PROSTATE CANCER STEM CELLS

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This report examines Prostate Cancer Stem cells as discussed in the recent literature. It examines the many variations in CSC characterization and then sets up a discussion regarding pathway dynamics in stem cell elements and non-stem cell offspring. It attempts to understand the specific characteristics of what constitutes a stem cell.

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1 PROSTATE STEM CELLS

There has been a great deal of work on stem cells. We may think of such cells as being part of the embryo, and in the placenta at birth. They are thought of as the universal cell generator. Theoretically the stem cell should become whatever cell type we may want it to be. In a more narrow sense there may be a variety of localized stem cells, namely cells which replenish local cells which are worn away such as on the skin or in the colon. It is not the mature cells which do the reproducing but it is the few stem cells which reside in say the basal layer of the skin which reproduce and create off spring which are just plain old keratinocytes.

In this note we examine in some detail the prostate stem cell, and in turn we generate the ability to consider the cancer stem cell issue in broader detail. We have discussed this issue in our draft volume on Prostate Cancer Genomics, and this is an additive section as that volume progresses.

The focus is on stem cells. It does not address the pathways which are different or activated. That in itself is a critical question. Namely what differentiates a stem cell from a mature non stem like cell when we examine the pathways? Thus when looking at PCa we see that pathway changes are then most likely pathway changes in the stem cell alone, yet if the agglomeration of stem cells is such that the non-stem constituents reflect the genetic makeup of the stem cell, then we would expect some parity in pathway dynamics. This will be an issue we examine in a later report.

The cancer stem cell theory has been developed over the past decade or so. For many years the theory was that cancer was clonal, namely one single cell was at fault and its progeny were the direct result of that genetically modified parent, a single parent, and that as the cancer evolved there may be increased genetic defects but again all were from a single parent.

Cancer stems cells are a construct which predicates the development of mature cells in a cell line as coming from a set of stem cells, akin to the blood cells arising from the bone. In contrast to the linear model of Vogelstein, say in the colon, the epithelial cell of the colon wall has some genetic disruption, and after multiple disruptions this epithelial cell becomes cancerous, dividing without bounds and failing to remain where is was supposed to. Typically an adenoma develops which after the final genetic hit becomes an adenocarcinoma.

For example, we have examined the prostate cancer cell, and in so doing have used a non CSC model, namely it is a basal or luminal cell which becomes genetically changed. If however we are wrong and there is an equivalent prostate cancer stem cell, as some have conjectured, then management of cancer of the prostate is quite a different thing. As we have expressed before, if one has diffuse HGPIN in the prostate and then after several high density prostate biopsies it disappears, is that inferentially valid for a prostate CSC?

The cancer stem cell construct is fundamentally different. It is not a mature cell which takes the genetic hits but the stem cell. The malignant stem cell acts almost as a force at a distance, and can impact other cells as the stem cell itself can reproduce, albeit at a somewhat slower rate than what it may influence.

Arguably if one can remove the stem cell then one removes any future malignancy, even to the extent of having other cells enter apoptosis for failure of having an active stem cell.

As Weinberg notes, there is the theory of clonal development of cancer which states that the cancer cells are pluripotent and have developed from a single source and that they have the capability of reproducing and do so in an autonomous manner[1]. Then there is the theory of the cancer stem cell, the theory which states that there is the equivalent of a stem cell as we know in blood cells, which have the capability but that the majority of malignant cells do not necessarily have that capacity.

The NCI presents an excellent summary of Cancer stem cell, CSC, research[2]:

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

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

As we shall discuss herein, the CSC does not yet have a steady state definition or description. Furthermore it is also difficult to tag and identify. In the above definition, there is the issue of what makes the stem cell different and how many are there and how do we identify it. The CSC is in one sense the single cell which can regenerate a full cancer growth. But does that mean in vivo or in vitro or both? Murine models have been used extensively but there are serious questions regarding their extensibility.

We shall discuss some of these issues in this report. Now the NCI goes on to say:

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

"The [stem cell] model has not been adequately tested in most cancers," said Dr. Sean Morrison, who directs the Center for Stem Cell Biology at the University of Michigan. "I think

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

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

that there are some cancers that do clearly follow a cancer stem cell model…But it will be more complicated than what's been presented so far."

They continue by noting a significant conclusion of the CSC theory, the fact that the CSC is the controlling cell, not just any cell. Specifically they state:

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

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

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

The above has substantial positive and negative impact. A single stem cell may control everything, for a while. If however it undergoes mitosis then we may have many stem cells. Or we may keep a single one. For example if a stem cell in mitosis reproduces a single stem cell plus a non-stem cancer cell, then we maintain single CSCs, while we multiply the malignant non CSC cells. However, if the CSC in mitosis just multiples itself for a while, then we end up with a collection of very powerful and spreadable bombs of CSCs.

The NCI also continues:

"The reason why it's so much stronger for hematologic malignancies are because hematopoiesis research goes back 40 or 50 years and it's very stem cell-based," said Dr. Jean Wang, a stem cell researcher at the University of Toronto. "Whereas in solid tumors, there's less of a foundation for identifying the normal cellular hierarchies and for [cell-surface] markers that identify different populations of cells like stem cells and progenitors."

The above comment has some merit but one must also recognize that the hematopoietic cells are fundamentally generated in a specific location, the bone, and there may very well be no such locations specificity for the many other cells we are considering. Nevertheless, we continue:

Even so, Dr. Wang believes the existence of CSCs is pretty well demonstrated for breast and brain cancers. But, she cautioned, "I don't know if it applies to all cancers. In a lot [of cancers] it does seem to apply. But most of the markers we have right now are still very rough."

Despite the evidence for CSC-like cells in a growing number of cancers, the theory clearly has its skeptics, who point to problems such as shortcomings in the mouse xenograft assay and the variable specificity of the cell-surface markers used to demarcate a CSC from a non-CSC.

"I still feel that it's a concept yet to be proven," said Dr. Barbara Vonderhaar, who, along with colleagues in NCI's Center for Cancer Research, recently published a study identifying a population of CSC-like cells in estrogen receptor-negative breast cancer. "It's certainly a good idea, but it's only a hypothesis at this point. We still don't have definitive proof that cancer stem cells exist."

The CSC concept is "a work in transition," said Dr. William Matsui, from the Johns Hopkins School of Medicine, whose lab studies the role of stem cells in hematologic cancers. "To me, as a clinical person, the ideal model is one where you can find something that is going to work in humans. We're far from that."

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

For example:

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

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

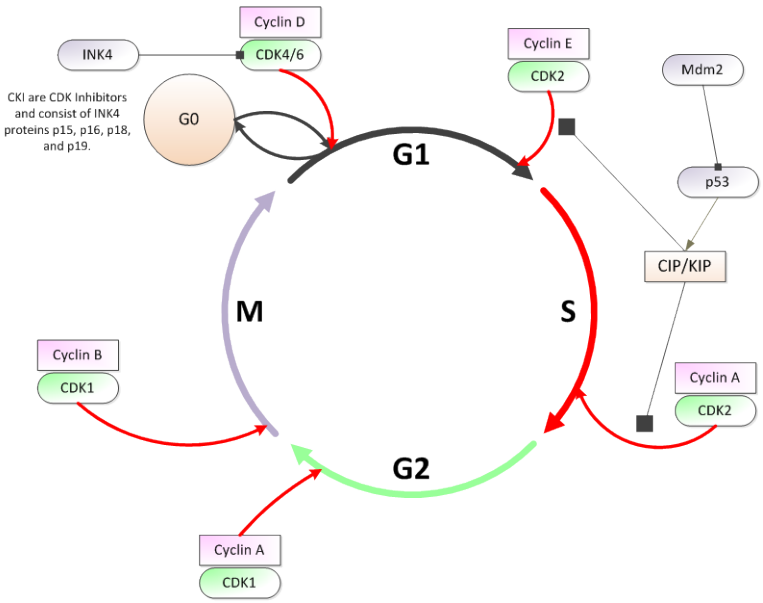
Many other such issues will arise and we discuss the CSC idea here and we return to it later in the work.

Now we can view the stem cells as shown below. There is a stem cell which can give rise to a new stem cell of ultimately a Post Mitotic Differentiated Cancer Cell. The PMDC cannot replicate, whereas the stem cell can. For metastasis it is thus necessary to send out a few stem cells, not PMDC cells.

2 THE STEM CELL PARADIGM

The first issue is a definition of a stem cell. We may understand stem cell from the hematopoietic stem cells found in the bone which give rise to a variety of blood cells and other types of cells. In fact almost all cells in the body which require some form of replenishment have such stem cells. Consider the skin. The basal layer has stem cells to generate the keratinocytes. In fact it may be argued that melanocytes have their own stem cells as well.

Cells are reproducing via the cell cycle as we show below and discuss in Appendix B. With a stem cell, it is only that cell which does the mitotic division; all other cells are just mature functioning cells subject to normal cell death or apoptosis.



The question is however, which cells. Which cells are the stem cells? Are all cells reproducing or just some select class of cells. The concept of stem cells makes the issue one of a small select group of cells. These are the stem cells.

As Alberts et al state (pp 1417-1421):

Humans renew the outer layers of their epidermis a thousand times over in the course of a lifetime. In the basal layer, there have to be cells that can remain undifferentiated and carry on dividing for this whole period, continually throwing off descendants that commit to differentiation, leave the basal layer, and are eventually discarded.

The process can be maintained only if the basal cell population is self-renewing. It must therefore contain some cells that generate a mixture of progeny, including daughters that remain undifferentiated like their parent, as well as daughters that differentiate. Cells with this property are called ***stem cells****.*

They have so important a role in such a variety of tissues that it is useful to have a formal definition. The defining properties of a stem cell are as follows:

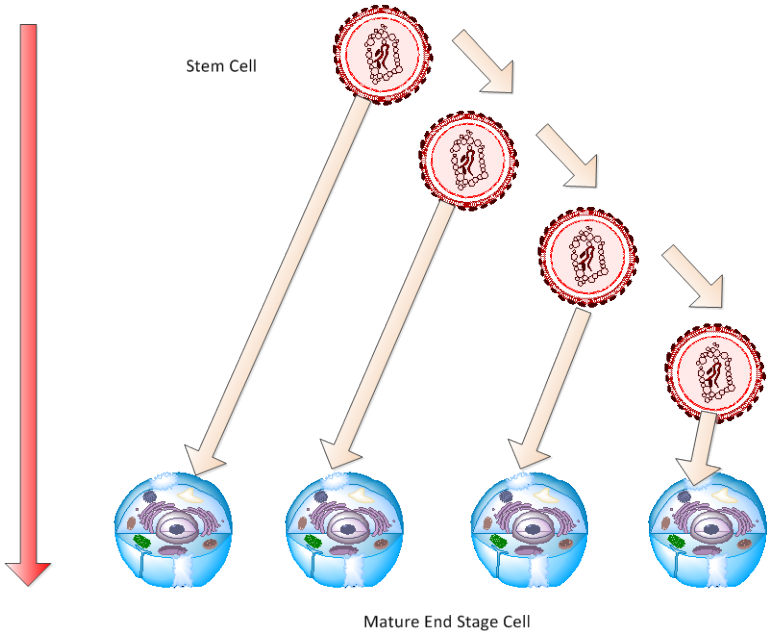
1. It is not itself terminally differentiated (that is, it is not at the end of a pathway of differentiation).

