

# CANCER STEM CELLS AND CANCER CELL OF ORIGIN REDUX

There is an ongoing debate regarding the Cancer Cell of Origin and the Cancer Stem Cell in PCa. Recent work from the Tang Lab at MD Anderson has proposed a basal cell whereas work from Columbia posits the luminal cell. We examine these approaches and detail some of our approach to this issue. Copyright 2016 Terrence P. McGarty, all rights reserved.

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## 1 INTRODUCTION

Cancer Stem Cells (“CSC”) and the related cells of origin, and similar cells have been examined in details by many researchers. Prostate Cancer, PCa, provides a unique target for examining these cells and at the same time has provided a fertile ground for disputes. Thus there is a strong disagreement as to whether the basal or luminal cell is the cell of origin and therein the CSC issue also arises. In a recent paper from the Tang Lab at MD Anderson they have present conclusions supporting a basal origin. In contrast Shen at Columbia has focused on luminal cells. In this note we attempt to bring an update to what we wrote in 2012 and provide some basis for comparing the various claims<sup>1</sup>. Fundamentally, we take no position in this debate<sup>2</sup>.

Zhang et al state:

*The prostate gland mainly contains basal and luminal cells constructed as a pseudostratified epithelium. Annotation of prostate epithelial transcriptomes provides a foundation for discoveries that can impact disease understanding and treatment. Here we describe a genome-wide transcriptome analysis of human benign prostatic basal and luminal epithelial populations using deep RNA sequencing. Through molecular and biological characterizations, we show that the differential gene-expression profiles account for their distinct functional properties. Strikingly, basal cells preferentially express gene categories associated with stem cells, neurogenesis and ribosomal RNA (rRNA) biogenesis.*

*Consistent with this profile, basal cells functionally exhibit intrinsic stem-like and neurogenic properties with enhanced rRNA transcription activity. Of clinical relevance, the basal cell gene-expression profile is enriched in advanced, anaplastic, castration-resistant and metastatic prostate cancers. Therefore, we link the cell-type-specific gene signatures to aggressive subtypes of prostate cancer and identify gene signatures associated with adverse clinical features.*

This is an argument for the basal cell being the origin of the CSC. They continue:

*The current study has made the following significant findings.*

*First, our study uncovers unique SC- and EMT-enriched gene-expression profile in unperturbed basal cells that support the long-held hypothesis that the human prostate basal cell layer harbors primitive SCs.*

*Second, we report the surprising finding that basal cells are enriched in genes normally associated with neurogenesis. In contrast, luminal cells preferentially express proneural genes*

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<sup>1</sup> See Telmarc White Paper 85, Stem Cells, <http://www.telmarc.com/Documents/White%20Papers/85%20Prostate%20Stem%20Cells.pdf> and [https://www.researchgate.net/publication/301222986\\_Prostate\\_Cancer\\_Stem\\_Cells?ev=prf\\_pub](https://www.researchgate.net/publication/301222986_Prostate_Cancer_Stem_Cells?ev=prf_pub)

<sup>2</sup> The reader is referenced to the White Papers referenced in this documents for details on specific topics. Also see Prostate Cancer: A Systems Approach by the author.

*involved in neural signal response and processing. Consistently, primary basal cells can spontaneously or be induced to undergo 'neural' development in vitro, generating NSC-like cells. Combined with the SC features, these transcriptional programs provide a molecular understanding for the reported basal cell plasticity.*

*Third, basal cells express high levels of Pol I-associated rRNA biogenesis genes regulated, at least in part, by the MYC transcriptional programme. MYC is often found overexpressed in PCa, especially metastatic PCa. Increased transcription of rRNA genes by Pol I is a common feature of human cancer. Thus, our data may suggest a rationale for treating anaplastic PCa and CRPC with Pol I inhibition, as well as targeting MYC and the MYC-mediated transcriptional programme as a therapy for PCa.*

*Fourth, our deep RNA-Seq data provide a rich resource for epithelial lineage specific genes and markers in the human prostate.*

*Fifth, distinct transcriptomes in basal and luminal cells also suggest cross communications between the two epithelial cell types, as well as between the epithelial compartment and the underlying stroma. Understanding such crosstalk will be instrumental for understanding the normal development and tumorigenesis of prostate. Although many of the signaling pathways mentioned in this study are poorly investigated in normal prostate epithelial biology, their functional involvement in PCa development and progression has been widely documented.*

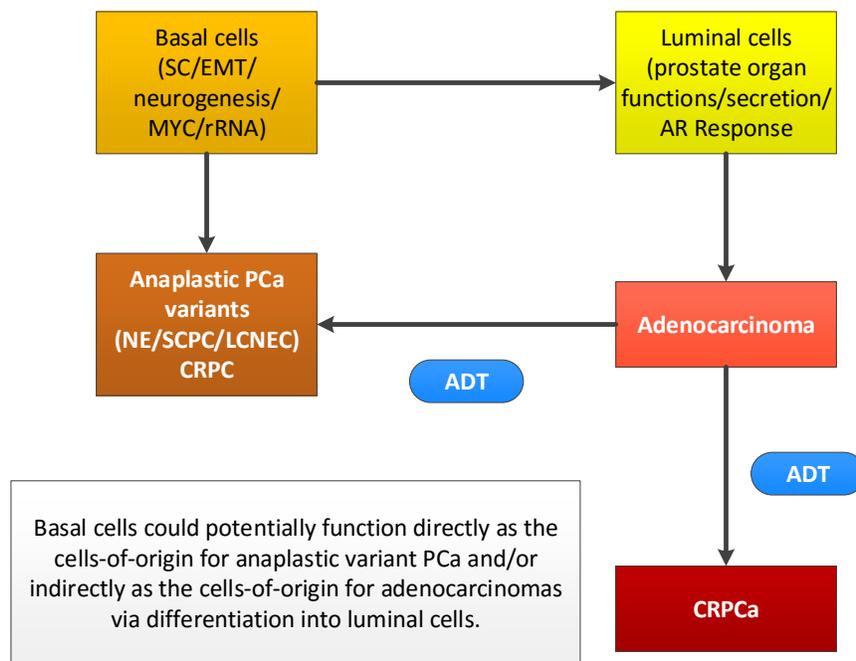
*Last, the basal cell gene-expression profile is linked to adverse clinical features of PCa, indicating a 'biomarker' value of basal cell gene signature for aggressive PCa. Importantly, the molecular resemblance of basal cells to anaplastic PCa and CRPC provides a common molecular understanding of these diverse and poorly characterized aggressive PCa subtypes and implicates basal cells as the cell-of-origin for these variant PCa.*

We present the summary of the Tang Lab model. The driver is a basal cell and the luminal cells seem to act if and only if driven by a basal cell process. Furthermore, the neuroendocrine case is shown as a direct and indirect result of the basal driver. We have recently discussed the neuroendocrine prostate cancers in our discussions of pro-NPY<sup>3</sup>.

The Figure below is modified from the Wang et al paper and summarizes their concept.

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<sup>3</sup> See [https://www.researchgate.net/publication/292978295\\_pro-NPY\\_PCa\\_and\\_Neuroendocrine\\_Tumors](https://www.researchgate.net/publication/292978295_pro-NPY_PCa_and_Neuroendocrine_Tumors)



The above indicates the origin is from basal and then to luminal or the neuroendocrine cells, the latter being substantially less common.

Now the CSC and CCO debate, especially that related to PCa can be viewed in almost classical terms. In the 14<sup>th</sup> century as Medical Schools at Montpellier, Bologna, Paris and Oxford studied Galen and other then classic medical texts, the use of logic was compelling and demanded. The Trivium, Grammar, Logic and Rhetoric, was required of any student studying the field. This was because studying Galen demanded logic. Processes that were diagnostic or prognostic demanded logical consistency more than phenomenological verification. Strangely in the case of the CSC perhaps logical consistency is *pari passu* with that phenomenon. One of the major problems is defining the terms in such a manner that they can be consistently phenomenologically compared.

## 2 DEFINITIONS

As we have indicated, one of the more difficult issues when discussing CSCs is the definition. Phenomenological observations have been reduced to definitions and in turn the definitions have been used to search for CSCs. This can be a bit circular at times and may very well be one of the sources of confusion. Let us begin with the paper by Jordan et al from a decade ago in NEJM:

*Many studies performed over the past 30 to 40 years, when viewed collectively, have shown that the characteristics of stem-cell systems, the specific stem-cell properties described above, or both, are relevant to some forms of human cancer. Biologically distinct and relatively rare populations of “tumor-initiating” cells have been identified in cancers of the hematopoietic system, brain, and breast.*

*Cells of this type have the capacity for self-renewal, the potential to develop into any cell in the overall tumor population, and the proliferative ability to drive continued expansion of the population of malignant cells. Accordingly, the properties of tumor-initiating cells closely parallel the three features that define normal stem cells. Malignant cells with these functional properties have been termed “cancer stem cells”.*

This frankly is a cumbersome definition. They describe stem cells; a necessary part of the definition as follows:

*Stem cells occur in many different somatic tissues and are important participants in their physiology. Populations of cells that derive from stem cells are organized in a hierarchical fashion, with the stem cell residing at the apex of the developmental pathway. Stem cells have three distinctive properties: self-renewal (i.e., at cell division, one or both daughter cells retain the same biologic properties as the parent cell), the capability to develop into multiple lineages, and the potential to proliferate extensively. The combination of these three properties makes stem cells unique. The attribute of self-renewal is especially notable, because its subversion is highly relevant to oncogenesis and malignancy. Aberrantly increased self-renewal, in combination with the intrinsic growth potential of stem cells, may account for much of what is considered a malignant phenotype.*

Thus we could ask; do all organs have stem cells which are organ specific? We know the skin continually reproduces cells, specifically keratinocytes. Colon cancer has a stem cell element<sup>4</sup>.

### 2.1 DESCRIPTIVE

In the paper by Navin and Hicks they present a taxonomy of possible cancer cell propagation. It is worth examining this before driving towards a definition. The facts will ultimately determine the definition. They present the following five categories:

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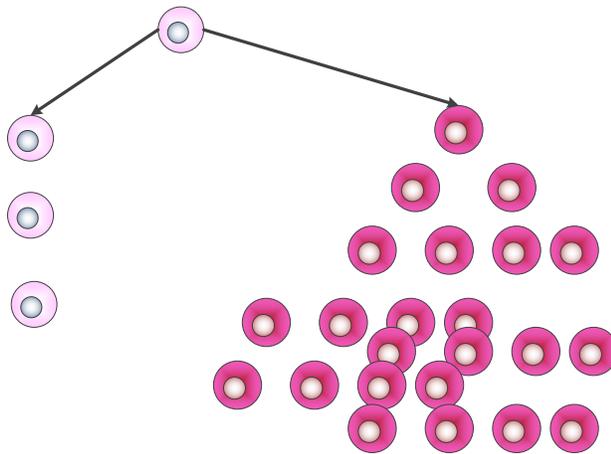
<sup>4</sup> See Rajasekhar p 274.

1. Clonal: This case is a single cell gets a malignant character and then proceeds with uncontrolled growth. Thus one would expect that each cell in the tumor would reflect this by having genetic homogeneity across any tumor sample. We know that this is clearly not the case.
2. Polyclonal: This is the first case but with a twist. Namely there are several clones clustering together. Thus across any section of the tumor there would be different clones but the clones would be locally consistent. Again this is not the case.
3. Polyclonal via Self Seeding: This is polyclonal but now the different clones appear across the blood stream as separate but homogeneous entities. Again we know that even as hematopoietic spread occurs there is non uniform genetic types.
4. Multiple Mutator: This type is total genetic diversity. Here we go from one genotype to another across almost every cell. Thus the genetic diversity is a maximum complexity. Again we know that this is not the case.
5. Cancer Stem Cell: This is the hypothesis of a cancer stem cell. Namely a single genotype both self-replicates and also generates a malignant progeny. The progeny cells are basically the same and they can replicate but do not drive the malignancy. Somehow if one were to remove the CSC the other cells would stop. This may be what happens in certain types of HGPIN as we have speculated based upon HGPIN saturation biopsies resulting in return to full benign state.

Now there is a sixth case not discussed by Navin and Hicks which we have proposed and analyzed using limited data. This is the Markov chain model wherein cells have a genotype but the genotype is subject to random changes at random instants, albeit changes driven by location and complexity of the tumor state. We refer the reader to the results elsewhere.

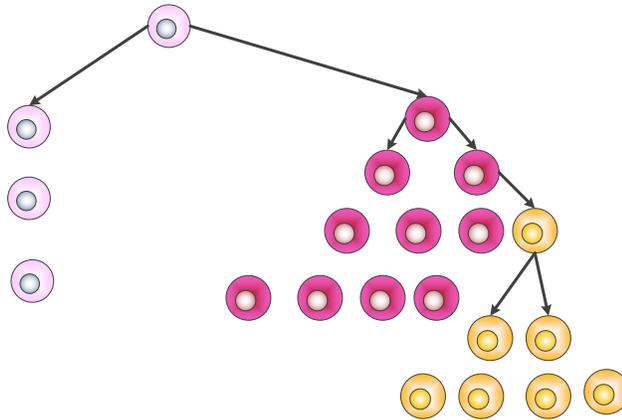
We now demonstrate graphically each of the five cases below:

Case 1: The Clonal Model shows a single mutation and then all subsequent cells are just clones. We have examined this in the case of MDS, Myelodysplastic Syndrome. We know that the malignancy most related to this is AML. We also know that AML appears clonal like but MDS is not the same in all cases. Some have proliferation in red cells while others in platelets. Others may be neutrophil driven. Thus the CSC if there is one is not at the cell base of a core stem cell but at a level above that. Then when AML occurs one may see the CSC moving downward.



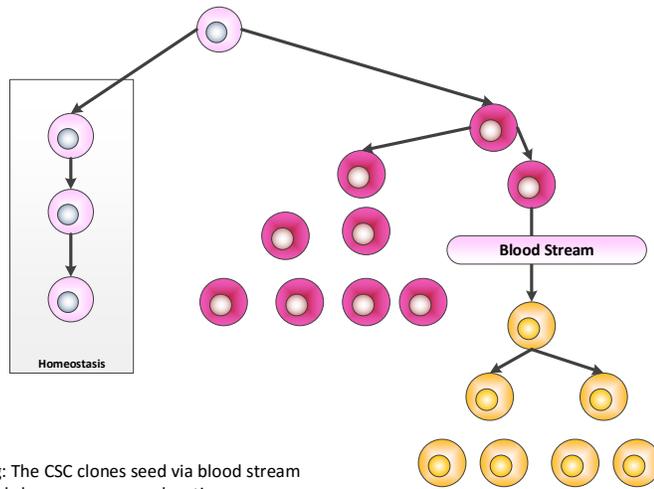
Monoclonal Evolution: Assumes a single malignant clone which continues to replicate

Case 2: Polyclonal. The second model below shows a multiple set of clones. The assumption is that cells continue to mutate but that one an active mutation occurs then the now clone takes off. One would expect to see clusters of common clones.



