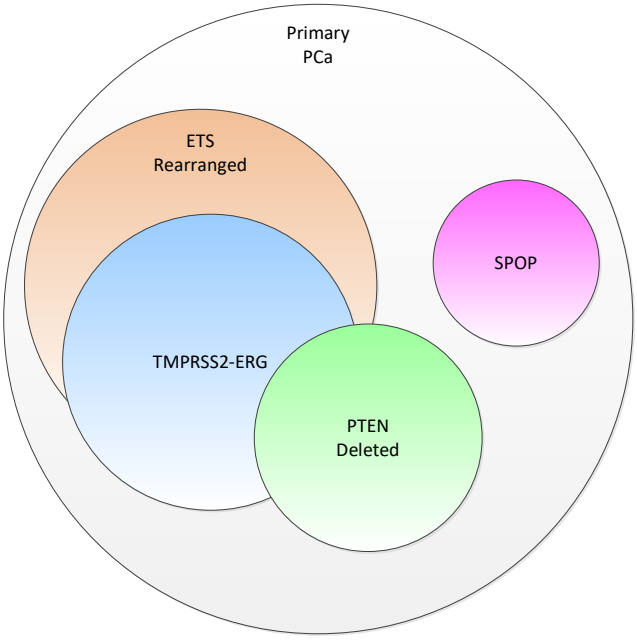
Now upon the activation of Smothered a set of processes are activated and one product is a protein called the zinc finger transcription factor Gli, which when mutually supported by SPOP allows movement to the nucleus as a transcription factor activating the DNA to transcribe[23]. From Barbieri et al we have the following putative relationships:

de=NIL&evidence_code=IAE&evidence_code=IC&evidence_code=IDA&evidence_code=IFC&evidence_code=IGI&evidence_code=IMP&evidence_code=IOS&evidence_code=IPI&evidence_code=RCA&evidence_code=RGE&evidence_code=TAS&output-format=graphic&Submit=Go

[22] See Marks et al p 210-212.

[23] See Pecorino, p. 168-170.

See Barbieri et al



The authors argue that SPOP is a separate and significant marker for PCa. The pathway involved is somewhat understood and is a transcription driven pathway initiated by Hedgehog activation and Patched suppression with Smothered activation. From the NCI pathway databases we have a putative requirement that SPOP is needed to activate GLI for subsequent transcription and cell reproduction.

Specifically Barbieri et al state:

As demonstrated by a subsequent analysis of significantly more genomes, there are only a few truly recurrent non-synonymous mutations in PCa (Barbieri, Rubin, Garraway and Chinnaiyan, submitted). The most common recurrent non-synonymous mutation in PCa involves SPOP. The SPOP gene encodes for the substrate-recognition component of a Cullin3-based E3-ubiquitin ligase. Mutations in SPOP in PCa were reported originally in two systematic sequencing studies.[12, 13] We have now identified the presence of recurrent mutations in SPOP in 6–13% of human PCas in multiple independent patient cohorts (C Barbieri and MA Rubin, unpublished data).

Recurrent missense mutations are found exclusively in the structurally defined substrate-binding cleft of SPOP, and structural analysis suggests that these mutations will inactivate SPOP function by disrupting SPOP–substrate interaction.[75] Further, we found that loss of SPOP function in prostate cell lines resulted in increased invasion and altered gene expression; evidence of this expression signature was identified in primary tumours harbouring SPOP mutation. Importantly, all SPOP mutations occurred in tumours that were negative for ERG rearrangement; these tumours displayed characteristic somatic copy number aberrations. Taken together, these findings support a distinct molecular class of PCa.

In a recent Nature Medicine article the same authors relate[24]:

Prostate cancer is the second most common cancer in men worldwide and causes over 250,000 deaths each year. Overtreatment of indolent disease also results in significant morbidity. Common genetic alterations in prostate cancer include losses of NKX3.1 (8p21) and PTEN (10q23), gains of AR (the androgen receptor gene) and fusion of ETS family transcription factor genes with androgen-responsive promoters.

Recurrent somatic base-pair substitutions are believed to be less contributory in prostate tumorigenesis but have not been systematically analyzed in large cohorts. Here, we sequenced the exomes of 112 prostate tumor and normal tissue pairs. New recurrent mutations were identified in multiple genes, including MED12 and FOXA1. SPOP was the most frequently mutated gene, with mutations involving the SPOP substrate-binding cleft in 6–15% of tumors across multiple independent cohorts.

Prostate cancers with mutant SPOP lacked ETS family gene rearrangements and showed a distinct pattern of genomic alterations. Thus, SPOP mutations may define a new molecular subtype of prostate cancer.

This just adds another gene in the mix for PCa. Namely they authors argue that it is a different type. We would still ask the same questions:

1. What is the issue regarding the presence or absence of a CSC stem cell in PCa.

2. When does this mutation occur?

3. What causes the mutation?

4. SPOP is not a true kinase so what type of blocking would be possible to mitigate the presence of a mutant.

The following also is noted from a Cell Reports article[25]:

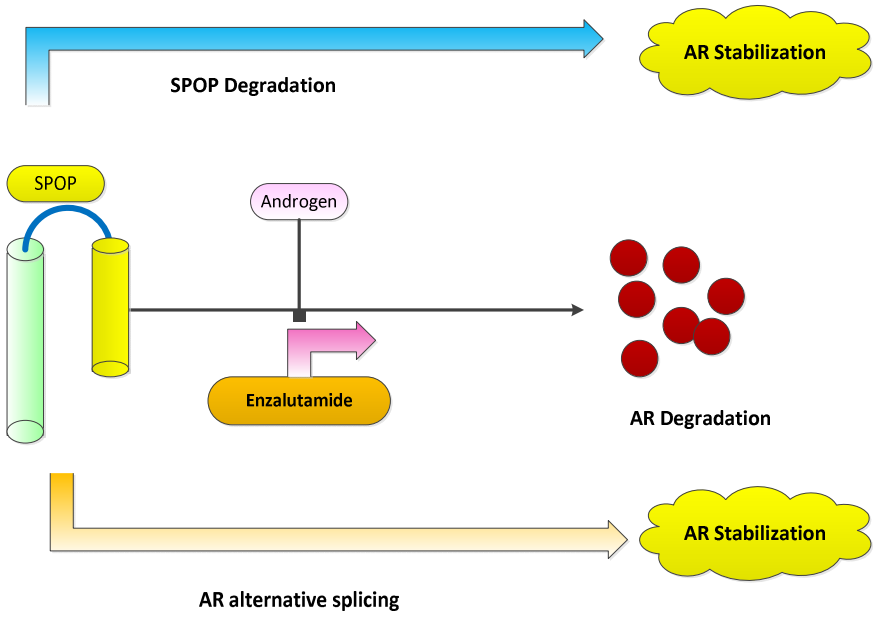
The SPOP E3 ubiquitin ligase gene is frequently mutated in human prostate cancers. Here, we demonstrate that SPOP recognizes a Ser/Thr-rich degron in the hinge domain of androgen receptor (AR) and induces degradation of full-length AR and inhibition of AR-mediated gene transcription and prostate cancer cell growth. AR splicing variants, most of which lack the hinge domain, escape SPOP- mediated degradation.

Prostate-cancer-associated mutants of SPOP cannot bind to and promote AR destruction. Furthermore, androgens antagonize SPOP-mediated degradation of AR, whereas anti-androgens promote this process. This study identifies AR as a bona fide substrate of SPOP and

[24] http://www.nature.com/ng/journal/vaop/ncurrent/full/ng.2279.html

[25] http://download.cell.com/cell-reports/pdf/PIIS2211124714000308.pdf?intermediate=true

elucidates a role of SPOP mutations in prostate cancer, thus implying the importance of this pathway in resistance to antiandrogen therapy of prostate cancer



In a discussion of some prior SPOP research it is noted[26]:

… researchers have shed light on a new mechanism by which prostate cancer develops in men. Central to development of nearly all prostate cancer cases are malfunctions in the androgen receptor — the cellular component that binds to male hormones.

The research team has shown that SPOP, a protein that is most frequently mutated in human prostate cancers, is a key regulator of androgen receptor activity that prevents uncontrolled growth of cells in the prostate and thus helps prevent cancer. The findings appear in the journal Cell Reports.

"By uncovering this new and important pathway of androgen receptor destruction, we may one day be able to develop more effective treatments for a substantial proportion of prostate cancer patients who have developed resistance to standard antiandrogen therapy,"

SPOP mutations have been detected in approximately 15 percent of prostate cancer cases. In addition, it has been shown that in about 35 percent of prostate cancers, the SPOP protein is expressed at abnormally low levels. Despite its prevalence in prostate cancer, it was not known whether or how SPOP defects contributed to tumor development. What the research team discovered is that SPOP is an enzyme that selectively destroys androgen receptor protein.

[26] http://www.healthcanal.com/cancers/prostate-cancer/47500-mayo-clinic-identifies-a-key-cellular-pathway-in-prostate-cancer.html

Failure to do so due to alterations in SPOP results in overabundance of androgen receptor, a master regulator of prostate cancer cell growth.

The above mentioned Mayo Clinic research team made four major discoveries:

1. *The antiandrogen receptor is a bona fide degradation substrate of SPOP.*
2. *Androgen receptor splicing variants are resistant to SPOP-mediated degradation.*
3. *Prostate cancer-associated SPOP mutants cannot bind to and promote androgen receptor degradation.*
4. *Androgens antagonize, but antiandrogens promote SPOP-mediated degradation of androgen receptor.*

It is noted and well known that the Androgen receptor (AR) is essential for normal prostate cell growth and survival. It is also important for initiation and progression of prostate cancer. Androgen deprivation therapy, including chemical castration and/or antiandrogen therapy, is the mainstay for treating advanced/disseminated prostate cancer. However, tumors almost always reoccur two to three years after initial response and relapse into a disease called castration-resistant prostate cancer. Development of this therapy-resistant symptom is related to a persistent activation of androgen receptor.

As Medical Express states concerning the work on SPOP[27]:

The gene SPOP is mutated in up to 15 percent of all cases of prostate cancer, making it one of the most mutated genes in the disease. However, when the gene is functioning properly, it acts as a tumor suppressor. Despite what's known about SPOP, scientists have not been able to determine exactly how the gene is able to halt the progression of disease.

In a paper published in 2012, a large study analyzed mutations in prostate cancer tumors and found that the SPOP gene was the most frequently mutated among genes identified in this cohort, suggesting that tumors exhibiting a mutation of SPOP could be characterized as a specific subtype of the disease. Further studies found several proteins that interact with SPOP, but this information still failed to explain exactly how SPOP is able to suppress tumors….

The Zhang laboratory began to unravel this mystery by determining if there was a connection between SPOP and senescence. Indeed, they were able to show that SPOP was found in higher concentrations in senescent cells. Next, they compared samples of wild-type (not mutated) SPOP with their mutated counterparts, which were associated with cancer. Wild-type SPOP samples showed senescent behavior, whereas their cancer-associated mutants were impaired in their ability to induce senescence.

In this study, the research team directly linked this behavior of SPOP to an enzyme called SENP7. The function of SENP7 is not entirely clear, but this study showed just how important it is with regard to SPOP. When SPOP is not mutated, SENP7 remains in check and senescent cells are able to keep cancer activity at bay.

[27] http://medicalxpress.com/news/2015-10-scientists-frequently-mutated-prostate-cancer.html

To test what happens when SPOP is not functioning properly, the researchers inactivated the gene and observed the effect this had on SENP7. They found that the levels of SENP7 increase enough that cells are able to overcome senescence and become cancerous. Notably, when SENP7 activity was inhibited, prostate cancer cells showed senescent behavior and stopped growing, suggesting that SENP7 might be an important therapeutic target.

As Zhu et al note[28]:

The SPOP gene, which encodes an E3 ubiquitin ligase adaptor, is frequently mutated in a number of cancer types.

However, the mechanisms by which SPOP functions as a tumor suppressor remain poorly understood. Here, we show that SPOP promotes senescence, an important tumor suppression mechanism, by targeting the SENP7 deSUMOylase for degradation. SPOP is upregulated during senescence.

This correlates with ubiquitin-mediated degradation of SENP7, which promotes senescence by increasing HP1α sumoylation and the associated epigenetic gene silencing.

Ectopic wild-type SPOP, but not its cancer-associated mutants, drives senescence. Conversely, SPOP knockdown overcomes senescence. These phenotypes correlate with ubiquitination and degradation of SENP7 and HP1α sumoylation, subcellular re-localization, and its associated gene silencing.

From NCBI we note regarding SENP7:

The reversible posttranslational modification of proteins by the addition of small ubiquitin-like SUMO proteins is required for many cellular processes. SUMO-specific proteases, such as SENP7, process SUMO precursors to generate a C-terminal diglycine motif required for the conjugation reaction. They also display isopeptidase activity for deconjugation of SUMO-conjugated substrates.

As we have shown before SUMO and SPOP all play a role in degrading via ubiquination. The degrading process is a part of normal homeostasis. The loss of such functionality is often noted in PCa. However it is not at all clear that these can or should be therapeutic targets.

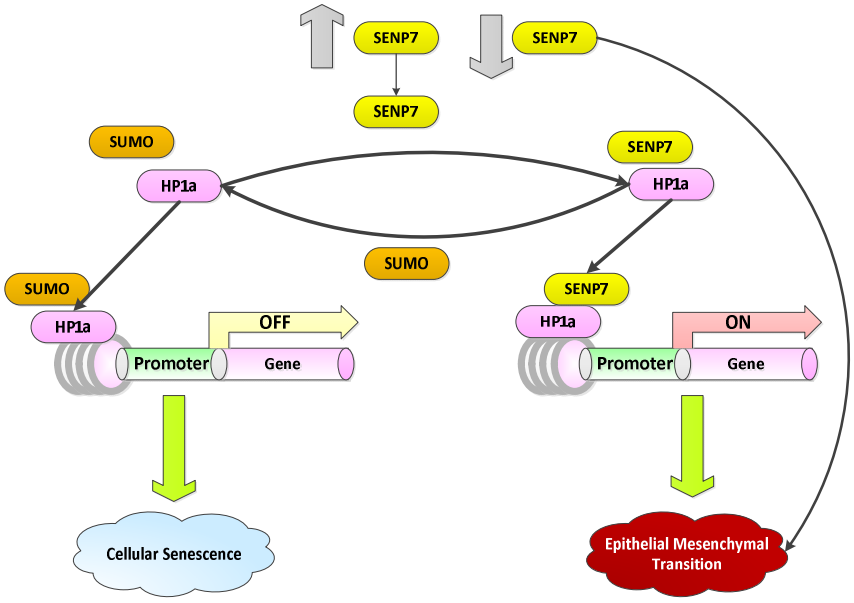
As Bawa-Khalfe et al state:

SENP7L levels dictate PCa cells' choice between senescence and EMT. Onset of cancer in breast epithelia decreases the SENP7S splice variant and increases SENP7L, which expresses an HP1α-interaction motif. Loss of SENP7LHP1α interaction causes HP1α hyper-SUMOylation, an enrichment of HP1α at E2F-responsive and mesenchymal gene promoters, silences transcription of these genes, and elicits cellular senescence. Induction of SENP7Lmaintains hypo-

[28] http://www.cell.com/cell-reports/abstract/S2211-1247%2815%2901137-7

SUMOylated HP1α, which relieves HP1α-mediated repression of proliferation promoting E2F-responsive genes as well as mesenchymal genes. SENP7L decreases epithelial gene expression via an unidentified HP1α-independent pathway, and concurrently with the HP1α-dependent pathway promotes dedifferentiation.

We demonstrate this below:



3.4.4 ARE

As Tan et al note:

In the nucleus, receptor dimers bind to androgen response elements (AREs) in the promoter regions of target genes, such as prostate specific antigen (PSA) and transmembrane protease serine 2 (TMPRSS2), etc, to which they recruit various coregulatory proteins to facilitate transcription, leading to responses such as growth and survival…

The DBD (residues 556–623) is a cysteine-rich region that is highly conserved among steroid hormone receptors. According to the crystal structure of the AR DBD, each DBD monomer has a core composed of two zinc fingers (PDB: 1R4I), each of which consists of four cysteine residues that coordinate a zinc ion. The AR functions as a dimer that, like other steroid receptors, binds to promoter DNA response elements consisting of two equal, common hexameric half-sites (5'-AGAACA-3') separated by a 3 base-pair spacer (IR3). The α-helix of the N-terminal zinc finger (the "recognition helix") interacts directly with nucleotides in the hormone response element in the DNA major groove .

Three amino acid residues at the N terminus of this α-helix, named the P(roximal) box [glycine-serine-valine] (amino acids 577–581; GSCKV), are identical in the PR, GR and MR and are responsible for the specific recognition of the DNA response element. A question that persisted

was how steroid receptors achieve target specificity if the AR, PR, GR, and MR bind a common DNA response element. Studies have identified selective androgen response elements (AREs) (eg, 5'-GGTTCT-3') that allow specific AR activation. AREs have hexameric half-sites in a direct repeat orientation. Structural studies have confirmed that selectivity is achieved by receptor dimerization in a "head-to-head" fashion through the D(istal) box region (amino acid 596–600; ASRND), which allows the AR to bind to direct repeat half-sites in its.

Because the DBD domains are highly conserved among the different steroid receptors, the reason why other steroid receptors do not recognize selective AREs is still a matter of debate. Based on crystallographic data, it was speculated that the AR contains an additional interface that stabilizes the AR dimer/ARE complex. In contrast, the dimerization strength of other steroid receptors would not be sufficient to retain stable binding to selective AREs

From Wilson et al:

Sequence motifs are short, recurring patterns in DNA that can mediate sequence-specific binding for proteins such as transcription factors or DNA modifying enzymes.

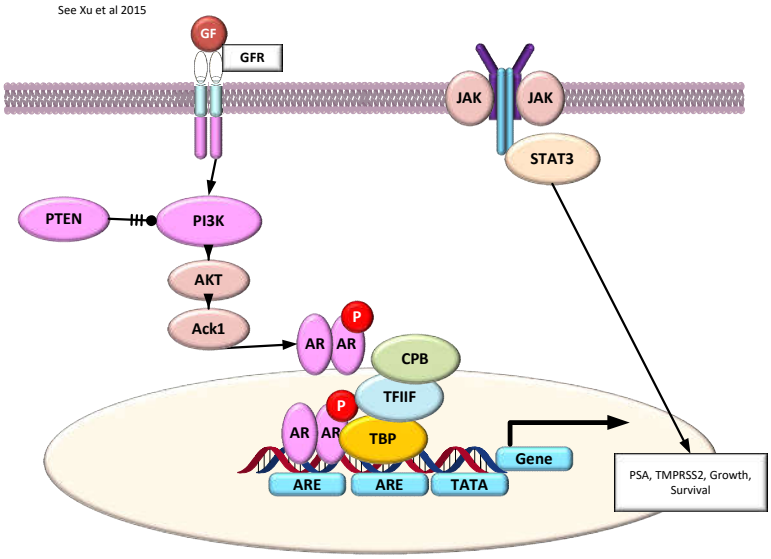
The androgen response element (ARE) is a palindromic, dihexameric motif present in promoters or enhancers of genes targeted by the androgen receptor (AR).

Using chromatin immunoprecipitation sequencing (ChIP-Seq) we refined AR-binding and AREs at a genome-scale in androgen-insensitive and androgen-responsive prostate cancer cell lines. Model-based searches identified more than 120,000 ChIP-Seq motifs allowing for expansion and refinement of the ARE. We classified AREs according to their degeneracy and their transcriptional involvement. Additionally, we quantified ARE utilization in response to somatic copy number amplifications, AR splice-variants, and steroid treatment. Although imperfect AREs make up 99.9% of the motifs, the degree of degeneracy correlates negatively with validated transcriptional outcome.

Weaker AREs, particularly ARE half sites, benefit from neighboring motifs or cooperating transcription factors in regulating gene expression. Taken together, ARE full sites generate a reliable transcriptional outcome in AR positive cells, despite their low genome-wide abundance. In contrast, the transcriptional influence of ARE half sites can be modulated by cooperating factors….

AREs are well studied but poorly defined and have been shown to contain two hexamers with a three base-pair spacer with an inverted repeat in the second hexamer. The sequence elements similar to this canonical ARE have been identified in some ChIP-Seq studies, whereas half AREs or tandem repeats of two hexamers were also found in other ChIP-Seq or ChIP-on-chip studies. In the past, studies revealed binding motifs adjacent to the AR binding sites but belonging to other transcription factor families such as the forkhead box A1 protein (FOXA1, GeneBank: 3169). Cooperative interactions facilitate chromatin binding of the AR and contribute to a promiscuous behavior of AREs23,24,25. AREs and adjacent transcription binding motifs have been well described in LNCaP cells but remain to be defined in CWR22Rv1 cells.

Therefore, the purpose of our AR ChIP-Seq study is to further characterize the ARE and identify cooperation with adjacent transcription binding motifs in androgen-responsive and androgen-insensitive prostate cancer cell lines.

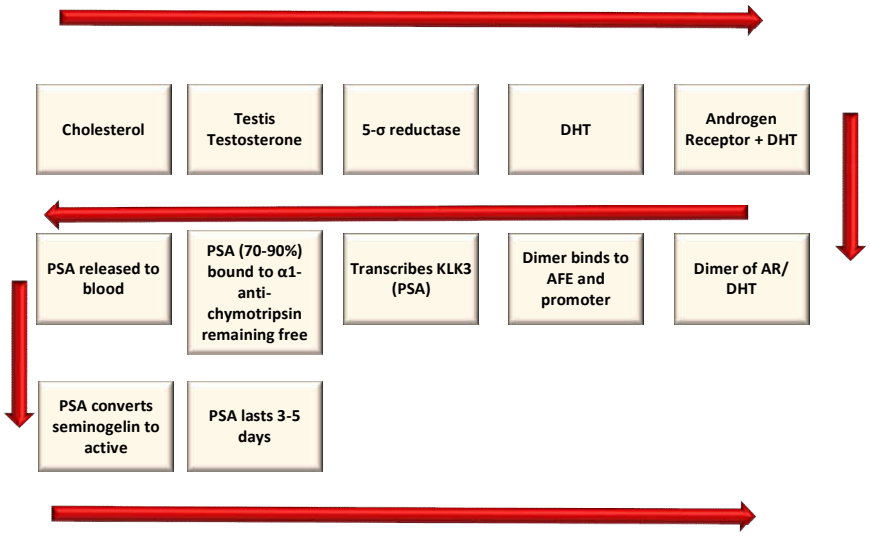


4 AR AND PSA

AR and PSA are the most recognized paid in PCa.

4.1 THE PSA PATHWAY

Let us reiterate the AR pathway from cholesterol to PSA activity as shown below. This is but one of many such paths about which AR plays a critical role.