2. It can divide without limit (or at least for the lifetime of the animal).

3. When it divides, each daughter has a choice: it can either remain a stem cell, or it can embark on a course that commits it to terminal differentiation.

Stem cells are required wherever there is a recurring need to replace differentiated cells that cannot themselves divide. The stem cell itself has to be able to divide—that is part of the definition—but it should be noted that it does not necessarily have to divide rapidly; in fact, stem cells usually divide at a relatively slow rate.

We present below a simplified example of a specialized stem cell. The stem cell is the only one of its kind to divide. The mature cells do not generally divide; they are just functional and proceed to mature. The stem cell always produces at least one of its own kinds, another stem cell, and then one of the mature like cells. Note the initial stem cell. In this example we allow it to divide and produce one stem cell and one maturing cell. Thus at some point this process just keeps the number of stem cells constant but can produce an ever growing number of maturing cells.



Now when we examine the above we can see that if the stem cell divides once every hour, and the life of a mature cell is say 24 hours, then we have a growth effect. We must have a cell stability of one replenishment per one destroyed. During a growth state however, the stem cells

are reproducing quickly and cells are added. The stem cell responds to surface stimulants to enter into cell cycle production.

As Tang et al state:

Normal adult stem cells (SC) have several fundamental properties: they are generally very rare, can self-renew, have tremendous proliferative potential but normally (i.e., in their niches) are quiescent, and can differentiate along one or several different cell lineages.

The most defining property of a SC is its ability to self-renew while being able to differentiate into all different lineages of progeny and even to reconstitute an organ, as exemplified by a single hematopoietic SC (HSC) to reconstitute the whole blood and rescue an irradiated mouse. SC development is a continuous and dynamic process, in which cells with distinct self-renewal, proliferative, and differentiation abilities may co-exist.

For example, mouse HSC are heterogeneous populations of cells containing long-term HSC (LT-HSC), which can sustain life-long self-renewal and reconstitution, and short-term HSC (ST-HSC), which can sustain self-renewal and reconstitution for only 8 wk. The ST-HSC generate multi-potent progenitor (MPP) cells exhibiting only limited self-renewal capacity, which then further develop into lineage-restricted progenitor (or precursor) cells that have lost self-renewal ability.

Although this paradigm of LT-HSCST-HSC early progenitors (MPP) late progenitors differentiated cells in mouse bone marrow can, in principle, be applied to other SC developmental processes, in reality, little is known about most tissue SC lineages and we often name the subsets of cells in a specific tissue/organ with certain self-renewal and differentiation abilities simply stem/progenitor cells. Such is the case with the putative prostate epithelial stem and progenitor cells.

Consequently, throughout this review, we shall frequently use the term '(prostate) stem/ progenitor cells.'

The above feature of maturing into various lineages is clearly seen in blood cells but one may question just where it functions say in prostate cells. Is there a single stem cell which generates either a basal or luminal cell or if so where does it reside, and how does this differentiation occur? This is the point made by Tang et al towards the end of the above quote.

1.1 THE STEM CELL THEORY

Cancer stem cells are a variant of the benign stem cell. Namely a cancer stem cell is a cell which behaves like a stem cell in terms of cell proliferation but now has genetic changes which reflect malignant behavior. In an NIH report the authors define cancer stem cells as follows:

A consensus panel convened by the American Association of Cancer Research has defined a CSC as "a cell within a tumor that possesses the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor." It should be noted that this

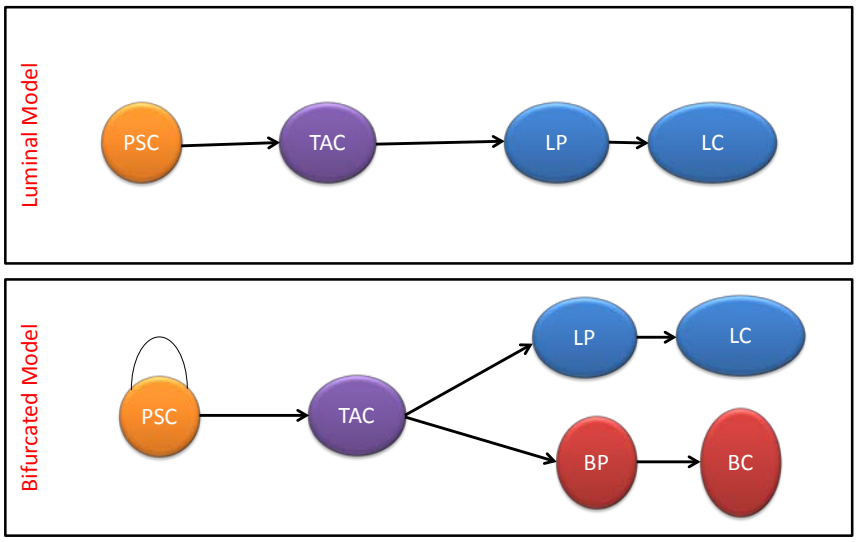
definition does not indicate the source of these cells—these tumor-forming cells could hypothetically originate from stem, progenitor, or differentiated cells.

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

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

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

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



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

As a report in the UK based newspaper The Guardian states:

An emerging, although highly controversial, answer to this question is that cancer's immortality, too, is borrowed from normal physiology. The human embryo and many of our adult organs possess a tiny population of stem cells that are capable of immortal regeneration. Stem cells are the body's reservoir of renewal. The entirety of human blood, for instance, can arise from a single, highly potent blood-forming stem cell (called a hematopoietic stem cell), which typically lives buried inside the bone marrow. Under normal conditions, only a fraction of these blood-forming stem cells are active; the rest are deeply quiescent – asleep.

But if blood is suddenly depleted, by injury or chemotherapy, say, then the stem cells awaken and begin to divide with awe-inspiring fecundity, generating cells that generate thousands upon thousands of blood cells. In weeks, a single hematopoietic stem cell can replenish the entire human organism with new blood - and then, through yet unknown mechanisms, lull itself back to sleep.

Something akin to this process, a few researchers believe, is constantly occurring in cancer – or at least in leukemia. In the mid-1990s, John Dick, a Canadian biologist working in Toronto, postulated that a small population of cells in human leukemias also possess this infinite self-renewing behavior.

These "cancer stem cells" act as the persistent reservoir of cancer – generating and regenerating cancer infinitely. When chemotherapy kills the bulk of cancer cells, a small remnant population of these stem cells, thought to be intrinsically more resistant to death, regenerate and renew the cancer, thus precipitating the common relapses of cancer after chemotherapy. Indeed, cancer stem cells have acquired the behavior of normal stem cells by activating the same genes and pathways that make normal stem cells immortal – except, unlike normal stem cells, they cannot be lulled back into physiological sleep.

Cancer, then, is quite literally trying to emulate a regenerating organ – or perhaps, more disturbingly, the regenerating organism. Its quest for immortality mirrors our own.

We quote Lawson and Witte as follows:

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

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

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

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

Furthermore, CD44+CD24- cells express genes known to be important in stem cell maintenance, such as BMI-1 and Oct-3/4. Moreover, we can maintain CD44+CD24- prostate stem-like cells as non-adherent spheres in serum-replacement media without substantially shifting gene

expression. Addition of serum results in adherence to plastic and shifts gene expression patterns to resemble the differentiated parental cells.

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

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

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

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

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

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

As Delarbra et al state:

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

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

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

The statement starts with the accepted monoclonal hypothesis and then departs to a polyclonal alternative view. It retains the CSC, cancer stem cell, paradigm for solid tumors as well. In the context of HGPIN we see a change in the cells and we have heard the argument that they have made one or several of the unchangeable steps towards PCa. Thus using the CSC theory one would expect that it would be from one or several of these cells that PCa would arise. In

addition, we could assume that there is no unique pathway mutations or changes which result in PCa but a plethora of them. Simply stated, cancer is complex, it finds ways to migrate forward no matter what the path.

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A recent study by Deleyrolle et al has focused on the stem cell and its dynamics[3]. The reviewers state:

The method, published in the online journal PLoS ONE in January, may rev up efforts to develop stem cell therapies for Alzheimer's, Parkinson's and other diseases. It may also help get to the root of the cancer-stem cell theory, which puts forth the idea that a tiny percentage of loner cancer cells gives rise to tumors.

"Math is going to be the new microscope of the 21st century because it is going to allow us to see things in biology that we cannot see any other way," said Brent Reynolds, Ph.D., an associate professor of neurosurgery at UF's McKnight Brain Institute and a member of the UF Shands Cancer Center. "Stem cells and the cells that drive cancer may be as infrequent as one in 10,000 or one in 100,000 cells. The problem is how do you understand the biology of something whose frequency is so low?"

Inspired by a 2004 essay by Joel E. Cohen, Ph.D., of The Rockefeller University and Columbia University that described the explosive synergy between mathematics and biology, Reynolds and postdoctoral associate Loic P. Deleyrolle set out to build an algorithm that could determine the rate stem cells and cancer stem cells divide.

High hopes to treat or prevent diseases have been pinned on these indistinguishable cells, which are often adrift in populations of millions of other cells. Scientists know stem cells exist mainly because their handiwork is everywhere — tissues heal and regenerate because of stem cells, and somehow cancer may reappear years after it was thought to be completely eliminated.

Nature has an interesting poster on the cancer stem cell, CSC[4]. The poster states:

The concept of the cancer stem cell (CSC) has taken off rapidly over the past 10 years. CSCs are cells with properties that are similar to those described for tissue stem cells: self-renewal and

[3] http://www.eurekalert.org/pub_releases/2011-01/uof-gfm012011.php

[4] http://www.nature.com/nrc/posters/cancerstemcells/csc_poster.pdf

asymmetric division resulting in the generation of daughter cells destined to differentiate, enabling the regeneration of a tissue. Initial research into the properties of CSCs was based on identifying and verifying markers of this subset of cancer cells.

However, most studies have now moved on to understanding the biology of CSCs and the cancers in which they maintain tumour growth, as well as how and why they are able to serially generate a tumour. It is thought that a key element regulating the biology of stem cells is their niche — cells and extracellular matrix that support self-renewal and survival. As we begin to understand the pathways that are crucial for the properties of CSCs, including signals provided by the niche, we will hopefully be able to effectively target this cell population.

Linked to the identification of CSCs is the cell of origin. These are cells that when mutated are able to give rise to a tumour. Although these cells may share properties with CSCs, in most cases it is not yet clear whether these cells are one and the same. This poster highlights some of the recent findings regarding the biology of CSCs and the identification of cell types from which cancers can arise.