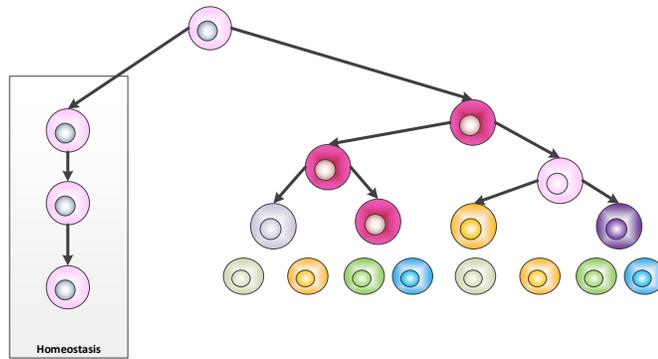
Polyclonal Evolution: Assumes a multiple malignant clones which continues to replicate

Case 3: Self Seeding. The model below is what they call the self-seeding. This is a polyclonal variant where the clone can change as it moves throughout the body.



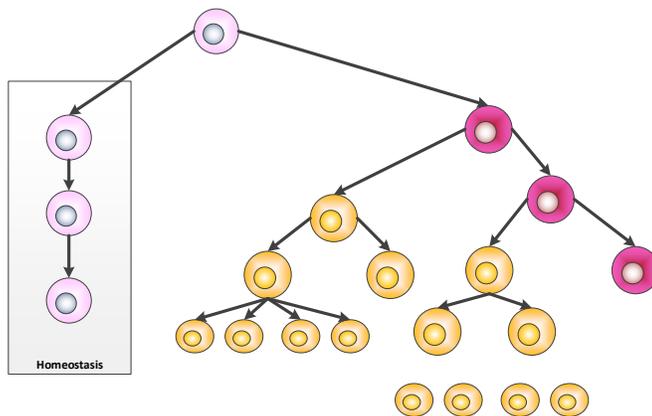
Self Seeding: The CSC clones seed via blood stream and change as per new location.

Case 4: This is the Mutator model wherein cells keep changing is shown below. The end result is a tumor with almost no genomic consistency.



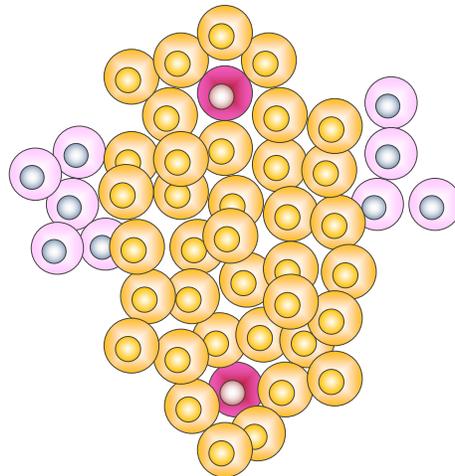
Mutator Phenotype: Generates many variants.

Case 5: This is the case of the CSC. Namely the one cell that starts everything off and keeps it going. We show that below.



Cancer Stem Cell: A single cell self replicates and also produces malignant but non-driving cells.

Thus if we accept the Navin and Hicks description of the CSC we would expect to see a tumor as shown below. Normal cells at a periphery in a normal homeostatic state, then a large collection of non-stem malignant cells (namely cells which can and do proliferate), and a few CSC which somehow drive the process. One could assume the CSCs drive the proliferation of the non-CSC malignant cells. However, that is open for debate. Furthermore, however, if we were to take the CSC away then in most CSC theories the other malignant cells would undergo apoptosis or some form of cell death.



## 2.2 PHENOMENOLOGICAL

The previous section used a purely logical descriptive approach for CSC classification. As we noted it did have deficiencies that we have explored elsewhere. However, it does not tell us what a CSC is. There are phenomenological

*As it is not experimentally feasible to investigate the potential existence of CSCs in human tumors solely on the basis of these theoretical definitions, CSCs are instead defined in practical terms through the use of several functional assays. The most frequently used methodology involves xenotransplantation of flowsorted 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. Using this assay, early studies identified CSC populations in hematological malignancies, such as the CD34+CD38- population in acute myeloid leukemia.*

*Similar approaches were subsequently applied to solid tumors, leading to the identification of candidate CSC populations that were prospectively enriched using specific markers in breast (CD44+CD24-Lin-), brain (CD133+) and colon cancers (CD133+). Overall, however, the available evidence supporting the identification of CSCs in solid tumors has been less convincing, at least in part because solid tumor cells exist in a complex microenvironment that is not readily modeled by xenotransplantation.*

Wang and Shen then continue to discuss the issue of definitions:

*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.*

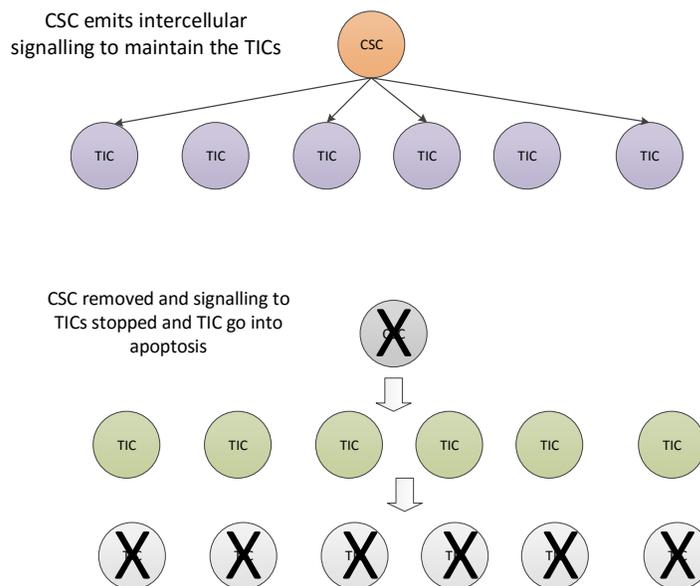
*A similar confusion arises with respect to the cell of origin for cancer, which corresponds to a normal tissue cell that is the target for the initiating events of tumorigenesis. In principle, a normal adult stem cell could be a logical cell of origin for cancer, as it would retain the ability to self-renew 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. For example, both hematopoietic stem cells as well as committed progenitor cells can initiate leukemia after transformation. More recently, activation of canonical Wnt signaling has been shown to transform mouse intestinal stem cells to give rise to adenocarcinomas.*

Thus we have as a start three concepts:

1. Cancer Stem Cell: Also the CSC. This is the self-renewing cell from which the TIC cells arise and which provides the necessary signaling to the TICs to continue their proliferation. Transplanting a CSC will cause a tumor to grow.
2. Tumor Initiating Cell (TIC)<sup>5</sup>: These are cells which are the proliferative cells. They are not the CSC. Transplanting a TIC will result in no growth of a tumor unless accompanied by a CSC.
3. Cell of Origin: Also the Cancer Cell of Origin, CCO. This is the cell from which the CSC was derived. Thus the debate in PCa is often the question; basal or luminal?

This collection leads to a model as shown below:



## 2.3 DEFINITIONS AGAIN

There is a debate about the existence of CSCs for all cancers as well as how one identifies the CSC if indeed it exists for cancers of specific type. We examine this issue again since in much of the literature there are a multiplicity of definitions.

From Nature we have the following definition<sup>6</sup>:

<sup>5</sup> Weinberg pp 460-463 discusses the CSC and what he terms the Transit Amplifying/Progenitor Cells.

<sup>6</sup> See: <http://www.nature.com/nature/focus/cancerstemcells/>

*Cancer stem cells are defined as those cells within a tumour that can self-renew and drive tumorigenesis. Rare cancer stem cells have been isolated from a number of human tumours, including hematopoietic, brain, colon and breast cancers. The cancer stem-cell concept has important implications for cancer therapy. However, the generality of the cancer stem-cell hypothesis has also been challenged...*

In a similar manner we have the Tumor initiating cell and its relationship to the CSC and the CCO.

From Agarwal et al we have for TIC:

*Tumor-initiating cells (TICs), defined by clonal tumor initiation from transplanted cells, have not been analyzed in primary prostate cancers, partly due to the poor transplantation ability of single-cell suspensions of human prostate cancers and low-grade mouse tumors. This may be due to the fragility of fractionated prostate tumor cells, to a high percentage of indolent cells in primary tumors, to a strict requirement for the proper microenvironment, or to other unknown reasons.*

Definitions are important. In mathematics and law, the definition will determine the outcome. In engineering we define certain parameters and we design accordingly. If there is a concern that we spend a great deal of time on the definition, that concern should realize that defining something so that it is replicable is a key to scientific study. In cancer studies the term "cancer stem cell" has been introduced but it seems to have been used somewhat loosely.

Definitions should be clear and they should be actionable. Namely the definition should present a way to ascertain through objective measures readily understood by someone trained in the science or art to determine if what is presented satisfies the definition. Namely we should with a good definition know if what we have is a cancer stem cell.

The results below are a sample of what seems to be definitions from the literature. Reading these one can readily see what the complexity is in understanding this topic.

<i>Author</i>	<i>Definition</i>
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<i>Author</i>	<i>Definition</i>
Ailles, and Weissman	<i>Cancer stem cells (CSCs) are cells that drive tumorigenesis, as well as giving rise to a large population of differentiated progeny that make up the bulk of the tumor, but that lack tumorigenic potential. CSCs have been identified in a variety of human tumors, as assayed by their ability to initiate tumor growth in immunocompromised mice... In addition, specific signaling pathways play a functional role in CSC self-renewal and/or differentiation, and early studies indicate that CSCs are associated with a microenvironmental niche... several important biological properties of CSCs: first, what is the cell of origin for a given tumor? Second, what are the signaling pathways that drive self-renewal and/or differentiation of CSCs? Third, are there molecules uniquely expressed on CSCs, regardless of whether they are functional, that will allow targeted therapies to be developed? Fourth, what are the mechanisms by which CSCs escape conventional therapies and can we defeat these mechanisms?</i>
Badeux and Tang (in Rajasekhar)	<i>To fulfill the obligate criteria of a cancer, stem cell (CSC) a cell must be capable of both self-renewal and differentiation, of regenerating and of generating anew...The term cancer stem cell is often replaced by or used synonymously with the phrase tumor initiating cell (TIC).</i>
Burgess	<i>Should stem mitotic activity become unregulated or uncontrolled, a tumorigenic and perhaps malignant phenotype may result hence the term cancer stem cell...tumor initiating cells that have malignant properties have been referred to as CSCs...</i>

<i>Author</i>	<i>Definition</i>
Dalerba et al	<p data-bbox="448 268 1065 300"><i>Stem cells are defined by three main properties:</i></p> <ol data-bbox="448 342 1373 779" style="list-style-type: none"> <li data-bbox="448 342 1373 485"><i>1. differentiation—the ability to give rise to a heterogeneous progeny of cells, which progressively diversify and specialize according to a hierarchical process, constantly replenishing the tissue of short-lived, mature elements;</i></li> <li data-bbox="448 527 1373 632"><i>2. self-renewal—the ability to form new stem cells with identical, intact potential for proliferation, expansion, and differentiation, thus maintaining the stem cell pool;</i></li> <li data-bbox="448 674 1373 779"><i>3. homeostatic control—the ability to modulate and balance differentiation and self-renewal according to environmental stimuli and genetic constraints</i></li> </ol> <p data-bbox="448 821 1373 1031"><i>Like their normal tissue counterparts, tumors are composed of heterogeneous populations of cells that differ in their apparent state of differentiation. Indeed, the differentiation features of a tumor, morphological and architectural, are the key parameter used in routine clinical practice by surgical pathologists to define a tumor’s primary anatomical origin.</i></p> <p data-bbox="448 1073 1373 1440"><i>This simple observation suggests that tumors are not mere monoclonal expansions of cells but might actually be akin to “abnormal organs,” sustained by a diseased “cancer stem cell” (CSC) population, which is endowed with the ability to self-renew and undergo aberrant differentiation (1, 2). This hypothesis is further reinforced by the fact that cancer is known to result from the accumulation of multiple genetic mutations in a single target cell, sometimes over a period of many years (3). Because stem cells are the only long-lived cells in many tissues, they are the natural candidates in which early transforming mutations may accumulate.</i></p>

<i>Author</i>	<i>Definition</i>
Dubrovskaja, A., et al	<i>One possible explanation for the initial positive response to therapy followed by androgen-refractory disease is that although current therapies eliminate the bulk of the tumor, they fail to eliminate cancer stem cells (CSCs) or tumor-initiating cells (TICs). In fact, it has been argued that many cancers are maintained in a hierarchical organization of rare CSCs, rapidly dividing cells, and differentiated tumor cells; the CSCs are not only a renewable source of tumor cells but are also a source of tumor resistance leading to tumor recurrence, metastasis, and tumor progression. Support for this hypothesis came with the identification of TICs in leukemia in 1994 and, subsequently, in a variety of cancers, including solid tumors. In addition, cancer cell lines have been shown to harbor cancer stem-like cells and are a promising model for CSC research because these progenitors can be readily expanded under anchorage independent (sphere formation) serum-free conditions</i>
Fang et al,	<i>Recent studies suggest that cancer can arise from a cancer stem cell (CSC), a tumor-initiating cell that has properties similar to those of stem cells. CSCs have been identified in several malignancies, including those of blood, brain, and breast.</i>
Hurt et al	<i>The cancer stem cell hypothesis suggests the existence of a small subpopulation of cells within the tumour that give rise to differentiated tumour cells. It is thought that the cancer stem cells survive conventional treatment to later re-emerge more resistant to therapy. To date, putative cancer stem cells have been identified in blood, brain, breast, lung, skin, pancreas, colon, and prostate</i>

<i>Author</i>	<i>Definition</i>
Jordan et al	<p><i>Stem cells have three distinctive properties: self-renewal (i.e., at cell division, one or both daughter cells retain the same biologic properties as the parent cell), the capability to develop into multiple lineages, and the potential to proliferate extensively. The combination of these three properties makes stem cells unique. The attribute of self-renewal is especially notable, because its subversion is highly relevant to oncogenesis and malignancy. Aberrantly increased self-renewal, in combination with the intrinsic growth potential of stem cells, may account for much of what is considered a malignant phenotype. Biologically distinct and relatively rare populations of “tumor-initiating” cells have been identified in cancers of the hematopoietic system, brain, and breast. Cells of this type have the capacity for self-renewal, the potential to develop into any cell in the overall tumor population, and the proliferative ability to drive continued expansion of the population of malignant cells. Accordingly, the properties of tumor-initiating cells closely parallel the three features that define normal stem cells. Malignant cells with these functional properties have been termed “cancer stem cells”</i></p>
Lawson and Witte	<p><i>Two theories were proposed to explain this paradox. The stochastic theory suggested that all cancer cells are equally malignant but only clones that randomly possess favorable biological properties will grow upon transplantation. An alternative theory predicted that tumors are hierarchical like normal tissues and only the rare subpopulation of cells at the pinnacle of that hierarchy have the unique biological properties necessary for tumor initiation (8, 9). Studies by John Dick and colleagues provided evidence for the hierarchy model. This group demonstrated that only the small subpopulation (0.1%–1.0%) of Lin<sup>−</sup>CD34<sup>+</sup>CD38<sup>−</sup> cells within human acute myelogenous leukemia samples were capable of initiating disease when transplanted into immune-deficient mice (10). These cells possessed the same antigenic profile as normal human HSCs, which are at the pinnacle of the normal hematopoietic hierarchy. This population also had the unique capacity to self-renew to propagate the disease as well as differentiate to produce the many leukemic cell types represented in the original leukemia. Since these cancer cells possess properties unique to normal tissue stem cells, they have been termed “cancer stem cells” (CSCs).</i></p>
Lobo et al	<p><i>Stem cell: a primitive cell defined by its capacity to self-renew and differentiate into at least one mature cell type</i></p> <p><i>Cancer stem cell: a self-renewing cell within a tumor that has the capacity to regenerate the phenotypic diversity of the original tumor</i></p>

<i>Author</i>	<i>Definition</i>
NCI	<i>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.</i>
Pavlovic and Balint	<i>As the stem cells that created the tumor to begin with are so few in number, scans following treatment usually fail to identify populations of CSCs in this limited population....<sup>7</sup></i>
Perego et al	<i>Although there is no definitive consensus on the phenotype and frequency of CSCs in the majority of human tumors, much experimental evidence supports the contentions that many tumors of both epithelial and nonepithelial origin have operationally defined CSCs (cells able to propagate tumors in immunodeficient mice) and that the presence of these CSCs affects tumor biology.</i>
Rajasekhar	<i>The "cancer stem cell model" CSC ...envisions tumors as "pathological organs" sustained in their aberrant growth by a mutated population of stem cells, in which normal homeostatic controls on tissue expansion have been lost.</i>
Roesch et al	<i>The CSC concept postulates a unidirectional hierarchy of tumor cells...According to the traditional CSC concept, tumor initiation is regarded as an exclusive characteristic of CSCs</i>

<sup>7</sup> This book is near incomprehensible. It is impossible to find a definition, only secondary referral characteristics at best!