4.2 5α-REDUCTASE

From Zhu and Sun we have:

5α-reductases convert testosterone to dihydrotestosterone (DHT).

There are two 5α-reductase isozymes, type 1 and type 2 in humans and animals. Mutations in type 2 isozyme with decreased enzymatic activity cause male pseudohermaphroditism. The affected 46XY individuals have high normal or elevated plasma testosterone levels with low normal or decreased DHT levels, resulting in an elevated testosterone/DHT ratios. They are born with ambiguous external genitalia and normal Wolffian differentiation.

Their prostate is small and rudimentary, and plasma levels of prostate specific antigen (PSA) are low or undetectable in adulthood.

Prostate cancer and benign prostate hyperplasia (BPH) have never been reported in these patients.

Similar defects in prostate development are observed in animals with either 5α-reductase-2 or 5α-reductase-2 plus 5α-reductase-1 gene knockout, and in animals treated with specific 5α-reductase inhibitor. 5α-reductase isozymes are expressed in multiple tissues, and the predominant isozyme in human prostate is 5α-reductase-2.

The expression of 5α-reductase-2 gene in prostate cells is regulated by various factors. A high dietary fat intake, a risk factor of prostate cancer, induces prostate 5α-reductase-2 gene expression and subsequently stimulates prostate growth, which is blocked by genistein, a phytoestrogen. Inhibition of 5α-reductase activity by medication is used in the treatment of BPH and male-pattern baldness, while its use in prostate cancer prevention is still controversial although it can decrease the incidence of prostate cancer. The analyses of 5α-reductases in humans and animals highlight the differences between testosterone and DHT, and the significance of DHT in male sexual differentiation and prostate physiology and pathophysiology.

As Azzouni et al note:

Testosterone (T) is the most abundant androgen in serum.

Approximately 97% of T is bound to albumen and sexhormone binding globulin and the remaining 3% is free and biologically active.

T is synthesized by the Leydig cells of the testes under the control of the hypothalamus and anterior pituitary gland.

In male fetuses, T stimulates the differentiation of the Wolffian duct into male internal genitalia (epididymis, vas deferens, and seminal vesicles) and development of libido, enlargement of the vocal cords, skeletal muscles, penis, and scrotum and the initiation of spermatogenesis at puberty.

T is taken from circulation to cells through processes that remain poorly understood. Intracellular T is converted to dihydrotestosterone (DHT), the preferred ligand for androgen receptor (AR) transactivation, by the enzyme 5 alpha-reductase (5α- R).

Upon ligand binding and transactivation, the DHTAR complex translocates from cytoplasm to nucleus and activates the transcription of certain genes (the androgen receptor-regulated genes, ARRG). DHT is important for in utero differentiation and growth of the prostate gland, male external genitalia (penis and scrotum), and pubertal growth of facial and body hair. DHT plays an important role in several human diseases, which include acne, hirsutism, male pattern baldness, benign prostate hyperplasia (BPH), and prostate cancer (CaP) .

The role of DHT was discovered after the description of 5α-R2 deficiency in a group of males from the Dominican Republic .

DHT has 2–5 times higher binding affinity for AR than T, and 10-fold higher potency of inducing AR signaling than T , which means that their effects are different but complementary .

Three isozymes of 5α-R are known to exist (5α-R1-3) and two other proteins exhibit 5-alpha reducing capabilities, glycoprotein synaptic 2 (GPSN2), and glycoprotein synaptic 2-like (GPSN2L) proteins.

Only one 5 beta-reductase (5β- R) enzyme has been identified. Its products, 5β-isomers, are labeled as epi-product, such as 5β-DHT (epi-DHT) . Several compounds have been developed to inhibit the 5α-R enzyme system and they play an important role in the prevention and treatment of many common diseases . This review describes the basic biochemical properties, functions, tissue distribution, chromosomal location, and clinical significance of this enzyme family.

4.3 KALLIKREINS

As Lawrence et al note:

The 15 members of the kallikrein-related serine peptidase (KLK) family have diverse tissue-specific expression profiles and putative proteolytic functions.

The kallikrein family is also emerging as a rich source of disease biomarkers with KLK3, commonly known as prostate-specific antigen, being the current serum biomarker for prostate cancer.

The kallikrein locus is also notable because it is extraordinarily responsive to steroids and other hormones. Indeed, at least 14 functional hormone response elements have been identified in the kallikrein locus. A more comprehensive understanding of the transcriptional regulation of kallikreins may help the field make more informed hypotheses about the physiological functions of kallikreins and their effectiveness as biomarkers. In this review, we describe the organization of the kallikrein locus and the structure of kallikrein genes and proteins.

We also focus on the transcriptional regulation of kallikreins by androgens, progestins, glucocorticoids, mineralocorticoids, estrogens, and other hormones in animal models and human prostate, breast, and reproductive tract tissues. The interaction of the androgen receptor with androgen response elements in the promoter and enhancer of KLK2 and KLK3 is also summarized in detail. There is evidence that all kallikreins are regulated by multiple nuclear receptors.

Yet, apart from KLK2 and KLK3, it is not clear whether all kallikreins are direct transcriptional targets. Therefore, we argue that gaining more detailed information about the mechanisms that regulate kallikrein expression should be a priority of future studies and that the kallikrein locus will continue to be an important model in the era of genome-wide analyses.

4.4 PSA FUNCTIONS

As Lawrence et al note:

Androgens regulate the prostatic expression of several human kallikreins, in particular KLK2 and KLK3.

The earliest evidence for androgen-regulated KLK3 expression came from immunohistochemistry experiments showing that prostatic KLK3 levels mirror serum testosterone concentrations: low in prenatal development and childhood, greater in puberty, and highest in adulthood. Soon after the KLK2 and KLK3 genes were cloned, their androgen responsiveness was confirmed at the mRNA level using Northern blots of androgen-treated LNCaP prostate cancer cells. These observations were verified with a range of in vitro and in vivo experiments.

Numerous studies have since used KLK2 and KLK3 as prototypical AR target genes to investigate different aspects of androgen signaling in prostate cells. KLK3 levels are also monitored in patients undergoing androgen ablation therapy for prostate cancer because KLK3 is re-expressed when AR signaling is reactivated in castrate-resistant tumors. KLK3 levels, however, are highly heterogeneous in castrate-resistant prostate cancer and do not directly correlate with tumor growth. This variability may be due to the different ways that tumors adapt to castrate androgen levels including overexpression and mutation of the AR, up-regulation of transcriptional coactivators, and intratumoral steroidogenesis. …

AREs were identified within the promoter of KLK3 soon after its androgen-dependent expression was established.

… the KLK3 promoter is bound by nuclear proteins in LNCaP cells.

They then identified the first KLK3 ARE, AREI (AGAACAgcaAGTGCT), at 170 to 156 bp from the TSS using a series of promoter deletion and mutation constructs.

Other groups confirmed this finding using similar reporter experiments and EMSAs. The results from reporter assays suggested that another ARE might be present between 320 and 539 bp from the KLK3 TSS.

Subsequently, AREII (GGATCAgggAGTCTC) was identified at 400 bp from the TSS and found to be a low-affinity AR binding site that cooperates with AREI. This was confirmed by other studies that also suggested that Fos-related complexes, distinct from AP-1, might be important in mediating AR transactivation of the KLK3 promoter …

Kallikreins can be used as markers of particular cell types, especially when their patterns of tissue-specific expression and hormonal regulation converge. KLK3 is a good example because it is one of the most highly expressed genes in the prostate. This means that KLK3 may have several clinical applications in prostate cancer. In addition to its use as the serum biomarker, KLK3 has been tested as a marker of circulating tumor cells, as an antigen to prime dendritic

cells for targeted immunotherapy, and as an enzyme to activate cytotoxic prodrugs. Furthermore, the KLK3 promoter and enhancer have been used to design prostate-specific expression vectors for gene therapy. KLK3 is more precisely a marker of terminally differentiated luminal epithelial cells of the prostate.

It is not produced by stem, transit amplifying, or intermediate cells, which make up the basal layer of the epithelium and express little or no AR.

Although the prostate stroma is androgen-responsive, it does not express KLK3.

This suggests that KLK3 expression in luminal epithelial cells depends on more than just androgens and AR.

Recent genome-wide ChIP studies have shown that epigenetic marks, such as histone 3 lysine 4 methylation and pioneer coactivators guide hormone receptors to enhancers of tissue-specific target genes. This holds true for AR-mediated expression of KLK3.

Prostate cancer cell lines that express endogenous KLK3 have high levels of di- and trimethylated histone 3 lysine 4 at the promoter and enhancer of KLK3.

Furthermore, pioneer factors like GATA2 bind to the KLK3 enhancer in prostate cells and are re quired for maximum androgen-regulated gene expression. Within the prostate, GATA2 and KLK3 are both produced by luminal epithelial cells, but not the stroma. As previously noted, KLK3 is expressed in some other tissues, but at much lower levels. Presumably, these tissues lack the precise combination of methylation, coactivator expression, and AR activity that stimulates such high levels of KLK3 in the prostate.

As Kalinska et al note:

The majority of studies on kallikreins and their physiological functions have focused on KLK3, also known as Prostate Specific Antigen (PSA). Since its identification and characterization in the 1970s, KLK3 has been investigated extensively with respect to its biochemical and cellular functions as an enzyme and prostate cancer biomarker.

Investigations into KLK3 as a prostate cancer marker, performed mostly by pharmaceutical companies, were fueled by its tissue specificity and incredibly high expression in prostate cancer.

These studies eventually led to development of diagnostic kits for the detection of the prostate cancer. However, KLK3 elevation needs to be considered in a broader biological context since it leads to frequent false-positive diagnoses followed by unnecessary treatments that have resulted in some instances in treatment-associated health problems.

Originally, the physiological activity of KLK3 was associated with its ability to perform semen liquefaction and enhance sperm motility, which it achieves by cleaving fibronectin and seminal-gel-forming proteins semenogelin 1 and semenogelin .

Recent reports, however, highlight the expression and mechanisms of action of other kallikreins in semen liquefaction, including proKLK3 (pro-PSA) activation by KLK-4, -5, -14, and -15, as well as the direct proteolytic activity of KLK-14 and -5 .

Following its activation, KLK3 degrades numerous proteins [extracellular matrix proteins, insulin-like growth factor (IGF)- binding proteins 3 and 5, and parathyroid-hormone-related protein (PTHRP)] facilitating metastasis of prostate cancer cells.

KLK2 is the second-best-characterized kallikrein biomarker used in prostate cancer diagnosis.

Despite its relatively low expression compared with PSA, utilization of KLK2 as a secondary biomarker increases the specificity and sensitivity of cancer detection . To date, the only known protein substrate of KLK2 is the ARA70 - the androgen receptor coregulator , suggesting that KLK2 has potential function in maintaining tissue balance in the testis. Another kallikrein highly expressed in prostate cancer is KLK4, an androgen regulated enzyme . Along with PSA, KLK4 facilitates metastasis of prostate cancer to the bone because it facilitates the degradation of extracellular matrix proteins.

5 CRPC

Ultimately PCa becomes independent of testosterone. This is called castrate resistant prostate cancer, CRPC. As Xu and Melcher note:

AR and castration-resistant prostate cancer (CRPC) Patients on androgen deprivation therapy remain in longterm remission of the disease. However, the development of a castration-resistant disease is inevitable.

This form of prostate cancer is lethal, and patients no longer respond to first-line androgen deprivation therapy.

***CRPC patients are usually treated with chemotherapy including the anti-mitotic compound docetaxel, which has been demonstrated to confer a survival advantage**.*

The mechanisms of castration resistance remain unclear but are thought to be diverse. For a comprehensive review of the mechanisms of CRPC development, the reader is referred to other excellent reviews.

Briefly, there are four possible mechanisms of CRPC development:

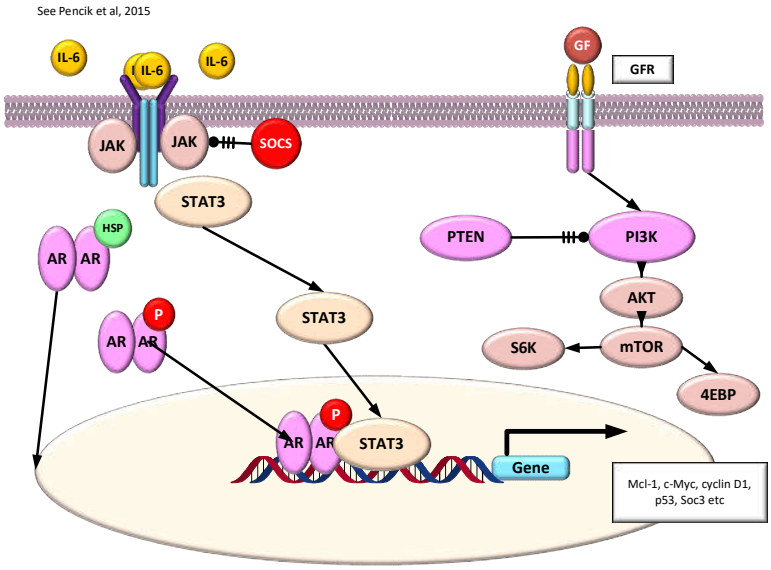
1) Increased sensitivity of the AR to its agonists,

2) AR mutations that render the receptor responsive to alternate, non-androgen ligands,

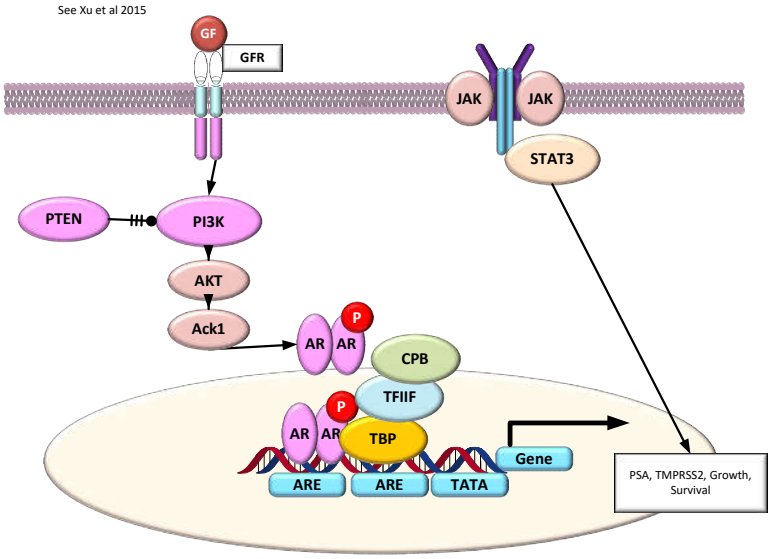
3) ligand-independent AR activation, and

4) AR-independent mechanisms.

We repeat in the graphic below the AR drive of proliferation but here we show a phosphorylated AR instead of the DHT AR. This graphic attempts to demonstrate a CRPC active lesion.



A second view shows STAT3 of the JAK-STAT pathway separately activating growth and survival.



Patients on androgen deprivation therapy have lower levels of circulating androgens, which initially curb prostate cancer cell proliferation; however, the opposite happens in CRPC patients, who have increased tumor cell proliferation.

One of the underlying mechanisms of CRPC is an increase in the expression of AR in the cell. Koivisto et al showed that 28% of androgen-independent tumors that developed after androgen deprivation therapy had increased AR expression due to AR gene amplification.

These results indicate that CRPC cells may not be strictly androgen independent, but rather, they become more sensitive due to a lowered threshold for androgens.

Even under androgen deprivation therapy, androgen levels are sufficiently high to activate overexpressed AR, which is due to intratumoral in-situ synthesis and residual synthesis in the adrenal gland, along with decreased levels of the androgen inactivating enzymes CYP3A4, CYP3A5, and CYP3A7 in patient tissue samples.

Another mechanism for the development of CRPC is ligand promiscuity, which results from AR gene mutations that cause amino acid substitutions in the LBD that decrease specificity and selectivity for ligands (eg, T877A, L701H, W741L, and F876L).

These mutant AR proteins bind to other steroid hormones, such as estrogen, progesterone and glucocorticoids, which induce the activation of AR transcriptional activity resulting in prostate cancer growth.

In certain situations, AR mutations cause antagonists to induce an agonist conformation, resulting in AR activation rather than inhibition.

Early examples have been found in patients on flutamide treatment in combination with androgen blockade. Five of these 16 patients had the AR mutation T877A. Through luciferase assays, it was shown that the antagonist flutamide behaves like an agonist for these AR mutant proteins, and it was suggested that flutamide exerts a strong selective pressure for AR mutations.

The third mechanism of CRPC development is AR activation through ligand-independent mechanisms.

Studies have shown that tyrosine kinase receptor-activating ligands, such as insulin-like growth-factor-1 (IGF-1), keratinocyte growth factor (KGF), and epidermal growth factor (EGF), can activate the AR as a consequence of activating the downstream PI3K/ AKT/mTOR pathway, thus creating an 'outlaw receptor'. IGF-1 was able to cause AR activation, inducing a five-fold increase in PSA levels in LNCaP cells cultured in serum-free medium. Activation of the AR complex can also occur via crosstalk with other signaling pathways, such as those mediated by the non-receptor tyrosine kinases Src and Ack1.

Recently, various groups have described AR activation by binding of long non-coding RNAs (eg, PCGEM1 and PRNCR1) to the AR that can result in castration-resistant prostate cancer.

In addition, several AR variants that lack the LBD and act as negative regulators of the NTD have been described in CRPC. The AR NTD is constitutively active in the absence of the LBD and thus can promote androgen depletion-resistant growth.

The last pathway leading to CRPC bypasses AR signaling completely. It has been shown that castration therapy in mice triggers an inflammatory response released by the dying cells. Proinflammatory factors produced by dying prostate cancer cells cause the infiltration of B and T cells. Infiltrating B cells produce lymphotoxin and factors that increase Stat3 signaling, which is vital for promoting hormone-free survival of prostate cancer cells.

Similarly, upregulation of the anti-apoptotic protein Bcl-2 protects cancer cells from castration-induced apoptosis. Very recently, CRPC tumors were found to upregulate the expression of GR, another member of the nuclear receptor family. GR was shown to drive the expression of a subset of AR target genes necessary for cancer cell survival.

The various pathways mentioned may operate simultaneously to enhance AR activity.

Most evidence suggests that ADT failure may not result from a loss of androgen signaling but rather from the acquisition of genetic changes that lead to aberrant activation of the AR and its signaling axis. Thus, the AR remains a potential therapeutic target for prostate cancer therapy

From Schwiewer et al:

The androgen receptor (AR) is a ligand-dependent transcription factor that elicits context specific effects in the prostate. In the developing gland, active AR acts as a differentiation factor that is requisite for prostate function and maintenance. However, in prostate cancer (PCa), AR acquires a cell-autonomous function in actively promoting cancer cell growth and survival, mediated in part through exquisite dependence of this tumor type on AR function to induce cell cycle progression. Prior to ligand (testosterone or dihydrotestosterone, DHT) binding, the receptor is present diffusely throughout the cytoplasm and is held inactive through association with heat shock proteins.

Ligand binding induces rapid nuclear translocation and accumulation, chromatin association at multiple sites that govern gene expression (including those that contain canonical androgen response elements, AREs), recruitment of cofactors that influence downstream gene expression events, and initiation of a gene expression program that promotes tumor phenotypes. In addition, the product of a prostate-specific AR target gene (KLK3/PSA, prostate specific antigen), is used clinically to monitor prostate cancer development and progression. As PSA is secreted into and detected in human serum, quantification of serum PSA provides a clinical means to assess prostate cancer tumor burden.

Prostatic adenocarcinomas respond poorly to standard chemotherapy (including both cytostatic and cytotoxic agents); therefore, AR-directed therapeutics are utilized as the first line of intervention for non-organ confined disease. At the biochemical level, suppression of AR function is readily achieved through pharmacological methods that ablate testicular androgen synthesis (androgen deprivation therapy, ADT) and therefore deprive AR of circulating ligand.

Such modalities are frequently accompanied by adjuvant use of direct AR antagonists (e.g. bicalutamide), which not only compete with androgens for AR binding, but also induce recruitment of corepressors to the bicalutamide-bound AR complexes on chromatin. Efficacy is monitored biochemically through marked reduction of serum PSA levels, and clinically through radiographic evidence of tumor regression. Although the vast majority of patients respond to ADT and AR-directed therapeutics, these responses are transient – within a median time of 2–3 years, recurrent tumors develop which are almost invariably preceded by a rise in detectable PSA (referred to as "biochemical failure").

This stage of disease, for which there is no durable cure, is known as castrate-resistant prostate cancer (CRPC), and arises as a result of restored AR activity that is refractory to ADT and AR-directed therapeutics.

An extensive body of literature has addressed the multiple mechanisms by which AR is reactivated to promote therapeutic bypass, and these pathways have been recently reviewed.

At least five major, non-mutually exclusive categories have been identified through which cells adapt to ADT and AR-directed therapeutics.

Most frequently, deregulation of AR is observed, as can be achieved through amplification of the AR gene locus, alternative mechanisms that induce high level AR gene expression, and/or mechanisms that induce AR protein stabilization.

Significantly, it has been shown in multiple model systems that up-regulation of AR alone is sufficient to drive the transition to CRPC, and high nuclear AR levels are predictive for increased risk of death from prostate cancer.

Secondly, it has been recently shown that prostate cancers up-regulate enzymes that convert weak adrenal androgens to testosterone, and thus engage in intracrine androgen synthesis.

These events therefore supply the receptor with sufficient ligand to outcompete AR antagonists, restore AR activity, and promote CRPC growth. New pharmacological agents (e.g. abiraterone acetate) directed against this pathway show positive results in clinical trials.

Third, somatic mutation of AR, or development of splice variants, are known to facilitate CRPC. ADT is known to select for AR mutations that broaden the spectrum of ligands able to be utilized as agonists and/or convert antagonists into agonists.

Not surprisingly, these mutations generally cluster to the ligand binding domain. Alternatively, production of constitutively active AR splice variants that lack the ligand binding domain occurs in CRPC; these variants are not amenable to inhibition by ADT or established AR antagonists.

Fourth, alterations in pathways that regulate AR post-translational modifications that alter AR activity in a no or low ligand environment have been observed, and are thought to promote CRPC.

Finally, alterations in the levels and/or action of cofactors that modulate AR function have been reported, and play diverse roles in CRPC. Irrespective of the mechanism(s) utilized to bypass therapeutic intervention, AR activity resumes the capacity to drive cellular proliferation in CRPC.