As regards to prostate cancer they state:

In the normal prostate, epithelial cells with tissue-regenerating capacity that are Sca1+, CD49fhi, TROP2hi, CD44+, CD133+ and CD117+ (mouse) or CD133+, CD44+, CD49fhi and TROP2+ (human) seem to reside in the basal layer of the prostate. However, studies in mice indicate the existence of luminal cells with progenitor characteristics that can regenerate the prostate after androgen withdrawal. As castration resistance is also a property of basal stem cells in the prostate, it suggests a complex cellular hierarchy.

Studies in mice indicate that prostate tumours can arise after transformation of basal stem cells and luminal progenitor cells. A subset of cells that are CD133+, a2b1 + and CD44+ and have basal cell characteristics have been shown to be tumorigenic, but whether these cells can serially propagate tumours in mice has yet to be verified.

Again and interesting experiment can be performed:

1. Take biopsies from N men with HGPIN diagnosed on initial biopsies. Perform sampling from say 20 cores.

2. Wait 9 months, and rebiopsy, again with near saturation cores, 20+ .. There are three possible outcomes:

a. HGPIN remains
b. PCa has been determined
c. HGPIN regresses and only benign cells are left

3. The question is why did (c) above happen? What percent of the HGPIN have regressed? If the percent of HGPIN that have regressed equals the probability of having actually excised the

cancer stem cell or cells, we can calculate this, then by chance we have removed the CSC from the HGPIN and this would affirm its existence by inference.

Now a similar article appears in Science which speaks to colon cancer and the cancer stem cell theory[5]:

In normal colon tissue, intestinal stem cells (ISCs) that reside at the base of mucosal wells, named crypts, expand through mitosis and move upward toward the crypt tip. The cells then undergo cell cycle arrest and terminal differentiation, finally becoming the mucosal epithelium of the colon. In the recent study, the investigators identified in mouse ISCs a gene signature that was specifically marked by high expression of the ephrin type-B receptor 2 gene(Ephb2), which encodes a receptor tyrosine kinase, the leucine-rich repeat–containing G protein–coupled receptor 5 gene (Lgr5), which encodes a G-coupled protein receptor of unknown function, and ~50 other genes. This gene signature also defined a specific population of stem-like cells at the base of colorectal tumor structures in mice that were morphologically similar to normal mouse intestinal crypts. The authors then similarly inspected tumor samples from 340 colorectal patients and discovered a 10-fold increase in the relative risk of recurrence in patients whose tumors displayed high expression of the human counterparts of the mouse ISC genes, relative to patients whose tumors showed low expression of these genes.

To test whether the mouse colorectal tumor cells with the ISC gene signature were cancer stem cells; the investigators isolated the cells and introduced them into an immunodeficient mouse model. The stem-like cancer cells demonstrated both a tumor-initiating capacity and self-renewal capability in vivo. *These findings pinpoint potential markers that may allow a clinician to predict a patient's future with respect to recurrence. These differentially expressed genes also may give rise to therapeutic targets that quell cancer stem cells.*

What is clear is that the CSC is becoming a viable model for understanding cancer at another level.

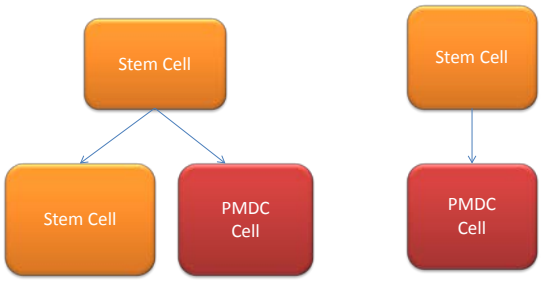
1.2 PROGRESSION AND REGRESSION

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

To look more closely we first return to the stem cell model for cancer which we developed earlier. The stem cell theory states that there are a certain number of cancer stem cells which in turn may replicate themselves but also create what are termed post mitotic differentiated cells. Not really stem cells but cells which exhibit the phenotypic characteristics of a cancer cell. One

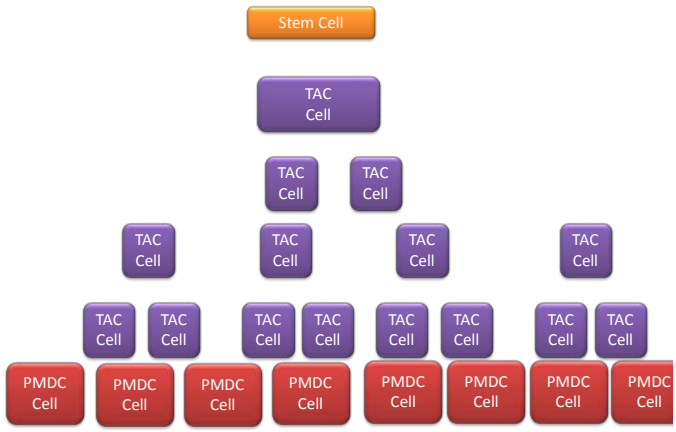
[5] http://stm.sciencemag.org/content/3/81/81ec64.short?rss=1

of the questions one may pose is do these PMDC exhibit a different genotypic character as well or are they controlled by some epigenetic factors. We show these examples below;



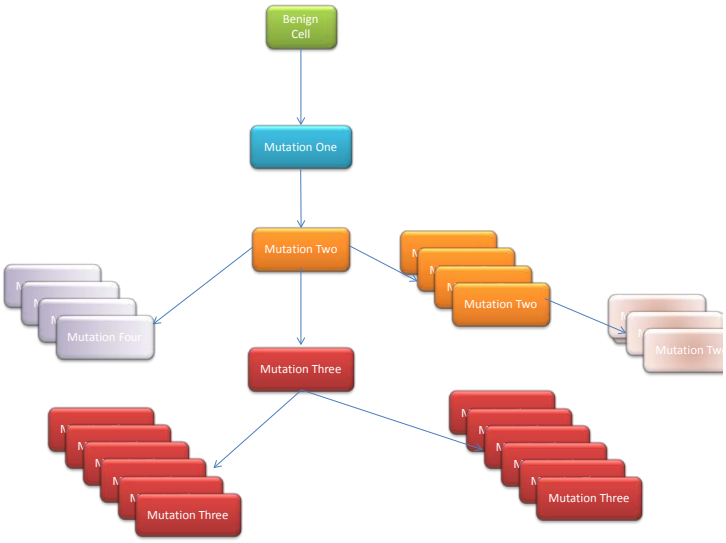
PMDC Cell: Post Mitotic Differentiated Cancer Cell

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

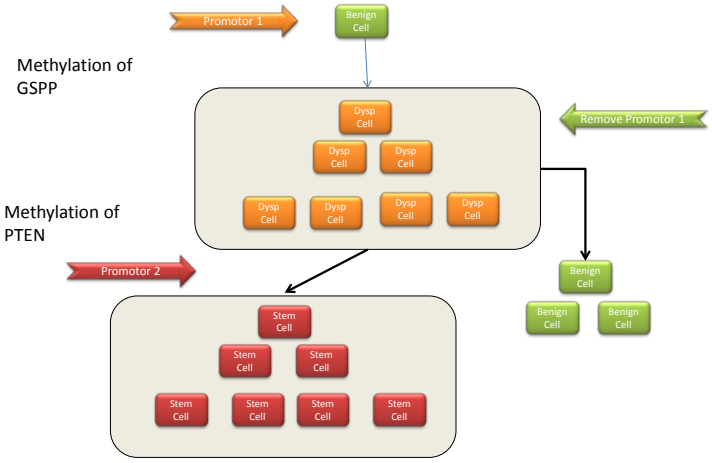


TAC: Transit Amplifying Cells

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



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



Wang and Shen have written a quite useful review of the cancer stem cell thesis for prostate cancer. There is no definitive conclusion but the review covers a wide path through what has been accomplished to date.

Recall as we have written before the cancer stem cell (CSC) model, and it is a model, hypothesizes that there are certain core cells which control the malignant growth of other cells and that the other cancerous type cells do not in and of themselves have the ability to continue to grow. In fact it could be concluded, although not part of the current theory, that removal of a

CSC from a tumor, say the only CSC, would result in the apoptosis of the remaining cells. Namely, a remission.

In contrast to the CSC model we have the clonal model which says that the cells have progressed through a set of pathway modifications that have resulted in a single cell which takes off and multiples and that the progeny have identical genetic makeup or further genetically modified makeup but all and equally malignant.

These are two fundamentally different views of cancer. One could also state that recent work with melanoma as we have discussed also posit that the CSC "communicates" to progeny to have them multiply and that arguably the loss of the CSC

There is a great deal of difficulty in identifying the CSC, usually attempting to do so via surface markers such as CD44 and the like.

Wang and Shen then discuss the controversy regarding the CSC concept. They state:

Much of the confusion in the literature arises through inconsistencies in nomenclature within the field. In particular, due to the wide use of xenotransplantation as a functional assay for CSCs, transformed cells that can initiate tumor formation in this assay are often referred to as CSCs in the literature. However, a tumor initiating cell (TIC) represents a different concept from that of a CSC, as TICs unquestionably exist within tumors and their identification does not by itself imply a hierarchical organization of a tumor.

Indeed, the majority of cells within a tumor could potentially possess TIC properties and nonetheless follow a clonal evolution model. Consequently, it is important to distinguish CSCs that have been strictly defined by their position and function within a lineage hierarchy in vivo from CSCs that have been identified as rare TICs in transplantation studies.

A similar confusion arises with respect to the cell of origin for cancer, which corresponds to a normal tissue cell that is the target for the initiating events of tumorigenesis. In principle, a normal adult stem cell could be a logical cell of origin for cancer, as it would retain the ability to self-renew and generate a hierarchy of differentiated lineages within a tumor. However, it is also possible that a cell of origin could correspond to a downstream progenitor cell or conceivably even a terminally differentiated cell that acquires stem cell properties during oncogenic transformation.

Our argument has been that the CSC may most likely exist and that it has undergone certain pathway changes and that as a result it may influence the growth of not identically genetically changed cells to multiply but not in and of themselves have the potential to multiply.

Wang and Shen continue:

The identification of normal cells that can serve as a cell of origin for prostate cancer is highly relevant for understanding the applicability of a CSC model, and is currently under intense investigation. The cell of origin may also have clinical significance, as in the case of breast

cancer, distinct tumor subtypes have been proposed to originate through transformation of different progenitors within the mammary epithelial lineage. Thus, it is conceivable that there may be distinct cells of origin for other epithelial cancers, and different cells of origin may give rise to clinically relevant subtypes that differ in their prognosis and treatment outcome.