<i>Author</i>	<i>Definition</i>
Rosen and Jordan	<i>Thus, the CSC paradigm refers to the ability of a subpopulation of cancer cells to initiate tumorigenesis by undergoing self-renewal and -differentiation, like normal stem cells, whereas the remaining majority of the cells are more “differentiated” and lack these properties.</i>
Soltysova, et al	<p><i>Normal stem cells in the adult organism are responsible for tissue renewal and repair of aged or damaged tissue. A substantial characteristic of stem cells is their ability for self-renewal without loss of proliferation capacity with each cell division. The stem cells are immortal, and rather resistant to action of drugs. They are able to differentiate and form specific types of tissue due to the influence of microenvironmental and some other factors. Stem cells divide asymmetrically producing two daughter cells – one is a new stem cell and the second is progenitor cell, which has the ability for differentiation and proliferation, but not the capability for self-renewal.</i></p> <p><i>Cancer stem cells are in many aspects similar to the stem cells. It has been proven that tumor cells are heterogeneous comprising rare tumor initiating cells and abundant non-tumor initiating cells. Tumor initiating cells – cancer stem cells have the ability of self-renewal and proliferation, are resistant to drugs, and express typical markers of stem cells. It is not clear whether cancer stem cells originate from normal stem cells in consequence of genetic and epigenetic changes and/or by redifferentiation from somatic tumor cells to the stem-like cells. Probably both mechanisms are involved in the origin of cancer stem cells. Dysregulation of stem cell self-renewal is a likely requirement for the development of cancer. Isolation and identification of cancer stem cells in human tumors and in tumor cell lines has been successful.</i></p>

<b>Author</b>	<b>Definition</b>
Visvader	<p><i>It is important to note that the cell of origin, the normal cell that acquires the first cancer-promoting mutation(s), is not necessarily related to the cancer stem cell (CSC), the cellular subset within the tumour that uniquely sustains malignant growth. That is, the cell-of-origin and CSC concepts refer to cancer-initiating cells and cancer-propagating cells, respectively (Fig. 1). Although the tumour-initiating cell and the CSC have been used interchangeably, the tumour-initiating cell more aptly denotes the cell of origin. There is considerable evidence that several diverse cancers, both leukaemias and solid tumours, are hierarchically organized and sustained by a subpopulation of self-renewing cells that can generate the full repertoire of tumour cells (both tumorigenic and non-tumorigenic cells)<sup>1</sup>. The cell of origin, the nature of the mutations acquired, and/ or the differentiation potential of the cancer cells are likely to determine whether a cancer follows a CSC model. In most instances, the phenotype of the cell of origin may differ substantially from that of the CSC.</i></p> <p><i>Normal cellular hierarchy comprising stem cells that progressively generate common and more restricted progenitor cells, yielding all the mature cell types that constitute a particular tissue. Although the cell of origin for a particular tumour could be an early precursor cell such as a common progenitor, the accumulation of further epigenetic mutations by a cell within the aberrant population (in this case expanded) during neoplastic progression may result in the emergence of a CSC. In this model, only the CSCs (and not other tumour cells) are capable of sustaining tumorigenesis. Thus, the cell of origin, in which tumorigenesis is initiated, may be distinct from the CSC, which propagates the tumour.</i></p>
Wang and Shen	<p><i>In its strictest form, the CSC model posits a hierarchical organization of tumors, with cancer stem cells at the top of the lineage hierarchy being capable of indefinite self-renewal, unlike their progeny, which undergoes an epigenetic program of differentiation and loss of tumorigenicity. In this view, rare CSCs may represent the driving force of tumor malignancy, and therefore effective treatment could be achieved by specific targeting of the CSC population. In contrast, the stochastic (clonal) evolution model proposes that most of the cancer cells within a tumor are highly tumorigenic and possess different genetic or epigenetic properties. 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.</i></p>

<i>Author</i>	<i>Definition</i>
Weinberg p 462	<i>...the tumor initiating cell, often termed a cancer stem cell (CSC), is self-renewing and has the ability to generate the countless neoplastic progeny that constitute a tumor. While the CSC and its progeny are genetically identical, the progeny, because they have lost self-renewing ability, have also lost tumor initiating ability.</i>

It does not take an extensive reading to see the overlap of ideas. Ideas of function and action.

## 2.4 CONFUSIONS

There is often a set of confusion regarding which cell does what. As we have discussed above the CSC is the driving cell for malignant growth. In contrast the CCO is the cell that originally underwent transformation. Is there a connection between them? Clearly the CSC must be some derivative of the CCO. But the CCO is reflective of where the initial genetic alteration occurred. As Tang et al state regarding CSC and CCO we have:

*A tumor originates from a normal cell that has undergone tumorigenic transformation as a result of genetic mutations.*

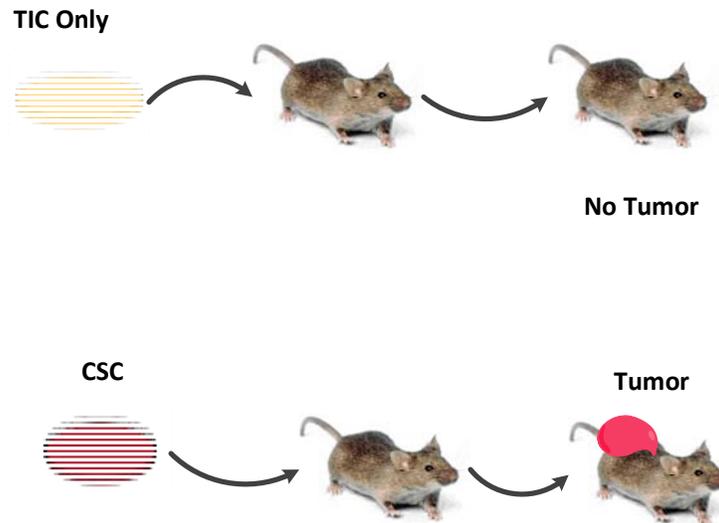
*This transformed cell is the cell-of-origin for the tumor.*

*In contrast, an established clinical tumor is sustained by subpopulations of self-renewing cancer cells operationally called cancer stem cells (CSC) that can generate, intraclonally, both tumorigenic and nontumorigenic cells.*

*Identifying and characterizing tumor cell-of-origin and CSCs should help elucidate tumor cell heterogeneity, which, in turn, should help understand tumor cell responses to clinical treatments, drug resistance, tumor relapse, and metastatic spread. Both tumor transplantation and lineage-tracing assays have been helpful in characterizing these cancer cell populations, although each system has its strengths and caveats.*

*In this article, we briefly review and summarize advantages and limitations of both assays in support of a combinatorial approach to accurately define the roles of both cancer-initiating and cancer-propagating cells. As an aside, we also wish to clarify the definitions of cancer cell-of-origin and CSCs, which are often interchangeably used by mistake.*

The CCO, cancer cell of origin, is distinct from the CSC. Below we depict a typical test. We select a set of tumor cells. We then mark them with some appropriate marker so that we can separate CSC and TIC cells as well as whatever else is in the mix. The markers are often based on what proteins each cell expresses. The we transplant them to a mouse and examine the result. If we have a CSC, then the tumor regrows. If TIC or benign cells, then no growth.



The above graphic is the approach often used. Namely take a cell which may be expressing a specific surface marker and then implant it in a mouse and observe the result. If the cell replicates the human tumor, then we have "found" the CSC. It is not clear that mice may not be primed for this. It is not clear how coincidental this may be. There should be a body of justification which is much more extensive.

### 3 A THOUGHT EXPERIMENT

The cancer stem cell concept is somewhat akin to the overall stem cell. Simply, a Cancer Stem Cell appears to be as a concept a single stem cell with some well-defined DNA structure which becomes capable during mitosis of;

- (i) regenerating itself consistently in some near immortal manner,
- (ii) while simultaneously generating another cell which is different from itself and which itself may duplicate itself exactly, subject to random genetic changes, and
- (iii) that such CSC if transplanted alone to some unaffected carrier will regenerate the tumor from which it was extracted.

This definition is an amalgam of the many attempts to define such a cell.

We know that such a process as the stem cell, albeit benign, appears to exist in hematopoiesis. Also it has been argued that such a cell is the basis for a variety of hematopoietic malignancies, such as MDS. MDS is especially interesting since it occurs not with the hematopoietic stem cell but somewhere along the line such a myelo or lympho line and that it involves methylation yet there is a CSC like behavior.

Let us begin with some facts:

1. All somatic cells have the same DNA. This is almost true. There are exceptions as follows:
  - a. There may have been some somatic mutation or translocation.
  - b. There may be some epigenetic changes due to methylation or miRNAs for example.
2. Mitosis of a single cell produces two identical offspring. There are some differences however:
  - a. First what do we mean by identical? They clearly have the same DNA but some DNA may be expressed slightly differently. Why is one cell expressing DNA differently than the other? Why is the other cell, if that be the case, working identically as its parent cell? Are the previous statements true?
  - b. Phenotypically there may be a significant difference in the cells.
3. A stem cell is defined in a certain manner. Essentially it is a self-replicating cell that can give rise to itself by definition and to other cells which may become mature cells in some terminal sense. However:

a. How does one identify a stem cell? Generally, it has been identified as a cell which when transplanted to a genetically primed target, a mouse for example, that it generates and reproduces the initial cancer. Furthermore, if it is silenced or removed the cancer ceases.

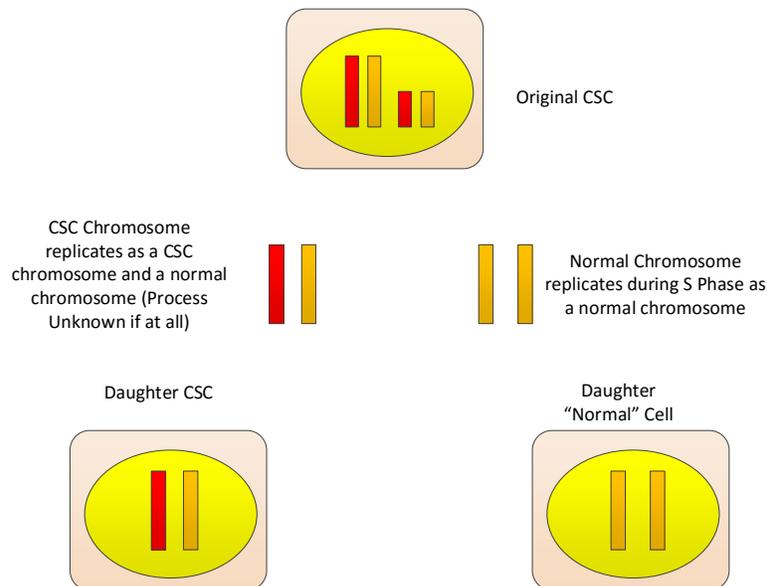
4. A cell of origin is a cell from which the original cancer arises. Yet:

a. What do we mean by the original cancer?

b. What is the relationship to the CSC?

5. A cancer stem cell, CSC, is a cell which can be defined as a self-replicating cell which also produces a second type of cell which is less self-replicating but which becomes the body of a tumor. The CSC somehow using the same DNA manages to go through the cell cycle yet produces two phenotypical cells which are also genotypically different in their expression albeit genotypically the same in toto.

We try to demonstrate this artifact below. One must note that there is as of yet no physical basis for this claim. It is merely a thought experiment.

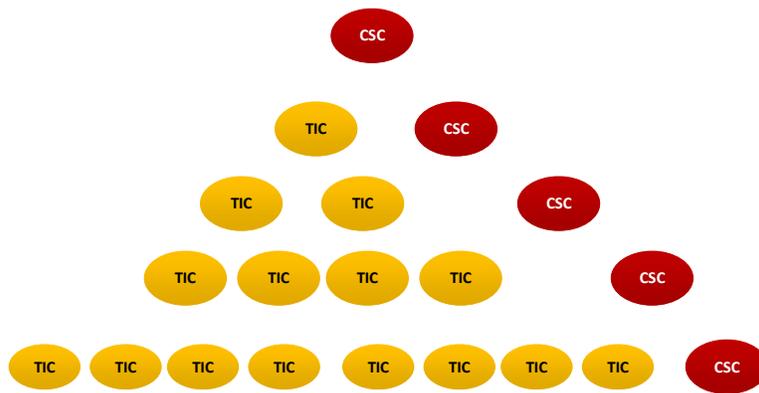


What do we have above? We have the following:

1. A cell with DNA that has somehow had some malignant alteration in two of the chromosomes. We have one chromosome marked as red which renders some semblance of immortality and a second chromosome which is marked orange which renders excess growth, albeit with limited mortality. Homologous orange chromosome cells are aggressive growers but can die off.

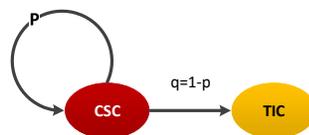
2. Now somehow, we really do not know but just posit a result, which makes this a thought experiment, the CSC goes through mitosis and produces two cells; a duplicate of itself and a daughter cell with homologous orange chromosomes.
3. The homologous cell goes on to replicate and then can go into apoptosis and die off.
4. The CSC can replicate again.
5. The CSC can replicate in one of two ways. First it can be deterministic, namely one CSC yields one CSC and a homologous cell. Second one CSC can regenerate itself with some probability and produce a homologous cell with another probability. The latter is the stochastic case.