As such, it is imperative to delineate the mechanisms by which AR governs cell cycle transitions in both early stage and castrate-resistant disease. As will be discussed herein, investigation of the mechanisms by which AR controls the cell cycle led to discovery of elegant crosstalk between

the AR signaling axis and the cell cycle machinery that, when altered, significantly influence tumor cell phenotypes and disease progression

Overall the understanding of CRPC is still a work in progress.

6 AR PRODUCTS

We have discussed the drivers and facilitators of AR. Now we summarize the products that result from AR activity. The list below represent some of the recent work but one suspects added and revised data will evolve.

6.1 SPECIFIC PRODUCTS

From the paper by The Cancer Genome Atlas Research Network, the following list reflects some of the primary products from the AR functions. Recall that KLK3 is PSA.

Product	Function
ABCC4	The protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MRP subfamily which is involved in multi-drug resistance. This family member plays a role in cellular detoxification as a pump for its substrate, organic anions. It may also function in prostaglandin-mediated cAMP signaling in ciliogenesis. Alternative splicing of this gene results in multiple transcript variants.
ACSL3	The protein encoded by this gene is an isozyme of the long-chain fatty-acid-coenzyme A ligase family. Although differing in substrate specificity, subcellular localization, and tissue distribution, all isozymes of this family convert free long-chain fatty acids into fatty acyl-CoA esters, and thereby play a key role in lipid biosynthesis and fatty acid degradation. This isozyme is highly expressed in brain, and preferentially utilizes myristate, arachidonate, and eicosapentaenoate as substrates. The amino acid sequence of this isozyme is 92% identical to that of rat homolog
CENPN	The protein encoded by this gene forms part of the nucleosome-associated complex and is important for kinetochore assembly. It is bound to kinetochores during S phase and G2 and recruits other proteins to the centromere. Pseudogenes of this gene are located on chromosome 2
EAF2	Enables transcription elongation regulator activity. Involved in positive regulation of transcription by RNA polymerase II and regulation of transcription elongation from RNA polymerase II promoter. Part of transcription elongation factor complex. Biomarker of prostate cancer
ELL2	Involved in snRNA transcription by RNA polymerase II. Located in nucleoplasm. Part of transcription elongation factor complex.

Product	Function
FKBP5	The protein encoded by this gene is a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. This encoded protein is a cis-trans prolyl isomerase that binds to the immunosuppressants FK506 and rapamycin. It is thought to mediate calcineurin inhibition. It also interacts functionally with mature hetero-oligomeric progesterone receptor complexes along with the 90 kDa heat shock protein and P23 protein. This gene has been found to have multiple polyadenylation sites.
GNMT	The protein encoded by this gene is an enzyme that catalyzes the conversion of S-adenosyl-L-methionine (along with glycine) to S-adenosyl-L-homocysteine and sarcosine. This protein is found in the cytoplasm and acts as a homotetramer. Defects in this gene are a cause of GNMT deficiency (hypermethioninemia). Alternative splicing results in multiple transcript variants. Naturally occurring readthrough transcription occurs between the upstream CNPY3
HERC3	This gene encodes a member the HERC ubiquitin ligase family. The encoded protein is located in the cytosol and binds ubiquitin via a HECT domain. Mutations in this gene have been associated with colorectal and gastric carcinomas
KLK2	This gene encodes a member of the grandular kallikrein protein family. Kallikreins are a subgroup of serine proteases that are clustered on chromosome 19. Members of this family are involved in a diverse array of biological functions. The protein encoded by this gene is a highly active trypsin-like serine protease that selectively cleaves at arginine residues. This protein is primarily expressed in prostatic tissue and is responsible for cleaving pro-prostate-specific antigen into its enzymatically active form. This gene is highly expressed in prostate tumor cells and may be a prognostic maker for prostate cancer risk.
KLK3	The gene is one of the fifteen kallikrein subfamily members located in a cluster on chromosome 19. It encodes a single-chain glycoprotein, a protease which is synthesized in the epithelial cells of the prostate gland, and is present in seminal plasma. It is thought to function normally in the liquefaction of seminal coagulum, presumably by hydrolysis of the high molecular mass seminal vesicle protein. The serum level of this protein, called PSA in the clinical setting, is useful in the diagnosis and monitoring of prostatic carcinoma. Alternate splicing of this gene generates several transcript variants encoding different isoforms

Product	Function
MAF	The protein encoded by this gene is a DNA-binding, leucine zipper-containing transcription factor that acts as a homodimer or as a heterodimer. Depending on the binding site and binding partner, the encoded protein can be a transcriptional activator or repressor. This protein plays a role in the regulation of several cellular processes, including embryonic lens fiber cell development, increased T-cell susceptibility to apoptosis, and chondrocyte terminal differentiation. Defects in this gene are a cause of juvenile-onset pulverulent cataract as well as congenital cerulean cataract 4 (CCA4)
MED28	Predicted to enable actin binding activity. Predicted to act upstream of or within negative regulation of smooth muscle cell differentiation and stem cell population maintenance. Located in nucleoplasm.
MPHOSPH9	Located in Golgi apparatus and centriole. Implicated in multiple sclerosis
NKX3−1	This gene encodes a homeobox-containing transcription factor. This transcription factor functions as a negative regulator of epithelial cell growth in prostate tissue. Aberrant expression of this gene is associated with prostate tumor progression. Alternate splicing results in multiple transcript variants of this gene
NNMT	N-methylation is one method by which drug and other xenobiotic compounds are metabolized by the liver. This gene encodes the protein responsible for this enzymatic activity which uses S-adenosyl methionine as the methyl donor
PMEPA1	This gene encodes a transmembrane protein that contains a Smad interacting motif (SIM). Expression of this gene is induced by androgens and transforming growth factor beta, and the encoded protein suppresses the androgen receptor and transforming growth factor beta signaling pathways though interactions with Smad proteins. Overexpression of this gene may play a role in multiple types of cancer.
PTGER4	The protein encoded by this gene is a member of the G-protein coupled receptor family. This protein is one of four receptors identified for prostaglandin E2 (PGE2). This receptor can activate T-cell factor signaling. It has been shown to mediate PGE2 induced expression of early growth response 1 (EGR1), regulate the level and stability of cyclooxygenase-2 mRNA, and lead to the phosphorylation of glycogen synthase kinase-3. Knockout studies in mice suggest that this receptor may be involved in the neonatal adaptation of circulatory system, osteoporosis, as well as initiation of skin immune responses

Product	Function
TMPRSS2	This gene encodes a protein that belongs to the serine protease family. The encoded protein contains a type II transmembrane domain, a receptor class A domain, a scavenger receptor cysteine-rich domain and a protease domain. Serine proteases are known to be involved in many physiological and pathological processes. This gene was demonstrated to be up-regulated by androgenic hormones in prostate cancer cells and down-regulated in androgen-independent prostate cancer tissue. The protease domain of this protein is thought to be cleaved and secreted into cell media after autocleavage. This protein also facilitates entry of viruses into host cells by proteolytically cleaving and activating viral envelope glycoproteins. Viruses found to use this protein for cell entry include Influenza virus and the human coronaviruses HCoV-229E, MERS-CoV, SARS-CoV and SARS-CoV-2 (COVID-19 virus).
ZBTB10	Predicted to enable RNA polymerase II transcription regulatory region sequence-specific DNA binding activity. Predicted to be involved in regulation of transcription by RNA polymerase II. Located in nucleoplasm

6.2 MUTATIONS

From the same paper we have the following set of genes seen as mutated in PCa:

Gene	Gene	Gene
AKT1	FOXA1	PTEN
ATM	HRAS	RB1
BRAF	IDH1	SPINK1
BRCA2	KMT2C	SPOP
CDK12	KMT2D	TP53
CDKN1B	MED12	ZMYM3
CHD1	NKX3-1	ZNF770
CTNNB1	PIK3CA	

As the authors note:

Primary prostate cancers exhibit a wide range of androgen receptor activity.

This study demonstrates for the first time a direct association between mutations in SPOP or FOXA1 and increased AR-driven transcription in human prostate cancers.

Further studies in preclinical models, as well as in clinical trial settings, will be required to understand the implications of variable AR activity in the contexts of chemoprevention and prostate cancer-directed treatment strategies. Other, more immediately actionable opportunities for targeted therapy exist for the 19% of primary prostate cancers that have defects in DNA

repair and for the nearly equal number of cancers with altered key effectors of both PI3K and MAPK pathways.

While the numbers of DNA repair defects found in organ confined prostate tumors may be lower than those found in metastatic prostate cancer, an increase in the number of such defects with disease progression suggests a possible advantage to targeting DNA repair-deficient tumors at an earlier stage of disease, perhaps at initial diagnosis. Such strategies may include preventing DNA damage, as well as targeting deficient DNA repair (Ferguson et al., 2015).

Alterations in the PI3K/MTOR pathway also play an important role: beyond the frequent inactivation of PTEN, we document rare activation of PIK3CA, PIK3CB, AKT1, and MTOR, and of several small GTPases, including HRAS, as well as BRAF.

As DNA sequencing of tumor samples becomes more widely adopted earlier in the clinical care of cancer patients, such alterations may emerge as candidates for inclusion in clinical trials after front-line therapy

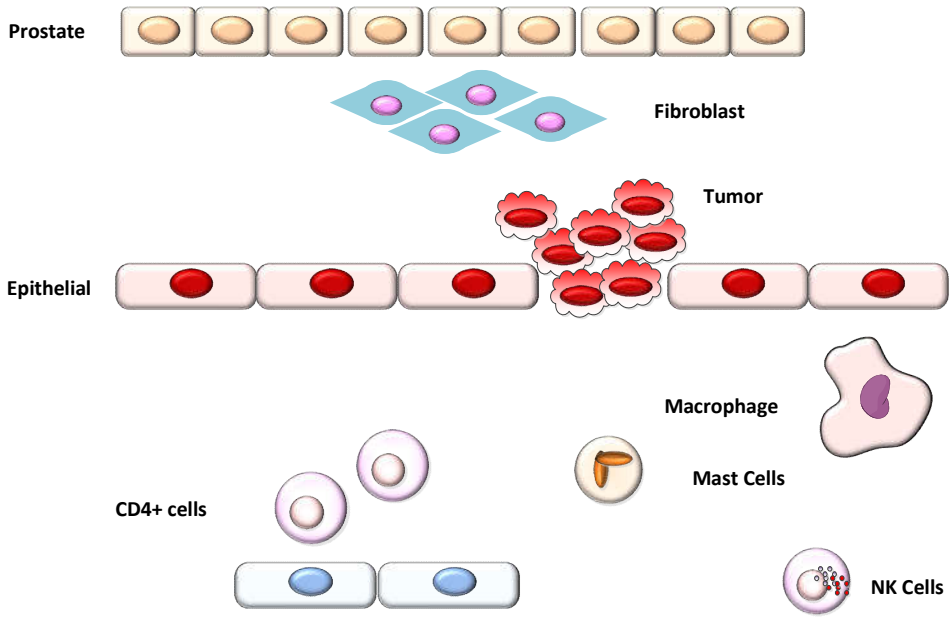
7 AR AND STROMA

The stroma is the "bed" upon which the prostate cells rest. It is the collection of supportive tissues and cells. What has become clear is that these tissues do not play some inactive role. Rather they often become co-conspirators in the development pf cancers. From Tang et al we have the following brief summary of some of these cells. We have also written extensively on most[29]:

Cell type	The role of AR	Effect on PCa
Endothelial cells	Is involved in TNF-induced apoptosis	Inhibits progression
	Induces VEGF-A and VCAM-1 expression, promotes proliferation	Promotes progression
CD4 T cells	Decreases IFN-y production and suppresses CD4 T cell proliferation	Inhibits progression
Fibroblasts	Upregulates FGF-2 and FGF-10 levels Decreases cell proliferation while increase cell migration of fibroblasts cells	Promotes initiation
	Negatively regulates the expression levels of CCL2, CCL8, M-CSF and IFN-y	Inhibits invasion
	Regulates cytokines secretion	Increases growth invasion
Macrophages	Negatively regulates the expression levels of CCL2	Inhibits invasion
	Promotes M2 macrophages polarization and regulates TREM-1 signaling	Promotes invasion

We graphically show these elements and their relationships below.

[29] https://www.researchgate.net/publication/341788660_Fibroblasts_and_Cancer_The_Wound_That_Would_Not_Heal , https://www.researchgate.net/publication/330222973_EMT_and_Cancers, https://www.researchgate.net/publication/321319216_Microbiome_Immune_System_and_Cancer, https://www.researchgate.net/publication/315374581_Extracellular_Matrix_vs_Intracellular_Pathways,



7.1 FIBROBLASTS

We have examined cancer associated fibroblasts, CAF, in PCa and other malignancies[30]. From Tang et al:

Cancer-associated fibroblasts (CAFs), one type of activated fibroblasts, typically express high levels of aSMA (a-smooth muscle actin) and fibroblast activation protein. Studies indicate that CAFs have a malignant property by affecting their surrounding compartments including cancerous cells and other stromal cells.

Compared to normal fibroblasts, CAFs have distinct genome-wide DNA methylation signatures at enhancers and promoters, causing aberrant expression of cancer-related genes to impact cancer fate. Abundant studies show that CAFs exist in the primary site of localized or metastatic PCa microenvironment and promote its initiation and progression. By comparing immortalized CAFs and normal prostate fibroblasts, researchers noticed that CAFs had stronger ability to promote malignant transformation of BPH-1 cells in vitro and in vivo, suggesting that CAF was a central driving source for PCa initiation.

In this process, AR in stromal fibroblasts but not in epithelium is necessary for prostatic epithelia malignant transformation.

According to this study, loss of stromal AR did not impair prostate homeostasis but decreased the expression levels of several stroma fibroblast-derived growth factors such as FGF-2 and FGF-10, impeding PCa carcinogenesis. Moreover, studies also indicated that CAFs had better

[30] https://www.researchgate.net/publication/341788660_Fibroblasts_and_Cancer_The_Wound_That_Would_Not_Heal

capacity to increase cell proliferation and invasion of LNCaP cells in vitro, compared to normal prostate fibroblasts.

The LNCaP xenografted mouse model also strengthened the tumor-promoting role of CAFs in PCa carcinogenesis. Consistent with this report, another study also found that CAFs significantly enhanced PCa growth and distant metastases when compared to normal prostate fibroblasts. Why are CAFs so effective in promoting PCa development?

Focus cytokine array showed that CAFs secreted more growth factors such as EGF, FGF, HGF, TGFb and VEGF, than normal prostate fibroblasts, providing survival advantage to PCa cells (Yu et al., 2017). How does AR contribute to fibroblast proliferation and migration? Results from mouse embryo fibroblasts NIH3T3 and fibrosarcoma HT1080 revealed that androgen stimulation (10 nM) could suppress proliferation while increasing migration of these two cell lines.

Mechanistic dissection unfolded that the androgen-stimulated AR/FlnA complex led to the activation of Rac1 as well as its downstream effector DYRK 1B, which phosphorylated p27 at Ser 10. The phosphorylated p27 at Ser 10 was stabilized and attributable to cell cycle arrest at G0/G1 of fibroblasts.

Meanwhile, the AR/FlnA complex also triggered the activation of focal adhesion kinase FAK, Rac1, and paxillin via recruiting integrin beta 1, promoting cell migration of NIH3T3, HT1080 cells, and PCa-derived fibroblasts. These findings suggest the therapeutic value of targeting the AR/FlnA complex to suppress the malignant activity of CAFs. Indeed, knockdown of FlnA with siRNAs or inhibition of AR by enzalutamide could abolish the migration and invasiveness of CAFs induced by androgen treatment.

Similarly, disruption of the AR/FlnA complex by drug-like compound Rh-2025u-stapled peptide, which was developed from the AR amino acid sequence required for the interaction with FlnA, abolished the androgen-induced migration and invasiveness of CAFs, consequently leading to less recruitment of CAFs to PCa and suppressing PCa-CAF organoid growth. How does AR in CAFs function to influence PCa growth and invasion?

Several studies have demonstrated that the reduced expression level of AR in CAFs was a prognostic indicator of PCa progression, suggesting that AR in CAFs serves as a negative regulator to determine PCa development.

A study showed that AR regulated extracellular matrix (ECM) components and maintained its inhibition on PCa cell invasion.

7.2 Tumor Associated Immune Cells

We have examined various tumor associated immune cells[31].

[31] https://www.researchgate.net/publication/336116071_Tumor_Associated_Immune_Cells_On_the_one_hand_and_on_the_other_hand

7.2.1 Macrophages

From Tang et al:

As the most predominant immune cells within the PCa microenvironment, macrophages play a central role in PCa development including cell survival, cell invasion, angiogenesis, lineage plasticity, and anti-androgen resistance.

Macrophages can be classified into pro-inflammatory/anti-tumoral M1 type and anti-inflammatory/pro-tumoral M2 type.

Typically, M2 macrophages, featured with high IL-10 production, increasingly infiltrate PCa, and this infiltration is strongly correlated with PCa aggressiveness. Studies demonstrated that PCa could promote differentiation and polarization of macrophages. PCa-derived IL-6, SDF1, and antimicrobial peptide LL-37 (leucin leucin 37) could promote M1–M2 differentiation/polarization, which in turn increased PCa invasiveness. Indeed, depletion of M2 macrophages remarkably inhibited tumor progression in various mouse tumor models, including PCa. Previous studies revealed that there was much more macrophage infiltration in PCa compared to matched normal tissues, monitored by the specific macrophage marker CD68, suggesting that macrophages may play a role in PCa tumorigenesis.

In an experimental setting mimicking in vivo cell–cell interaction…applied the coculture system using immortalized prostate epithelial cells (RWPE-1) and macrophages (THP-1) to induce prostate tumorigenesis. The results demonstrated that RWPE-1 cells cocultured with THP-1 cells could well differentiate into prostate spheres and had better ability to develop tumor in xenografted mouse models, and strengthening macrophage infiltration can promote PCa tumorigenesis.

Castration further increased the infiltration of M2 macrophages into PCa, and this recruitment of macrophages by ADT may be attributable to CCL2 production from PCa cells.

One publication illustrated that a transcriptional repressor of CCL2, SPDEF (SAM pointed domain-containing ETS transcription factor), was positively regulated by AR.

ADT inactivated AR transcriptional activity to reduce the transcription of SPDEF, which in turn promoted the expression of CCL2. CCL2 bound its receptor CCR2 on macrophages in a paracrine manner and enhanced their recruitment to PCa cells. Furthermore, the infiltrated macrophages would enhance PCa invasion/metastasis via downregulating AR and activating STAT3 signaling. Chang et al. found that coculture of THP-1 with PCa cells led to AR reduction in PCa cells. They also identified that PIAS3 was an AR-inducible gene, which was transcriptionally decreased in the presence of THP-1 cells.

Without the negative regulator PIAS3, STAT3 signaling was activated and drove PCa invasion/metastasis. Nevertheless, how AR is reduced when PCa cells receive the signals from macrophages remains unknown and requires additional investigation.

7.2.2 Natural Killer Cells

From Tang et al:

Natural killer (NK) cells are cytotoxic lymphocytes which play an important role in the regulation of innate immune response. Accumulating evidence suggests that the infiltration of NK cells is greater in PCa than that in normal prostate tissues and castration amplifies this phenomenon. By using the coculture system, …confirmed that PCa cells had a stronger ability to enhance IL-15-mediated expansion and cytotoxicity of NK cells than non-cancerous cell lines (PNT2 and WPMY-1), suggesting that the human body applies a protective strategy against PCa by activating NK cells.

Accordantly, another study showed that AR could transcriptionally regulate the expression of NK inhibitory ligand LLT1 (lectin-like transcript 1) in PCa cells. Castration led to a short decline of AR activity so that the LLT1 level was reduced, eventually contributing to the expansion and activation of NK cells. Specifically, the recruited NK cells could suppress the progression of CRPC by selectively degrading ARv7, the most important AR variant determining CRPC growth and drug resistance.

NK cells could not only sensitize CRPC cells to anti-androgen treatment but also inhibit cell invasion of CRPC cells.

More importantly, the suppression effect of NK cells on CRPC progression could be attenuated by the introduction of ARv7 into PCa cells. NK cells allowed CRPC cells to express high levels of miR-34 and miR-449, which bound the 30-UTR of ARv7 and caused its degradation. As the direct downstream effect of ARv7, EZH2 was reduced upon NK cell treatment and was the key causal factor controlling PCa invasion. All these data indicate that targeting ARv7 or EZH2 may overcome CRPC progression

7.2.3 Mast Cells

From Tang et al:

Mast cells are immune cells originated from myeloid stem cells. The wide distribution of mast cells across various tissues suggests that they act as key players to regulate a variety of physiological and pathological functions. Typically, mast cells can be classified into intra-tumoral and peri-tumoral mast cells based on their location in specific tissues. Intra-tumoral mast cells exert a protective role to prevent cancer development while peri-tumoral mast cells support tumor growth. Evidence supports the tumor-promoting role of mast cells in PCa owing to their peri-tumoral addiction. Castration with ADT stimulates mast cell recruitment. …

Interestingly, the expression levels of these cytokines were tightly regulated by AR. Castration mediated AR inhibition increased the expression levels of IL8, AM, and CCL8 in PCa cells so that more mast cells were attracted. Their results also revealed that the recruited mast cells

could drive neuroendocrine (NE) and stemness differentiation of PCa cells, promoting cancer invasion and tumor metastasis.

According to their data, miR-32 upregulation in PCa after AR inhibition was the causal factor determining NE differentiation. However, the targets of miR-32, potentially the direct downstream effectors regulating NE differentiation, are still not yet identified. Evidence also revealed that infiltrating mast cells could enhance the occupation of the HOTAIR/PRC2-suppressing complex at the upstream promoter region of the AR gene locus, leading to the suppressive transcription of AR.

The exact role of AR in mast cells in regard to PCa growth and invasion/metastasis is another direction for further investigation

8 AR AND CELL CYCLE

The cell cycle is the foundation of proliferation. We now examine the influence of AE upon the cell cycle.

8.1 CELL CYCLE BASICS

Cancer is basically uncontrolled cell growth, replication, and failure for cells to die off, normal apoptosis. It may also include loss of location stability and metabolic enhancement, but let us start with the key issue, replication. Then we examine two other major factors; apoptosis or cell death and cell to cell adhesion, or simply cells being where they should be. All of this examination is to be focused on the cell cycle. This section is a discussion of what is necessary to understand the importance of the cell cycle. The cycle is what often is broken in cancer cells, namely the cell reproduces again and again.

Cancer in many ways is a loss of the three factors:

1. Cell Replication: This is the normal or abnormal cell cycle.

2. Cell Death: This is normal cell death or apoptosis.

3. Cell Localization: The establishment and maintenance of a cells relative position and function.

We shall thus begin with the control of the cell cycle and then work upwards in terms of the cells control mechanism.