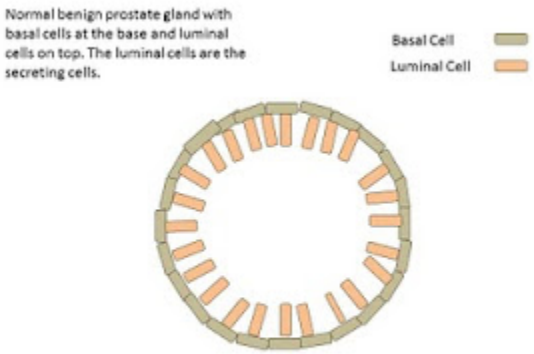
Thus there are either basal cells or luminal cells as the cell of origin. Goldstein et al in Witte's lab had developed a murine model demonstrating the basal cell as the cell of origin. However there may be strong issue regarding this model as applied to human prostate cancer. It represents a viable pathway but not necessarily the only. The issue is one of pathways as well as one of intercellular communications with debilitated pathways.

Now to follow the Wang and Shen model we have the following. Fist we show a normal prostate gland with basal and luminal cells.

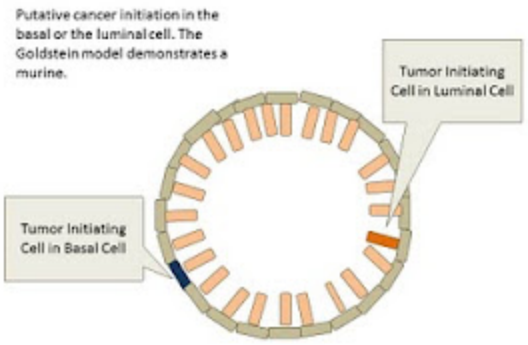
Then we show their view of a Tumor Initiating Cell in either the basal or luminal layer. The Goldstein et al murine model argue for the basal layer and there are others arguing for the luminal.

The Wang and Shen model is as follows.

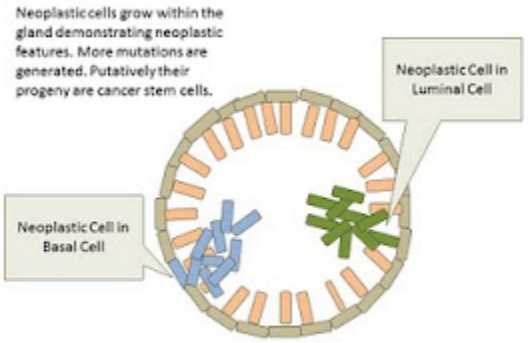
1. A normal prostate cell has both luminal and basal cells.



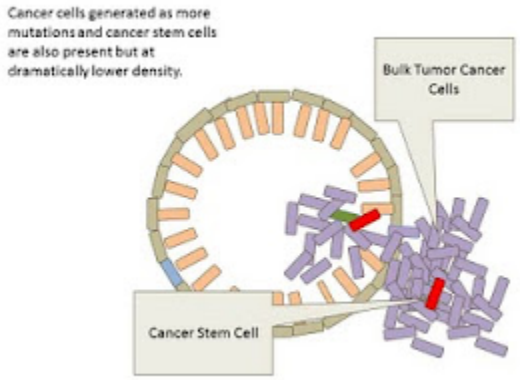
2. TICs may be formed in either basal or luminal cells.



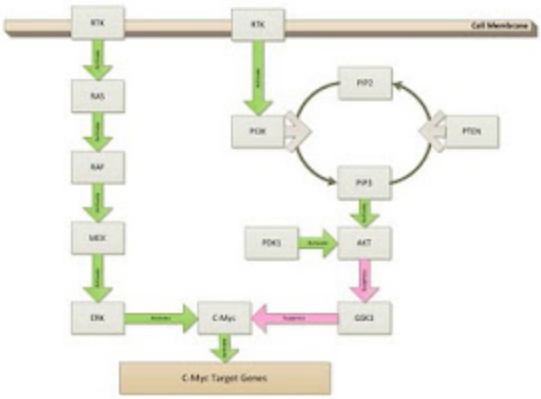
3. Neoplasia starts with intro acinar proliferation.



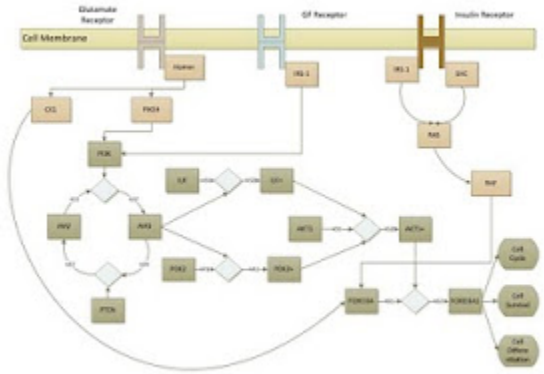
4. Carcinoma starts when it expands beyond the gland and starts up its own quasi-glandular structures.



Now what causes this? Genetic changes result in pathway changes. We show two pathways below. We lose PTEN and we may activate myc and other parts of the pathway control mechanism.



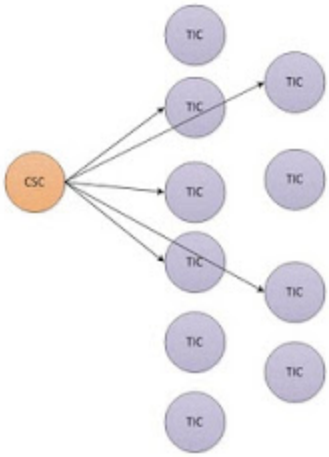
And the following pathway:



We now make a different argument. If there exists a true PCa CSC then perhaps one may putatively validate it as follows. The logic then is:

1. Assume a PCa CSC exists.

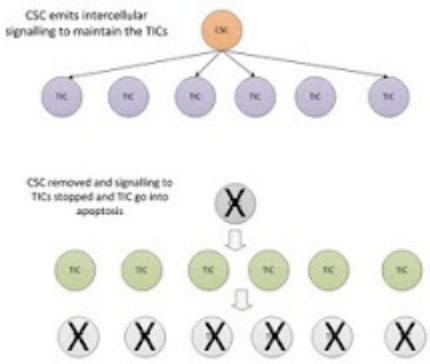
2. Assume that the PCa CSC replicates its CSC self at a low rate and is initially confined to the prostate gland.



3. Assume that the PCa CSC can influence the growth of TIC which themselves cannot sustain a malignancy. Specifically we assume that the TICs require the CSC for continued growth and further the CSC does so via cell growth as well as intercellular communications.

4. Now let us assume we have performed an 18 core biopsy on a 60 cc prostate gland and find histologically extensive high grade focal prostatic intraepithelial neoplasia. According to Wang and Shen they are most likely TICs and furthermore there may be a CSC somewhere so that eventually we see a PCa. There may be one or a few CSC in one or all of the glands yet we have no definitive marker to indicate as such.

5. Now assume we perform a second multi core biopsy on the gland and say do 22 cores in a 60 cc gland. This is the same gland but say 9 months later. We would arguably expect one of two possible outcomes. First that the HGPIN remains in place and possibly has expanded. Second that there was a CSC and the HGPIN had become classic PCa with say Gleason 2 or 3 at a minimum about the HGPIN clusters.



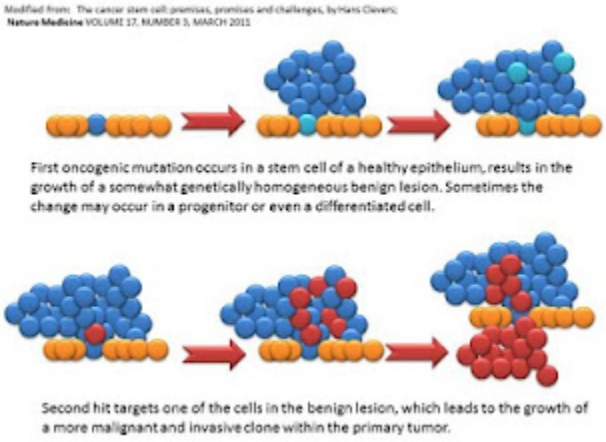
6. If however, we examine the cores and find no evidence of any neoplasia or PCa, namely the gland has totally reverted to benign histology, we may have a reasonable argument that perhaps the CSC was present initially, and it was somehow removed along with the HGPIN in the initial

biopsy leaving the TIC alone behind. Thus the TICs requiring a CSC to survive go into an apoptotic state and are removed from the prostate. Perhaps.

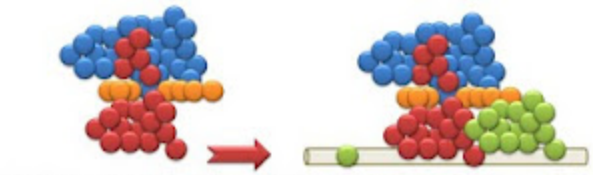
We have seen that specific situation occur and one could then argue that the Wang and Shen model for CSCs may be a viable model and further if such can be shown more extensively than we may have a basis for PCa progression.

There is an interesting article by Clevers in Nature Medicine which is an up to date review of the cancer stem cell issue. In light of the flurry of reports stating the wonders of having identified genes which appear in many tumors, prostate being the case, and my previous remarks that perhaps is the CSC is in fact existent, that then one should be identifying it and its genetic makeup as well as the dynamics of its pathways.

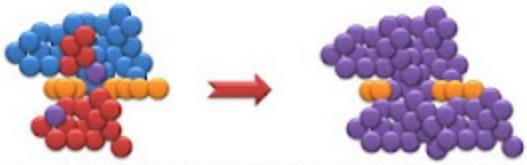
Now Clevers suggests a four step process, albeit with limited experimental evidence, but a superb start. It is as follows:



The above are the first two steps. Perhaps a dysplasia or neoplasia but with the kernel of a stem cell. This is the first "hit" theory. The epithelium starts to grow in a strange manner. Say a polyp in the colon or HGPIN in the prostate. Then we see a second hit and the formation of extraepithelial growth.



Third hit in a cell within the malignant subclone causes further transformation, visualized as entry into a blood vessel for distant metastasis.



Fourth hit leads to tumor being entirely taken over by cells that behave as cancer stem cells. Tumors and subclones within tumors contain some cells that behave as CSCs. The final hit causes all cells to behave as CSCs. Thus tumors cells and CSC are identical.

Then the third hit for the author and we see transmission via the blood stream. Then the fourth hit and the explosion from a few to almost all cancer stem cells.

Whether this is a good or bad model is yet to be seen. As Clevers states:

Central to the cancer stem cell (CSC) concept is the observation that not all cells in tumors are equal. The CSC concept postulates that, similar to the growth of normal proliferative tissues such as bone marrow, skin or intestinal epithelium, the growth of tumors is fueled by limited numbers of dedicated stem cells that are capable of self-renewal. The bulk of a tumor consists of rapidly proliferating cells as well as postmitotic, differentiated cells. As neither of these latter two classes of cells has the capacity to self-renew, the contribution of these non-CSC tumor cells to the long-term sustenance of the tumor is negligible.