We demonstrate the deterministic below:



Deterministic: The CSC replicates itself each time and the TIC also replicates but it doubles. Thus we see a single CSC while the TICs double.

We demonstrate the stochastic as follows:



Stochastic: The CSC can split into another CSC with a probability  $p$  or a TIC with probability  $q$ . If, for example, it splits into 2 TIC then the CSC could die off. It also could split into two CSC which would then cause added growth.

With the above model one can determine the distribution of cells as a function of time. For a linear progression the split is always 50:50 and otherwise we would have a probability that the CSC itself could extinguish. Even if  $p$  approaches 1.0. and never really reaches it then there is a minute but possible extinction. We do have examples of tumor regression. The classic case is in melanoma and in Rosenberg's early observations. We also know that in the case of HGPIN, that most likely we have some form of stem cell and that HGPIN also regresses in a finite number of cases<sup>8</sup>.

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<sup>8</sup> It is worth reading some of the cases we have discussed in the White Papers. There is a recent case where we saw total extinction.

## 4 CSC DYNAMICS

A great deal of work has been done examining the dynamics of CSCs. Part of that efforts pertains to establishing some means to identify them. We examine a small subset of the models here but there are many studies worth examining. Fundamentally the studies all seem to reflect the approach which starts with a stem cell and examines the products resulting therefrom. We have argued elsewhere and summarize latter herein that there is an alternative approach which frankly eschews the CSC and examines a collection of cells each sub-collection having a specific genetic expression state. That approach only looks at the cancer as a separate organism from the host and tries to understand it holistically as a spatio-temporal collection of interdependent genetic expression states evolving over time.

Many authors have examined the mathematical dynamics of the stem cell and the CSC. Stukalin et al have developed models for the fluctuations in cell populations. In a sense this is always a significant issue since the CSC growth is complex and does not reflect a simple deterministic model. Dhawan et al have examined the tumor control mechanisms in dynamic CSC environments. This is one of many ways that the CSM paradigm could be used in the control of cancers. Shahriyari et al have examined mathematical models for the stochastic dynamics of the CSC environment. They look at multiple mutations and effects on non-symmetric changes. Zhang and Wolynes use the many-body paradigm to explore stability points in complex CSC models. These are but a few of the approaches taken in modelling the CSC environment.

### 4.1 SOME BASICS

Let us begin with some simple fundamentals. As Dingli and Pacheco note:

*Tissues have evolved an architecture where most cells have a relatively short lifetime and undergo continuous turnover, and this mitigates the accumulation and retention of mutant cells.*

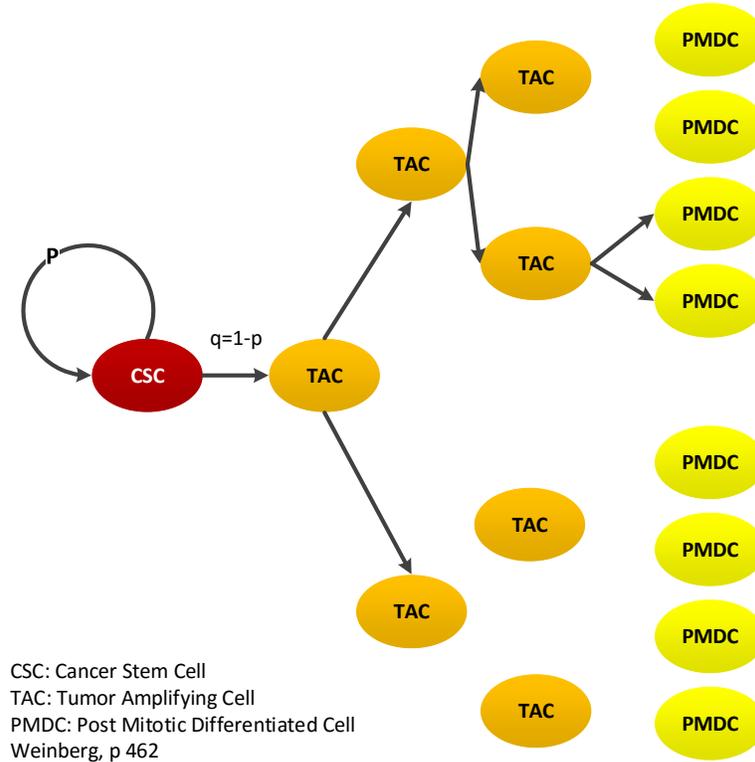
*At the root of this process are the stem cells that are able to maintain tissue integrity because of a dual phenotypic characteristic: self-renewal and production of progeny that can differentiate into various cell lineages that together constitute tissues and organs.*

*One can visualize tissues as having a tree-like organization of cells with stem cells at one extreme and mature, non-dividing cells at the other extreme.*

*Intermediate cells divide, often at relatively high rates, but live for relatively short periods of time. Although mutations can occur at every level of this cell hierarchy, the relatively short lifetime of more mature cell stages means that, in effect, the real risk of long-lasting oncogenic mutations is restricted to the small population of stem cells and early progenitor cells that maintain a given tissue.*

*This, in turn, effectively reduces the probability of the occurrence of mutations, given the small population of cells at risk, despite the fact that a mutation arising in a stem cell can persist for a long time. It is important to point out that the relevance of a mutation is cell context-dependent – a mutation in a gene that is not expressed in a cell is of no consequence to that cell but expression of the gene in more committed cells, downstream of the cell that is the source of the mutation, may lead to a phenotype associated with disease.*

From Weinberg we have the following model which reflects the above:



Note that in the above model we have the long lasting CSC and then we have proliferating intermediaries and ultimately the non-proliferating end stage cells. As the above authors note that since this is stochastic then there is a multiplicity of end states. At one extreme the CSC may actually die off, it may not reproduce and thus the cancer may just regress. We have argued that in certain cases of HGPIN followed by high saturation prostate biopsy that one may actually capture the CSC in a single core and thus deprive the nascent malignancy of its growth potential. Also one could imagine the immune system performing a similar function.

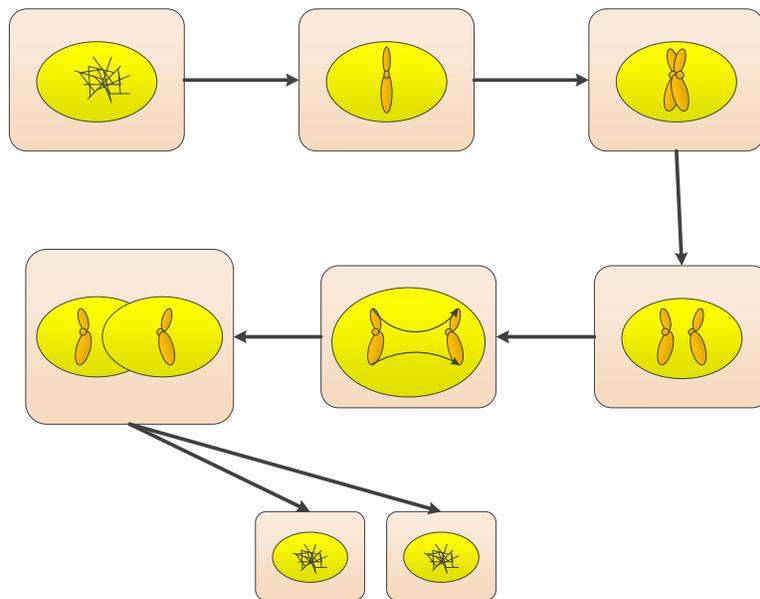
Bogdan et al report:

*Stem cell division times exhibit non-stationary behavior. Besides the heterogeneous structure of stem cells population, we also observe that the empirical PDF estimated from stem cell DTs exhibits a pronounced time dependent behavior...*

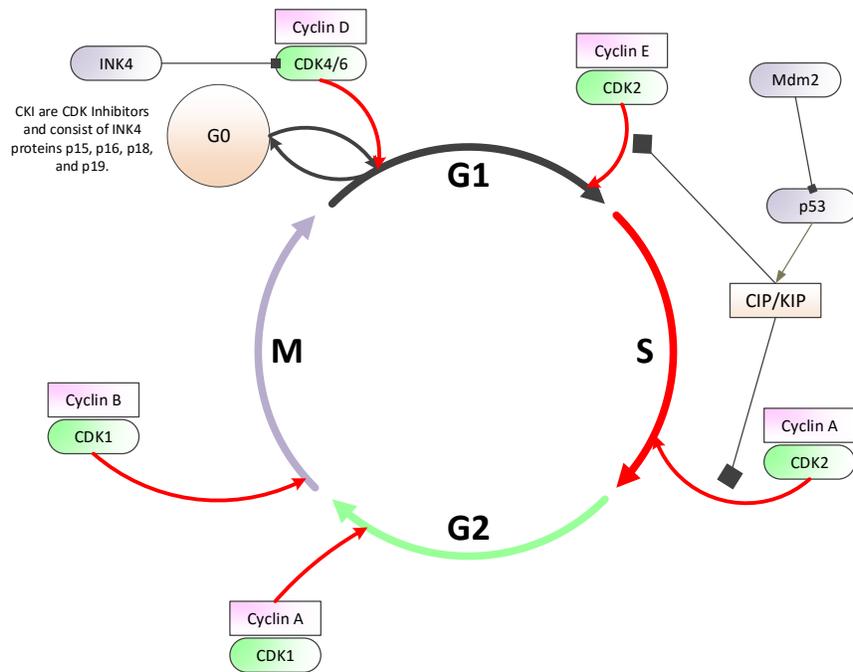
*Stem cell growth rates possesses multi-fractal characteristics. For a comprehensive investigation of the heteroscedastic dynamics of stem cell growth, we investigate the relationship between the higher order moments of stem cells dynamics and their order; we also estimate both the multi-fractal spectrum and generalized Hurst exponent function*

## 4.2 THE CELL CYCLE

A fundamental element of the understanding of cancer dynamics and the issues related to CSCs is the cell cycle itself. We start with a simplified description of mitosis. The intent here is not to present mitosis which is well documented in a multiplicity of places but to place a focus on some of the issues of the CSC. The simplified cycle is below:

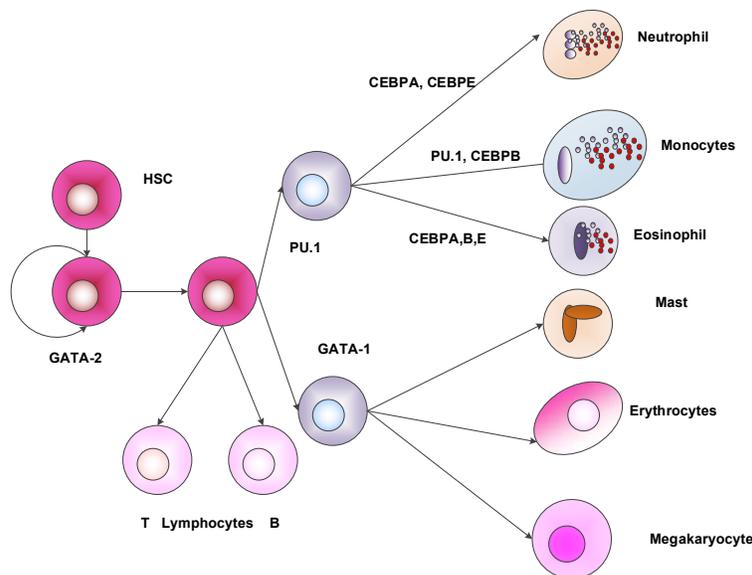


Now note the key step is in the reproduction or duplication of the DNA in the S phase. That is the last step on the top, we see a doubling of the chromosome. We detail the cycle below for reference.



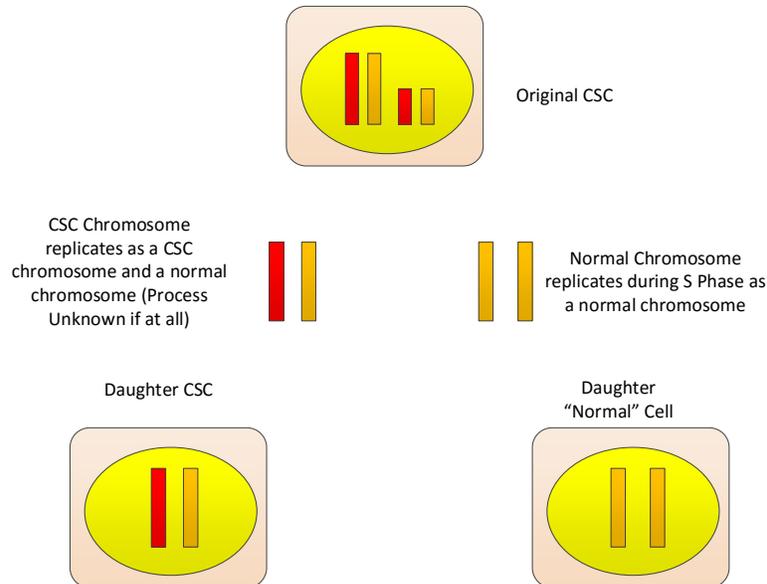
Now let us consider two processes in which this occurs:

1. Hematopoiesis: As the stem cell for the various blood lines evolve as shown below we have a cell move along but it changes based on what its local environment presents. The stem cell in the bone produces two stem cells, one which stays put, I am assuming a deterministic model, and another moves, and as it moves it encounters ligands that attach to receptors and the cell begins to change. As it changes it goes through mitosis again perhaps and it again encounters more ligands and changes some more.



This process continues until complete maturity.

2. CSC: This model is problematic Recall the assumption below:



There is the issue of recreating the CSC while also creating a new cell where the S phase appears to have some asymmetry. This is problematic. There is no well-known process whereby this can occur.

### 4.3 IDENTIFYING CSCs

There are currently several ways to identify CSCs. The primary one is via cell surface markers and in the case of PCa one specific one is CD44. Karsten and Goletz present a recent review of a collection of such markers. As they note:

*In recent years' considerable effort has been invested in the detection and characterization of stem cell markers. The result is that there are now an overwhelming and steadily increasing number of such marker molecules. Some markers are indeed more or less specific for different types of stem cells, for example, markers that differentiate embryonic from adult stem cells or pluripotent from progenitor cells. With the exception of pluripotent embryonic stem cells all other stem cells carry, in addition, lineage-specific markers.*

*Stem cells are also defined by the absence of certain markers. Contemplating these data, several questions arise. First, as already mentioned, almost all markers of normal stem cells are also found on cancer stem cells. This, of course, poses a problem with respect to their potential use as therapeutic targets. Ectopic (non-lineage) expression of stem cell markers on cancer cells does*

*not resolve the therapeutic dilemma. Currently the best option for a therapeutic target would be to rely on onco-fetal stem cell markers which are not expressed on normal adult stem cells. Otherwise there is at present no clear-cut distinction available between normal and cancer stem cell markers. Even at the level of regulatory miRNA clusters, identical patterns were observed*

They continue:

*These data and other more general considerations led us to propose the following hypothesis.*

- 1. During the process of malignant transformation from a normal stem or progenitor cell to a cancer stem cell, stem cell glycoprotein markers undergo alterations in their glycosylation.*
- 2. As a consequence, cancer stem cells carry cancer specific glycans.*
- 3. This appears to be a selective process. Accordingly, these cancer-specific glycans are CSC makers.*
- 4. Changes in stem cell marker glycosylation contribute to the altered biological behavior of these cells.*

*In brief, we propose that cancer stem cell markers differ from their normal counterparts by the expression of tumor-specific glycans.*

We have seen the glycan presence previously. But the change in glycosylation may be a change in energy utilization which we have also seen in the Warburg process. Thus the glycan markers may logically be targeted as markers. The logic and data in this paper may add more to the understanding of the CSC dynamics.