The following Figure presents a simple view of how cell signalling functions. There are six functions described, and not all must be present in any cell function. The steps are generally:

1. Ligand: There is some external activator that floats about and ultimately finds its home on the surface of a cell. Now the issue is not that there is one such protein floating about that eventually may find itself attached to the surface of a cell. The protein may be from afar or it may be from the very same cell. We could then consider the concentration of the protein as well, and its flow across cells themselves as well. This issue is a complex one and all too often it is treated like a simple one protein to one receptor issue. In reality it is a distributed random process.

2. Receptor: The ligand seeks and may ultimately find a receptor. The receptor is a protein on the cell surface. A cell produces the protein and the number of such receptors may be significant as well. Thus there exists a concentration in space of the ligands and they can attach to and activate receptors, proteins, on cell surfaces.

3. Adaptor: The Receptor when connected to a ligand effects a response and there may be an adaptor protein which then gets connected and starts the inter-cell communications process.

4. Transducer: The transducer, such as RAS or PI3K, converts the signal to the receptor as displayed by the adaptor into the beginning of a chain down through the cytoplasm. This is a highly controlled and redundant chain which can become unstable if certain genes are affected and the controlling proteins disabled.

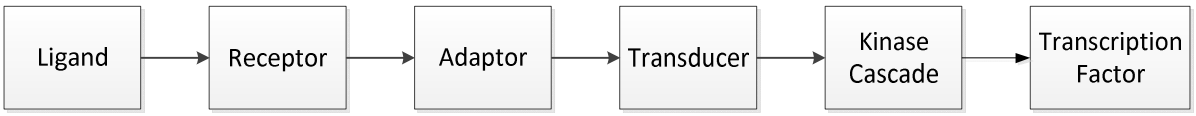
5. Kinase Cascade: This is the chain of protein communicating links and effectors from the Transducer to the cell nucleus and includes the initiation of the targeted transcription factor. As with the Transduce this kinase chain is controlled by redundant checks but if they become defective then the chain internal controls can be lost and the result become unstable.

6. Transcription Factor: This is the protein which has been activated within the nucleus which then commences transcription of the targeted sets of genes for the purpose of producing the resulting product.

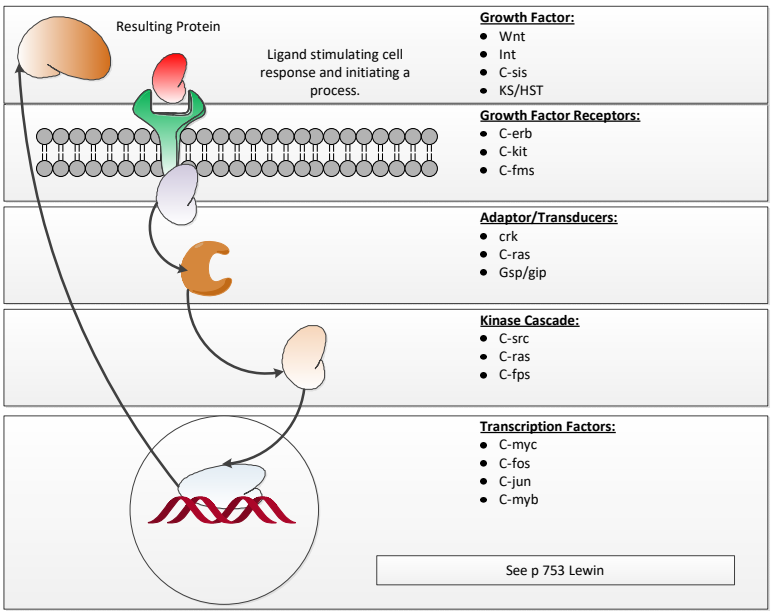
Note that this is a complex process.

Ligand	PDGF	Insulin	Growth Hormone	IL-1β	TGF-β
Receptor	PDGF Receptor	Insulin Receptor	GH Receptor	IL Receptor	TGF Receptor
Adaptor	SHP2/Grb2	IRS 1			
Transducer	SOS/Ras	PI3K	JAK	JAK	Type 1 Receptor
Kinase Cascade	MAPK	Akt			
Transcription Factor	Ternanry complex factors	FOXO	STATs	STATs	SMADs

See p 818 Lewin



The following depicts the process at several levels in a cell.



Now there are two major states a cell finds itself in; stasis and reproduction. A third, apoptosis, is natural cell death, we shall consider later. In stasis the cell is in G0 and producing proteins generally in response to external ligands or through normal internal processes. Unlike most standard biological models, we look at the proteins generally in terms of their concentrations and thus look at cell kinetics as well.

A cell in stasis is a little protein production factory, and each cell is pumping out the proteins and they then are in some extracellular balance. The cells in stasis communicate with one another via their respective ligands. In contrast when a cell reproduces it is standing out from the crowd if one will and looking out for itself.

We now examine first gene operations and then cell replication.

8.1.1 Cell Replication

We first address cell replication. First we examine the cell cycle from a generic perspective. We then examine the details on the pathways which may result in unstable cell reproduction.

The cell replication cycle goes through 4 stages. The dormant stage, G0, is not part of this process. The stages in cell reproduction are:

G0: This is the resting phase. It is during this phase that the cell is producing proteins via normal transcription processes. G0 may be resting related to the reproductive mitotic activities but the cell is quite active as a protein generating factory.

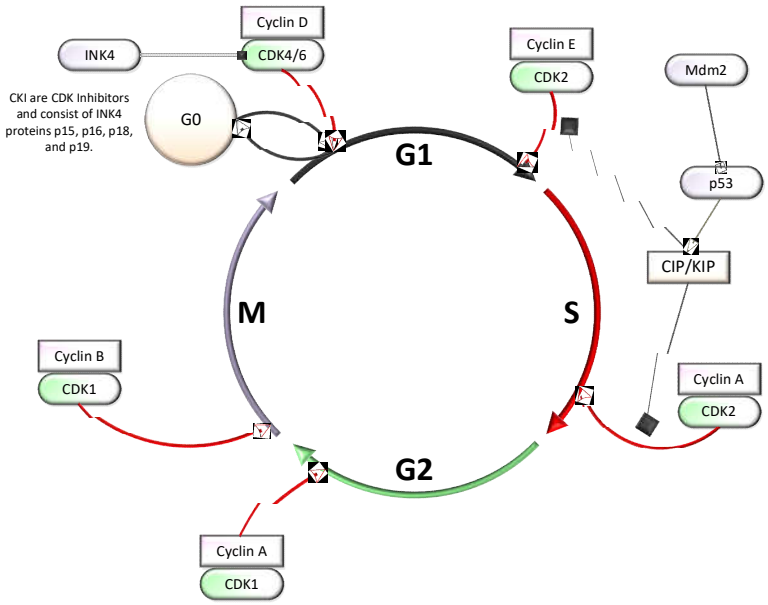
G1: Once the cell begins the G1 phase it is on its way to reproducing via mitosis.

S: The S phase is the phase where the DNA is duplicated. This is a sensitive stage; any error here can be propagated forward albeit there may still be checks available.

G2: This is the second gap phase.

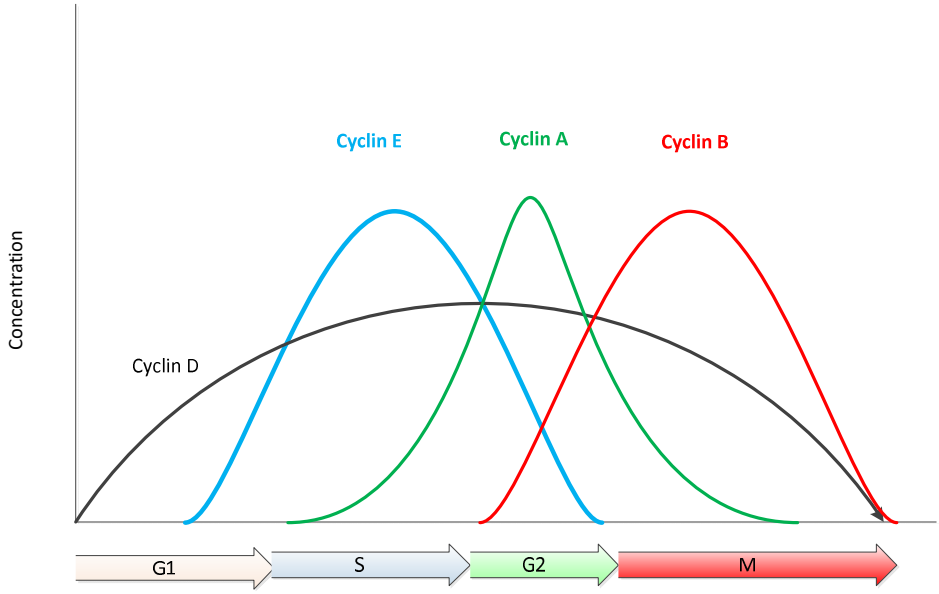
M: M phase includes mitosis and cytokinesis, namely the creation of two identical new cells.

Now the cell starts G1 by being instigated by a bound pair of a cyclin and a CDK, a cyclin dependent kinase. In this specific case we start with a binding of cyclin D and CDK4/6. This is the initiating event moving into G1 from senescence in G0. We depict these processes below (from McKinnell et al p. 169.):



The cyclins in each stage grow in concentration and as such move the cell along in each of its reproductive stages.

The following shows the phases and the relevant concentrations of cyclin bound to CDKs. Note the increase in concentration activates a change or movement along the mitotic path.



Note in the above the concentration of a specific cyclin above a level of a previous cyclin initiates the next step in mitosis. The details as to how and why this happens are detailed in Morgan (Chapter 3).

Protein[32]	***Gene***	***Function***[33]
Cyclin A (also CCN1; CCNA, CCNA2, Cyclin A2)	4q25-q31	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through 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. In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in all tissues tested. This cyclin binds and activates CDC2 or CDK2 kinases, and thus promotes both cell cycle G1/S and G2/M transitions.
Cyclin B1 (CCNB1)	5q12	The protein encoded by this gene is a regulatory protein involved in mitosis. The gene product complexes with p34 (cdc2) to form the maturation-promoting factor (MPF). Two alternative transcripts have been found, a constitutively expressed transcript and a cell cycle-regulated transcript that is expressed predominantly during G2/M phase. The different transcripts result from the use of alternate transcription initiation sites.

[32] http://www.ncbi.nlm.nih.gov/gene/983

[33] From http://www.ncbi.nlm.nih.gov/gene/595 data bases as a source.

Protein[32]	***Gene***	***Function***[33]
Cyclin B2 (CCNB2)	15q22.2	Cyclin B2 is a member of the cyclin family, specifically the B-type cyclins. The B-type cyclins, B1 and B2, associate with p34cdc2 and are essential components of the cell cycle regulatory machinery. B1 and B2 differ in their subcellular localization. Cyclin B1 co-localizes with microtubules, whereas cyclin B2 is primarily associated with the Golgi region. Cyclin B2 also binds to transforming growth factor beta RII and thus cyclin B2/cdc2 may play a key role in transforming growth factor beta-mediated cell cycle control.
Cyclin C (CCNC)	6q21	The protein encoded by this gene is a member of the cyclin family of proteins. The encoded protein interacts with cyclin-dependent kinase 8 and induces the phophorylation of the carboxy-terminal domain of the large subunit of RNA polymerase II. The level of mRNAs for this gene peaks in the G1 phase of the cell cycle. Two transcript variants encoding different isoforms have been found for this gene.
Cyclin D (Cyclin D1)	11q13	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. Mutations, amplification and overexpression of this gene, which alters cell cycle progression, is observed frequently in a variety of tumors and may contribute to tumorigenesis.

Protein[32]	*Gene*	*Function*[33]
Cyclin E (CCNE1)[34]	19q12	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through 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 CDK2, whose activity is, required for cell cycle G1/S transition. This protein accumulates at the G1-S phase boundary and is degraded as cells progress through S phase. Overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. This protein was found to associate with, and be involved in, the phosphorylation of NPAT protein (nuclear protein mapped to the ATM locus), which participates in cell-cycle regulated histone gene expression and plays a critical role in promoting cell-cycle progression in the absence of pRB. Two alternatively spliced transcript variants of this gene, which encode distinct isoforms, have been described.

The CDKs involved are:

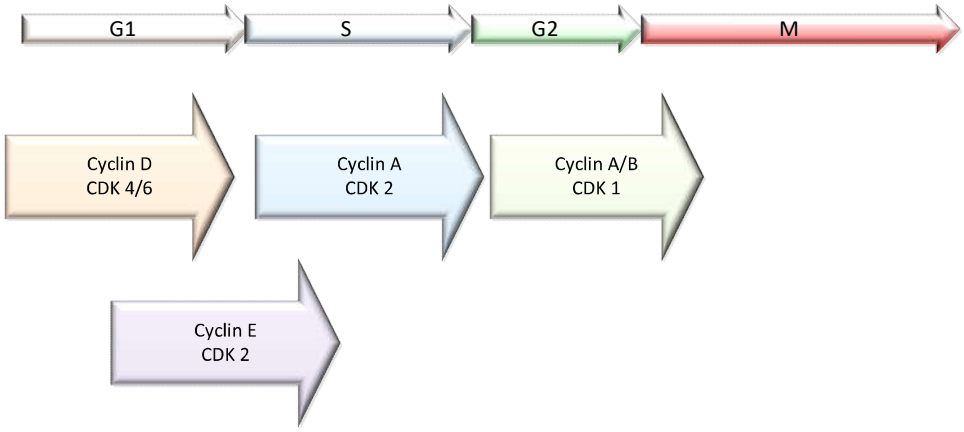
[34] http://www.ncbi.nlm.nih.gov/gene/898

Protein[35]	*Gene*	*Function*[36]
CDK 1 (also known as CDC2; CDC28A; P34CDC2)	10q21.1	This protein is a catalytic subunit of the highly conserved protein kinase complex known as M-phase promoting factor (MPF), which is essential for G1/S and G2/M phase transitions of eukaryotic cell cycle. Mitotic cyclins stably associate with this protein and function as regulatory subunits. The kinase activity of this protein is controlled by cyclin accumulation and destruction through the cell cycle. The phosphorylation and dephosphorylation of this protein also play important regulatory roles in cell cycle control.
CDK 2 (also called p33)	12q13	It is a catalytic subunit of the cyclin-dependent protein kinase complex, whose activity is restricted to the G1-S phase, and essential for cell cycle G1/S phase transition. This protein associates with and regulated by the regulatory subunits of the complex including cyclin A or E, CDK inhibitor p21Cip1 (CDKN1A) and p27Kip1 (CDKN1B). Its activity is also regulated by its protein phosphorylation.
CDK 3	17q22	This gene encodes a member of the cyclin-dependent protein kinase family. The protein promotes entry into S phase, in part by activating members of the E2F family of transcription factors. The protein also associates with cyclin C and phosphorylates the retinoblastoma 1 protein to promote exit from G0.
CDK 4 (also CMM3; PSK-J3)	12q14	This protein is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. The activity of this kinase is restricted to the G1-S phase, which is controlled by the regulatory subunits D-type cyclins and CDK inhibitor p16 (INK4a). This kinase was shown to be responsible for the phosphorylation of retinoblastoma gene product (Rb). Mutations in this gene as well as in its related proteins including D-type cyclins, p16 (INK4a) and Rb were all found to be associated with tumorigenesis of a variety of cancers.
CDK 6 (also PLSTIRE)	7q21-22	The protein encoded by this gene is a member of the cyclin-dependent protein kinase (CDK) family. CDK family members are known to be important regulators of cell cycle progression. This kinase is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression and G1/S transition. The activity of this k+inase first appears in mid-G1 phase, which is controlled by the regulatory subunits including D-type cyclins and members of INK4 family of CDK inhibitors. This kinase, as well as CDK4, has been shown to phosphorylate, and thus regulate the activity of, tumor suppressor protein Rb. Expression of this gene is up-regulated in some types of cancer.

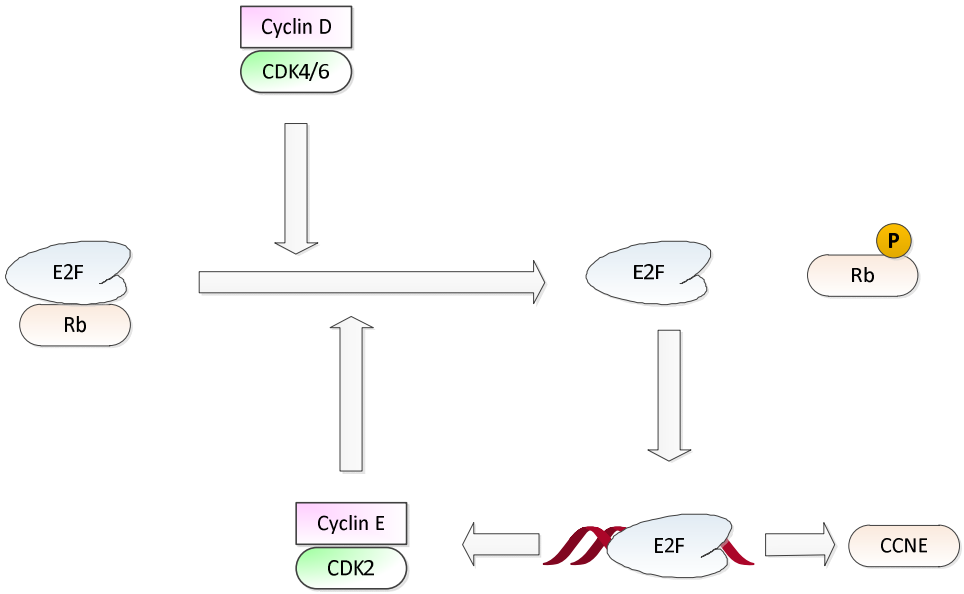
[35] http://www.ncbi.nlm.nih.gov/gene/983

[36] From http://www.ncbi.nlm.nih.gov/gene/595 data bases as a source.

Now the question is what activates these proteins, the cyclins and the CDKs, to make the cell cycle progress. This begins the creep upward in this pathway concern. We can redraw this process as follows and it will help to focus:



Now we ask what activates these proteins. We look at the activation of Cyclin E as shown by Bunz (p 219) below:



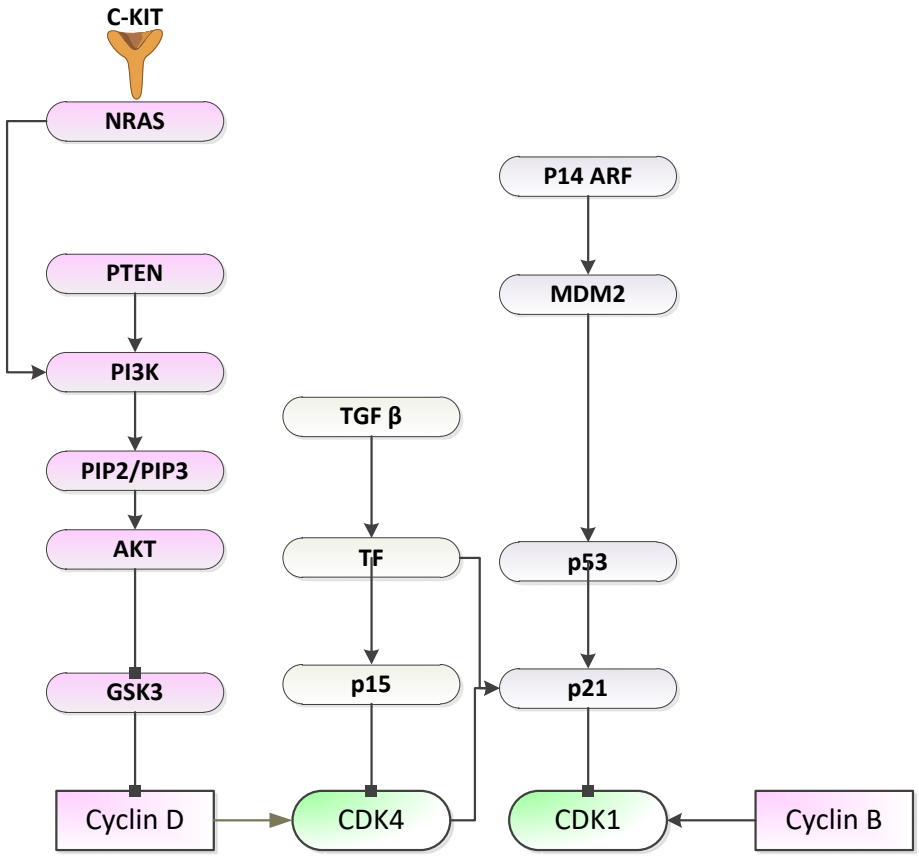
This is a feedback type reaction initiated by Rb the retinoblastoma gene protein. This feedback generates cyclin E which drives the cell through G1 and into the S cycle.

Gene	*Location*	*Function*
E2F1[37] (also RBP3; E2F-1; RBAP1; RBBP3)	20q11.2	The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming proteins of small DNA tumor viruses. The E2F proteins contain several evolutionally conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain which is embedded within the transactivation domain. This protein and another 2 members, E2F2 and E2F3, have an additional cyclin binding domain. This protein binds preferentially to retinoblastoma protein pRB in a cell-cycle dependent manner. It can mediate both cell proliferation and p53-dependent/independent apoptosis.
RB 1[38] (also RB; pRb; OSRC; pp110; p105-Rb)	13q14.2	The protein encoded by this gene is a negative regulator of the cell cycle and was the first tumor suppressor gene found. The encoded protein also stabilizes constitutive heterochromatin to maintain the overall chromatin structure. The active, hypophosphorylated form of the protein binds transcription factor E2F1. Defects in this gene are a cause of childhood cancer retinoblastoma (RB), bladder cancer, and osteogenic sarcoma.
CCNE1[39]	19q12	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through 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 CDK2, whose activity is, required for cell cycle G1/S transition. This protein accumulates at the G1-S phase boundary and is degraded as cells progress through S phase. Overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. This protein was found to associate with, and be involved in, the phosphorylation of NPAT protein (nuclear protein mapped to the ATM locus), which participates in cell-cycle regulated histone gene expression and plays a critical role in promoting cell-cycle progression in the absence of pRB. Two alternatively spliced transcript variants of this gene, which encode distinct isoforms, have been described.