The increased focus on the CSC is truly needed because if it is indeed a key paradigm in cancer then it and not large tumor masses should be examined. Clevers concludes with:

Epilogue: are CSCs and clonal evolution mutually exclusive?

To date, the CSC field has treated tumors as genetically homogeneous entities, by and large ignoring the fact that the observed tumor heterogeneity may result from underlying genetic differences. However, it is well known that most solid tumors show extensive genomic instability. Moreover, genetic defects in a large variety of molecules that are involved in the maintenance of the integrity of the genome are well-known drivers of oncogenesis. Even in a disease like CML, so clearly driven by stem cells, clonal evolution can be seen at work when imatinib is administered: the malignancy becomes tumor-resistant through the emergence of clones that carry mutations in the target of imatinib, the BCR-ABL1 fusion gene75. And the progression of CML into ALL blast crisis is caused by the emergence of subclones that harbor inactivating lesions in the cyclin-dependent kinase inhibitor 2A (CDKN2A, also known as ARF) gene in addition to the BCR-ABL1 translocation76. The evidence for clonal evolution in the pathogenesis of cancer is so overwhelming that it appears inescapable that all models should be integrated with it.

The recent rapid advances in DNA sequencing are now allowing the global analysis of genomic changes of cancer cells. These analyses have confirmed many previously known common genetic alterations in cancer, and they have also revealed some new common mutations as well as unexpectedly large numbers of rare mutations. As a next step, this technology can be applied to chart genetic heterogeneity within individual tumors as well as between primary tumors and their local recurrences and metastases.

It should thus be possible to map, in both space and time, the genetic evolution of a tumor.

The last sentence is the most compelling. Cancer may be more than just a cellular disease; it may require the spatial domain as well. This is an exceptionally good review and should be a focus for future research.

3 PCA STEM CELL RECOGNITION

Recent work by Qin et al. examine the more detailed nature of the prostate cancer stem cell (PCa CSC). We here look at that as a starting point and then examine some of the surrounding literature to see if the results from that work can be extensible. The cancer stem cell model is one which akin to the stem cell model above states that there are a class of stem like cells which have been mutated and the development of cancer results from the turning on of these cells.

Before proceeding let us review a few issues. It should be noted that we are simplifying the analysis to intensify several points and let the reader focus on the literature to assist in resolving some of the lost complexities. Now:

1. Stem cells have certain characteristic and the only one we focus on here is that for the most part they are the only cells of a class which have the ability to reproduce. In a stable environment, the stem cells reproduce at a rate equal to the loss of mature functional cells. Thus in the skin, the basal stem cells reproduce at a rate equivalent to the death and loss of the keratinocytes, no more or less. Let there be an injury then they produce more by being activated by some ligand on some receptor on the stem cell. Cells reproduce until equilibrium is reached.

2. Mature cells, derivative from stem cells, do not reproduce. They just do what they were intended to do, no more or less.

As Wang and Shen state in a recent article (2011):

The cancer stem cell (CSC) model proposes that cells within a tumor are organized in a hierarchical lineage relationship and display different tumorigenic potential, suggesting that effective therapeutics should target rare CSCs that sustain tumor malignancy…CSCs are instead defined in practical terms through the use of several functional assays. The most frequently used methodology involves xenotransplantation of flow sorted populations of primary cancer cells into immunodeficient mice. In this assay, CSCs are defined as a subpopulation of cells within a primary tumor that can initiate tumor formation in mice following transplantation, unlike the remaining tumor cells

This is a definition limited to the assay produced. It is not a broad based definition.

Wang and Shen then discuss the types of prostate cells:

In human and mouse, the normal prostate gland epithelium contains three primary differentiated cell types.

1. *Luminal cells are columnar epithelial cells that express secretory proteins as well as markers such as cytokeratin 8 (CK8), CK18, Nkx3.1, prostate-specific antigen and high levels of androgen receptor (AR).*

2. *Basal cells are localized beneath the luminal layer and express markers including CK5, CK14 and p63, but express low levels of AR.*

3. *A rare third type of cells termed neuroendocrine cells express endocrine markers such as synaptophysin and chromogranin A, but do not express AR.*

Then they allege:

Prostatic intraepithelial neoplasia (PIN) is often considered a precursor of prostate cancer, and is characterized histologically by luminal epithelial hyperplasia and a progressive loss of basal cells …

Here we have previously expressed concern regarding counter-examples. Namely it is known that there are patients where a diffuse HGPIN may be present upon a high density sampling and then after a second high grade sampling the HGPIN is totally gone. The question is why? If as many agree HGPIN is the precursor of PCa and if moreover HGPIN is already a representation of a CSC mutation, then what has reversed the mutation. Perhaps it was the fortuitous removal on the CSC in the initial sampling? We have argued that such may be inductively deduced from examining the number of times this occurred related to the statistical chance of such happening.

In a recent paper, Qin et al state[6]:

Prostate cancer (PCa) is heterogeneous and contains both differentiated and undifferentiated tumor cells, but the relative functional contribution of these two cell populations remains unclear. Here we report distinct molecular, cellular, and tumor-propagating properties of PCa cells that express high (PSA^{+}) and low ($PSA^{-/lo}$) levels of the differentiation marker PSA. $PSA^{-/lo}$ PCa cells are quiescent and refractory to stresses including androgen deprivation, exhibit high clonogenic potential, and possess long-term tumor-propagating capacity.

They preferentially express stem cell genes and can undergo asymmetric cell division to generate PSA^{+} cells.

Importantly, $PSA^{-/lo}$ PCa cells can initiate robust tumor development and resist androgen ablation in castrated hosts, and they harbor highly tumorigenic castration-resistant PCa cells that can be prospectively enriched using $ALDH^{+}CD44^{+}\alpha2\beta1^{+}$ phenotype.

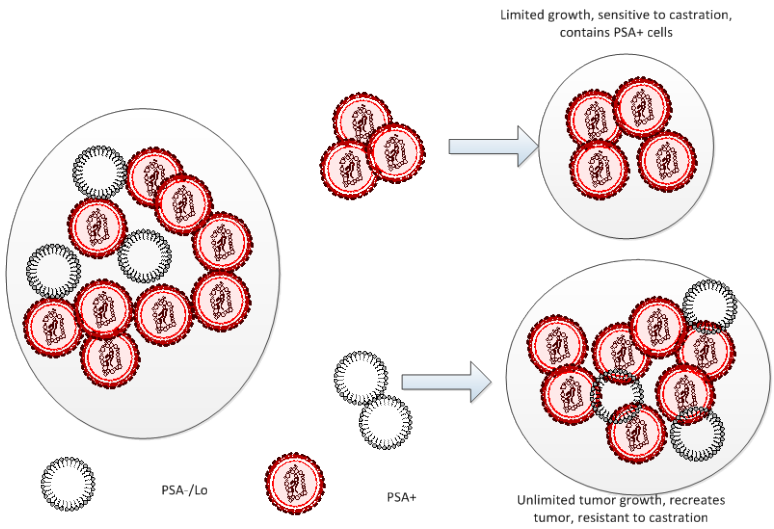
In contrast, PSA^{+} PCa cells possess more limited tumor-propagating capacity, undergo symmetric division, and are sensitive to castration. Altogether, our study suggests that $PSA^{-/lo}$ cells may represent a critical source of castration-resistant PCa cells.

Specifically:

1. *$PSA^{-/lo}$ PCa cells are quiescent and refractory to anti-androgen and chemotherapy*
2. *These cells express stem cell genes and can undergo asymmetric cell division*
3. *They also possess long-term tumor-propagating capacity in intact male mice*
4. *$PSA^{-/lo}$ PCa cells are highly tumorigenic and resist androgen ablation in vivo*

[6] http://www.cell.com/cell-stem-cell/abstract/S1934-5909%2812%2900126-9

We depict the details from the paper and show it below:



As Merville states in commenting on the work of Qin et al[7]:

In cell lines and mouse model experiments, the low-PSA cells resisted chemotherapy and thrived under hormone deprivation, the two main prostate cancer drug treatments, the researchers found.

Low-PSA cells were found to be both self-renewing and capable of differentiating into other prostate cancer cell types upon division, a hallmark of stem cells called asymmetric cell division. "Asymmetric cell division is the gold standard feature of normal stem cells," Tang said. "Using time-lapse fluorescent microscopy, we were able to show asymmetric cell division by filming a low-PSA cell dividing into one high-PSA cell and one low-PSA cell."

When the team implanted the two cell types in hormonally intact male mice, the rapidly reproducing PSA-positive cells caused faster growth and larger tumors in the first generation. However, after that the low-PSA cells generated larger, faster-growing tumors and tumor incidence in the high-PSA cells dropped.

In fact, the low-PSA prostate cancer cells possess indefinite tumor-propagating capacity. In contrast, when implanted in the castrated mice, the low-PSA prostate cancer cells developed much larger tumors than the corresponding high-PSA cells. In another experiment, mice with tumors generated by either cell type were then castrated and treated with hormonal therapy.

Low-PSA tumors grew better in these doubly androgen-deprived mice than the high-PSA tumors. "These findings closely resemble progression observed in patients after androgen-deprivation treatment and reflect reduced PSA-producing cells in patient tumors after androgen depletion," Tang said.

[7] http://www.eurekalert.org/pub_releases/2012-05/uotm-sip050412.php

As Jeet et al state regarding their view of the prostate related stem cell:

Stem cells are unspecialized cells that can self-renew and differentiate to yield a diverse range of specialized cell types of a tissue or organ. The mouse prostate comprises dorsal, lateral, ventral, and anterior lobes, each containing three regions of proliferating cells—distal, intermediate, and proximal. It has been suggested that the prostatic stem cells reside in the proximal region of the mouse prostate.

These findings, together with tissue recombination approaches (that allow the study of mesenchymal-epithelial interactions in developing tissues), led to the elegant work that developed a new prostate regeneration system by combining CD117 (a prostate stem cell marker predominantly expressed in the proximal region) positive fractions from C57BL/6 mouse donors with rat embryonic urogenital sinus mesenchymal stromal cells. These cells were then placed under the renal capsule of athymic nu/nu mouse hosts to generate functional, secretion-producing prostates. This is the first model to demonstrate the ability of mesenchyme to trigger prostate genesis thus opening up possibilities for developing insights into the earliest changes that evolve into cancer.

Jeet et al argue that their worked demonstrates the ability of these identified stem cells to have a form of prostate related pluripotency. They like many others have been using cell markers as a means of tracking the stem cell. One may then ask what is the cell receptors and activating ligands which result in the stem cell ability to perform its regenerative functions.

As Zhang stated:

Importantly, Staege and Max also noted that tumor stem cells in EFT have been identified. These tumor stem cells expressed some markers of embryonic stem cells. There are cell populations with the phenotype of embryonic stem cells in the adult body. It remains unclear as to whether such cell populations are permissive for EWSR1-FLI1 induced transformation and whether EFT is derived from these cell populations.