## 5 PATHWAY ISSUES

We briefly examine some of the key pathways that have been argued as critical in the CSC evolution. Although we present them we however do not attribute anything specific to them herein.

As Zhang et al note:

*IPA uncovered important signaling pathways enriched in basal cells including*

1. *TGF- $\beta$ ,*
2. *NOTCH,*
3. *WNT/TCF,*
4. *IGF,*
5. *FGF,*
6. *STAT3/IL6 and others.*

*For instance, immunofluorescence of FGFR3 validated our RNA-Seq data and revealed its expression preferentially in the basal layer. We systematically investigated some of these pathways in regulating primary basal stem/progenitor activities.*

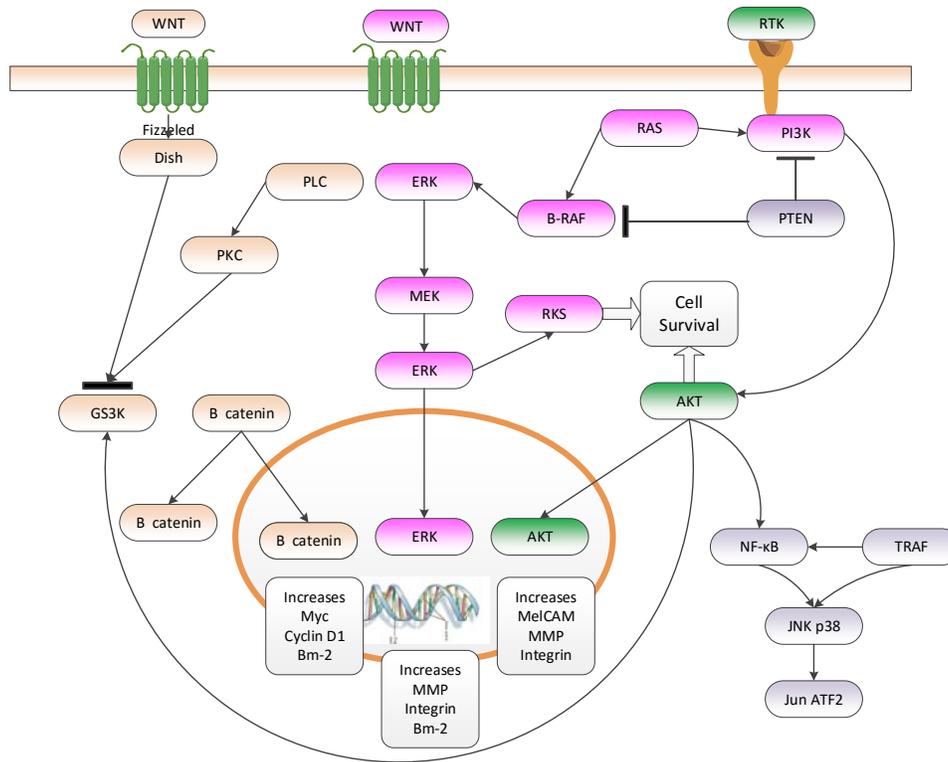
*Given that each pathway has a large number of components, we first used the pathway-specific pharmacological inhibitors to interrogate their roles in regulating basal cell activity. For pathways of particular interest, small interfering RNA (siRNA)-mediated knock-down experiments were performed to validate the inhibitor results.*

### 5.1 WNT

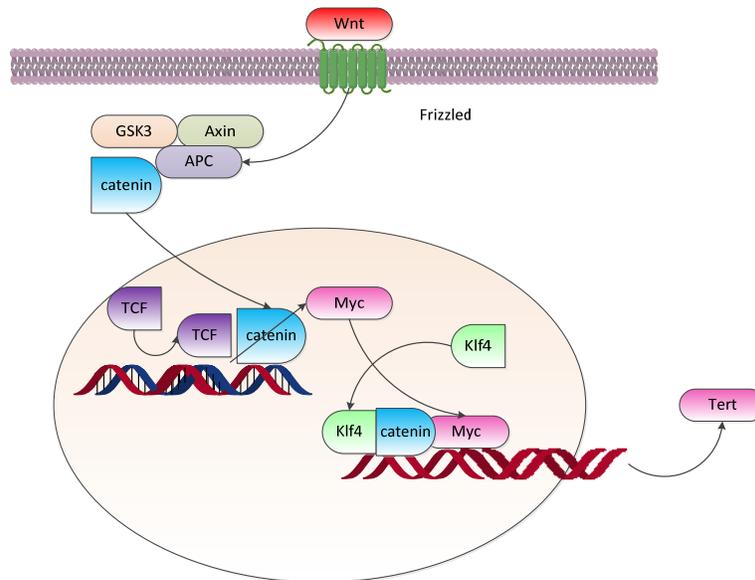
We briefly re-examine each of these. First we show the WNT pathways below. This is a well know process and we have examined it extensively previously<sup>9</sup>.

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<sup>9</sup> Specifically see the reference by Goss and Kahn.



Signaling pathways in the cells have been a major focus on study for the past decade or so. The focus generally has been on what protein or gene influences what other protein or gene. A recent article in [Science](http://science.sciencemag.org/content/336/6088/1519) presents some interesting work on Wnt and TERT<sup>10</sup>.



<sup>10</sup> <http://science.sciencemag.org/content/336/6088/1519>

Wnt is an extra cellular signaling protein and it attaches to Frizzled a receptor and sets off a cascade that moves B catenin into the nucleus and generates Myc which is a transcription protein with together with catenin and other transcription proteins generates Tert from TERT.

To quote from [NCBI<sup>11</sup>](#):

*Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.*

As the Science article states:

*Maintaining the length of telomere, the ends of chromosomes, is essential for all cells that divide many times. The enzyme telomerase lengthens these ends, counterbalancing their shortening that occurs each time chromosomes are copied. Telomerase is essential for cell viability, and loss of its function from the loss of only one of two copies of the encoding gene can lead to the failure of stem cell renewal that is seen in premature aging conditions such as dyskeratosis congenita, aplastic anemia, and pulmonary fibrosis. Conversely, telomerase activity is increased in many cancers and may be required for cancer cells to maintain their telomere length...*

They continue is a rather interesting wording:

*Because of the importance of telomerase expression, the signaling pathways that control TERT transcription have been extensively studied. Remarkably, many different transcription factors, including c-Myc, Sp1, nuclear factor of activated T cells (NFAT), activating protein 2B, nuclear factor  $\kappa$ B (NF- $\kappa$ B), Myb, activating transcription factor, nuclear factor 1 (NF1), and the estrogen receptor (ER), bind to the 330–base pair minimal TERT promoter and regulate transcription. In addition, a number of negative regulators bind the TERT promoter, including CTCF, elongation factor 2, p53, Ets, Mad1, Men1, and Wt1. Adding  $\beta$ -catenin and Klf4 to the many regulators that bind the TERT promoter is like adding one more guest to a crowded table at a dinner party.*

They conclude:

*It is reasonable to propose that Wnt regulates TERT given that Wnt signaling plays an essential role in stem cell self-renewal and that TERT is needed for the long-term growth of stem cells. TERT regulation seems to require not one, but two master transcriptional regulators to assure that there is neither too much, which may allow the growth of cancer cells, nor too little, which might lead to stem cell failure. The finding by Hoffmeyer et al. that both  $\beta$ -catenin and Klf4 are*

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<sup>11</sup> <http://www.ncbi.nlm.nih.gov/gene/7015>

*required to activate TERT expression puts the horse (Wnt) before the cart (TERT) and provides a foundation for linking telomerase levels and self-renewal.*

The observation of the inter-cellular signaling with Wnt and its control over TERT and the telomere process is quite interesting. This may be an interesting way to incorporate many of the Turing models we have been discussing as well.

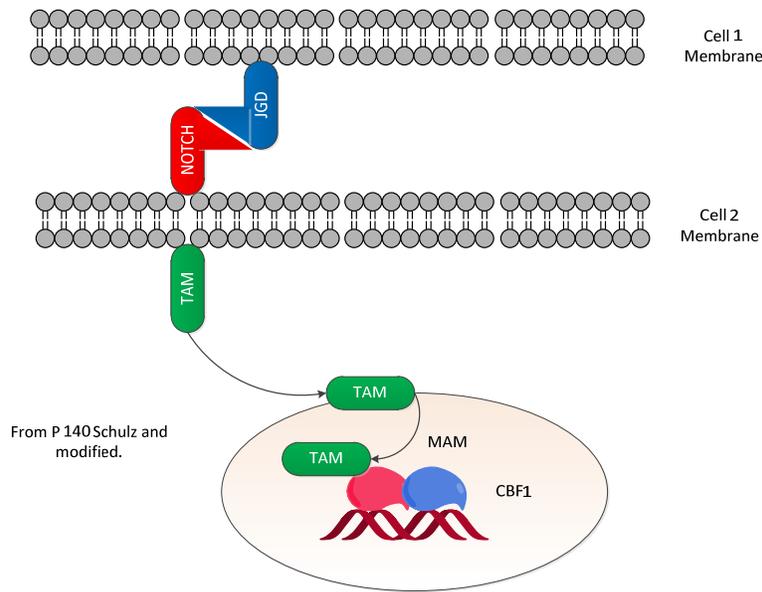
## 5.2 NOTCH

Notched is a bit of an amalgam of the above discussion. The notched pathway is characterized as follows.

The notch protein sits like a trigger spanning the cell membrane, with part of it inside and part outside. Ligand proteins binding to the extracellular domain induce proteolytic cleavage and release of the intracellular domain, which enters the cell nucleus to alter gene expression. The notch signaling pathway is important for cell-cell communication, which involves gene regulation mechanisms that control multiple cell differentiation processes during embryonic and adult life. Notch signaling also has a role in the following processes:

1. neuronal function and development
2. stabilization of arterial endothelial fate and angiogenesis
3. regulation of crucial cell communication events between endocardium and myocardium during both the formation of the valve primordial and ventricular development and differentiation
4. cardiac valve homeostasis, as well as implications in other human disorders involving the cardiovascular system
5. timely cell lineage specification of both endocrine and exocrine pancreas
6. influencing of binary fate decisions of cells that must choose between the secretory and absorptive lineages in the gut
7. expansion of the hematopoietic stem cell compartment during bone development and participation in commitment to the osteoblastic lineage, suggesting a potential therapeutic role for notch in bone regeneration and osteoporosis
8. T cell lineage commitment from common lymphoid precursor
9. regulation of cell-fate decision in mammary glands at several distinct development stages
10. possibly some non-nuclear mechanisms, such as control of the actin cytoskeleton through the tyrosine kinase Ab

We demonstrate Notched and its counterpart Jagged in the following Figure. On the cell surface we have Notched and on the other cell surface we have Jagged. When they bond, in a sense as surface proteins but with a communicating capability, Notched release or activates Tam which is a transcription factor facilitator.



Notch signaling is dysregulated in many cancers.

### 5.3 FGF

FGFR is a Receptor and this gene encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence being highly conserved between members and among divergent species. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance.

As we have noted elsewhere FGF is one of many such receptors as shown below:

<i>Models</i>	<i>Genes regulated</i>	<i>Prostate phenotype</i>
<b>Hormone receptors</b>	Androgen receptor	HGPIN
	Retinoic acid receptor $\alpha/\gamma$	Squamous metaplasia and pre-neoplastic lesions
	Estrogen receptor $\alpha/\beta$	No marked phenotype
<b>Growth factors and receptors</b>	FGF8b	HGPIN
	FGFreceptor1	PIN with reversible hyperplasia
	FGF7	Prostate epithelial dysplasia
	FGFR2iiib	Hyperplasia/dysplasia
	IGF-1	PIN and spontaneous tumor growth

<i>Models</i>	<i>Genes regulated</i>	<i>Prostate phenotype</i>
	TGFR- $\beta$	PIN and invasive adenocarcinoma
	HER-2/Neu	PIN and invasive carcinoma
<b>Tumor suppressors, cell cycle, and signaling pathways</b>	p53Rb	PIN with reduced apoptotic potential Focal hyperplasia
	Nkx3.1	Hyperplasia followed by PIN
	H-Ras	LGPIN and intestinal metaplasia
	APC	PIN and invasive adenocarcinoma
	Pten	PIN and metastatic adenocarcinoma
	Bcl-2	No overt phenotype
	Akt-1	Focal regions of PIN
	C-MYC	PIN and locally invasive adenocarcinoma
<b>Genomic instability</b>	Eco RI	HGPIN
	c-fos	No significant pathology
<b>Composite transgenic mice</b>	Ink4a/Arf+/-/Pten+/-	Rapid growth of PIN lesion
	Nkx3.1/Pten	PIN and metastatic spread of invasive tumors to lymph nodes
	Pten+/-/Akt1-/-	Akt1-/- repressed prostate tumor growth
	Pten+/-/p27kip1-/-	Rapid progression of invasive carcinoma
	Pten-/-/p53-/-	Early onset of invasive tumors
	PTEN+/-/TRAMP	Increased rate of tumor development
	P53-/-/Rb-/-	Highly metastatic adenocarcinoma
	Pten+/-/FGF8b	Metastatic adenocarcinoma
	Bcl-2/TRAMP	Multi step prostate carcinogenesis

## 5.4 TGF-B/EMT

As we had noted previously<sup>12</sup>:

*TGFB1: This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth factors. The secreted protein is cleaved into a latency associated peptide (LAP) and a mature TGFB1 peptide, and is found in either a latent form composed of a TGFB1 homodimer, a LAP homodimer, and a latent TGFB1-binding protein, or in an active form composed of a TGFB1 homodimer. The mature peptide may also form heterodimers with other TGFB family members. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease.*

<sup>12</sup> See White Paper No. 133 LY6 and Prognostic Markers (February 2016)

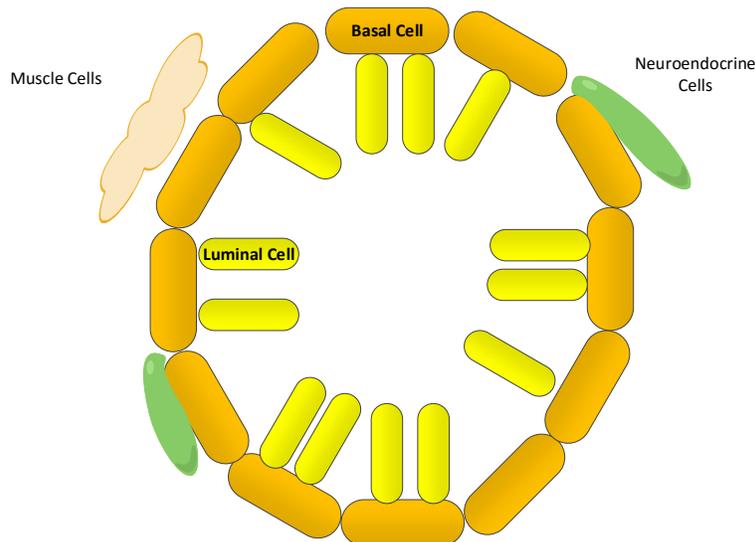
## 6 PROSTATE CANCER STEM CELLS

We now focus on the issue of prostate cancer, PCa, and its stem cell as well as its cancer cell of origin, CSC and CCO.