[37] http://www.ncbi.nlm.nih.gov/gene/1869

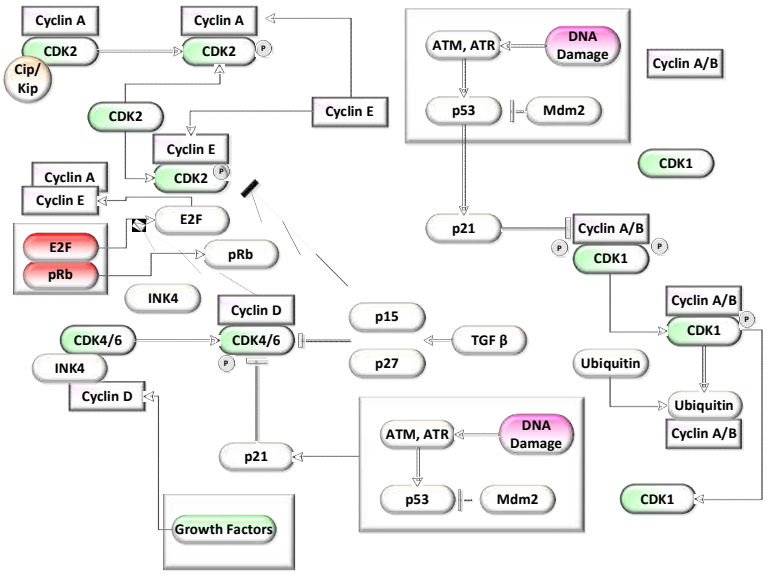
[38] http://www.ncbi.nlm.nih.gov/gene/5925

Now this establishes one base line for understanding cancer at the base of cell reproduction. Namely what can cause this process to continue unabated?



A more details analysis has been by Vermulen et al almost a decade ago. We shall use this as a baseline and then add to what we have learned in that period. The Vermulen network is shown as follows:

[39] http://www.ncbi.nlm.nih.gov/gene/898



Now in the Vermulen configuration we have the following elements:

1. CDKs: These are the cyclin dependent kinases we have been discussing.

2. Cyclins:

3. CDK Activating Enzymes:

4. CKI or CK Inhibitors

The following is a detailed list of some major CKIs or Cyclin Kinase Inhibitors. We have discussed them briefly before but they play a critical role in managing cell reproduction.

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
INK4 Family	p15[40] (also P15; MTS2; TP15; CDK4I; INK4B; p15INK4b)	INK-4b	9p21	This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases, thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition.
	p16[41] (also ARF; MLM; P14; P16; P19; CMM2; INK4; MTS1; TP16; CDK4I; CDKN2; INK4A; MTS-1; P14ARF; P19ARF; P16INK4; P16INK4A; P16-INK4A)	INK-4a	9p21	This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, MDM1, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control.

[40] http://www.ncbi.nlm.nih.gov/gene/1030

[41] http://www.ncbi.nlm.nih.gov/gene/1029

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p18[42]	INK-4c	1p32	The protein encoded by this gene is a member of the INK4 family of cyclin-dependent kinase inhibitors. This protein has been shown to interact with CDK4 or CDK6, and prevent the activation of the CDK kinases, thus function as a cell growth regulator that controls cell cycle G1 progression. Ectopic expression of this gene was shown to suppress the growth of human cells in a manner that appears to correlate with the presence of a wild-type RB1 function. Studies in the knockout mice suggested the roles of this gene in regulating spermatogenesis, as well as in suppressing tumorigenesis.
	p19[43]	INK-4d	19p13	The protein encoded by this gene is a member of the INK4 family of cyclin-dependent kinase inhibitors. This protein has been shown to form a stable complex with CDK4 or CDK6, and prevent the activation of the CDK kinases, thus function as a cell growth regulator that controls cell cycle G1 progression. The abundance of the transcript of this gene was found to oscillate in a cell-cycle dependent manner with the lowest expression at mid G1 and a maximal expression during S phase. The negative regulation of the cell cycle involved in this protein was shown to participate in repressing neuronal proliferation, as well as spermatogenesis.

[42] http://www.ncbi.nlm.nih.gov/gene/1031

[43] http://www.ncbi.nlm.nih.gov/gene/1032

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
Cip-Kip Family	p21[44] also P21; CIP1; SDI1; WAF1; CAP20; CDKN1; MDA-6; p21CIP1	Waf1, Cip1	6p21.2	This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation.
	p27[45] also p27; Rpn4	Cip2	12q24.31-q24.32	The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes a non-ATPase subunit of the 19S regulator.

[44] http://www.ncbi.nlm.nih.gov/gene/1026

[45] http://www.ncbi.nlm.nih.gov/gene/5715

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p57[46] also BWS; WBS; p57; BWCR; KIP2	Kip2	11p15.5	This gene is imprinted, with preferential expression of the maternal allele. The encoded protein is a tight-binding, strong inhibitor of several G1 cyclin/Cdk complexes and a negative regulator of cell proliferation. Mutations in this gene are implicated in sporadic cancers and Beckwith-Wiedemann syndrome, suggesting that this gene is a tumor suppressor candidate.

The following genes are elements of cell cycle control.

Gene	*Location*	*Function*
Jun[47]	1p32-p31	This gene is the putative transforming gene of avian sarcoma virus 17. It encodes a protein which is highly similar to the viral protein, and which interacts directly with specific target DNA sequences to regulate gene expression. This gene is intronless and is mapped to 1p32-p31, a chromosomal region involved in both translocations and deletions in human malignancies.
Fos[48]	14q24.3	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation. In some cases, expression of the FOS gene has also been associated with apoptotic cell death.

[46] http://www.ncbi.nlm.nih.gov/gene/1028

[47] http://www.ncbi.nlm.nih.gov/gene/3725

[48] http://www.ncbi.nlm.nih.gov/gene/2353

Gene	*Location*	*Function*
Myc[49]	8q24.21	The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma. There is evidence to show that alternative translation initiations from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site result in the production of two isoforms with distinct N-termini. The synthesis of non-AUG initiated protein is suppressed in Burkitt's lymphomas, suggesting its importance in the normal function of this gene

8.1.2 Other Factors in the Cell Cycle

In a recent paper by Solimini et al the authors discuss the concepts of STOP and GO genes and carcinogenesis[50]. The paper reports on some extensive experimental results focusing on the issue of proliferation and the loss of certain sets of gene sites, the STP and GO sites.

The authors begin by discussing the current concepts of changes in oncogenes and tumor suppressor genes, some of the key pathway elements that we examine in analyzing intracellular pathway dynamics. They state:

Cancer progression is directed by alterations in oncogenes and tumor suppressor genes (TSGs) that provide a competitive advantage to increase proliferation, survival, and metastasis. The cancer genome is riddled with amplifications, deletions, rearrangements, point mutations, loss of heterozygosity (LOH), and epigenetic changes that collectively result in tumorigenesis.

How these changes contribute to the disease is a central question in cancer biology. In his "two-hit hypothesis," Knudson proposed that two mutations in the same gene are required for tumorigenesis, indicating a recessive disease. In addition, there are now several examples of haploinsufficient TSGs.

Current models do not explain the recent observation that hemizygous recurrent deletions are found in most tumors. Whether multiple genes within such regions contribute to the tumorigenic phenotype remains to be elucidated…

[49] http://www.ncbi.nlm.nih.gov/gene/4609

[50] Solimini, N., et al, Recurrent Hemizygous Deletions in Cancers May Optimize Proliferative Potential, Science, 6 JULY 2012 VOL 337, p 104.

The last sentence regarding the inability to explain the presence of hemizygous deletions under the current model is the main driver for this effort. Thus they argue and demonstrate experimentally that:

Tumors exhibit numerous recurrent hemizygous focal deletions that contain no known tumor suppressors and are poorly understood. To investigate whether these regions contribute to tumorigenesis, we searched genetically for genes with cancer-relevant properties within these hemizygous deletions.

We identified STOP and GO genes, which negatively and positively regulate proliferation, respectively.

STOP genes include many known tumor suppressors, whereas GO genes are enriched for essential genes.

Analysis of their chromosomal distribution revealed that recurring deletions preferentially over-represent STOP genes and under-represent GO genes.

*We propose a hypothesis called the **cancer gene island model**, whereby gene islands encompassing high densities of STOP genes and low densities of GO genes are hemizygously deleted to maximize proliferative fitness through cumulative haploinsufficiencies.*

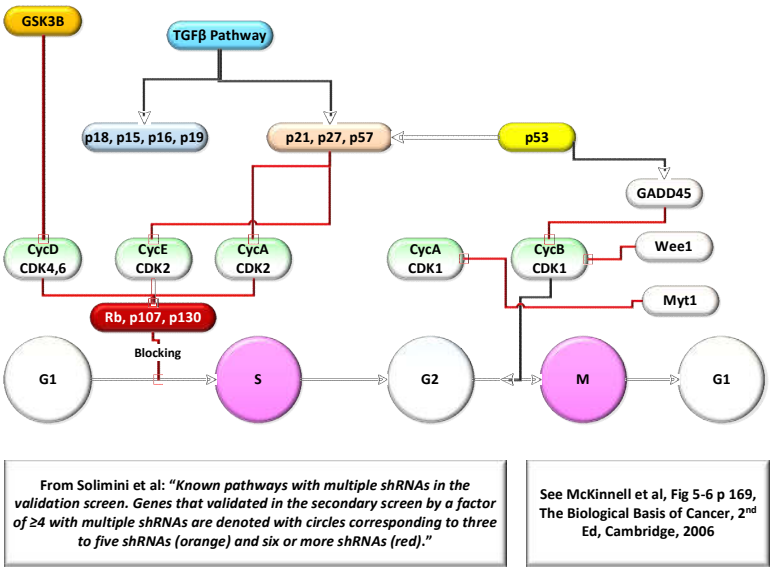
Because hundreds to thousands of genes are hemizygously deleted per tumor, this mechanism may help to drive tumorigenesis across many cancer types.

This is an intriguing hypothesis. It adds more pieces to an already complex puzzle. The Cancer Gene Island, CGI, hypothesis seems to indicate the complex changes in multiple gene sites. In particular there was a deletion of the STOP genes in preference to the GO genes. Unfortunately there did not seem to be a mechanism for these deletions, however the experimental evidence does indicate the phenomenon.

In their experimental analysis they have observed certain in vitro results which compel their hypothesis. They state:

This in silico analysis suggests that the loss of a single copy of GO genes has a negative impact on cellular fitness. To independently test this hypothesis, we turned to the other arm of our screen that identified candidate GO genes whose depletion limits proliferation and survival. Because both normal and cancer cells are dependent on these essential GO genes, we analyzed data from proliferation screens on HMECs, one normal prostate epithelial cell line, and seven breast or prostate cancer cell lines

They provide an interesting pathway model as shown below (as modified, and also not that they have short hairpin RNAs (shRNAs)).



From Solimini et al: "*Known pathways with multiple shRNAs in the validation screen. Genes that validated in the secondary screen by a factor of ≥4 with multiple shRNAs are denoted with circles corresponding to three to five shRNAs (orange) and six or more shRNAs (red).*"

See McKinnell et al, Fig 5-6 p 169, The Biological Basis of Cancer, 2nd Ed, Cambridge, 2006

They conclude as follows:

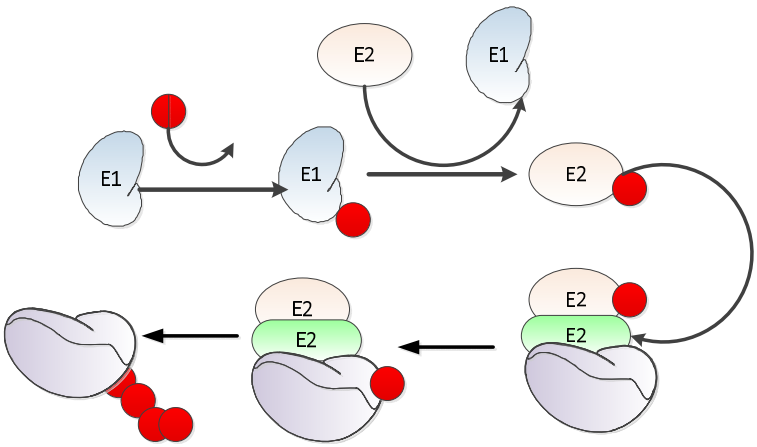
The enrichment for genes localized to deletions suggests that we have identified dozens of new TSGs in recurrent deletions. We have also likely identified more TSGs outside of these regions because the STOP gene set is (i) enriched for known TSGs, many of which are not found in recurrent deletions, and (ii) enriched for genes that undergo somatic loss-of-function mutation.

Finally, this work suggests that cells possess a substantial number of genes that restrain proliferation in vitro, which could be inactivated to promote clonal expansion during tumorigenesis in addition to the traditional driver genes currently known. Given the prevalence of multiple, large, recurring hemizygous deletions encompassing skewed distributions of growth control genes in tumors, we propose that the elimination of cancer gene islands that optimize fitness through cumulative haplo-insufficiencies may play an important role in driving tumorigenesis, with implications for the way in which we think about cancer evolution.

As with many such works this raises as many questions as it seems to answer. However the control or lack thereof of proliferation and the cell cycle is a critical issue in carcinogenesis.

8.1.3 Ubiquination

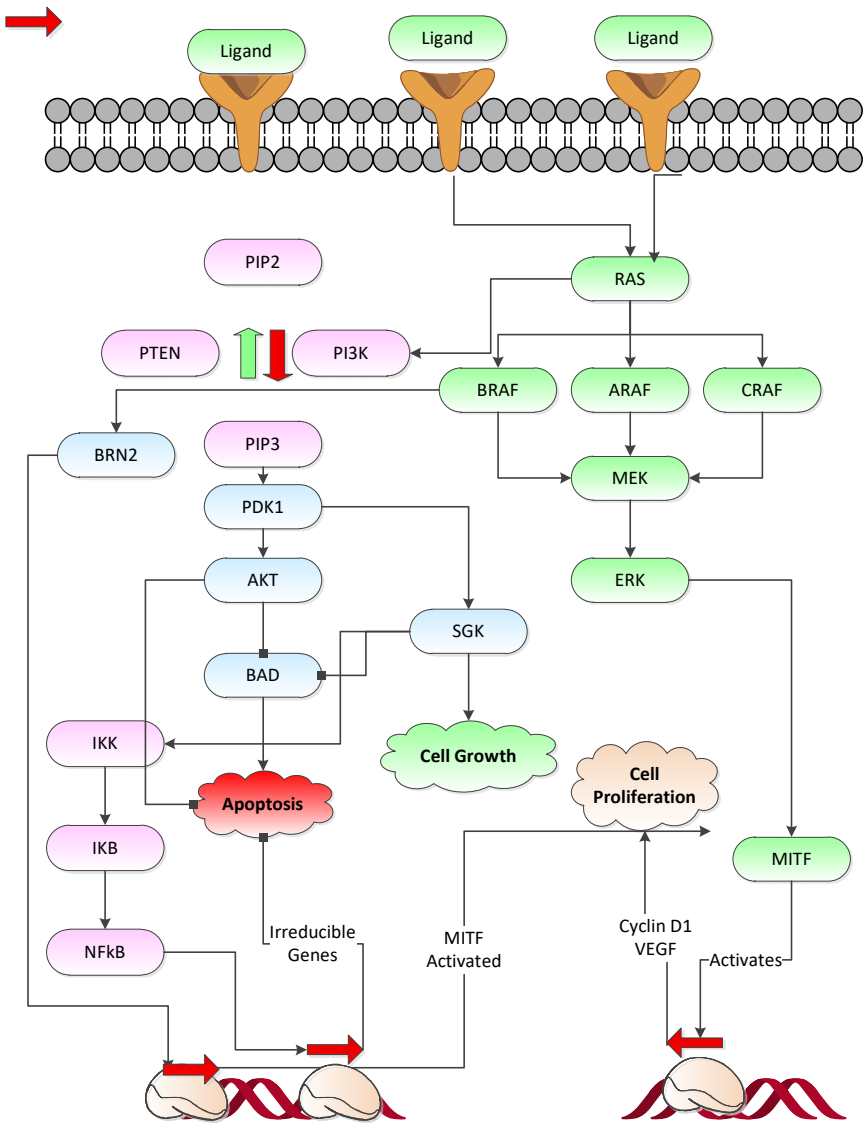
Ubiquitin is a small protein which acts with three related proteins; E1, E2, and E3. E1 is also called the ubiquitin activating enzyme, E2 the ubiquitin conjugating enzyme, and E3 ubiquitin ligase. Together they act to attach ubiquitin to a target protein and mark it for digestion and elimination. The process is shown below in general graphic form.



Ubiquination is an essential process within a cell to eliminate used or excess proteins. Although we will not discuss tis in detail, it is an essential process and the reader should refer to standard texts[51].

The following Figure depicts some of the mechanics in terms of genetic flow and control as to how Ubiquination occurs.

[51] See: Cassimeris et al p 688, Weinberg, p 242, Alberts et al, p 1065.



Simply there are three end states:

1. Cell Proliferation or Cell Cycle Mitosis

2. Cell Growth or the expansion and operations of a single cell outside of mitosis.

3. Apoptosis or cell death.

Now in the simplified model above we have several feedback loops, many driven by external ligands.

In this section we briefly review the issue of cellular growth. What makes cells reproduce? If we first examine skin cells, one of the many cells in the body which reproduce all the time, like blood cells, we can gain some insight.

Skin cells are reproducing all the time. Mostly the keratinocytes and getting sloughed off at the surface or rebuilding after a wound. The melanocytes frequently do not reproduce. They are neural crest derives and often just remain in the G0 state. They produce such products as melanosomes, and other proteins required for homeostasis. There are times when they may reproduce to a cluster state, such as found in lentigenes. This is a common response to excessive sun exposure. Namely we may see heavily pigmented areas of clustered melanocytes. Then we may have a nevus, the raised collection of melanocytes. In both cases the melanocytes tend to stay attached to the cluster, thus having functional E cadherin molecules.

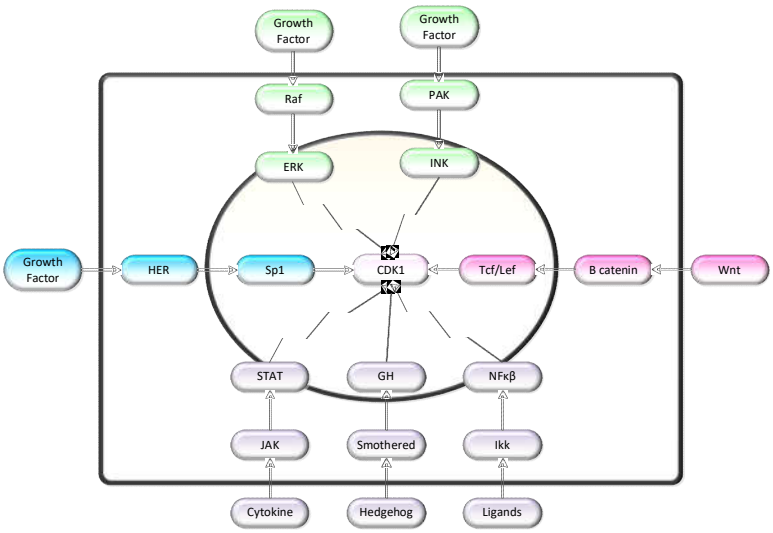
Now what of prostate cells, they do not reproduce as quickly. The glands are generally stable and often reproduce after some nominal lifetime of the basal or luminal cell. However a cell is stressed, for example by some external driver as inflammation, or other external attack, and then the cells may regenerate and thus reproduce. Perhaps that is one of the mechanism which underlies indolent PCa. Melanoma for example is highly aggressive in any form, most likely driven by the aggressive growth medium. However, as is known, melanocytes alone are indolent. This is one of those "on the one hand, on the other hand" arguments.

8.1.4 Kinetics of Cell Cycles

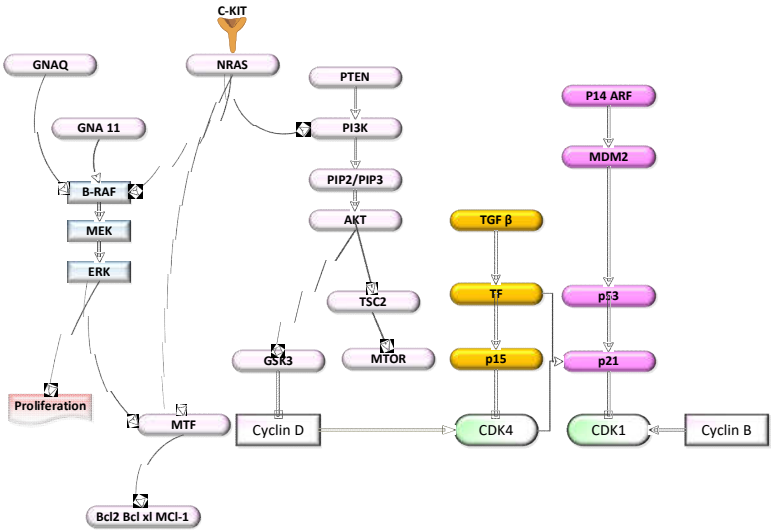
One of the questions we may ask is related to the kinetics of these processes. For example in many cancers the cell doubling time is highly variable at different locations and at different times and with different cells. There have been a few studies regarding the kinetics, namely what facilitates and accelerates the cell cycle but there does not appear at this time to be a definitive conclusion.

We have presented a high level summary of the DNA activity and the resulting cell cycle in mitotic activity. The cell cycle play an important role in cancer since inherent in any cancer is uncontrolled cell reproduction. The cyclins are at the heart of that process. It will be useful to go back to these basic ideas from time to time yet we do not consider the cell cycle as an integral part of our control model. Generally we try to take actions which prevent it from ever being entered. However it may become more critical to examine the cell cycle as a control point.

We show the interconnectivity below:



And the details in the following:



8.2 AR AND CELL CYCLE

We now examine the impact of AR on the cell cycle. From Schwiewer et al:

The means by which ligand-activated AR initiates the cell cycle has been generally defined. ADT-sensitive cells deprived of androgen exit the cell cycle and arrest in G0.

Transitions into and within the cell cycle are controlled by cyclin/cyclin-dependent kinase (cdk) complexes, which act sequentially to maintain ordered progression from G1 to mitosis. Typically, cdk activity is influenced by limited availability of the required cyclin subunit and/or the presence of cdk inhibitors, and both processes assist in controlling androgen-dependent cell cycle progression.

In early G1, cdk4 or cdk6 activity is induced in most cell types, as achieved by growth factor-mediated D-type cyclin accumulation. Indeed, expression of major D-type cyclins (cyclins D1 and D3) is suppressed in ADT-responsive prostate cancer cells after steroid deprivation, and contributes to cell cycle arrest. There is little evidence that androgen deprivation alters p16ink4a levels, a known cdk4/6 inhibitor.

As such, it is thought that androgen regulation of D-cyclin accumulation serves as the major underpinning means by which AR regulates cdk4/6 activity and early G1 transitions.

Accordingly, androgen stimulation induces mTOR-dependent translation of D-cyclins, resulting in protein accumulation sufficient to activate cdk4/6.

