ERG, TMPRSS2 and Prostate Cancer

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White Paper No110
January 2014

We examine some recent research on ERG in HGPIN. The recent work considers the merged TMPRSS2:ERG fusion and its presence in HGPIN as highly prognostic of aggressive PCa. We discuss the considerable body of work on the topic as it stands and then examine some dimensions worth considering in light of know regression of HGPIN.

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1 INTRODUCTION

Prostate Cancer is a complex disease. One has estimated that well in excess of those found to have PCa, even at autopsy, have an indolent form. The PCa remains in the capsule and the PSA may remain elevated but does not progress. We have recently examined a multiple set of gene profiles that putatively separate indolent and aggressive forms. The profiles are totally distinct. The development of an elevated PSA, and more often an increase in PSA velocity may warrant a biopsy. Often the biopsy, assuming a high density core biopsy of 24 or more cores by an expert Urologist, yields extensive HGPIN. Typically a second biopsy after 9 months is performed and a non-inconsequential percent, 25%, may have progressed to PCa. At the other extreme a small but not inconsequential group upon a second high density core biopsy are found to be HGPIN free. There are many clinical questions arising from these observations. One is; what genetic test on HGPIN is or value. We examine a recent paper to discuss that issue.

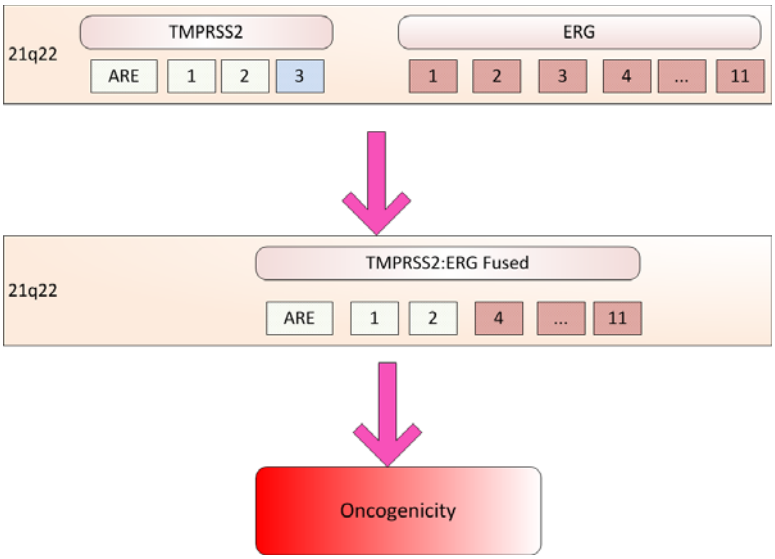
ERG is also known as The "**E**TS **R**elated **G**ene". The ETS family is the "**E**rythroblast **T**ransformation **S**pecific" gene, which is a family of genes[1]. The ETS family of genes is highly important in the formation and maintenance of tissues. ETS factors, gene products, are phosphorylated to become active and this is done by various MAP kinases which we have discussed before. Thus ETS activation is linked closely to MAP kinase pathway activation.

ERG produces a protein what is a transcriptional regulator in the nucleus. ERG is also known for its movement from its base location 21q.22.3 and binds to TMPRSS2 at 21q22.3[2][3]. This is effect a gene fusion and is frequently found in Androgen Resistant PCa. We demonstrate this change below, by showing the exons of TPMRSS2 and ERG and how they get fused producing a new gene with deleted exons but producing an oncogene product. In essence TMPRSS2 is androgen activated and the ERG gene becomes a promoter more fully activated via the TMPRSS2 association. In a sense it is not a true translocation, namely the genes have not been moved from the original chromosome like that in CML but a section is removed and they are joined.

[1] Marks et al, pp 404-406.

[2] For TMPRSS2 see http://www.ncbi.nlm.nih.gov/gene/7113

[3] For ERG see http://www.ncbi.nlm.nih.gov/gene/2078



In addition there has been extensive discussion of HGPIN, high grade prostatic intraepithelial neoplasia, which is a confined excess growth of cells in the prostate glands. HGPIN has been associated with progression to PCa. However, as we have shown before, there are times when HGPIN resolves itself, especially after extensive high density core biopsies. The answer to why such a resolution occurs is open to speculation.