### 6.1 PROSTATE MORPHOLOGY

The prostate is an interesting organ. It is a collection of glandular cell segments and the glands contain a circumference of basal cells and a collection of luminal cells extending into the lumen, the empty space. Around the periphery and in the connective space are a multiplicity of other cells of various types; muscle cells, neuroendocrine cells and the like. The prostate tends to grow or enlarge as a man gets older and thus can grow from a typical size of 40 cc to at times well over 100 cc. In simple age related benign growth the prostate duplicates itself in an ever enlarging glandular network and generally appears somewhat uniform. Inflammation may occur as well as hyperplasia.

The hyperplasia generally appears as masses of excess and somewhat disordered luminal cells in the lumen, and the organization of the lumen begins to become distorted. In the extreme case of High Grade Prostatic Intraepithelial Hyperplasia, HGPIN, the gland appears almost filled with luminal cells. Some have argued that this is a precancerous state and irreversible. We however have seen cases where it is totally reversible and thus this existence proof of non-inevitability is questionable.



As Agarwal et al note:

*Prostate glands are composed of:*

1. *an outer layer of basal cells expressing KRT5, KRT14, and TP63,*
2. *an inner layer of secretory, luminal cells expressing KRT8, KRT18, and AR,*
3. *and rare SYP and CHGA positive neuroendocrine cells.*

*TP63 is a marker of prostate basal epithelial and stem cells and is required for prostate development. Lineage tracing studies based upon cytokeratin drivers have established a number of principles for stem cell hierarchies in the developing and adult prostate.*

*The majority of regenerative adult stem cells appear to be unipotent. In addition, studies using other lineage tracing schemes have described minor populations of multipotent progenitor cells that have not been captured with KRT-specific drivers.*

*Using an inducible NKX3.1-specific CRE driver, a rare (0.7%) population of bipotential luminal cells in the castrate prostate (CARNs) has been described (Wang et al., 2009). In addition, the existence of KRT5<sup>neg</sup>, KRT14<sup>-</sup>, TP63<sup>+</sup> cells has been observed, as well as the ability of TP63 lineage marked cells to generate luminal epithelial cells in the adult*

As PCa develops it initially appears as a multiplicity of poorly formed glandular structures, and although looking somewhat like the benign normal glands it starts to lose structure as it develops. The question then is; what cells is the basis for this change, basal or luminal or other, and as the PCa starts to expand which cell is and/or becomes a CSC?

From White and Lowry, we have a summary of the issue regarding the cell of origin for PCa. They note:

*Models for both murine and human prostate cancers have produced conflicting conclusions within the field as to whether the CCO is of basal or luminal origin. Debate has arisen as to whether the stem cells of the prostate reside in either the basal or luminal populations.*

*Using a broader range of lineage tracing alleles, it was suggested that a multipotent population arises from the basal population, while separate unipotent progenitors populate the neuroendocrine and luminal pools. The lack of a consensus on the identity of ASCs of the prostate has also clouded the interpretation of CCO studies for the prostate. Similar to the discrepancies observed for SCC/BCC, much of the debate regarding CCOs for prostate cancer centers on the fact that prostate tumors typically adopt a morphology consistent with a luminal origin, while experimental data often point towards a basal source for CCOs.*

*Human prostatic epithelial transplantation studies, which do not include a native stromal and immune component, indicated a basal CCO with MYC, AKT or ERG as oncogenic drivers. By contrast, genetically modified mouse models that used Pten deletion implicated both basal and luminal cells as CCOs, depending on the targeting alleles and tumorigenic strategies used.*

*In addition, one study showed that initiation from human basal cells generates transformed luminal-like cells that are able to propagate the tumor. Together, these results suggest that the identity of the CCO for prostate cancer could be dependent on cellular, genetic, and environmental contexts, and further work will be needed to address whether differences exist between human and mouse models systems or whether the differences are caused by nonequivalent cell-intrinsic and cell-extrinsic stimuli.*

*Heterogeneity of tumor initiators and tumor phenotypes The experimental models described here have proven to yield important insights into tumor initiation and CCOs. However, there are technical limitations to these models that ignore the heterogeneity of bona fide cancer initiation. Tumors are thought to be initiated in a clonal fashion as a result of mutations.*

We thus examine this CCO and corresponding CSC issue for PCa.

## 6.2 CELL OF ORIGIN

The Cell of Origin is akin to the CSC and has been the focus of debate in PCa. As White and Lowry have noted:

*Significant progress has been made to identify the cells at the foundation of tumorigenesis, the **cancer cell of origin (CCO)**. The majority of data points towards resident **adult stem cells (ASCs) or primitive progenitors as the CCO for those cancers studied, highlighting the importance of stem cells not only as propagators but also as initiators of cancer**. Recent data suggest tumor initiation at the CCOs can be regulated through both intrinsic and extrinsic signals and that the identity of the CCOs and their propensity to initiate tumorigenesis is context dependent. In this review, we summarize some of the recent findings regarding CCOs and solid tumor initiation and highlight its relation with bona fide human cancer.*

*Cancer is a complex disease due to the wide variety of cellular and molecular mechanisms associated with its initiation and progression. It is accepted that cancer cells divide and proliferate uncontrollably because of the accumulation of somatic mutations in normal tissue, which confers a selective growth advantage in the mutated progeny.*

*However, the cells that make up a tumor are heterogeneous; often making it difficult to determine the **CCO, which is the normal cell that acquires the mutational load necessary to first initiate cancerous proliferation**. Furthermore, since cancer is a transformative process, the cells composing advanced cancers may no longer contain morphological or molecular characteristics of the CCO. The identity of the CCO could be critical to the generation of more effective treatments and preventative strategies.*

*If CCOs can be identified and targeted specifically, it would be possible to stop cancer before it has a chance to undergo expansion. Molecular or physiological attributes specific to CCOs could be exploited to slow or block progression, thus avoiding treatments that simply kill dividing cells. This has led to significant recent efforts to define CCOs for all types of cancers, and numerous lines of evidence point towards ASCs as possible CCOs.*

They continue:

*ASCs are found in many of the major adult organs and are essential for tissue homeostasis as well as regeneration in response to injury.*

*Most ASCs were discovered on the basis of their relative quiescence and their ability to reconstitute differentiated cell lineages of the tissue or organ in which they. Either upon activation by natural turnover/cycling or in the case of regeneration due to injury, ASCs give rise to multilineage restricted progenitors or, as they are often called, transit amplifying cells (TACs).*

*These cells divide rapidly and then differentiate to generate the bulk of cells required for tissue turnover or regeneration. Due to their rapid division, TACs are also targeted by chemotherapeutics that act on cell division pathways to kill cancer cells*

In the above we have seen defined three entities:

1. CCO: The cancer cell of origin.
2. ASC: Adult stem cells.
3. TAC: Transit amplifying cells

Wang and Shen note:

*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.*

Thus we even here have some confusion as to the CCO, cancer cell of origin.

### **6.3 BASAL CELL ARGUMENTS**

One of the sets of arguments presents the basal cell as the cell of origin. As Wang and Shen note:

*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*

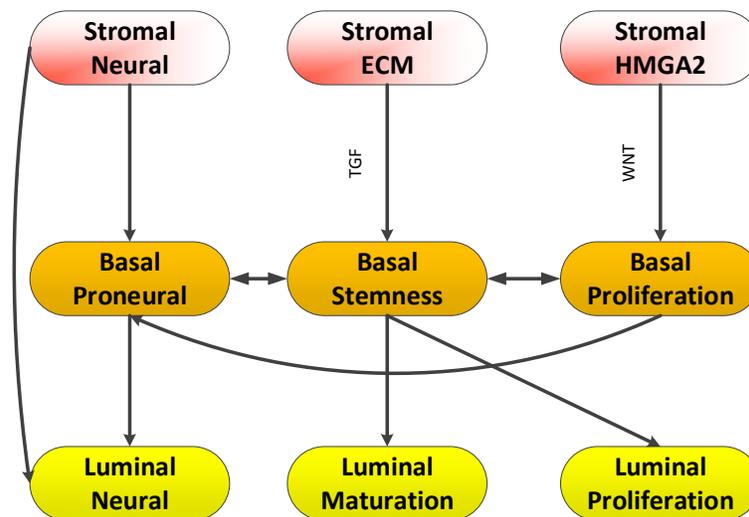
*driver allele has been shown to result in a basal cell expansion accompanied by increased number of intermediate cells, suggesting a basal cell of origin.*

In a paper by Goldstein et al they note:

*Luminal cells are believed to be the cells of origin for human prostate cancer, because the disease is characterized by luminal cell expansion and the absence of basal cells. Yet functional studies addressing the origin of human prostate cancer have not previously been reported because of a lack of relevant in vivo human models. Here we show that basal cells from primary benign human prostate tissue can initiate prostate cancer in immunodeficient mice.*

*The cooperative effects of AKT, ERG, and androgen receptor in basal cells recapitulated the histological and molecular features of human prostate cancer, with loss of basal cells and expansion of luminal cells expressing prostate-specific antigen and alpha-methylacyl-CoA racemase. Our results demonstrate that histological characterization of cancers does not necessarily correlate with the cellular origins of the disease.*

We had examined this in some detail when it first appeared some six years ago. The problems were that it was murine related and one could argue that CSC in a mouse is not CSC in human. Suggestive but not a definitive proof.



From Moscatelli and Wilson we have the arguments:

*In a recent paper in Science (3), Goldstein et al. describe a model system in which questions about the cell of origin and oncogenic pathways of human prostate cancers can be addressed. Using two cell surface antigens, Trop2 (TACSTD2) and CD49f (integrin  $\alpha 6$ ), Goldstein et al. (3) separated luminal (Trop2<sup>+</sup>/CD49f<sup>-</sup>) from basal (Trop2<sup>+</sup>/CD49f<sup>+</sup>) cells in digests of benign human prostate tissue.*

*When each of these populations, along with urogenital sinus mesenchyme cells that promote the proliferation of primitive prostate cells, was injected subcutaneously into immunodeficient (NODSCID- IL2R $\gamma$ -/-) mice, the basal cell population gave rise to prostate-like structures containing both basal and luminal cells, whereas the luminal population did not grow, confirming observations from mouse prostate (4) that the basal layer contains prostatic epithelial stem cells.*

*Goldstein et al. (3) then used lentiviral vectors to transform these cells with genes encoding activated Akt and ERG, which are commonly associated with human prostate cancers. When transplanted into the mouse, the transformed basal cells formed tissues that resembled prostatic intraepithelial neoplasia (PIN) (that is, microscopic groups of atypical epithelial cells that represent a premalignant state), containing both basal and luminal cells, whereas transformed luminal cells did not grow.*

*Finally, addition of the androgen receptor gene, which is often up-regulated in prostate cancer, to the genes expressing activated Akt and ERG in the basal cells gave rise to frank adenocarcinomas with an expanded luminal cell population and an absence of basal cells, whereas expression of these same genes in luminal cells did not generate any prostatic tissue.*

*The authors conclude that basal stem cells are the target of transformation in the generation of prostate tumors.*

Finally, in the recent study by Zhang et al they conclude:

*The current study has made the following significant findings (see Supplementary Discussion).*

*First, our study uncovers unique SC- and EMT-enriched gene-expression profile in unperturbed basal cells that support the long-held hypothesis that the human prostate basal cell layer harbors primitive SCs.*

*Second, we report the surprising finding that basal cells are enriched in genes normally associated with neurogenesis. In contrast, luminal cells preferentially express proneural genes involved in neural signal response and processing. Consistently, primary basal cells can spontaneously or be induced to undergo 'neural' development in vitro, generating NSC-like cells. Combined with the SC features, these transcriptional programs provide a molecular understanding for the reported basal cell plasticity.*

*Third, basal cells express high levels of Pol I-associated rRNA biogenesis genes regulated, at least in part, by the MYC transcriptional programme. MYC is often found overexpressed in PCa, especially metastatic PCa. Increased transcription of rRNA genes by Pol I is a common feature of human cancer. Thus, our data may suggest a rationale for treating anaplastic PCa and CRPC with Pol I inhibition, as well as targeting MYC and the MYC-mediated transcriptional programme as a therapy for PCa.*

*Fourth, our deep RNA-Seq data provide a rich resource for epithelial lineage specific genes and markers in the human prostate.*

*Fifth, distinct transcriptomes in basal and luminal cells also suggest cross communications between the two epithelial cell types, as well as between the epithelial compartment and the underlying stroma. Understanding such crosstalk will be instrumental for understanding the normal development and tumorigenesis of prostate. Although many of the signaling pathways mentioned in this study are poorly investigated in normal prostate epithelial biology, their functional involvement in PCa development and progression has been widely documented<sup>3</sup>.*

*Last, the basal cell gene-expression profile is linked to adverse clinical features of PCa, indicating a 'biomarker' value of basal cell gene signature for aggressive PCa.*

*Importantly, the molecular resemblance of basal cells to anaplastic PCa and CRPC provides a common molecular understanding of these diverse and poorly characterized aggressive PCa subtypes and implicates basal cells as the cell-of-origin for these variant PCa. It should be noted that while this manuscript was under review, another paper reported similar findings in linking the basal cell gene expression to aggressive PCa*

The above by Zhang et al appears to be the most comprehensive argument for CCO as basal.

#### **6.4 LUMINAL CELL ARGUMENTS**

There is a set of counter proposals for the luminal cells as the CCO.