These events are independent of D-cyclin gene expression; distinct from what is observed in breast cancer cells, D-cyclin mRNA levels are unchanged by hormone deprivation in prostate cancer cells. Thus, AR regulates early G1 progression primarily through controlling Dtype cyclin protein levels.

Activated D-cyclin/cdk4 or 6 complexes initiate phosphorylation/inactivation of the retinoblastoma tumor suppressor (RB), which negatively regulates cell cycle transitions and the onset of DNA replication. RB function is envisaged as a "rheostat" to govern all stages of the cell cycle, wherein cdk4/6-mediated phosphorylation events compromise RB activity, and subsequent cdks increase RB phosphorylation status as a function of cell cycle progression…

Active RB is recruited to chromatin at sites that regulate expression of genes important for cellular proliferation, including genes essential for DNA replication (e.g. MCM7) and S-phase entry (e.g. cyclin A2).

Many RB-regulated genes are activated by the E2F family of transcription factors, and RB counterbalances E2F-mediated gene expression by assembling transcriptional repressor complexes that dampen transcriptional transactivation. Thus, a major function of G1 cdk complexes is to attenuate these functions of RB through direct phosphorylation and inactivation. Toward this end, cdk2 activity promotes completion of G1 and transitions into S-phase, as mediated by sequential partnering with cyclins E1 and A2. Similar to what is observed with cdk4/6, androgen ablation or stimulation has little influence on cdk2 protein levels.

However, at least two gatekeepers place cdk2 activity under AR control.

First, while cyclin E1 protein levels are unchanged by the presence or absence of androgen, cyclin E1/cdk2 activity is strongly suppressed by ADT in vitro.

This effect is likely attributed to androgen-mediated regulation of p27kip1, a potent suppressor of cdk2 activity and bona fide tumor suppressor protein. p27kip1 levels are induced by androgen deprivation …

conversely, androgen stimulation is known to promote rapid p27kip1 degradation. Although the mechanisms by which androgen promotes p27kip1 degradation and subsequent cdk2 activity is

incompletely defined, it is thought that these events serve to promote G1 progression and commitment to the mitotic cell cycle.

A related protein with no known tumor suppressor function, p21cip1, exerts both pro-proliferative effects (as mediated through its capacity to assist in assembly of D-cyclin/cdk4 complexes) and anti-proliferative effects (through association with and suppression of cdk2 complexes) on the cell cycle. Interestingly, p21 levels are directly up-regulated by androgen, indicating that p21cip1 may serve to facilitate G1 progression in this tumor type.

Second, androgen deprivation reduces cyclin A2 levels, as a result of ADT-mediated suppression of cdk4/6 function and resultant engagement of RB transcriptional repressive capacity.

Recently, it has been shown that the DNA replication factor Cdc6 is under direct control of AR activity, indicating that both G1 and S-phase components of the cell cycle machinery may be under AR regulation.

Based on these collective findings, it is evident that a major function of AR is to control the G1-S transition. Additionally, AR could potentially serve to assist in DNA replication licensing, as in some systems, AR is degraded in mitosis. Whether the putative link between AR and the mechanics of DNA synthesis control impinge upon cell cycle transitions mediated by endogenous AR or in an in vivo setting will be of interest to discern.

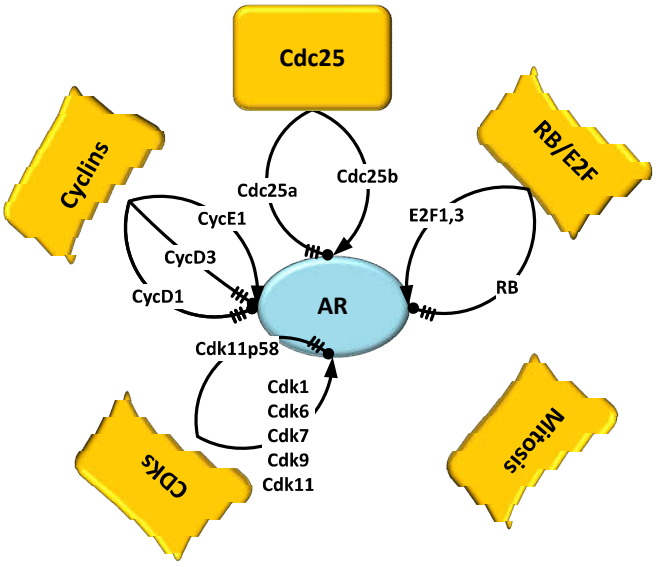
While androgen utilizes divergent mechanisms to induce cdk4/6 and cdk2 activation, suppress RB activity, and thereby permit commitment to the mitotic cell cycle in ADT sensitive cells, distinct mechanisms may be invoked in CRPC which further enhance AR dependent cell cycle progression. Through genome-wide analyses of AR binding in CRPC cells, novel AR occupancy sites were observed near the regulatory loci of genes associated with mitotic progression. Consistent with the postulate that the AR program is altered in CRPC so as to strengthen the capacity of AR to drive cellular proliferation, AR was shown to up-regulate expression of UBE2C, whose gene product has been associated with regulation of cyclin levels. Upregulation of UBE2C was not sufficient to induce castrate-resistant cell growth in ADT-responsive cells, but silencing of UBE2C slowed cell proliferation rates in CRPC cells.

These findings suggest that CRPC cells develop additional means to foster AR-dependent cell cycle control, and further delineation of these mechanisms may be of benefit for the design of novel therapeutic agents.

Cell cycle feedback regulation of AR activity Investigation of the mechanisms by which AR governs cell cycle control led to the unexpected discovery of feedback pathways that influence AR activity. Delineation of the interplay between AR and cell cycle machinery has both illuminated the cellular consequence of pathway crosstalk and underscored the importance of cdk and cyclin functions in transcriptional control.

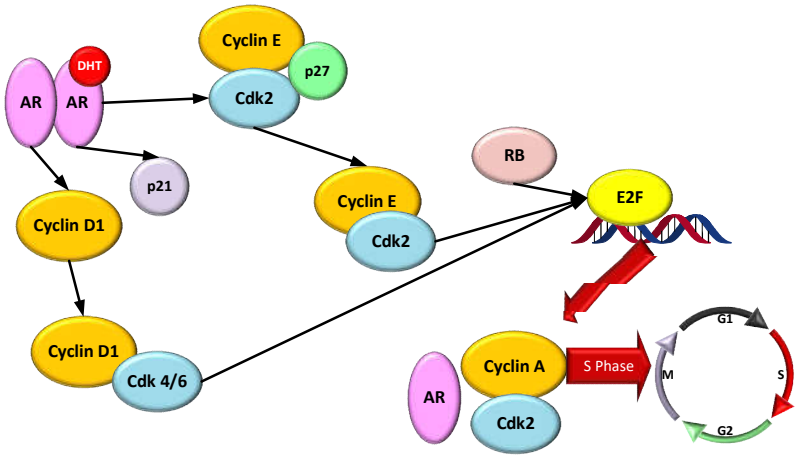
Firs we show the relationships between AR and cell cycle factors below:

See Schiewer et al



Now the pathway controls can be shown below. AR acts as a transcription factor and it that transcription that gives rise to cell cycle proteins.

See Schiewer et al

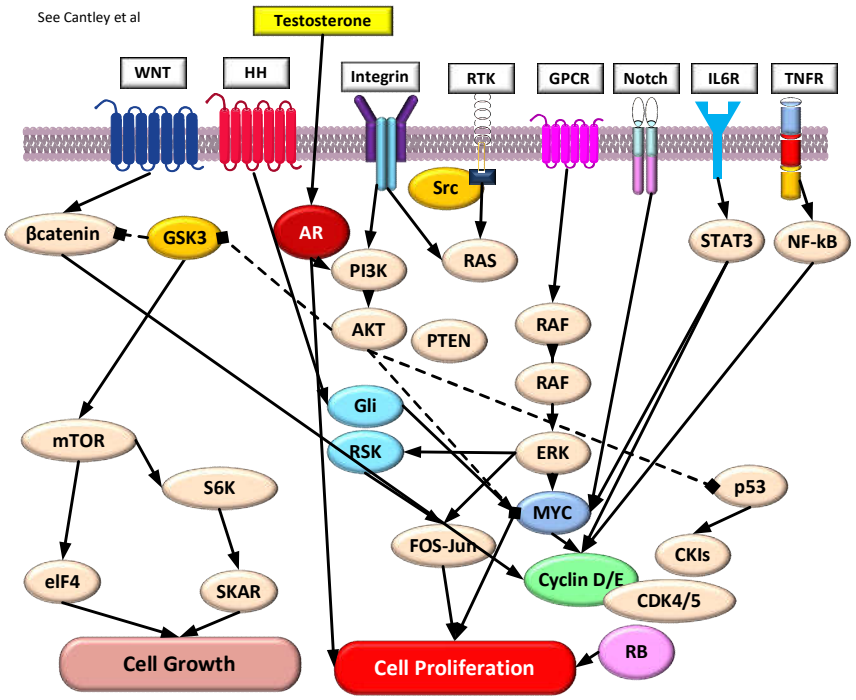


From Schiewer et al:

Regulation of cell cycle by the androgen receptor. Liganded AR induces the translation and accumulation of D-type cyclins by engaging the mammalian Target of Rapamycin (mTOR) complex, which mediates cdk4/6 activation and subsequent phosphorylation and inactivation of the retinoblastoma tumor suppressor (RB).

Concomitantly, AR further impinges on G1-S progression by inducing expression of p21cip1 and degradation of p27kip1, promoting enhanced cdk4/6 and cdk2 dependent inactivation of RB and progression through G1. Pathways requisite for entry into S phase are initiated upon RB inactivation, which induce the activity of the E2F family of transcription factors responsible for the production cyclin A, activation of cdk2 (via cyclin A binding), and entry into S-phase. Additionally, E2F1 directly induces the expression of AR itself, potentially enhancing progression through the cell cycle.

From Cantley et al:



8.3 AR AND RB

The retinoblastoma gene, Rb, plays a significant role in PCa and the AR. As Winman notes in an early paper (1993):

The retinoblastoma (RB) gene is the prototype tumor suppressor gene.

It encodes a nuclear protein that acts as a cell cycle control checkpoint at the G1 phase.

Deletion or inactivation of both RB alleles plays an essential, rate-limiting role in retinoblastoma and in the osteosarcomas that arise within families that carry a mutated RB gene. RB inactivation is also found in other sarcomas, small cell carcinoma of the lung, and in carcinoma of the breast, bladder, and prostate. Transforming proteins encoded by SV40, and the transforming or tumor-associated subtypes of adenoviruses and human papilloma viruses (HPV) can bind RB, thereby blocking its normal function. The EBNA-5 protein of Epstein-Barr virus (EBV) is also able to bind RB in vitro.

In addition, RB can interact with several cellular proteins, including the transcription factor E2F.

RB gene knock-out mice die in utero around day 14 of gestation. The embryos show disturbed neural and hematopoietic differentiation, indicating that RB is vitally important for these processes. This notion is further supported by studies demonstrating that RB expression in mouse embryo tissues is highest in cells undergoing differentiation, and that RB is required for MyoD-induced muscle differentiation.

As Knudsen and Knudsen note:

In cancer it is well accepted that tumour cells invoke multiple mechanisms to bypass proliferative control. A crucial junction in the control of cellular proliferation is linked to the retinoblastoma (RB) tumour suppressor protein, whose primary function is to prevent unscheduled entry into the mitotic cell cycle.

RB exerts its antiproliferative effects, at least in part, through the ability to mediate the transcriptional repression of genes required for DNA replication and mitosis

Through these actions, RB impinges on a sophisticated network of target genes to limit cell-cycle progression. Mitogens must counteract this action of RB, and do so through signals that promote activation of cyclin-dependent kinase (CDK)–cyclin complexes, which phosphorylate RB and attenuate its capacity to induce transcriptional repression. Typically, RB remains in this inactive state until passage through mitosis, at which point it is re-engaged through the action of a phosphatase. Alternatively, RB action can be invoked during an active cycle in response to specific cellular stresses (for example, genotoxic insult) and induce cell-cycle arrest, thus protecting against continued inappropriate proliferation.

Collectively, these functions of RB are thought to be crucial in preventing tumour formation, on the basis of several criteria. First, loss of heterozygosity of RB1 contributes to tumour formation in the human retina. Second, mutations that disrupt RB-mediated transcriptional regulation or genetic events that facilitate RB phosphorylation (for example, amplification of cyclin D1) are frequently observed in tumours. Third, RB inactivation is mediated by and cooperates with oncogenes that contribute to human cancer.

These observations support a significant role for RB-mediated cell-cycle control in human tumours and predict that RB deficiency serves to confer a common proliferative advantage. However, recent studies support the hypothesis that the consequence of RB inactivation is quite complex, and can result in disparate outcomes dependent on tumour type. Moreover, it is apparent that different mechanisms used by tumour cells to disrupt the tumour suppressive function of RB are not synonymous in consequence, suggesting that both tissue type-specific and lesion-specific variances exist with regard to cellular outcome.

The findings, reviewed herein, demonstrate that RB inactivation evokes specific responses to cancer therapeutics and suggest that RB status could be developed as a metric to direct

therapeutic agents. The contemporary model of RB function in cell-cycle control and tumour suppression is wellfounded based on investigation in multiple model systems (FIG. 1). In the absence of mitogenic stimuli, RB activity is engaged to inhibit cell-cycle progression. Although this function of RB can be ascribed to multiple mechanisms, it is clear that RB serves to inhibit the transcription of multiple genes required for S-phase entry.

The best-studied of these target genes are regulated by the E2F family of transcription factors. In this context, RB mediates transcriptional repression dependent on histone deacetylases, SWI/SNF chromatin remodelling enzymes and additional chromatin modifiers. Mitogens reverse transcriptional inhibition of E2F–dependent promoters through sequential activation of CDK–cyclin complexes, which phosphorylate RB and attenuate its transcriptional co-repressor capability.

The D-type cyclins (cyclins D1, D2 and D3) are considered focal points of this process, as the majority of mitogens signal for D-cyclin accumulation and concomitant formation of complexes between cyclin D and CDK4 or CDK6. Active CDK4 and CDK6 kinases initiate RB phosphorylation26,30, thus relaxing E2F target gene suppression. Unbiased gene expression analyses revealed that the RB–E2F regulatory targets consist of approximately 150–200 genes, many of which are involved in S phase and mitosis.

Now for PCa specifically the authors note:

Prostate cancers are exquisitely dependent on androgen receptor (AR) activity for growth and progression1.

Given the poor response of this tumour type to cytotoxic therapeutic agents, strategies to ablate AR function (achieved through the use of androgen depletion strategies or direct AR antagonists) are the first line of treatment for disseminated prostate tumours. In cell culture models of androgen-dependent prostate cancer, such therapeutics lead to cell cycle arrest in G1 that is accompanied by reduced expression of D-cyclins and efficient RB dephosphorylation. Given these observations, there has been a concerted interest in delineating the mechanisms by which the cyclin D1–p16INK4A–RB axis may be perturbed in the transition to androgen independence and thus promote therapeutic resistance. With regard to p16INK4A, although overexpression can potently arrest prostate cancer cell lines, there is little evidence that p16INK4A induction participates in cell-cycle exit following AR antagonism.

Similarly, although D-cyclins are induced by androgen through post-translational mechanisms, cyclin D1 expression is not sufficient to restore cellular proliferation in cultured prostate cancer cells challenged with androgen ablation or androgen antagonists. In this cell type, accumulated cyclin D1 markedly antagonizes AR function and can impede subsequent rounds of cellular proliferation through kinase-independent mechanisms that have been well defined. Thus, cyclin D1 has a general antiproliferative role in such models.

By contrast, emerging evidence suggests that disruption of RB itself may have a significant consequence on prostate cancer therapies. Initially, it was observed that viral oncoproteins with the capacity to inhibit RB function were sufficient to promote cell-cycle progression in the

absence of androgen or presence of androgen antagonists. Subsequent analyses of isogenic, AR-positive cancer cells revealed that RB depletion alone rendered no discernable proliferative advantage to prostate cancer cells in the presence of androgen.

However, RB depletion in prostate cancer cells was sufficient to sustain cell-cycle progression after challenge with androgen ablation and/or AR antagonist strategies that mimic therapeutic intervention.

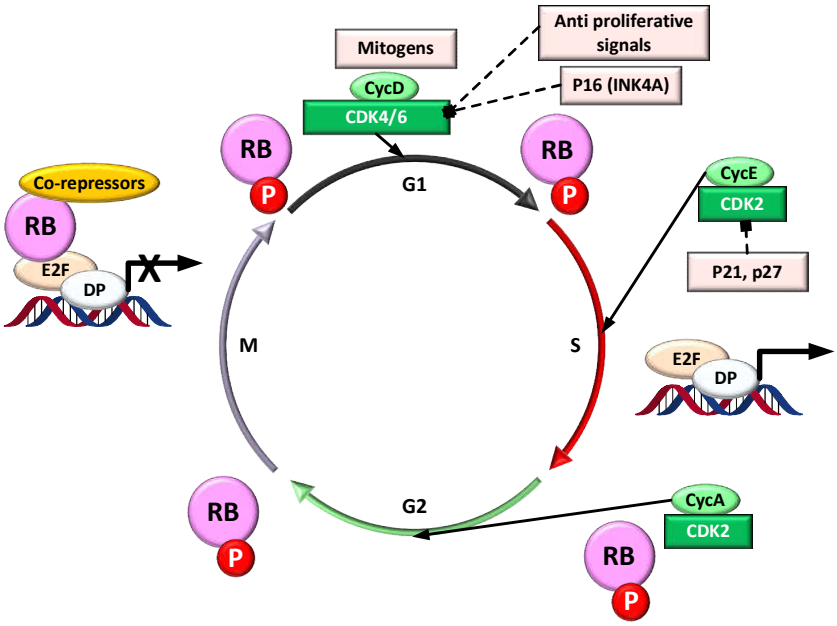
Based on these observations, it was suspected that aberrations in RB itself (rather than loss of p16INK4A or overexpression of cyclin D1) may contribute to hormone resistance. Despite these findings, clinical studies examining the impact of cyclin D1, p16INK4A or RB as determinants of therapeutic outcome have been only preliminarily considered. p16INK4A loss is infrequently observed; conversely, increased p16INK4A levels are associated with a poor prognosis, similar to that observed in breast cancer. Thus, p16INK4A function appears to be maintained in the majority of prostate cancers, and p16INK4A loss does not appear to have a role in the transition to androgen independence.

A role for cyclin D1 in this process is similarly obscure, and the function(s) of cyclin D1 in this tissue type incompletely defined. Several reports have demonstrated that cyclin D1 expression is rare or infrequent in primary disease, supporting the idea that excessive cyclin D1 expression is probably not a major factor in disease development or progression. Furthermore, recent analyses showed that cyclin D1 is low or mislocalized to the cytoplasm in a significant fraction of prostate cancers obtained from radical prostatectomy.

However, a subset of tumours, either associated with high p21CIP1 levels or associated with bone metastases, do show enhanced nuclear cyclin D1, suggesting that cyclin D1 function is complex and dependent on molecular milieu. Surprisingly, there have been few studies of RB loss in prostate cancer specimens; however, RB loss is overrepresented in recurrent prostate cancers, which are resistant to hormone therapy.

Furthermore, the gene expression profile generated by viral oncoproteins that disrupt RB function is associated with poor disease outcome in prostate cancer. These findings are consistent with functional analyses of RB loss in cultured prostate cancer cells, and indicate that RB loss may have a specific role in the acquisition of androgen independence

They represent these facts as shown below:



Where the authors note:

Mitogenic signals stimulate the expression of D-type cyclins (Cyc) and a concomitant increase in cyclin-dependent kinase 4 (CDK4) and CDK6 activity.

These factors initiate RB phosphorylation, which is augmented by the activity of CDK2 complexes with cyclins A and E.

The phosphorylation of RB disrupts its association with E2F.

This inactivation of RB allows for the expression of a transcriptional programme that enables progression through S-phase and mitosis. At the transition from mitosis to G1, RB is dephosphorylated through the action of phosphatases.

Importantly, a large number of anti-mitogenic signals function to prevent RB phosphorylation either by limiting the activity of CDK4, CDK6 and CDK2 complexes or by inducing the activity of CDK inhibitors

As Ben-Salem et al note:

The central protein in cell cycle regulation is RB, as phosphorylation of RB induces cell division. RB's prevention of tumor development relies in large part on its capacity to modulate the activity of E2F family transcription factor activity, which controls the production of proteins that are important for cellular replication.

Under normal circumstances, unphosphorylated RB binds and thereby inhibits E2F family members. Loss of heterozygosity (LOH) at the RB locus is an early event that promotes the

initiation and progression of Ca and is significantly more prevalent in CRPC. RB loss causes acquired treatment resistance and the emergence of NEPC. Therefore, loss of RB function is associated with poor clinical outcome. Moreover, restoration of WT RB in DU145 CaP cells that express a truncated short RB form prevented these cells to form tumors in nude mice.

Of note, some RB mutations can also alter the affinity for RB binding proteins such as E2F without affecting the level of RB expression Consistent with these findings, levels of E2F1, the best-studied E2F family member in CaP, increased in the transition from benign prostate to localized CaP, to metastatic lymph nodes from treatment-naïve patients, and to CRPC. Increased expression of E2F1 was associated with CaP growth, cell survival and treatment resistance.

In a transgenic mice model, overexpression of E2F1 caused prostate hyperplasia while its loss in CaP xenograft models delayed CaP growth. Recently, a novel E2F1 cistrome was observed after RB loss that diverged considerably from the canonically described E2F1 binding patterns after phosphorylation-induced RB functional inactivation, suggesting that RB loss might reprogram the E2F1 cistrome). In addition, in phosphoproteomic analysis of CRPC specimens, members of E2F family and their target genes were found to be significantly enriched and in transcriptomics analyses of NEPC patient-derived xenografts and circulation tumor cell-derived explant models, E2F pathways were upregulated, verifying the importance of E2F action for aggressive CaP progression.

They then focus on the critical role of AR:

AR regulation of cell cycle

The core machinery that drives the cell cycle is well conserved among cell and tissue types although the signals that dictate commitment to the cell cycle are often cell type-specific. Androgen and AR activation have been recognized as such lineagespecific signals to drive growth in benign and malignant prostate glands. The increased proliferation rate in CaP compared to benign glands coincides with a shift in AR's function from transcription factor only in normal gland to both transcription factor and DNA replication factor in CaP, and from paracrine stimulator of benign growth to autocrine growth regulator in CaP.