Zhang has extended this identification somewhat but the issue of good markers remains.

Yet as Gupta et al state:

Some of the controversy surrounding the CSC model seems to arise from confusion regarding the definition of CSCs, leading to two key objections against the use of this term.

The first objection derives from the fact that, unlike the case for normal stem cells, which are usually oligo or multipotent, it is currently unclear whether CSCs can give rise to multiple differentiated cell types….

A second key objection to the CSC model is that it is currently unclear whether the normal cellular precursors of CSCs are, in fact, bona fide stem cells. It is clear, however, that the traits used to define CSCs do not rely on knowledge of their cellular origins within normal tissues. Accordingly, the CSC model must stand or fall on the basis of experimental characterizations of cancer cell populations

The Gupta et al observations are quite important. Namely, is a stem cell born or made. Namely is there an unbroken lineage from stem cell to stem cell? Also his first observation is the pluripotency issue, namely, are stem cells able to generate a broad number of cells or are stem cells cell-specific? The current nature of the Gupta et al observations do raise issues as to how well we understand the stem cell model.

As Tang et al conclude:

The hypothetical model of hierarchical organization of PCa cells has several important implications. Above all, it can help explain how the tremendous heterogeneity associated with the PCa can be generated. The rare PCa SC that persist in a tumor will continue to generate a repertoire of progenitor cells that in turn will develop into a spectrum of cells at different stages of differentiation , thus engendering the heterogeneous phenotype of the tumor. The model posits that the tumorigenic stem/progenitor cells are mostly undifferentiated cells as supported by the observations that most CD44 and CD133 cells are AR. The model also implies that most differentiated, luminal-like cells, which constitute the bulk of the tumor, might be much less or even non-tumorigenic (Figure 6A). In support, prospectively purified CD57 cells are non-clonogenic and non-invasive [44] and prospectively purified PSA$_b$ cells are less tumorigenic than the isogenic PSA_ cells.

They also note the positive and negative PSA in this paper.

4 CELLS OF ORIGIN

There is a great deal of concern as regards to where the stem cells come from. Namely the issue of the cells of origin. Previously we had reviewed the Goldstein model, where they had indicate a basal stem source as compared to a luminal cell source.

Wang and Shen state:

The identification of normal cells that can serve as a cell of origin for prostate cancer is highly relevant for understanding the applicability of a CSC model, and is currently under intense investigation. The cell of origin may also have clinical significance, as in the case of breast cancer, distinct tumor subtypes have been proposed to originate through transformation of different progenitors within the mammary epithelial lineage hierarchy. Thus, it is conceivable that there may be distinct cells of origin for other epithelial cancers, and different cells of origin may give rise to clinically relevant subtypes that differ in their prognosis and treatment outcome.

They consider several sources. For basal cells they state:

Although prostate tumors display a strongly luminal phenotype, this does not exclude the possibility that basal cells could be a cell of origin for prostate cancer. In particular, it is possible that transformed basal cells could differentiate to generate large numbers of luminal cancer cells. For example, prostate-specific conditional deletion of Pten by a probasin-Cre driver allele has been shown to result in a basal cell expansion accompanied by increased number of intermediate cells, suggesting a basal cell of origin … An important recent study from the Witte laboratory has used similar approaches with primary human prostate tissues to show that basal cells are a cell of origin for human prostate cancer

The Witte lab results are those of Goldstein et al which we have discussed at length (See Appendix A).

In contrast we have luminal cell origin as stated as follows:

Other studies have provided evidence that luminal cells can serve as cells of origin for prostate cancer. For example, pathological analysis of high-grade PIN samples, which still retain basal cells, suggest that molecular events associated with human prostate cancer initiation such as upregulation of c-MYC and shortening of telomere length occur exclusively in luminal cells but not their basal neighbors …

In Moscatelli and Wilson, the authors state:

There is nothing inherently contradictory in the results described by Wang et al. and Goldstein et al., because it is possible that both basal and luminal stem/progenitor cells may independently serve as cells of origin for prostate cancer.

Indeed, it is also possible that oncogenic stimuli may differ in their effectiveness in transforming distinct cell populations. The tumors that arise from different target cells may also vary in their biological behavior and genetic profiles.

There are also indications that normal prostate stem cells may reside in both the basal and the luminal compartments. Thus, if stem cells are preferentially targeted during malignant transformation, both compartments may contain cells of origin for prostate cancer.

Most of the scientific evidence indicates that prostate stem cells reside in the basal layer and give rise to the secretory luminal cells via transit-amplifying cells, which are intermediate in phenotype between stem cells and terminally differentiated cells.

There is definitive evidence that

(i) secretory cells of the adult murine prostate derive from cells that express p63, a transcription factor that is expressed by all basal cells in the prostate , and

(ii) p63- expressing basal cells are required for prostate development. In addition, prostate basal cells (human and murine) have greater proliferative activity in vitro and in vivo than luminal cells.

The molecular signature of prostate stem cells also identifies a basal-like phenotype, as they express cytokeratins 5/14, p63, and integrin ⍺6 (11). There is also evidence, however, that the luminal compartment may contain stem/progenitor cells and that these give rise to basal cells.

Experiments involving labeling cells with the synthetic nucleoside bromo-deoxy-uridine to detect those that are proliferating indicate that slow-cycling stem cells are concentrated in the proximal region of prostatic ducts adjacent to the urethra and that both basal and luminal compartments contain slow-cycling cells. Cells from this region have substantial growth potential in vivo and in vitro and can be serially passaged in vivo at least four times. It is not known whether CARNs are concentrated in the proximal region, but if so, CARNs may comprise some of the slow-cycling proximal luminal cell population.

These results provide a possible means to address the CSC signature issue. However, it is not clear that the result is definitive nor of immediate clinical use.

5 CONCLUSIONS

Stem cells are known in hematopoietic cell generation. They are isolated, separate and their ability to develop the full plethora of blood cells is well known. The stem cell concept applied to say prostate cells or skin cells is of more recent structure and is in many ways still open for debate. Taking that construct one step further and considering a cancer stem cell is possible even more of a conjecture. We can accept the concept of a cancer stem cell in the many blood cancers. We know that CML may very well have a translocation, as is found in other leukemias. Yet the establishment of the same for say prostate and melanoma malignancies is I believe still a work in progress.

For example as Jeet et al state:

Different stages of prostate cancer progression: (a) prostatic intraepithelial neoplasia, a premalignant lesion considered to be a precursor to invasive carcinoma; (b) primary localized adenocarcinoma, dependent on androgen stimulus and can be treated by androgen ablation; (c) androgen-independent prostate cancer, tumor then becomes androgen independent and metastasizes to other organs (e.g., lung, bone, and lymph node)

The linear progression we have disputed in prior writings based upon clinical observations. The reason is that we have observed the remission of diffuse HGPIN in patients at first biopsy and then the absence in subsequent. Not just reduction of HGPIN, but total elimination. Our hypothesis is that there has been the presence of a stem cell and its removal during the first extensive core biopsy, usually 16 or more cores, not classic sextant biopsy.

Stem cells are a powerful paradigm which may very well align with the clonal model. For if it is the stem cell which has suffered the genetic change then if this cell has the controlling powers attributed to it, then the stem cell model will also tell us a great deal regarding treatment, and our inability to do so.

For example, a stem cell will itself generate other stem cells as well as non-stem cells.

There are many questions still posed regarding the cancer stem cell:

1. What are the pathway dynamics and are they the same in the non-stem like cells?

2. What is the driver for the kinetics of a CSC? Namely do we have a dramatically different set of kinetics?

3. What is the mechanism for the progression of subsequent mutations in a CSC?

4. How do we identify the CSC in a sample biopsy? Are there specific cell markers and are they consistent or do they change?

5. What are the driving ligands which activate a CSC?

6. Do stem cells have true pluripotency or are they cell specific?

7. What are the stem cell surface ligands and receptors which promote mitosis and how are they transmitted across a group of cells?

8. What causes a stem cell, specifically a CSC, to evolve and how does that occur?

We can continue with a great number of these types of questions. However if one hopes to be able to model cancer pathway dynamics one must first address the issue of the CSC, for if the CSC has the definitive characteristics that we have discussed then it and it alone is what should be focused upon. Furthermore the examination of cells for pathway markers may very well have to be done only on the CSC, which then argues that we need sophisticated techniques to identify them and extract them as well.

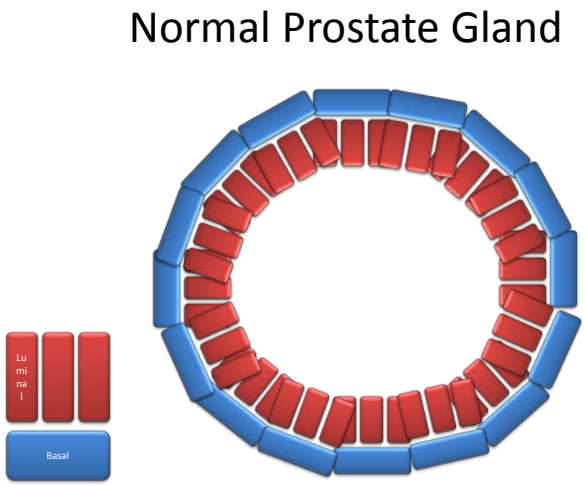
6 APPENDIX A: THE GOLDSTEIN MODEL

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

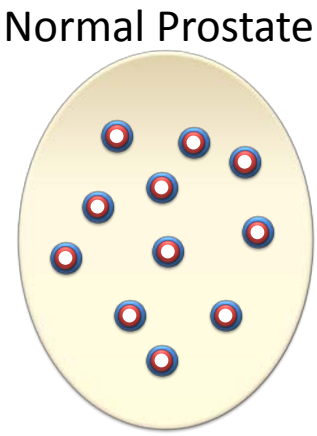
Simply what they did was to take two types of prostate cells, the basal and the luminal, tag them with surface tags, inject them into a mouse, and saw that only the basal cells grew, then they added two genes encoding for putative cancer pathways, and they saw that the basal cells grew to basal and luminal,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

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

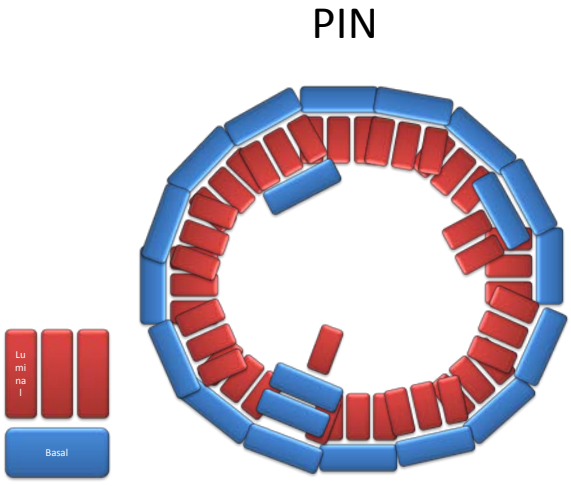
1. First the prostate has cell collections which act as glands with basal cells at the base and luminal cells on top. The luminal cells secret to the gland, the luminal space. This we show below.