Specifically, another set of arguments defends the luminal cell, namely Wang and Shen have noted:

*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 mouse models, a recent study using a prostate-specific antigen-Cre, PtenloxP/loxP prostate cancer model reported that the initial hyperplastic cells were all luminal. Finally, our laboratory has shown that targeted deletion of Pten in CARNs resulted in high-grade PIN and carcinoma, indicating that CARNs are a cell of origin. At present, however, it is unknown whether CARNs exist in the hormonally intact prostate epithelium, and if so, whether these cells can serve as cells of origin. Indeed, if CARNs correspond to facultative stem cells, as discussed above, they may correspond to a cell state that is only acquired in the regressed epithelium.*

Also from Moscatelli and Wilson we have the arguments:

*At first glance, these findings seem to be in conflict with those in a recent paper from Wang et al. (5) that concludes that a luminal epithelial stem cell is the target of transformation in prostate*

*cancer. This conclusion relies on lineage-tracing studies in the mouse prostate. Wang et al. (5) found that expression of a prostate-specific homeobox gene, Nkx3-1, marked rare luminal epithelial cells but was never observed in basal cells in prostates after castration-induced involution. When mice are castrated to abolish the production of testicular androgens, the prostate involutes, resulting in a reduction in size due to apoptosis of most luminal cells and of a small fraction of basal cells.*

*When androgens are readministered, the prostate regenerates. When castration-resistant Nkx3-1-expressing cells (CARNs) marked with yellow fluorescent protein (YFP) were followed, it was found that these cells expanded over ninefold during regeneration of the prostate after androgen replenishment and gave rise to luminal, basal, and neuroendocrine cells.*

*Reimplantation of single YFP-marked CARNs, along with urogenital sinus mesenchyme, under the renal capsule (a fibrous layer surrounding the kidney) of immunodeficient (nude) mice generated prostatic ducts containing both basal and luminal cells that were completely YFP positive.*

*Specifically deleting the tumor suppressor gene PTEN (which regulates the Akt signaling pathway and is often inactivated in human prostate cancer) in CARN cells led to the rapid development of tumors with a luminal phenotype and an absence of basal cells upon prostate regeneration. These results suggested that CARNs are prostate stem/progenitor cells and targets of transformation.*

Similarly, in a recent (2015) paper by Agarwal et al the authors note:

*Primary prostate cancer almost always has a luminal phenotype. However, little is known about the stem/ progenitor properties of transformed cells within tumors. Using the aggressive Pten/Tp53-null mouse model of prostate cancer, we show that two classes of luminal progenitors exist within a tumor. Not only did tumors contain previously described multipotent progenitors, but also a major population of committed luminal progenitors.*

*Luminal cells, sorted directly from tumors or grown as organoids, initiated tumors of adenocarcinoma or multilineage histological phenotypes, which is consistent with luminal and multipotent differentiation potentials, respectively.*

*Moreover, using organoids we show that the ability of luminal-committed progenitors to self-renew is a tumor-specific property, absent in benign luminal cells.*

*Finally, a significant fraction of luminal progenitors survived in vivo castration. In all, these data reveal two luminal tumor populations with different stem/progenitor cell capacities, providing insight into prostate cancer cells that initiate tumors and can influence treatment response.*

Thus using this model, we again see an argument for luminal cells.



## 7 OBSERVATIONS

The various sides of the arguments presented herein most likely continue. As much as murine models have value they also are a substantially different species.

### 7.1 HOW CLOSE IS CLOSE?

The issue of how close we should be examining the tumors is a critical one. As Gudem et al have noted the PCa tumors are very genetically heterogeneous. In the development of a metastatic state the original tumor spreads and optimizes itself to the environment in which it is best suited. Thus as is frequently the case the PCa tumors seek presence in the bone and restructure the bone in their own liking. The question is then; what are the spatio temporal changes we see and can they become elements of therapeutic targets?

To understand this better, we again examine the literature. In the conclusion to the Navin and Hicks paper they state:

*Biological models are by definition built upon incomplete information. At best, these explicit models for tumor progression provide guideposts for further exploration. As technology continues to evolve, the analysis of cancer samples of complex mixtures will give way to methods aimed at the individual cell.*

*Such methods will enable single cancer cells to be tracked as they progress to form the primary tumor and traced as they migrate through the body to seed the metastasis. In the near future the cost of deep sequencing a mammalian genome, whether from a tumor sample or a few disseminated cells will be approximately equivalent to the current price of a microarray experiment. Single cell genomes are also ideal for constructing detailed lineages of tumor progression, because individual mutations in a genome can be traced as they are inherited and expanded in subpopulations.*

*As we bring the magnifying glass closer, we may also be able to track the genetic stepping stones for tumor growth, or follow the genetic changes in circulating tumor cells as they progress from the primary to metastasis. Perhaps, we will find evidence that individual circulating tumor cells return to the primary tumor after developing offsite as the self-seeding model suggests. It is then that these predictive genetic models will have realized their full value.*

It is reasonable to consider that examining the cell by cell profile of a cancer will be exceptionally enlightening. In addition to understand from the tumor progression how the malignancy changes in time and place is also critical. The issues as to what causes a cell to proliferate and mutate is essential to understanding how to target the cell. Perhaps if the CSC model is correct and that if we target the CSC itself then the other cells just die off.

### 7.2 CELL IMPORT

What cell should we focus on and how do we identify it? As much as we have gathered about PCa and its genetics, we are still often in the dark because we lack the equivalent of the simplicity of a set of Newton's Laws. The state of a PCa cell is stochastic and does not follow the ballistic parabolic flight of a Newtonian projectile. Thus "Moon Shots" are problematic at best. We may still be hurling stones from Roman like launches.

From Agarwal et al:

*This study characterizes primary prostate tumors initiated by loss of the common tumor suppressors, Pten and Tp53, for stem/progenitor phenotypes as assayed by in vitro organoid cultures and in vivo tumor-initiating activity.*

*It has not been routinely possible to culture luminal stem/progenitor cells, which has prevented ex vivo analysis of these important cells in primary prostate tumors, biasing most studies toward primary basal cells or human prostate cancer cell lines.*

*We have observed two classes of self-renewing luminal progenitors in Pten/Tp53-null tumors, a minor population giving rise to multilineage organoids (multipotent progenitors) and a major population producing luminal-only organoids (luminal committed progenitors). Of particular interest is the observation that multilineage organoids give rise to self-renewing luminal organoids, providing additional insight into progenitor subpopulations, lineage stages leading to luminal commitment, and one route of prostate adenocarcinoma mitogenesis.*

*We suggest that combined loss of Pten and Tp53 either in the luminal multipotent progenitor or a precursor has revealed a naturally transient population, possibly by inhibiting the normal rate of differentiation. This interpretation is consistent with considerable evidence linking Tp53 to the regulation of differentiation in stem cells.*

*To date, luminal multipotent progenitor cells have not been observed in lineage tracing experiments, except in the case of rare CARN's, prompting questions about the significance of the multipotent progenitors revealed in organoid cultures. We show the existence of multipotent and luminal-committed TICs isolated directly from tumors, producing either adenosquamous carcinoma or adenocarcinoma, respectively. Importantly, the TIC assays used here measured autonomous differentiation potential in the absence of inductive embryonic urogenital mesenchyme. Endogenous adenosquamous prostate carcinoma is observed in a fraction of PB-CRE4; Pten<sup>fl/fl</sup>;Tp53<sup>fl/fl</sup> mice, supporting the concept that transformed multipotent progenitors exist in vivo and can differentiate to both basal and luminal lineages in tumors in situ.*

*It seems likely that the microenvironment will influence lineage commitment, and we note that organoids and TIC assays are performed in the absence of stromal cells. Therefore, it is possible in these assays that the extent of basal cell commitment by multilineage progenitors may be increased relative to the endogenous microenvironment.*

*Although engineered models of prostate cancer are often used to analyze the consequences of combined genetic mutations, the effect upon stem/progenitor populations has not been commonly considered. We show here for PB-CRE4-initiated genetic changes that Tp53 in combination with*

*Pten loss demonstrated significantly different stem/ progenitor populations compared to Pten loss alone.*

*Specifically, Tp53 loss leads to the presence of luminal multipotent stem/progenitor cells and a self-renewing luminal population, correlated with accelerated adenocarcinoma development, that is absent in Pten-null prostates. In addition, it is possible that Tp53 loss primes for lineage plasticity, similarly to the phenotypic dedifferentiation of luminal mammary epithelium following Brca1 loss. Analyses of stem/progenitor populations contribute fundamental knowledge for molecular and pathological comparisons of GEM models and for interpretation of target populations responding to therapeutics...*

*Due to a lack of biomarkers, the extent of innate stem/progenitor subpopulation heterogeneity in human prostate cancer is not known.*

The metaphor of launching stones is apropos. We cannot truly identify the targets and we do not have the predictive tools of Newton.

### 7.3 ALTERNATIVE VIEWS

It appears that most if not all of the work on understanding the cancer dynamics has been from the cell upwards<sup>13</sup>. The CSC has become a focal point, and paradigm for the bench work from which possibly prognostic, diagnostic and therapeutic approaches could evolve. We have on the other hand examined the process from the top down. Namely we looked at the gross characteristics of cumulative collections of common cell states. We have defined a metric which is the local cell density of a cell having a specific genetic state, which may also include a specific epigenetic state as well. Namely we define:

$$\bar{n}_j(x,t) = E[n_j(x,t)]$$

Where j is a genetic state which may be for example:

$$j = \{all \text{ cells such that genes } k=1,\dots,N \text{ are functional and genes belonging to the set } \Omega(j) \text{ are present}\}$$

$$\Omega(j) = \{\text{set of all genes when gene } G_j \text{ is aberrant, ie BRAF V600}\}$$

Thus if we admit a total of J states and we admit that states can transition and that each state has a growth mechanism as well as a flow and diffusion mechanism then we can determine the spatio-temporal values for the average densities simply by solving:

<sup>13</sup> One should examine the work by Tan et al. Although they do not pose the problem as we have they do start examining the work from the perspective of a state space with spatial and temporal complexity. Regrettably their focus is on the mathematics and not the phenomenology.

$$\frac{\partial \overline{n(x,t)}}{\partial t} = \widetilde{L} \overline{n(x,t)} + \Lambda \overline{n(x,t)}$$

where

$$\widetilde{L} = [\widetilde{L}_1, \dots, \widetilde{L}_N]$$

and

$$\Lambda = \begin{pmatrix} -\lambda_{11} & \lambda_{12} & \lambda_{13} \\ \lambda_{21} & -\lambda_{22} & \lambda_{23} \\ \lambda_{31} & \lambda_{32} & -\lambda_{33} \end{pmatrix}$$

Where the L values are operators reflecting diffusion and flow while  $\Lambda$  is a growth related value. We assume that states transition with certain probabilities, which can be ascertained phenomenologically. Thus we have:

Let

$$p(i, x, t) = P[n(x, t) \in S_i] = p_i(x, t)$$

$$S_i = \text{Gene State}(i)$$

Assume

$$p_j(x, t + \Delta) = P_{j,i} p_i(x, t)$$

and

$$P_{j,i} = \begin{cases} P_{i,i}(1 - \Delta) = \\ \dots \\ P_{j,i} \Delta = \lambda_{i,j} \\ \dots \end{cases}$$

And we can conclude:

$$P = \begin{bmatrix} p_{1,1} \cdot p_{2,1} \cdot p_{2,2} \cdot p_{2,3} \cdot 0 \dots 0 \dots 0 \dots 0 \\ 0 \dots p_{2,2} \cdot p_{2,5} \cdot p_{2,6} \cdot p_{2,7} \cdot 0 \dots 0 \dots 0 \\ 0 \dots 0 \dots p_{3,3} \cdot p_{3,5} \cdot p_{3,6} \cdot p_{3,7} \cdot 0 \dots 0 \\ 0 \dots 0 \dots 0 \dots p_{4,4} \cdot p_{4,5} \cdot p_{4,6} \cdot p_{4,7} \dots 0 \\ 0 \dots 0 \dots 0 \dots 0 \dots p_{5,5} \cdot 0 \dots 0 \dots p_{5,8} \\ 0 \dots 0 \dots 0 \dots 0 \dots 0 \dots p_{6,8} \dots 0 \dots p_{6,8} \\ 0 \dots 0 \dots 0 \dots 0 \dots 0 \dots 0 \dots p_{7,8} \dots p_{7,8} \\ 0 \dots p_{8,8} \end{bmatrix}$$

Is the totality of these transitions.

We have demonstrated how one may actually estimate or identify the value of these gross parameters for any cancer. The recent work of Gudem et al has shown also how this works. We have further demonstrated an example of this for PCa.

In a recent paper by Smith et al the authors have a model somewhat akin to what we presented several years before. Namely they state:

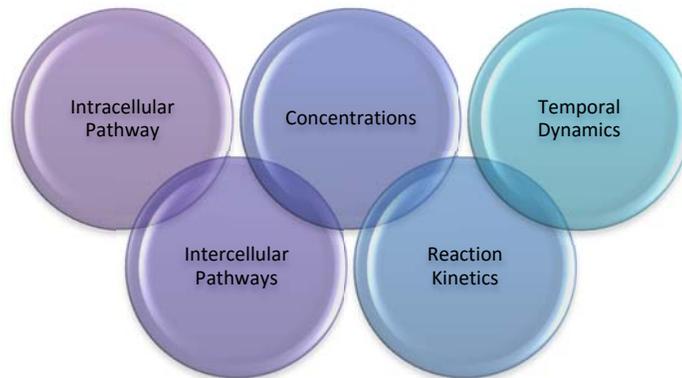
*By analyzing stem cell differentiation dynamics in many spatially defined microenvironments, we found strong stochastic behavior during the differentiation process. The composition of individual micropatterns varied dramatically over the time course of the differentiation. On smaller micropatterns, we observe that the most probable composition is either 100% stem cells or 100% differentiated cells.*

*Moreover, the physical dimensions of the microenvironment can influence stem cell differentiation in significant ways. We propose a stochastic differentiation model framework, and showed that stem cell differentiation probability is a strong function of local stem cell fraction within the immediate cell vicinity.*

*When stem cells are surrounded by other stem cells, the differentiation decision is slow; whereas, when differentiated cells surround stem cells, then the differentiation rate is faster by nearly threefold. This result is consistent with the previous proposal that there are feedback signals between differentiated cells and stem cells<sup>16</sup>. The proposed stochastic modeling framework should be applicable in other settings for understanding differentiation dynamics. We also found that the cell-cell interaction during differentiation is partially mediated by an E-cadherin governed signaling mechanism. Although, cell-cell interaction is not completely inhibited in our experimental conditions, we are able to manipulate, observe, and quantify variances in differentiation kinetics when the roles of cell contact in spatially confined domains are altered.*

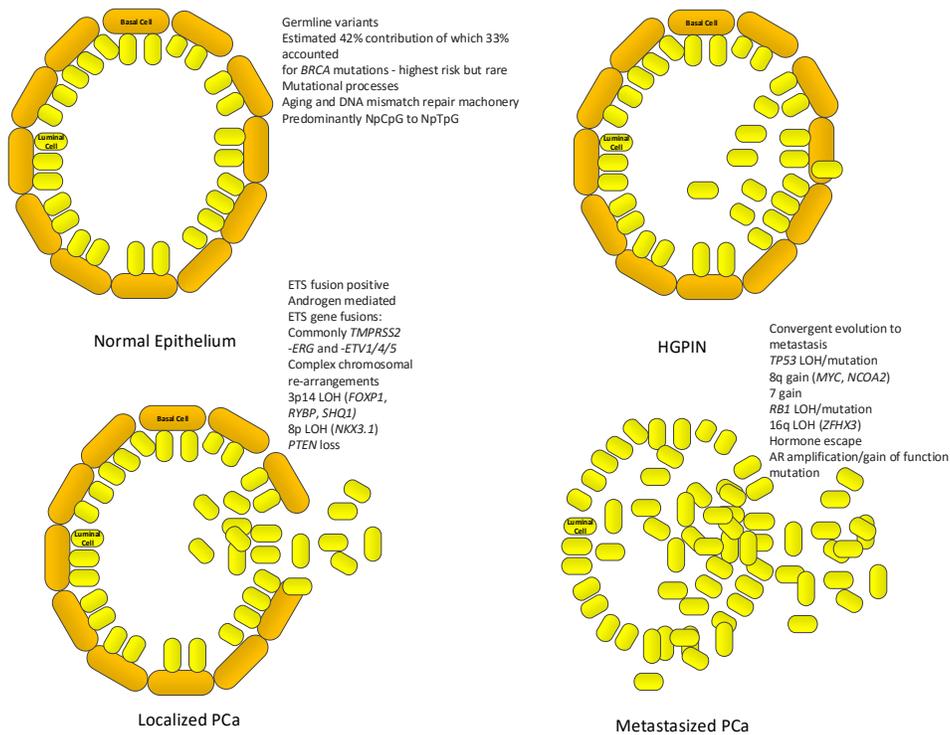
*It is possible that E-cadherin affects multiple sensing mechanisms in stem cells and there are redundant mechanisms that reinforce cell-cell interaction in stem cell niches.*

We have demonstrated a model containing the key elements shown below.