In treatment-naïve localized CaP cells, AR has been reported as a master regulator of G1–S phase progression, able to induce signals that promote G1 cyclin-dependent kinase (CDK) activity, induce phosphorylation/inactivation of RB, induce E2F activity and reduce p27 expression and thereby stimulate androgen-dependent CaP cell proliferation. AR remains a major regulator of CaP growth also in the majority of cases once one or more rounds of ADT have failed. Analyses of AR-dependent transcriptomes and cistromes have revealed a shift of AR control over G1–S checkpoint in treatment-naive CaP to G2-M checkpoints in CRPC.

While AR remains expressed in a significant fraction of NEPC, these cancers have been deemed AR-indifferent; the remaining portion of NEPC is even AR-negative, reducing the likelihood of similar control of AR over cell cycle in this CaP phenotype.

Interactions between cell cycle regulators and AR Several reports indicate also direct protein–protein interactions between AR and several cell cycle regulators, which may underlie AR's involvement in cell cycle control. In addition to AR's control over CDK and cyclin expression and activity, already referred to above, members of the cell cycle-related subfamily of CDKs such as CDK6 have been reported to bind directly to AR (Lim et al. 2005), and so have cyclins that are mostly relevant to cell cycle phase transition such as cyclin D (1 and 3) and cyclin E.

Moreover, Rb1 has been described to directly interact with AR.

It should be noted that these studies were mostly done in the context of AR transcriptional regulation, that is, they evaluated the coregulator and cofactor potential of cell cycle regulators to influence AR-dependent transcription. Such transcriptional involvement is consistent with CDK and cyclin family members having either selective, dual or preferential roles in cell cycle control or transcriptional regulation. In this respect, transcription-related subfamily members such as CDK11, 7 and 9, and cyclin H have also been reported as coregulators modulating AR's transcription output.

Cell cycle regulation of AR

The interactions between AR and select cell cycle regulators suggest that the stage of the cell cycle may influence the transactivation function of AR. This possibility is supported by other lines of evidence. For instance, AR activation and stability is regulated by phosphorylation by cell cycle regulators: for example, phosphorylation of AR on S83 by CDK1 enhances AR stability and transcriptional activity; while AR phosphorylation on S310 by CDK 1, 5 and 11 represses AR transcriptional activity and alters its localization during mitosis. These finding already suggests that CDKs that are not necessarily solely involved with cell cycle progression can modify these as well as other AR sites. Moreover, nonCDK cell cycle regulators such as Aurora A phosphorylate AR and impact its transcriptional output.

These data also indicate the possibility of regulation of AR activity in the stage of cell cycle where an AR-modifying regulator is most expressed and/or active.

9 AR AND APOPTOSIS

Apoptosis is a form of cell death which is a matter of course. As Reed notes:

Programmed cell death plays critical roles in a wide variety of physiological processes during fetal development and in adult tissues. In most cases, physiological cell death occurs by apoptosis as opposed to necrosis. Defects in apoptotic cell death regulation contribute to many diseases, including disorders where cell accumulation occurs (cancer, restenosis) or where cell loss ensues (stroke, heart failure, neurodegeneration, AIDS). In recent years, the molecular machinery responsible for apoptosis has been elucidated, revealing a family of intracellular proteases, the caspases, which are responsible directly or indirectly for the morphological and biochemical changes that characterize the phenomenon of apoptosis.

Diverse regulators of the caspases have also been discovered, including activators and inhibitors of these cell death proteases. Inputs from signal transduction pathways into the core of the cell death machinery have also been identified, demonstrating ways of linking environmental stimuli to cell death responses or cell survival maintenance. Knowledge of the molecular mechanisms of apoptosis is providing insights into the causes of multiple pathologies where aberrant cell death regulation occurs and is beginning to provide new approaches to the treatment of human diseases. …

Apoptosis is a morphological phenomenon. As viewed with the assistance of the light (or, preferably, the electron) microscope, the characteristics of the apoptotic cell include chromatin condensation and nuclear fragmentation (pyknosis), plasma membrane blebbing, and cell shrinkage. Eventually, the cells breaks into small membrane-surrounded fragments (apoptotic bodies), which are cleared by phagocytosis without inciting an inflammatory response. The release of apoptotic bodies is what inspired the term "apoptosis" from the Greek, meaning "to fall away from" and conjuring notions of the falling of leaves in the autumn from deciduous trees.

9.1 CELL DEATH TYPES

There are a variety of cell deaths. Green notes four prominent ones:

1. Apoptosis: As Elmore notes:

Apoptosis has since been recognized and accepted as a distinctive and important mode of "programmed" cell death, which involves the genetically determined elimination of cells. However, it is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered…

Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (Norbury and Hickson, 2001). Although there are a wide variety of stimuli and conditions, both physiological

and pathological, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus. Irradiation or drugs used for cancer chemotherapy results in DNA damage in some cells, which can lead to apoptotic death through a p53-dependent pathway. Some hormones, such as corticosteroids, may lead to apoptotic death in some cells (e.g., thymocytes) although other cells are unaffected or even stimulated

2. Autophagy: We have examined this in the context of PCa[52]. From Glick et al we have:

The term 'autophagy', derived from the Greek meaning 'eating of self', was first coined by Christian de Duve over 40 years ago, and was largely based on the observed degradation of mitochondria and other intra-cellular structures within lysosomes of rat liver perfused with the pancreatic hormone, glucagon . The mechanism of glucagon-induced autophagy in the liver is still not fully understood at the molecular level, other than that it requires cyclic AMP induced activation of protein kinase-A and is highly tissue-specific .

In recent years the scientific world has 'rediscovered' autophagy, with major contributions to our molecular understanding and appreciation of the physiological significance of this process coming from numerous laboratories There are three defined types of autophagy: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy, all of which promote proteolytic degradation of cytosolic components at the lysosome. Macro-autophagy delivers cytoplasmic cargo to the lysosome through the intermediary of a double membrane-bound vesicle, referred to as an autophagosome, that fuses with the lysosome to form an autolysosome. In micro-autophagy, by contrast, cytosolic components are directly taken up by the lysosome itself through invagination of the lysosomal membrane.

Both macro-and micro-autophagy are able to engulf large structures through both selective and non-selective mechanisms. In chaperone-mediated autophagy (CMA), targeted proteins are translocated across the lysosomal membrane in a complex with chaperone proteins (such as Hsc-70) that are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A (LAMP-2A), resulting in their unfolding and degradation . Due to recent and increased interest specifically in macroautophagy and its role in disease, this review focuses on molecular and cellular aspects of macro-autophagy (henceforth referred to as 'autophagy') and how it is regulated under both healthy and pathological conditions.

3. Necrosis: This type of cell death is when the cell expands and explodes leaving a mess of cellular elements to be removed.

4. Cornification: A special type of cell death that results in death of the nucleus and a binding or the proteins as in the surface of the skin.

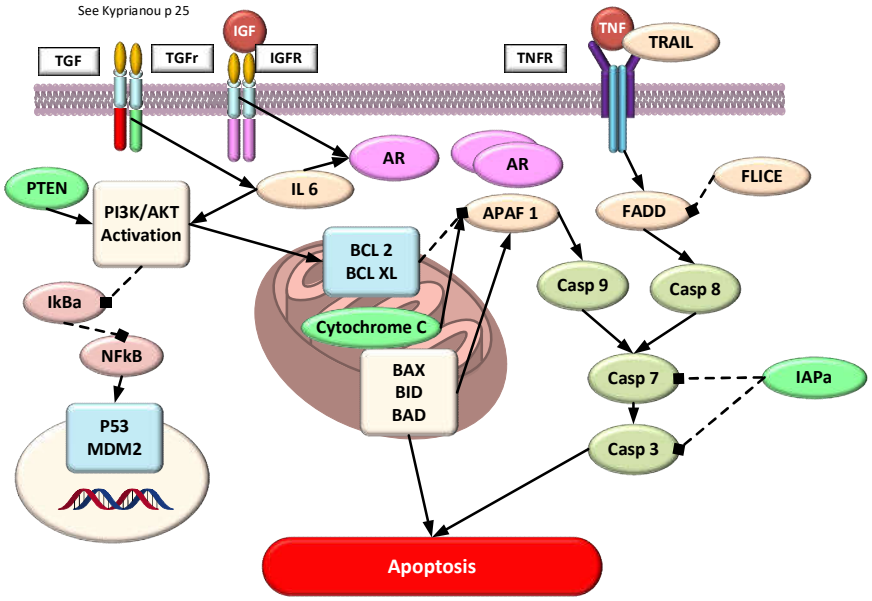
9.2 APOPTOSIS

According to Kyprianou, apoptosis in the context of PCa has two pathways.

[52] https://www.researchgate.net/publication/328278580_Autophagy_and_PCa_On_the_One_Hand_and_on_the_Other_Hand

Intrinsic: This is an internally generated and controlled pathway. It is initiated by some form of internal cellular stress such as DNA damage and targets the mitochondria. The principal player is ARF 1. We demonstrate this in a composite below.

Extrinsic: This is generated by the attaching of apoptosis generating ligands such as FAS or TRAIL. Again we show the composite below.



As Huang and Oliff had noted:

p53: an apoptosis-promoting tumor suppressor

One of the best-documented but still incompletely understood regulatory pathways of apoptosis is the p53 pathway. The p53 protein is a transcription factor and tumor suppressor that is lost during tumorigenesis in 30–70% of clinical tumor samples. The presence of the wild-type p53 gene in a tumor correlates with a favorable response to chemotherapy, and, in experimental systems, elevated p53 signaling contributes to the induction of apoptosis by DNAdamaging agents. The p53 protein potentiates….

AKT: an anti-apoptotic oncoprotein

One of the most attractive kinase targets relevant to apoptotic pathways in cancer cells is the serine/threonine kinase AKT (or PKB). AKT is negatively regulated by the tumor suppressor PTEN (or MMAC), which is frequently mutated in cancer cells.

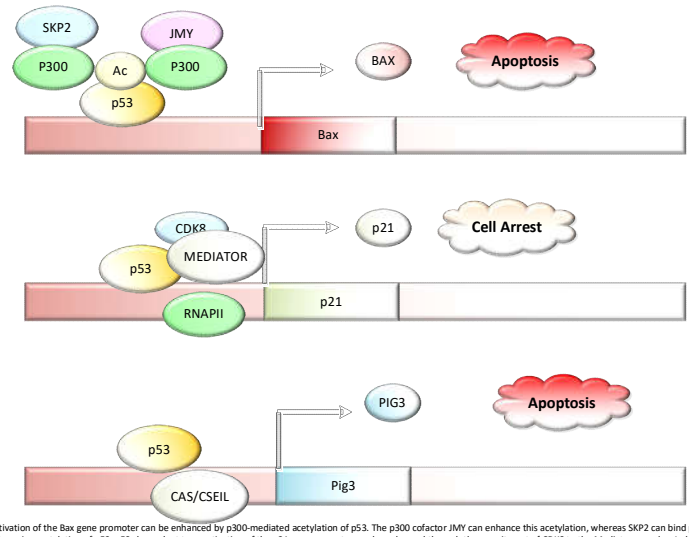
AKT has also been shown to function as a downstream effector of the oncoprotein her2/neu because the expression of a dominant-negative form of AKT will sensitize her-2/neu-overexpressing cells to tumor necrosis factor (TNF)-induced apoptosis. These observations suggest that an AKT inhibitor might inhibit the growth of breast tumors where her2/neu

signaling is increased. The overexpression of dominant-negative forms of AKT or exposure to an inhibitor of the AKT pathway (the phosphoinositide 3-kinase inhibitor wortmannin) can contribute to the release of cytochrome c from mitochondria and promote apoptosis.

Presumably, an inhibitor of the kinase function of AKT would also potentiate apoptosis. Activated AKT can interfere with p53-dependent apoptosis, indicating that these two pathways intersect. The anti-apoptotic effect of AKT might be distinct from that of Bcl-xL, which also protects cells from p53-dependent apoptosis. During apoptosis induced by growth-factor withdrawal, loss of glycolysis leads to loss of the mitochondrial membrane potential and caspase activation. Rescue from caspase activation and apoptosis by overexpressing Bcl-xL in this system occurs without restoration of glucose levels or protection against loss of mitochondrial membrane potential.

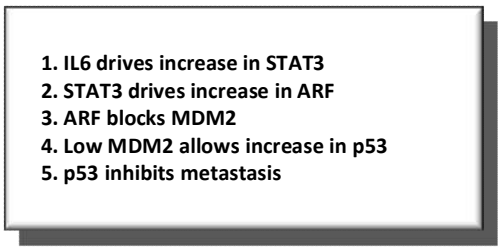
By contrast, when activated AKT rescues cells from apoptosis induced by growth factor withdrawal, glucose levels and mitochondrial membrane potential are maintained, suggesting that alternative rescue pathways can be used by cancer cells that are dependent on either AKT activation or Bcl-xL protein function.

The different types of gene controls are shown below for BAX, p21, and Pig3



Activation of the Bax gene promoter can be enhanced by p300-mediated acetylation of p53. The p300 cofactor JMY can enhance this acetylation, whereas SKP2 can bind p300 and antagonize acetylation of p53. p53-dependent transactivation of the p21 gene promoter can be enhanced through the recruitment of CDK8 to the Mediator complex. Independent binding of the long-range chromatin modifier CAS/CSE1L, which is the human orthologue of yeast Cse1, to promoters, such as the Pig3 gene promoter, can also enhance p53-dependent transcription.

STAT3 also plays a significant role. We demonstrate below the key drivers and effects. MDM2 has a critical role in this process.

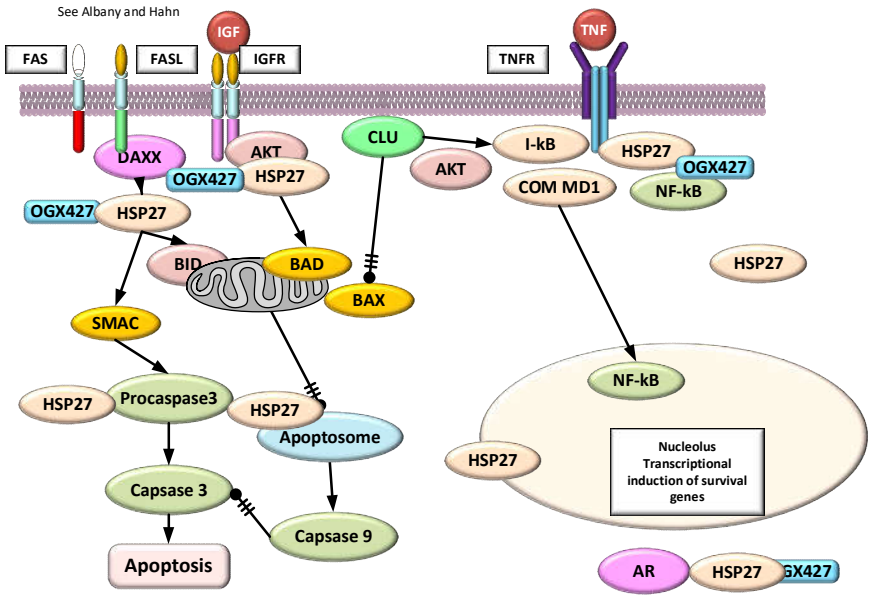


The above is a critical control pathway which plays a role in PCa.

9.3 Heat Shock Proteins and Apoptosis

As we noted from the above the role of AR is limited. However when examining the HSP we see that the AR can become a key player. From Albany and Hahn:

Defects within apoptotic pathways have been implicated in prostate cancer (PCa) tumorigenesis, metastatic progression and treatment resistance. A hallmark of cancers is the ability to derail apoptosis by inhibiting the apoptotic signal, reducing the expression of apoptotic proteins and/or amplifying survival signals through increased production of antiapoptotic molecule. This review describes associations between heat shock proteins (HSPs) and the human androgen receptor (AR), the role of HSPs and other stress-induced proteins in PCa development and emerging strategies in targeting these protective proteins to treat PCa



Role of Heat shock protein-27 (HSP27) and clusterin (CLU) in cancer cell survival. CLU increases cell survival through mechanisms involving inhibition of ER stress, suppressing Bax activation with mitochondrial sequestration of cytochrome C, and transcriptional induction of survival genes. Custirsen (OGX-011) acts at each of these points by decreasing clusterin expression. HSP27 inhibits apoptosis by integrating different signaling pathways, including extrinsic and intrinsic apoptosis pathways, as well as growth factor pathways. It enhances androgen receptor signaling and insulin-like growth factor 1-induced Bad phosphorylation. HSP27 inhibits the extrinsic and the intrinsic apoptosis pathway. OGX-427 reduces HSP27 expression leading to appoptosis. APAF1: apoptotic protease-activating factor-1; ER: endoplasmic reticulum.

10 OBSERVATIONS

We can make some extending observations based upon the previous expositions.

10.1 AR IS CLEARLY A CONTROL POINT FOR VARIOUS FORMS OF PCA

Our intent was to focus an examination of PCa around the AR and its modifications as the disease progresses. The other genes and gene products become elements of what is happening with AR. Clearly such an approach could be done with a variety of other genes and gene products yet AR presents a unique and powerful approach.

10.2 AR AND ITS DRIVERS AND FACILITATORS MAY PRESENT THERAPEUTIC TARGETS

Each element of the AR chain also presents as a potential therapeutic target.

10.3 AN INTEGRATED ANALYSIS OF ALL PATHWAYS ASSOCIATED WITH AR IS ESSENTIAL YET COMPLEX.

We have demonstrated on pathway, KLK3, and have highlighted several others. AR is a transcription factor and as such it does transcribe a multiplicity of other genes. Having a complete understanding of all these pathways may enhance therapeutic approaches.

10.4 THE CELL CYCLE APPEARS TO BE A CRITICAL TARGET GOT SUPPRESSING AR GENERATED PCA

Stopping the cell cycle on a targeted basis is critical. There are means to do so but unless targeted to specific cells leads to catastrophic results. However one may try a PSMA target with an added cell cycle inhibitor.

10.5 THERE DOES NOT APPEAR TO BE TARGETABLE SURFACE MARKERS RESULTING FROM ABERRANT AR

The use of CAR-T or CAR-NK or polyclonal Ab demands surface targets to identify the cells. It is not clear that any of the elements in AR functioning leads to such a result. However it is worth further examinations.

10.6 IF THE AR IS ON THE X GENE AND THUS INHERITED FROM THE MOTHER THEN WHY IS PCA RELATED TO THE FATHER'S STATE RELATIVE TO PCA?

The AR we argue is the lynchpin of PCa. However the AR gene is inherited from the mother. The father's PCa state is alleged to predispose the individual with PCa. If so is there another key element that comes from the father?

10.7 THERAPEUTIC OPTIONS DEMAND A DETAILED UNDERSTANDING OF THE PCA CELLS ONE AT A TIME AN INDIVIDUAL PROFILING.

Current therapeutic techniques have become very varied. On can attack pathway elements, which often can be quite useful but do have side effects. One can try immune techniques by suppressing such mitigators as PD-1. One can try CAR T or NK cells or polyclonal antibodies.

The challenge is truly understanding the cells, and it possible the stem cell. We now have the capacity to perform single cell analyses. This means when excising a tumor we can sequence a cell by cell collection and lay out everything from target genes to surface markers or targets. In many ways having unique surface targets allows for efficient targeting of a therapeutic via an antibody or a CAR type cell. Uniqueness of the target is essential to reduce the risk of such things as a cytokine storm.

If we now take the complexity of the AR and its surroundings then we have a multiplicity of options. Yet the surface targets are still quite limited. PSMA is one but to reduce the risk of adverse event one need more such targets.

11 LIST OF GENES AND RELATED PROTEINS

Element	*Type (Ligand, Receptor, Cell Surface, Pathway, Intra Nucleus, Transcription)*	*Function (See http://www.ncbi.nlm.nih.gov/gene/)*
ABL	Pathway	The *ABL1* proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response. Activity of ABL1 protein is negatively regulated by its SH3 domain, and deletion of the SH3 domain turns ABL1 into an oncogene.
AKT	Pathway	The serine-threonine protein kinase encoded by the AKT1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase.
APC	Pathway	The activity of one protein in particular, beta-catenin, is controlled by the APC protein (see: Wnt signaling pathway). Regulation of beta-catenin prevents genes that stimulate cell division from being turned on too often and prevents cell overgrowth.
ARF	Pathway	p14ARF is an alternate reading frame (ARF) product of the CDKN2A locus. Both p16INK4a and p14ARF are involved in cell cycle regulation. p14ARF inhibits mdm2, thus promoting p53, which promotes p21 activation, which then binds and inactivates certain cyclin-CDK complexes, which would otherwise promote transcription of genes that would carry the cell through the G_1/S checkpoint of the cell cycle. Loss of p14ARF by a homozygous mutation in the CDKN2A (INK4A) gene will lead to elevated levels in mdm2 and, therefore, loss of p53 function and cell cycle control.
AR	Receptor	The androgen receptor gene is more than 90 kb long and codes for a protein that has 3 major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. The protein functions as a steroid-hormone activated transcription factor. Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes. This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts in the N-terminal transactivation domain of its protein. Expansion of the polyglutamine tract causes spinal bulbar muscular atrophy (Kennedy disease). Mutations in this gene are also associated with complete androgen insensitivity (CAIS). Two alternatively spliced variants encoding distinct isoforms have been described.

BAD	Pathway	The Bcl-2-associated death promoter (BAD) protein is a pro-apoptotic member of the Bcl-2 gene family which is involved in initiating apoptosis. BAD is a member of the BH3-only family , a subfamily of the Bcl-2 family. It does not contain a C-terminal transmembrane domain for outer mitochondrial membrane and nuclear envelope targeting, unlike most other members of the Bcl-2 family. After activation, it is able to form a heterodimer with anti-apoptotic proteins and prevent them from stopping apoptosis.
CCNA2	Cell Cycle	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through 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. In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in all tissues tested. This cyclin binds and activates CDC2 or CDK2 kinases, and thus promotes both cell cycle G1/S and G2/M transitions.
CCND1	Transcription	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.
CDK4	Transcription	The protein encoded by this gene is a member of the Ser/Thr protein kinase family. This protein is highly similar to the gene products of S. cerevisiae cdc28 and S. pombe cdc2. It is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. The activity of this kinase is restricted to the G1-S phase, which is controlled by the regulatory subunits D-type cyclins and CDK inhibitor p16(INK4a).
Disheveled Dsh	Pathway	Dishevelled (Dsh) is a family of proteins involved in canonical and non-canonical Wnt signalling pathways. Dsh is a cytoplasmic phosphoprotein that acts directly downstream of frizzled receptors
E cadherin	Cell Surface	This gene is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. Mutations in this gene are correlated with gastric, breast, colorectal, thyroid and ovarian cancer. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis. The ectodomain of this protein mediates bacterial adhesion to mammalian cells and the cytoplasmic domain is required for internalization.
EGF	Ligand	This gene encodes a member of the epidermal growth factor superfamily. The encoded protein is synthesized as a large precursor molecule that is proteolytically cleaved to generate the 53-amino acid epidermal growth factor peptide. This protein acts a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of numerous cell types. This protein acts by binding the high affinity cell surface receptor, epidermal growth factor receptor. Defects in this gene are the cause of hypomagnesemia type 4. Dysregulation of this gene has been associated with the growth and progression of certain cancers.