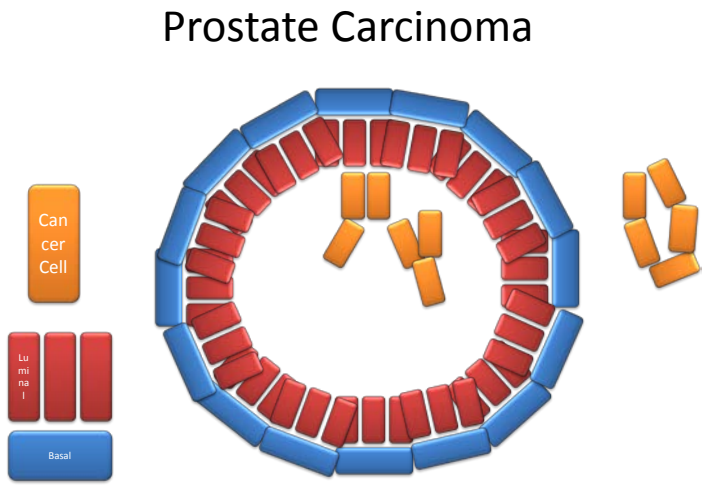
2. The normal prostate looks like what we show below, about 35-50 of these glands, and then surrounding material of muscle, blood supply, nerves, and lymphatics. The glands stand apart and they secret fluids into the lumen, the open parts of the gland. In between is the stroma composed of nerves, blood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



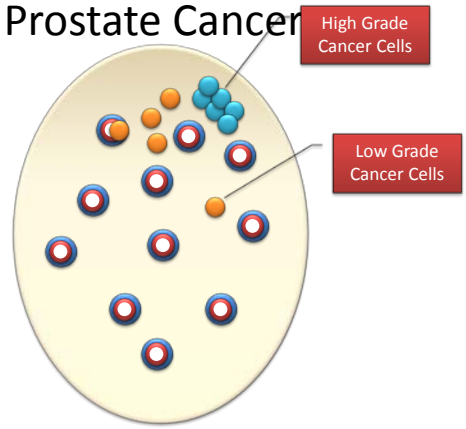
3. Now sometimes we see PIN, prostatic intraepithelial neoplasia, which is a growth of normal cells but not where they are to be. We may see the basal cells growing outwards and even some more luminal cells as well. The sign may be an increase in PSA since we have more luminal cells but the percent free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



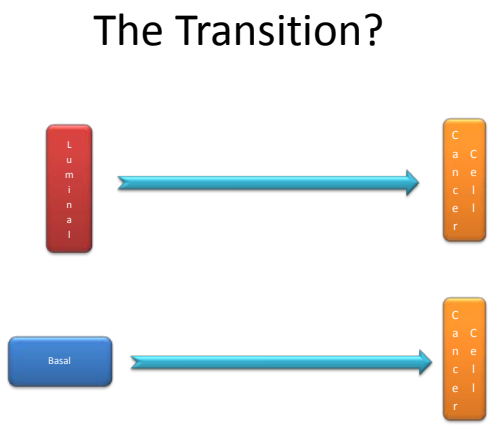
4. Then we may get prostate cancer, PCa, where the luminal cells types start to appear and grow without bound. The question is, where did these cells come from, other luminal cells or basal cells, or what. This is the question that the authors addressed with this elegant experiment. There is also the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



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

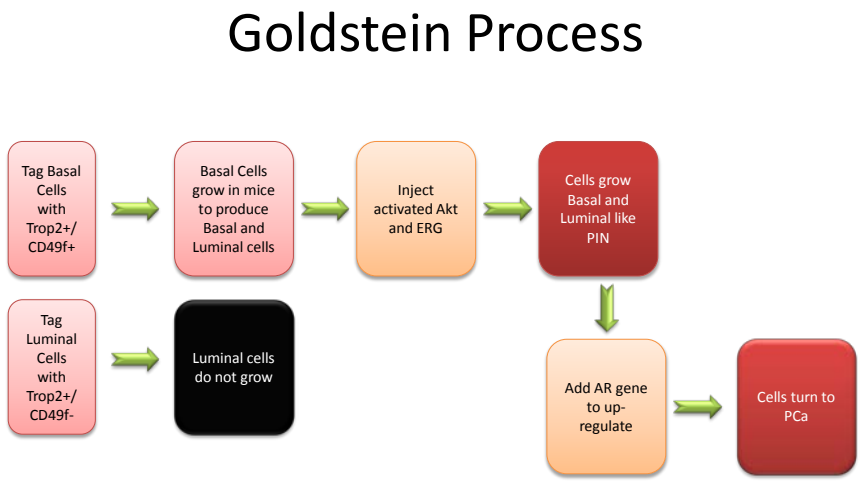


5. Thus the question posed by the authors was the one which asks from what cell does cancer begin. Their answer suggests the basal cell.



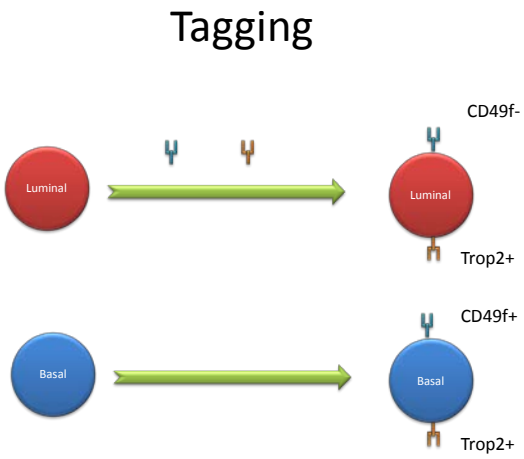
6. Pathways have been studied for PCa extensively and we shall discuss them in some detail.

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



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

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



As to the two initial genes we have:

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

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

This study still leaves several open questions:

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

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

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

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

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

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

7 APPENDIX B: THE CELL CYCLE: A BEGINNING FOR PATHWAYS

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.

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

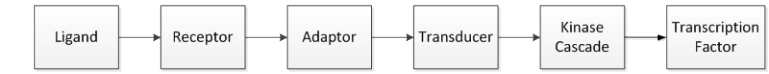
with the Transduce this kinase chain is controlled by redundant checks but if they become defective then the chain internal controls can be lost and the result become unstable.

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.

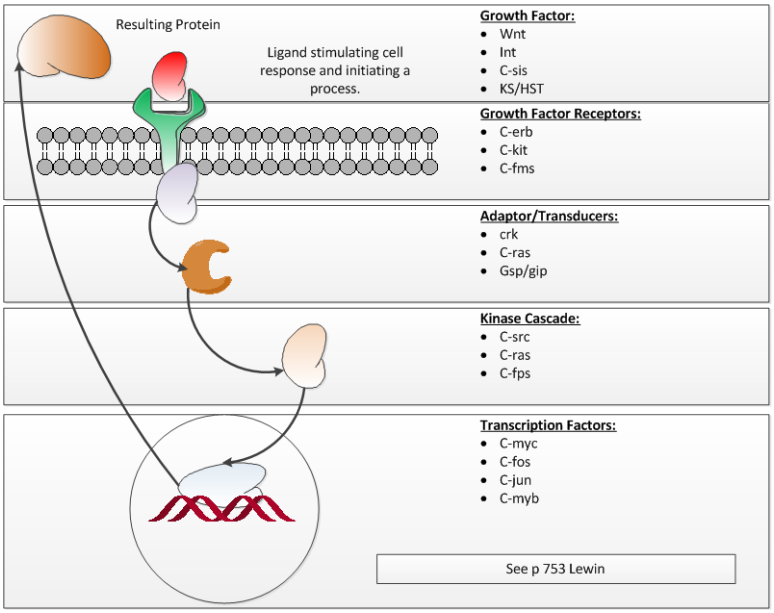
Note that this is a complex process.

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 cell replication.

7.1 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.

7.1.1 Cell Cycle

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.

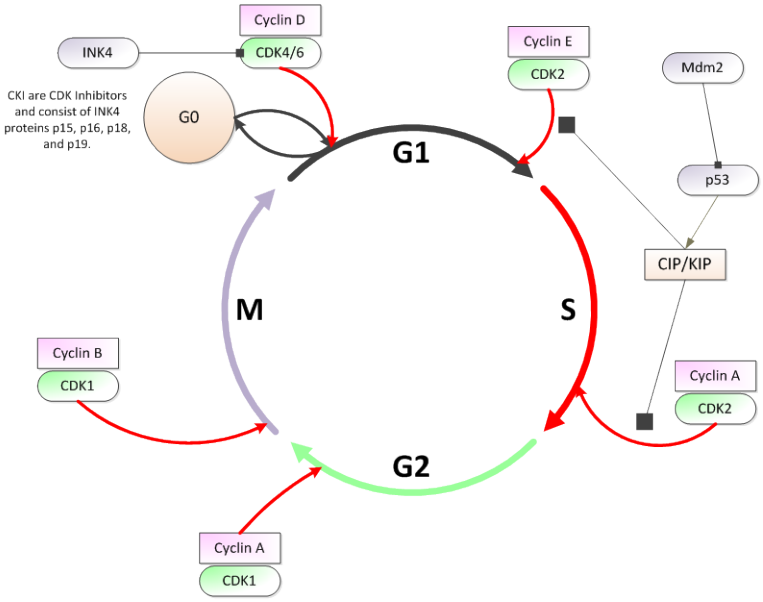
G1: Once the cell begins the G1 phase it is on its way to reproducing via mitosis.

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.

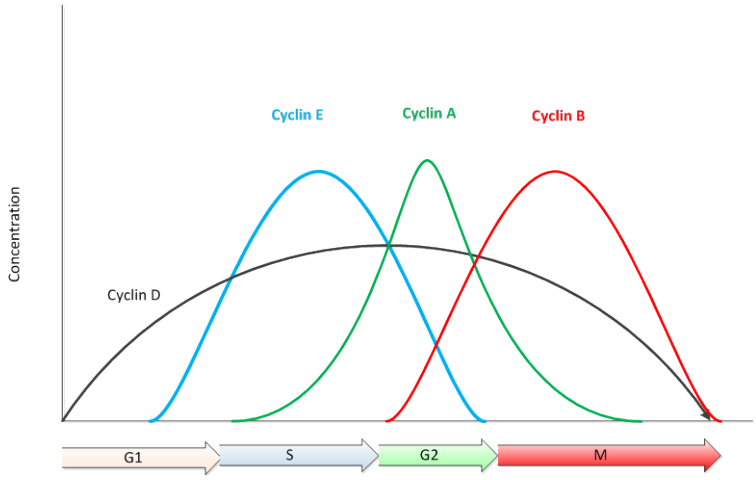
M: M phase includes mitosis and cytokinesis, namely the creation of two identical new cells.