In a sense this is also what Smith et al are trying to develop. We believe that by examining the cancer in a large scale stochastic manner we can utilize current knowledge and develop new understanding. The cancer in our model is considered almost as a separate entity existing in a human body, and it uses the characteristics of its carrier, the human, to facilitate its growth. The human is in homeostasis and the cancer entity is competing with the human for resources to survive and prosper.

Considerable understanding on the details of PCa cell complexity has become available recently (see Gudem et al and Mitchell and Neal) From Mitchell and Neal we have the following Figure:



The question then is: in this phenomenological complex, what is the role of the CCO and CSC? One can consider the gene expression changes, due to mutations, epigenetic factors or otherwise, then combined with ligands that prompt pathways to operate and for the gene expression changed cell to proliferate and/or produce other growth factors and/or impact the extracellular matrix changing adhesion to see this new "organism", the tumor mass, to spread and alter itself to maximize its growth potential.

In essence we have a Darwinian sub-process allowing this new "organism" to prosper. To counter this process, we must identify the control mechanisms, all, not just a few, and then suppress them.

## 8 REFERENCES

1. Agarwal, S., et al, Identification of Different Classes of Luminal Progenitor Cells within Prostate Tumors, 2015, *Cell Reports* 13, 2147–2158
2. Ailles, L and I. Weissman, Cancer stem cells in solid tumors, *Current Opinion in Biotechnology* 2007, 18:460–466
3. Alberts et al, *Molecular Biology of the Cell*, Garland (New York) 2010.
4. Bogdan, P. et al, Heterogeneous Structure of Stem Cells Dynamics: Statistical Models and Quantitative Predictions, *SCIENTIFIC REPORTS* 4 : 4826, 2014
5. Burgess, R., *Stem Cells*, Wiley (New York) 2016.
6. Ciccia, A., S. Ellege, The DNA Damage Response: Making It Safe to Play with Knives, *Molecular Cell* 40, October 22, 2010
7. Clevers, The Cancer Stem Cell: Premises, Promises, and Challenges, *Nature Med* V 17 March 2011, p 313.
8. Dalerba, P., Robert W. Cho, and Michael F. Clarke, Cancer Stem Cells: Models and Concepts, *Annu. Rev. Med.* 2007. 58:267–284.
9. Deleyrolle, et al, Determination of Somatic and Cancer Stem Cell Self-Renewing Symmetric Division Rate Using Sphere Assays, *PLOS* January 2011.
10. Dhawan, A., et al, Tumour Control Probability in Cancer Stem Cells Hypothesis, *PLOS* May 2014 Vol 9 Issue 5
11. Dingli, D., J. Pacheco, Stochastic dynamics and the evolution of mutations in stem cells, *BMC Biology* 2011, 9:41
12. Dingli, et al, Stochastic Dynamics of Hematopoietic Tumor Stem Cells, *Cell Cycle* 2007; Vol. 6 Issue 4
13. Dreesen, O., A., Brivanlou, Signalling Pathways in Cancer and Embryonic Stem Cells, *Stem Cell Rev*, 2007.
14. Dubrovskaya, A et al, The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations, *PNAS*, Jan 2009 V 106 N 1
15. Fang et al, A Tumorigenic Subpopulation with Stem Cell Properties in Melanomas, *Cancer Res* 2005; 65: (20). October 15, 2005
16. Floor, S., Cancer Cells in Epithelial to Mesenchymal Transition and Tumor Propagating Cancer Stem Cells: Distinct, Overlapping or Same Populations, *Oncogene* V 30, pp 4609-4621, 2011.
17. Goldstein, A. et al, Identification of a Cell of Origin for Human Prostate Cancer, *Science*, 2010 V 329, pp 568-571.
18. Goss, K., M. Kahn. *Targeting the Wnt Pathway in Cancer*, Springer, New York, 2011.

19. Gundem et al, The evolutionary history of lethal metastatic prostate cancer, *Nature* 2015.
20. Guo, W., et al, *Cancer Stem Cells*, *Pediatric Research* (2006) 59, 59R–64R
21. Gupta, P, et al, *Cancer Stem Cells*, *Mirage or Reality*, *Nature Medicine*, 2009, p. 1010.
22. Gupta, P., et al, Identification of Selective Inhibitors of Cancer Stem Cells by High Throughput Screening, *Cell*, V 138, pp 645-659, Aug 2009.
23. Hong, D., A Fountain of Cancer, *Sci Transl Med* 4 May 2011: Vol. 3, Issue 81, p. 81
24. Hurt et al, CD44<sup>+</sup>CD24<sup>-</sup>prostate cells are early cancer progenitor/stem cells that provide a model for patients with poor prognosis, *British Journal of Cancer* (2008) 98(4), 756 – 765
25. Hurt, E., et al, CD44<sup>+</sup>CD44<sup>-</sup> Prostate Cells are Early Cancer Progenitor Stem Cells that Provide a Model for Patients with Poor Prognosis, *Brit Jrl Can* 2008 pp 756-765.
26. Jeet, et al, Modeling prostate cancer: a perspective on transgenic mouse models, *Cancer Metastasis Rev* (2010) 29:123–142.
27. Jordan et al, *Cancer Stem Cells*, *NEJM*, Sept 21 2006.
28. Jordan, C., et al, *Cancer Stem Cells*, *NEJM* 2006.
29. Karsten, U., S. Goletz, What makes cancer stem cell markers different?, *SpringerPlus* 2013, 2:301
30. Lawson D., et al, Basal epithelial stem cells are efficient targets for prostate cancer initiation, *PNAS*, Feb 2010, B107 N 6.
31. Lawson, D., O. Witte, Stem Cells in Prostate Cancer Initiation and Progression, *Jrl Clin Inv*, 2007 pp 2044-2050.
32. Lawson, Witte, Stem cells in prostate cancer initiation and progression, *The Journal of Clinical Investigation* <http://www.jci.org> Volume 117 Number 8 August 2007
33. Leder, K., et al, The Therapeutic Implications of Plasticity of the Cancer Stem Cell Type, *PLOS One* V 5 Dec 2010.
34. Lobo, N., Yohei Shimono, Dalong Qian, and Michael F. Clarke, The Biology of Cancer Stem Cells, *Annu. Rev. Cell Dev. Biol.* 2007. 23:675–99.
35. McGarty, T., Prostate Cancer: A Systems Approach, see [https://www.researchgate.net/publication/264960277\\_Prostate\\_Cancer\\_A\\_Systems\\_Approach](https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach)
36. Mendelsohn, M., Cell Cycle Kinetics and Mitotically Linked Chemotherapy, *Can Res* V 29 pp 2390-2393, 1969.
37. Mitchell, T., D. Neal, The genomic evolution of human prostate cancer, *British Journal of Cancer* (2015) 113, 193–198
38. Moscatelli, D., F. Wilson, PINing Down the Origin of Prostate Cancer, *Science Translational Medicine*, 4 August 2010 Vol 2 Issue 43 pp 43-48.
39. Navin, N., J., Hicks, Tracing Tumor Lineage, *Molec Onco*, 2010.
40. Ni, et al, Cancer Stem Cells in Prostate Cancer Chemoresistance, *Current Cancer Drug Targets*, 2014, 14, 225-240.

41. Pavlovic, M., B. Balint, Bioengineering and Cancer Stem Cell Concept, Springer (New York) 2015
42. Perego et al, Heterogeneous Phenotype of Human Melanoma Cells with In Vitro and In Vivo Features of Tumor-Initiating Cells, Journal of Investigative Dermatology (2010), Volume 130
43. Qin, J., et al, The PSA-<sup>-</sup>/lo Prostate Cancer Cell Population Harbors Self-Renewing Long-Term Tumor-Propagating Cells that Resist Castration, Cell Stem Cell, Volume 10, Issue 5, 556-569, 4 May 2012.
44. Quintana, E., et al, Efficient tumour formation by single human melanoma cells, Nature, Vol 456, 4 December 2008
45. Rajasekhar, V., Cancer Stem Cells, Wiley (New York) 2014.
46. Reya, T., et al, Stem Cells, Cancer and Cancer Stem Cells, Nature, V 414 Nov 2001.
47. Reya, T., H. Clevers, Wnt Signalling in Stem Cells and Cancer, Nature V 434 p 843, 2005.
48. Risbridger, G., R. Taylor, Regulation of Prostatic Stem Cells by Stromal Niche in Health and Disease, Endocrinology 2008 149:4303-4306.
49. Roesch et al, A Temporarily Distinct Subpopulation of Slow-Cycling Melanoma Cells Is Required for Continuous Tumor Growth, Cell 141, 583–594, May 14, 2010
50. Rosen, J., C. Jordan, The Increasing Complexity of the Cancer Stem Cell Paradigm, Science, 26 JUNE 2009 VOL 324.
51. Rycaj, K., D. Tang, Cell-of-Origin of Cancer versus Cancer Stem Cells: Assays and Interpretations, Cancer Res October 1, 2015 75; 4003
52. Shahriyari, L., N. Komarova, Symmetric vs. Asymmetric Stem Cell Divisions: An Adaptation against Cancer?, PLOS Oct 2013 Vol 8 Issue 10
53. Smith, Q., et al, Stochasticity and Spatial Interaction Govern Stem Cell Differentiation Dynamics, Scientific Reports 5:12617, July 2015.
54. Sole, R., Cancer Stem Cells as the Engine of Tumor Progression, Journal of Theoretical Biology 253 (2008) 629– 637.
55. Soltysova, et al, Cancer stem cells, Neoplasma, Vol.52, p.435-440, 2005
56. Stukalin, E., et al, Age-dependent stochastic models for understanding population fluctuations in continuously cultured cells, Journal of the Royal Society, 2013.
57. Tan, W., et al, A stochastic and state space model for tumor growth and applications, Comp and Math Meth in Med, Vol 10, N 2, June 2009.
58. Tang, D., et al, Prostate Cancer Stem/Progenitor Cells: Identification, Characterization, and Implications, MOLECULAR CARCINOGENESIS 46:1–14 (2007).
59. Visvader, J., Cells of Origen in Cancer, Nature, V 469 20 Jan 2011.
60. Wang, Z., M. Shen, Revisiting the concept of cancer stem cells in prostate cancer, Oncogene (2011) 30, 1261–1271.

61. Weinberg, R., *Biology of Cancer* (1<sup>st</sup> Ed), Garland (New York) 2008.
62. Weinberg, R., *The Biology of Cancer*, 2<sup>nd</sup> Ed Garland (New York) 2014.
63. Werbowetski-Ogilvie, T., et al, Evidence for the Transition of Neoplastic Properties From Transformed to Normal Human Stem Cells, *Oncogene*, V 30, pp 4632-4644, 2011.
64. White, A., W. Lowry, Refining the role for adult stem cells as cancer cells of origin, *Trends in Cell Biology*, January 2015, Vol. 25, No. 1
65. Wolfram Kleeberger, G. Steven Bova, Matthew E. Nielsen, et al., Roles for the Stem Cell–Associated Intermediate Filament Nesting in Prostate Cancer Migration and Metastasis, *Cancer Res* 2007;67:9199-9206. Published online October 1, 2007.
66. Zhang et al, Stem cell and neurogenic gene-expression profiles link prostate basal cells, to aggressive prostate cancer, *Nature Communications*, Feb 2016.
67. Zhang, B., P. Wolynes, Stem Cell Differentiation as a Multi-body Problem, *PNAS*, July 2014 V 111 N 28

## 9 RELATED WHITE PAPERS

The following is a list of related White Papers that provide details expanding on what we have included herein.

- No. 135 Seven Types of PCa (April 2016)
- No. 134 CHK2, AR, and PCa (February 2016)
- No. 133 LY6 and Prognostic Markers (February 2016)
- No. 132 More PCa Markers (February 2016)
- No. 131 pro-NPY and PCa (February 2016)
- No. 130 SPOP and Prostate Cancer (November 2015)
- No. 129 TRUST BUT VERIFY: The Value of PSA (November 2015)
- No. 128 Prostate Cancer Prognostic Tests: PRE AND POST DIAGNOSIS (October 2015)
- No. 127 STAT3 and PCA: OF MICE AND MEN (August 2015)
- No.126. Prostate Cancer Metastasis: Some Simple Cases (July 2015)
- No. 125 CRISPR and Cancer: Revised (April 2015)
- No. 124 CRISPR Cas9: A Genomic Tool (April 2015)
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- No. 122 MDS and DNMT1 Pathway Control (January 2015)
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- No. 117 SPDEF, ETS Transcription Factors and PCa (October 2014)
- No. 116 Methylation, Prostate Cancer, Prognostics (August 2014)
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- No. 108 Cancer Cell Dynamics (January 2014)
- No. 107 Prostate Cancer Genetic Metrics (January 2014)
- No. 106 Divergent Transcription (December 2013)
- No. 104 Prostate Cancer and Blood Borne Markers (December 2013)
- No. 103 Prostate Cancer Indolence (December 2013)

- No. 102 MDS and Methylation (August 2013)
- No. 101 Exosomes and Cancer (August 2013)
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- No. 95 MER Tyrosine Kinase Receptors and Inhibition (June 2013)
- No. 93 Cancer Cell Dynamics Methylation and Cancer (April 2013)
- No. 91 Methylation and Cancer (March 2013)
- No. 88 Extracellular Matrix vs. Intracellular Pathways
- No. 87 Prostate Cancer Prognostic Markers
- No. 86 Cancer Models for Understanding, Prediction, and Control
- No. 85 Prostate Cancer Stem Cells
- No. 84 Epistemology of Cancer Genomics
- No. 83 Prostatic Intraepithelial Neoplasia
- No 82 Prostate Cancer: Metastatic Pathway Identification (February 2011)
- No 81 Backscatter Radiation and Cancer (December 2010)
- No 80 PSA Evaluation Methodologies (December 2010)
- No 79 The PSA Controversy (November 2010)