ERBB4	Receptor	Receptor tyrosine-protein kinase erbB-4 is an enzyme that in humans is encoded by the *ERBB4* gene. Alternatively spliced variants that encode different protein isoforms have been described; however, not all variants have been fully characterized. Receptor tyrosine-protein kinase erbB-4 is a receptor tyrosine kinase that is a member of the epidermal growth factor receptor subfamily. ERBB4 is a single-pass type I transmembrane protein with multiple furin-like cysteine rich domains, a tyrosine kinase domain, a phosphotidylinositol-3 kinase binding site and a PDZ domain binding motif. The protein binds to and is activated by neuregulins-2 and -3, heparin-binding EGF-like growth factor and betacellulin.
ERK	Pathway	Ephrin receptors and their ligands, the ephrins, mediate numerous developmental processes, particularly in the nervous system. Based on their structures and sequence relationships, ephrins are divided into the ephrin-A (EFNA) class, which are anchored to the membrane by a glycosylphosphatidylinositol linkage, and the ephrin-B (EFNB) class, which are transmembrane proteins. The Eph family of receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. Ephrin receptors make up the largest subgroup of the receptor tyrosine kinase (RTK) family.
ETV1	Transcription	ETS translocation variant 1 is a protein that in humans is encoded by the *ETV1* gene. This gene encodes a member of the ETS (E twenty-six) family of transcription factors. The ETS proteins regulate many target genes that modulate biological processes like cell growth, angiogenesis, migration, proliferation and differentiation. All ETS proteins contain an ETS DNA-binding domain that binds to DNA sequences containing the consensus 5'-CGGA[AT]-3'. The protein encoded by this gene contains a conserved short acidic transactivation domain (TAD) in the N-terminal region, in addition to the ETS DNA-binding domain in the C-terminal region.
FGFR	Receptor	This gene encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence being highly conserved between members and among divergent species. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance.
FOS	Transcription	c-Jun is the name of a gene and protein that, in combination with c-Fos, forms the AP-1 early response transcription factor. It was first identified as the Fos-binding protein p39 and only later rediscovered as the product of the c-jun gene. It is activated through double phosphorylation by the JNK pathway but has also a phosphorylation-independent function. c-Jun knockout is lethal, but transgenic animals with a mutated c-Jun that cannot be phosphorylated (termed c-JunAA) can survive.
Frizzled	Receptor	This gene encodes a member of the SFRP family that contains a cysteine-rich domain homologous to the putative Wnt-binding site of Frizzled proteins. Members of this family act as soluble modulators of Wnt signaling; epigenetic silencing of SFRP genes leads to deregulated activation of the Wnt-pathway which is associated with cancer. This gene may also be involved in determining the polarity of photoreceptor cells in the retina. NOTE: There are several “frizzled” genes.

GAS1	Pathway	Growth arrest-specific 1 plays a role in growth suppression. GAS1 blocks entry to S phase and prevents cycling of normal and transformed cells. Gas1 is a putative tumor suppressor gene.
GLI2	Transcription	GLI2 belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are characterized as transcription factors which bind DNA through zinc finger motifs
GOLPH3	Pathway	The Golgi complex plays a key role in the sorting and modification of proteins exported from the endoplasmic reticulum. The protein encoded by this gene is a peripheral membrane protein of the Golgi stack and may have a regulatory role in Golgi trafficking. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of these variants has not been determined.
GR	Receptor	Growth receptor
GSK-3β	Pathway	Glycogen synthase kinase-3 (GSK-3) is a proline-directed serine-threonine kinase that was initially identified as a phosphorylating and an inactivating agent of glycogen synthase. Two isoforms, alpha (GSK3A) and beta, show a high degree of amino acid homology. GSK3B is involved in energy metabolism, neuronal cell development, and body pattern formation
Hedgehog (Sonic)	Ligand	This gene encodes a protein that is instrumental in patterning the early embryo. It has been implicated as the key inductive signal in patterning of the ventral neural tube, the anterior-posterior limb axis, and the ventral somites. Of three human proteins showing sequence and functional similarity to the sonic hedgehog protein of Drosophila, this protein is the most similar. The protein is made as a precursor that is autocatalytically cleaved; the N-terminal portion is soluble and contains the signalling activity while the C-terminal portion is involved in precursor processing. More importantly, the C-terminal product covalently attaches a cholesterol moiety to the N-terminal product, restricting the N-terminal product to the cell surface and preventing it from freely diffusing throughout the developing embryo. Defects in this protein or in its signalling pathway are a cause of holoprosencephaly (HPE), a disorder in which the developing forebrain fails to correctly separate into right and left hemispheres. HPE is manifested by facial deformities. It is also thought that mutations in this gene or in its signalling pathway may be responsible for VACTERL syndrome, which is characterized by vertebral defects, anal atresia, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, cardiac anomalies, and limb abnormalities.
HGF	Ligand	Hepatocyte growth factor regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor. Hepatocyte growth factor is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells of mainly epithelial origin. Its ability to stimulate mitogenesis, cell motility, and matrix invasion gives it a central role in angiogenesis, tumorogenesis, and tissue regeneration. It is secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and 34-kDa beta-chain. A disulfide bond between the alpha and beta chains produces the active, heterodimeric molecule. The protein belongs to the plasminogen subfamily of S1 peptidases but has no detectable protease activity.
IGFBP7	Ligand	This gene encodes a member of the insulin-like growth factor (IGF)-binding protein (IGFBP) family. IGFBPs bind IGFs with high affinity, and regulate IGF availability in body fluids and tissues and modulate IGF binding to its receptors. This protein binds IGF-I and IGF-II with relatively low affinity, and belongs to a subfamily of low-affinity IGFBPs. It also stimulates prostacyclin production and cell adhesion.

INK4A	Transcription	This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, MDM1, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.
INSR	Receptor	Insulin receptor. This gene encodes a member of the receptor tyrosine kinase family of proteins. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that form a heterotetrameric receptor. Binding of insulin or other ligands to this receptor activates the insulin signaling pathway, which regulates glucose uptake and release, as well as the synthesis and storage of carbohydrates, lipids and protein. Mutations in this gene underlie the inherited severe insulin resistance syndromes including type A insulin resistance syndrome, Donohue syndrome and Rabson-Mendenhall syndrome. Alternative splicing results in multiple transcript variants.
JUN	Transcription	See FOS
KIF7		This gene encodes a cilia-associated protein belonging to the kinesin family. This protein plays a role in the sonic hedgehog (SHH) signaling pathway through the regulation of GLI transcription factors. It functions as a negative regulator of the SHH pathway by preventing inappropriate activation of GLI2 in the absence of ligand, and as a positive regulator by preventing the processing of GLI3 into its repressor form. Mutations in this gene have been associated with various ciliopathies.
KIT	Receptor	This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous lukemia, and piebaldism.
LEF	Transcription	This gene encodes a transcription factor belonging to a family of proteins that share homology with the high mobility group protein-1. The protein encoded by this gene can bind to a functionally important site in the T-cell receptor-alpha enhancer, thereby conferring maximal enhancer activity. This transcription factor is involved in the Wnt signaling pathway, and it may function in hair cell differentiation and follicle morphogenesis. Mutations in this gene have been found in somatic sebaceous tumors. This gene has also been linked to other cancers, including androgen-independent prostate cancer.
LKB1	Pathway	This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and functions as a tumor suppressor. Mutations in this gene have been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms. Alternate transcriptional splice variants of this gene have been observed but have not been thoroughly characterized.

MEK	Pathway	The protein encoded by this gene is a serine/threonine kinase and is part of some signal transduction cascades, including the ERK and JNK kinase pathways as well as the NF-kappa-B pathway. The encoded protein is activated by autophosphorylation and requires magnesium as a cofactor in phosphorylating other proteins
MITF	Transcription	Microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper transcription factor involved in melanocyte and osteoclast development
mTOR	Pathway	The mammalian target of rapamycin (mTOR) also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) is a protein which in humans is encoded by the *FRAP1* gene. mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription. mTOR belongs to the phosphatidylinositol 3-kinase-related kinase protein family.
MYC	Transcription	The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma. There is evidence to show that alternative translation initiations from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site result in the production of two isoforms with distinct N-termini. The synthesis of non-AUG initiated protein is suppressed in Burkitt's lymphomas, suggesting its importance in the normal function of this gene.
NEDD9	Pathway	CRK-associated substrate-related protein; Cas scaffolding protein family member 2; Crk-associated substrate related; NEDD-9; cas-like docking; dJ49G10.2 (Enhancer of Filamentation 1 (HEF1)); dJ761I2.1 (enhancer of filamentation (HEF1)); enhancer of filamentation 1; neural precursor cell expressed developmentally down-regulated protein 9; p105; renal carcinoma antigen NY-REN-12
NF1	Pathway	NF1 encodes the protein neurofibromin, which appears to be a negative regulator of the ras signal transduction pathway. NF1 is found within the mammalian postsynapse, where it is known to bind to the NMDA receptor complex. It has been found to lead to deficits in learning, and it is suspected that this is a result of its regulation of the Ras pathway. It is known to regulate the GTPase HRAS, causing the hydrolyzation of GTP and thereby inactivating it
NF-κB	Transcription	NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens
Notched	Receptor	

NRAS	Pathway	This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex. The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein. Mutations in this gene have been associated with somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia.
p15	Pathway	CDKN2B This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases, thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition. Two alternatively spliced transcript variants of this gene, which encode distinct proteins, have been reported.
p16	Pathway	CDKN2A This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, MDM1, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene
p21	Pathway	CDKN1A This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Multiple alternatively spliced variants have been found for this gene.

p27	Pathway	The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes a non-ATPase subunit of the 19S regulator. Three transcript variants encoding two different isoforms have been found for this gene.
p53	Pathway	This gene encodes tumor protein p53, which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. p53 protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. p53 is a DNA-binding protein containing transcription activation, DNA-binding, and oligomerization domains. It is postulated to bind to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mutants of p53 that frequently occur in a number of different human cancers fail to bind the consensus DNA binding site, and hence cause the loss of tumor suppressor activity. Alterations of this gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome. Multiple p53 variants due to alternative promoters and multiple alternative splicing have been found. These variants encode distinct isoforms, which can regulate p53 transcriptional activity
Patched	Receptor	This gene encodes a member of the patched gene family. The encoded protein is the receptor for sonic hedgehog, a secreted molecule implicated in the formation of embryonic structures and in tumorigenesis, as well as the desert hedgehog and indian hedgehog proteins. This gene functions as a tumor suppressor. Mutations of this gene have been associated with basal cell nevus syndrome, esophageal squamous cell carcinoma, trichoepitheliomas, transitional cell carcinomas of the bladder, as well as holoprosencephaly. Alternative splicing results in multiple transcript variants encoding different isoforms.
PI3K	Pathway	Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers.
PIP2 PIP3	Pathway	Phosphatidylinositol 4,5-bisphosphate (PIP2) is a minority phospholipid of the inner leaflet of plasma membranes. Many plasma membrane ion channels and ion transporters require PIP2 to function and can be turned off by signaling pathways that deplete PIP2. This review discusses the dependence of ion channels on phosphoinositides and considers possible mechanisms by which PIP2 and analogues regulate ion channel activity.

PREX2	Pathway	An activator of Rac, P-REX2, that is structurally related to the exchange factor PtdIns(3,4,5)-dependent Rac exchanger (P-REX1), but exhibits distinct tissue-specific expression. P-REX2 is spliced into two RNA species, approximately 3.5 and approximately 10 kb in size. The cDNA corresponding to the smaller transcript encodes a protein that exhibits strong similarity with P-REX1 within its N-terminal domains, but differs in the C-terminal region. P-REX2 promoted increased levels of GTP-bound Rac that could be further stimulated by enhancing PI-3K activity. Thus, P-REX2 may serve as a novel link between Rac activation and the PI-3 kinase pathway.
PTEN	Pathway	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, 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.
RAF	Pathway	This gene is the cellular homolog of viral raf gene (v-raf). The encoded protein is a MAP kinase kinase kinase (MAP3K), which functions downstream of the Ras family of membrane associated GTPases to which it binds directly. Once activated, the cellular RAF1 protein can phosphorylate to activate the dual specificity protein kinases MEK1 and MEK2, which in turn phosphorylate to activate the serine/threonine specific protein kinases, ERK1 and ERK2. Activated ERKs are pleiotropic effectors of cell physiology and play an important role in the control of gene expression involved in the cell division cycle, apoptosis, cell differentiation and cell migration.
RAS	Pathway	The protein encoded by this gene is located in the cytoplasm and is part of the GAP1 family of GTPase-activating proteins. The gene product stimulates the GTPase activity of normal RAS p21 but not its oncogenic counterpart. Acting as a suppressor of RAS function, the protein enhances the weak intrinsic GTPase activity of RAS proteins resulting in the inactive GDP-bound form of RAS, thereby allowing control of cellular proliferation and differentiation. Mutations leading to changes in the binding sites of either protein are associated with basal cell carcinomas. Mutations also have been associated with hereditary capillary malformations (CM) with or without arteriovenous malformations (AVM) and Parkes Weber syndrome. Alternative splicing results in two isoforms where the shorter isoform, lacking the N-terminal hydrophobic region but retaining the same activity, appears to be abundantly expressed in placental but not adult tissues.
SENP7	Pathway	The reversible posttranslational modification of proteins by the addition of small ubiquitin-like SUMO proteins (see SUMO1; MIM 601912) is required for many cellular processes. SUMO-specific proteases, such as SENP7, process SUMO precursors to generate a C-terminal diglycine motif required for the conjugation reaction. They also display isopeptidase activity for deconjugation of SUMO-conjugated substrates.
SMAD4	Pathway	SMADs are intracellular proteins that transduce extracellular signals from transforming growth factor beta ligands to the nucleus where they activate downstream TGF-β gene transcription. The SMADs, which form a trimer of two receptor-regulated SMADs and one co-SMAD, act as transcription factors that regulate the expression of certain genes

Smoothened	Receptor	The protein encoded by this gene is a G protein-coupled receptor that interacts with the patched protein, a receptor for hedgehog proteins. The encoded protein tranduces signals to other proteins after activation by a hedgehog protein/patched protein complex.
SPOP	Pathway	This gene encodes a protein that may modulate the transcriptional repression activities of death-associated protein 6 (DAXX), which interacts with histone deacetylase, core histones, and other histone-associated proteins. In mouse, the encoded protein binds to the putative leucine zipper domain of macroH2A1.2, a variant H2A histone that is enriched on inactivated X chromosomes. The BTB/POZ domain of this protein has been shown in other proteins to mediate transcriptional repression and to interact with components of histone deacetylase co-repressor complexes. Alternative splicing of this gene results in multiple transcript variants encoding the same protein.
SRC3		The protein encoded by this gene is a nuclear receptor coactivator that interacts with nuclear hormone receptors to enhance their transcriptional activator functions. The encoded protein has histone acetyltransferase activity and recruits p300/CBP-associated factor and CREB binding protein as part of a multisubunit coactivation complex. This protein is initially found in the cytoplasm but is translocated into the nucleus upon phosphorylation. Several transcript variants encoding different isoforms have been found for this gene. In addition, a polymorphic repeat region is found in the C-terminus of the encoded protein.
SUFU		The Hedgehog signaling pathway plays an important role in early human development. The pathway is a signaling cascade that plays a role in pattern formation and cellular proliferation during development. This gene encodes a negative regulator of the hedgehog signaling pathway. Defects in this gene are a cause of medulloblastoma. Alternative splicing results in multiple transcript variant
TCF	Transcription	The protein encoded by this gene is a nuclear transcription factor which binds DNA as a homodimer. The encoded protein controls the expression of several genes, including hepatocyte nuclear factor 1 alpha, a transcription factor which regulates the expression of several hepatic genes. This gene may play a role in development of the liver, kidney, and intestines. Mutations in this gene have been associated with monogenic autosomal dominant non-insulin-dependent diabetes mellitus type I. Alternative splicing of this gene results in multiple transcript variants encoding several different isoforms.
TGF	Ligand	This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth factors. The secreted protein is cleaved into a latency-associated peptide (LAP) and a mature TGFB1 peptide, and is found in either a latent form composed of a TGFB1 homodimer, a LAP homodimer, and a latent TGFB1-binding protein, or in an active form composed of a TGFB1 homodimer. The mature peptide may also form heterodimers with other TGFB family members. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease
β catenin	Pathway	Beta-catenin (or β-catenin) is a protein that in humans is encoded by the *CTNNB1* gene

12 LIST OF PATHWAYS

From Shtivelman et al as modified we have:

Genes and alterations	***Description***	***Alterations***	***Frequency in primary versus metastatic (when known)***	***PATHWAY***
AR	*Androgen receptor*	*Amplification Mutations Variant splicing*	*Only CRPC. in majority of tumors together with cofactors*	*Androgen receptor signaling*
AR cofactors and regulators NCOA1.2.3: NCOR1. NCOR2. TNK2 and more	*Regulation of the AR activity*	*Amplification Mutations*	*Infrequent in localized: 60-80° o CRPC*	
FOXA1	*Transcription, AR co-factor, prostate development*	*Mutations, ovcTexpression*	*5% mutations in localized, higher levels in CRPC*	
Androgen synthesis enzymes: CYP17 etc	*Steroidogenic/androgen synthesis*	*Activating mutations, copy gain*	*Uncommon in localized; very common in mCRPC*	
TMPRSS2:ERG. other ETS	*Gene fusion involving ERG; rarely other ETS family members*	*Translocation aud ovcrcxprcssion*	*50-60° o of localized and CRPC*	*Transcription, controlled by AR*
NKX3.1	*llomeobox, prostate specific, androgen regulated*	*Deletions*	*3-5° « mutations. 10-20% deletions in localized. 40-80% decreased expression in CRPC*	*Developmental lineage specific, transcription. AR pathway*
PTEN	*Phosphatase suppressor of PI3K*	*Deletions, rare mutations*	*40-50° o of primary, 80% CRPC*	*PI3K signal transduction Co-operates with AR pathway in pathogenesis of PCa*
MAGL2	*PTEN intcractor*	*Rearrangement*		
PIK3CA1 catalytic subimit	*PIP2 kinase*	*Ovcrcxprcssion. mutations*		

Genes and alterations	***Description***	***Alterations***	***Frequency in primary versus metastatic (when known)***	***PATHWAY***
PIILPP1/2	*Phosphatase, inhibits AKT*	*Deletion. down-regualtion*		
Aktl	*Central kinase in PI3K pathway*	*Point mutations (rare)*		
SPOP	*Spccklc-typc POZ domain ubiquitin ligase*	*Mutations*	*5-10% primary and metastatic*	*Degradation of AR cofactor NCOA3/SRC-3. and Gli factors*
SPINK 1	*Serine peptidase inhibitor*	*Ovcrcxpression*	*5-10%. mutually exclusive with ERG rearrangements*	*Unknown*
MYC	*Master of transcription regulation; opposes NKX3.1*	*Overexpressed in primary, amplified in metastatic and NEPC*	*20-30% with gain in metastatic disease*	*Transcription/ translation[1'] metabolism*
NMYC	*Transcriptional regulation*	*Ovcrcxprcssion. amplification*	*40% of neuroendocrine PCa; 5% overall*	*Transcription*
MED 12	*Regulatory component of mediator complex*	*Mutations*	*2-5%*	*Transcription*
EZH2	*Polycomb group*	*Elevated expression*	*Localized (poor prognosis) aud CRPC*	*Chromatin modification Transcriptional suppression*
BMI	*Polycomb group, transcriptional suppression*	*Elevated expression*	*Localized and metastatic*	
TP53	*Tumor suppressor*	*Loss. LOF*. GOF* mutations*	*30-100° •. mostly in metastatic*	*Cell cycle. apoptosis. metabolism*
Aurora A kinase	*Mitotic kinase*	*Ovcrcxprcssion. amplification*	*40% of neuroendocrine PCa; 5% overall*	*Cell Cycle*
BRAF. RAF	*Serine-threoniue kinases activating MAPK cascade*	*Rearrangements*	*1%, all*	*MAPK*

Genes and alterations	***Description***	***Alterations***	***Frequency in primary versus metastatic (when known)***	***PATHWAY***
CADM2	*Cell adhesion molecule*	*Rearrangements*	*Primary and metastatic*	*Cell polarity, potential tumor suppressor*
CHDI	*Nucleosome positioning*	*Mutations*	*8®o. mostly with SPOP mutations, in ETS normal*	*Chromatin remodeling*
MLL complex (MLL2. ASII2L and more)	*Epigenetic transcriptional activation*	*Mutations*	*9% CRPC*	
TAK1/MAP3K 7	*TGFp-activatcd kinase*	*Deletions*	*Deleted in 30% of primary and CRPC*	*Activation of NFkB and other not yet understood functions*
RBI	*Cell cycle*	*Loss. LOF*	*50% metastatic*	*Cell cycle*
ERCC2.4.5: ATM. XRCC4. PRKDC and more	*Various genes involved in DNA repair*	*Losses, mutations*	*Mostly in metastatic*	*DNA damage repair*
CTNNB1. APC. BMP7. WNT factors	*WNT developmental pathway*	*Losses, mutations*	*5% or more in CRPC*	*Developmental pathways*
Shh. Gli factors	*Hedgehog developmental pathway*	*Activation, elevated expression*	*CRPC*	
SOX9	*Prostate stem cells homcobox*	*Activation, elevated expression*	*CRPC*	
TGFβ. TGFβR	*TGFβ pathway*	*Activation, elevated expression*	*CRPC*	
SMAD4	*TGF'P pathway*	*Loss of expression*	*CRPC*	
FGF10. FGFR	*Developmental pathway, paracrine*	*Elevated expression*	*CRPC*	
EGFR. IGF1R, FGFR. MET	*Growth factor receptors*	*Activation*	*NA*	*Growth factor induced signaling. activation of PI3K and MAPK pathways. aud AR signaling*

Genes and alterations	***Description***	***Alterations***	***Frequency in primary versus metastatic (when known)***	***PATHWAY***
IL6-IL6R	*Cytokine receptor*	*Activation*	*NA*	*JAK-STAT3 pathway; activates AR*
SRC	*Tyrosine kinase*	*Activation*	*NA*	*Many signaling pathways*
HSP90, HSP27 Clusterin/TRP M2	*Maintain stability of various signaling proteins including AR and many others*	*Activation*	*NA*	*Protein Chaperons*

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