What has instigated this study was the Press Release ascribing to ERG in an HGPIN environment a significant prognostic value. Namely the release states[4]:

Investigators found that 53 percent of men whose prostate biopsies showed expression of ERG protein developed invasive prostate cancer, compared to 35 percent of men whose biopsies were ERG-negative. All of the biopsies were classified as having high-grade prostatic intraepithelial neoplasia (HGPIN), which are lesions that may or may not morph into cancer.

Actually this could have been better written. The class that the 53% was found in was all HGPIN. The ERG expression was the ERG expression found in a TMPRSS2:ERG fusion gene, not the ERG by itself, unfused. Frankly having a fusion product is in itself prognostic as has been demonstrated for the last few years. It is recognized that the fusion acts as an oncogene and presents a poor prognosis. Thus, although this paper does confirm a class of facts, many of them had already been confirmed by other researchers as well.

The general concern discussed herein is the whole construct of prognostic genetic tests. This test has clear and unambiguous validity based upon well know pathway factors. As we have discussed elsewhere, the tests that are based upon weighted genetic vectors reduced to a single metric may be prognostic but they are not based upon a clear genetic structure of cause and effect.

[4] http://www.sciencecodex.com/protein_in_prostate_biopsies_signals_increased_cancer_risk-124069

2 ERG, HGPIN AND PCA

To date there has been little evidence that ERG by itself, that is not fused as shown above, has any significance. However ERG fused with TMPRSS2 does have significant influence. TMPRSS2 is androgen activated and in the fusion some nucleic acids are cut from ERG and the resulting product appears to be oncogenic. In addition specific gene expression in HGPIN is also limited, and generally the fusion factor does not occur until androgen resistant PCa has been established. However, in a recent Press Release the Researchers work is reported as follows[5]:

Researchers at Weill Cornell Medical College are looking at specific changes within prostate cells, from an initial biopsy, to determine which men have a higher risk of prostate cancer development and need repeat biopsies or other types of monitoring. This study starts to fill in the picture for about 10 percent of prostate biopsies.

Investigators found that 53 percent of men whose prostate biopsies showed expression of ERG protein developed invasive prostate cancer, compared to 35 percent of men whose biopsies were ERG-negative. All of the biopsies were classified as having high-grade prostatic intraepithelial neoplasia (HGPIN), which are lesions that may or may not morph into cancer.

The findings mean that potentially thousands of men a year -- those with ERG-positive HGPIN biopsies -- may benefit from increased surveillance and early treatment of prostate cancer, while those whose HGPIN biopsies come back ERG-negative may be able to avoid unnecessary future biopsies, ...

Before continuing, it should be noted as we do later that what is measured is the ERG:TMPRSS2 fusion and not ERG expression alone. We return to this in a bit.

"What this study shows is that not all HGPIN is equal -- that is, clinically significant," Rubin adds. "When confirmed in larger studies, testing for ERG in these precancerous lesions may change clinical practice in how men are evaluated with abnormal biopsies and may lead to earlier cancer detection.".....

"About 1.3 million men will have a prostate biopsy each year, and this biopsy will be HGPIN-positive in more than 100,000 men. While HGPIN can be a precursor to prostate cancer, it's not really clear how often men with HGPIN will develop prostate cancer, or how quickly," Dr. Barbieri says....

"Now we have shown that men with HGPIN that expressed ERG were at higher risk for developing prostate cancer. This finding could potentially give urologists a better idea of who needs a repeat biopsy, and who is more likely to be at lower risk," ....

Cancer increases over time in ERG-positive biopsies. The prostate cancer-specific ERG protein overproduction results from the fusion of two genes, leading to a chimeric gene referred to as

[5] http://www.sciencecodex.com/protein_in_prostate_biopsies_signals_increased_cancer_risk-124069

TMPRSS2-ERG that is present in over half of the 230,000 prostate cancers diagnosed in the United States each year. …

Scientists believe approximately 15 percent of HGPIN express ERG. They have been examining the presence of ERG protein in prostate biopsies as a potential marker of future prostate cancer -- hence the development of tissue and blood ERG tests -- but studies have been small and their results unclear….

Investigators found ERG expression in about 11 percent of participants' biopsies, and over time, increasing numbers of these patients developed invasive prostate cancer -- about 15 percent within the first year of the three year-trial, 37 percent at year two, and 53 percent at year three.