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 is detailed in Morgan (Chapter 3).

Protein[8]	*Gene*	*Function*[9]
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.
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, are observed frequently in a variety of tumors and may contribute to tumorigenesis.

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

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

Protein[8]	***Gene***	***Function***[9]
Cyclin E (CCNE1)[10]	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:

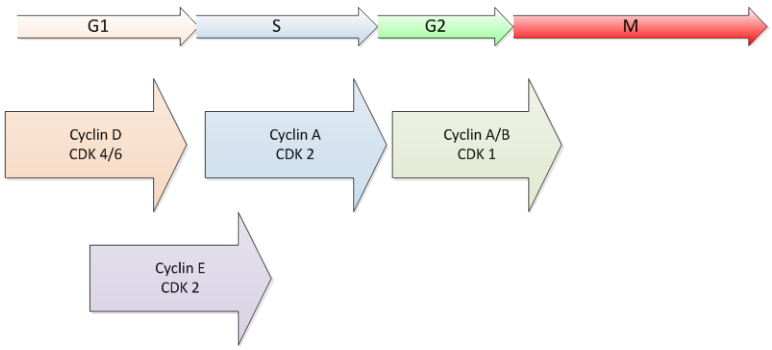
[10] http://www.ncbi.nlm.nih.gov/gene/898

Protein[11]	***Gene***	***Function***[12]
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 kinase 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.

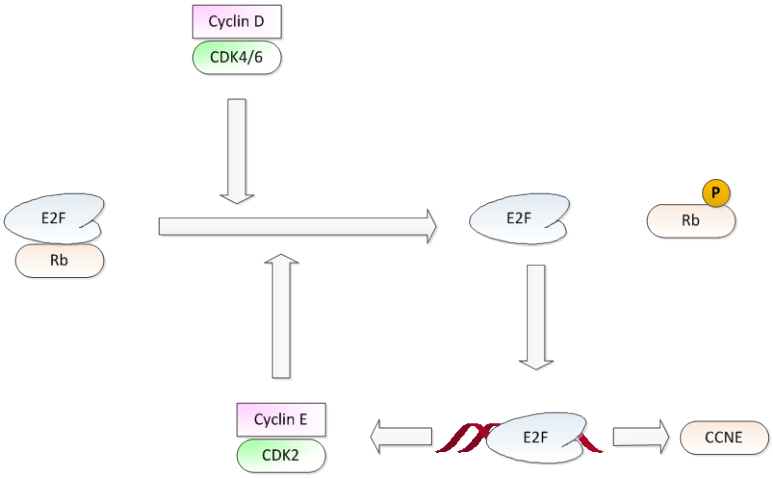
[11] http://www.ncbi.nlm.nih.gov/gene/983

[12] 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.

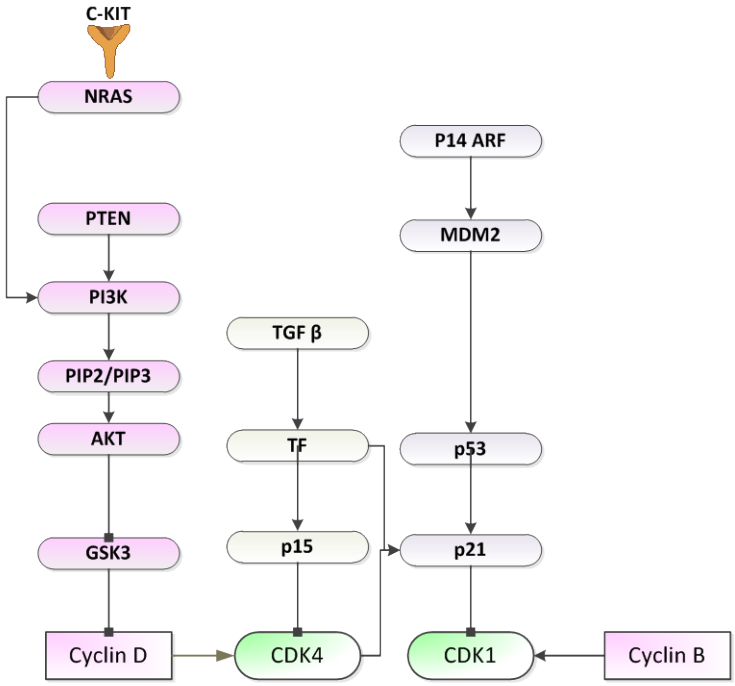
Gene	*Location*	*Function*
E2F1[13] (also RBP3; E2F-1; RBAP1; RBBP3)	20q11.2	The protein encoded by this gene is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle and action of tumor suppressor proteins and is also a target of the transforming 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[14] (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[15]	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.

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

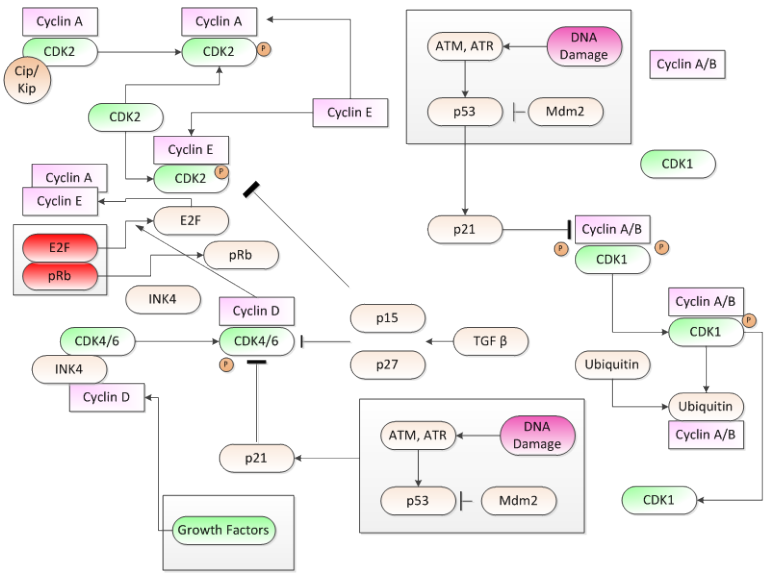
[14] http://www.ncbi.nlm.nih.gov/gene/5925

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

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:



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[16] (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[17] (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.

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

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

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p18[18]	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[19]	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.

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

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

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
Cip-Kip Family	p21[20] 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[21] 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.

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

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

CKI Family	*Member Name*	*Alternative Name*	*Gene*	*Function*
	p57[22] 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.

5. Substrates:

6. Checkpoint Proteins:

7.1.2 Cell Cycle Control

The following depicts cell cycle control:

Gene	*Location*	*Function*
Jun[23]	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[24]	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.

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

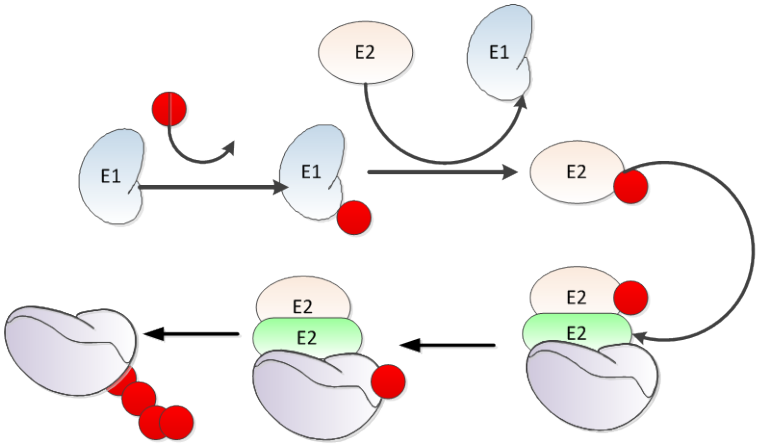
[23] http://www.ncbi.nlm.nih.gov/gene/3725

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

Gene	*Location*	*Function*
Myc[25]	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

7.2 UBIQUINATION

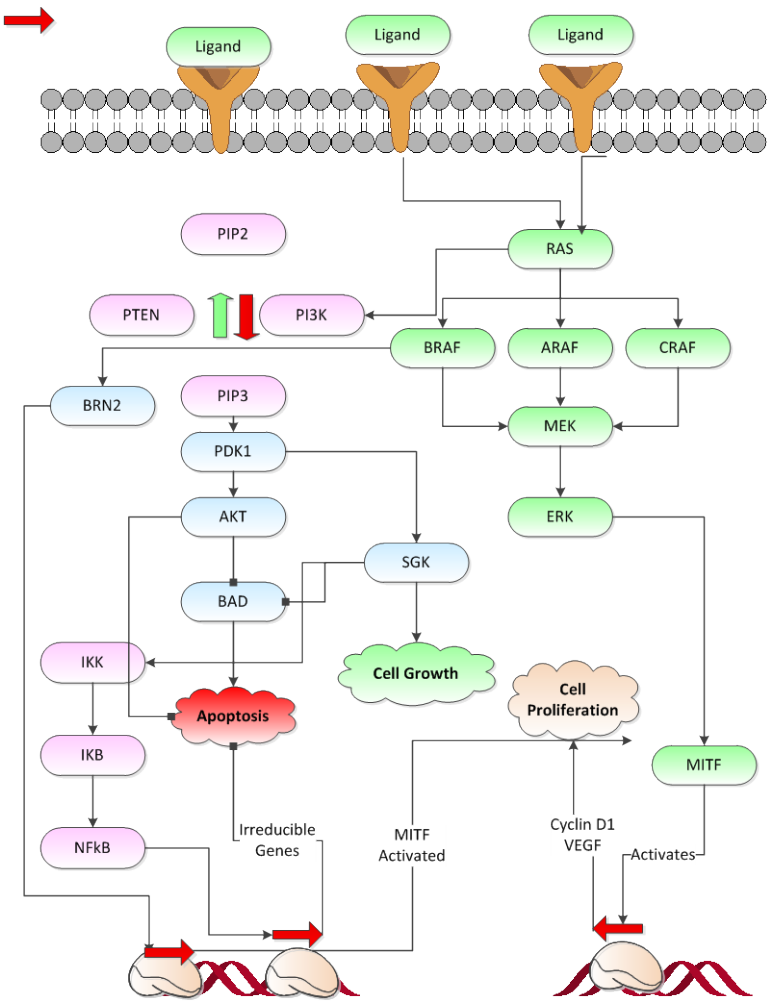
Ubiquitin is a small protein which acts with three related proteins; E1, E2, and E3. E1 is also called the ubiquitin activating enzyme, E2 the ubiquitin conjugating enzyme, and E3 ubiquitin ligase. Together they act to attach ubiquitin to a target protein and mark it for digestion and elimination. The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



7.3 CELL CYCLE

The following is an example of the prostate cell cycle.

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



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.

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