Now from the above referenced paper by Park et al we have the following[6]:

*High-grade prostatic intraepithelial neoplasia (HGPIN) is considered a precursor lesion of prostate cancer (PCa). The predictive value of **ERG gene fusion** in HGPIN for PCa was interrogated as a post hoc analysis in the context of a randomized clinical trial.*

First, what is important to note is that the study examined the ERG fusion, not just ERG expression. Namely, it is already known that the ERG:TMPRSS2 fusion is found commonly in advanced PCa and thus one would suspect that if such a fusion is found in HGPIN that a move to aggressive development would be expected. It is also of note we have examined this issue in detail and have found that although there is some correlation with HGPIN and PCa there is not a necessary progression. As noted later in their report, in just a bit more than a third in three years were converted over to PCa. One of the questions one always asks is what was the PSA and PSA velocity that were present at the commencement of biopsies. Also what specifically precipitated the biopsy? They continue:

… randomly assigned 1,590 men with biopsy-diagnosed HGPIN to receive toremifene or placebo for 3 years or until a diagnosis of PCa was made on prostate biopsy. As part of this phase III clinical trial, a central pathologist evaluated biopsies of patients with isolated HGPIN at baseline and 12, 24, and 36 months of follow-up. ERG immunohistochemistry was performed on biopsies from 461 patients and evaluated for protein overexpression.

First the use of toremifene as an estrogen suppressant in androgen resistant PCa appears as a cofactor in this analysis[7].

…ERG expression was detected in 11.1% of patients (51 of 461 patients) with isolated HGPIN. In the first year and during the 3-year clinical trial, 14.7% and 36.9% of 461 patients were diagnosed with PCa, respectively.

[6] http://jco.ascopubs.org/content/early/2013/12/02/JCO.2013.49.8386.abstract

[7] From the NCI: A nonsteroidal triphenylethylene antiestrogen. Chemically related to tamoxifen, toremifene is a selective estrogen receptor modulator (SERM). This agent binds competitively to estrogen receptors, thereby interfering with estrogen activity. Toremifene also has intrinsic estrogenic properties, which are manifested according to tissue type or species. See http://www.cancer.gov/drugdictionary?cdrid=41103

One assumes that in the initial 11.1% of the initial patients had HGPIN and elevated ERG and that at year 1 and 3 that in that group 14.7% had PCa in year 1 and 36.9% in year 3.

Patients with ERG expression were more likely to develop PCa, with 27 (53%) of 51 ERG-positive and 143 (35%) of 410 ERG-negative patients experiencing progression to PCa (P = .014, Fisher's exact test). ERG expression was not associated with age, baseline PSA, Gleason score, or tumor volume...This study underscores the necessity of more stringent follow-up for men with HGPIN that is also positive for ERG overexpression. Clinicians should consider molecular characterization of HGPIN as a means to improve risk stratification.

The above is the final set of numbers. Namely 53% of the ERG positive progressed whereas only 35% of the non-ERG progressed. One question that we have asked again and again is what percent had the HGPIN regress totally? We have observed that phenomenon. In fact, in those samples, albeit small, the regression remained after three years with a stable PSA.

We now examine some of the ERG and TMPRSS2 issues in some detail to gain a broader perspective of this effect.

3 ERG

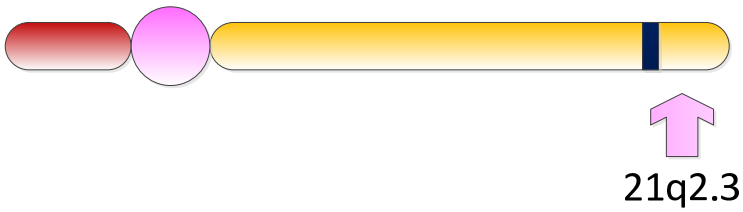
We begin with a study of ERG. As NCBI states[8]:

This gene encodes a member of the erythroblast transformation-specific (ETS) family of transcriptions factors. All members of this family are key regulators of embryonic development, cell proliferation, differentiation, angiogenesis, inflammation, and apoptosis. The protein encoded by this gene is mainly expressed in the nucleus. It contains an ETS DNA-binding domain and a PNT (pointed) domain which is implicated in the self-association of chimeric oncoproteins.

This protein is required for platelet adhesion to the subendothelium, inducing vascular cell remodeling. It also regulates hematopoesis, and the differentiation and maturation of megakaryocytic cells.

This gene is involved in chromosomal translocations, resulting in different fusion gene products, such as TMPSSR2-ERG and NDRG1-ERG in prostate cancer, EWS-ERG in Ewing's sarcoma and FUS-ERG in acute myeloid leukemia. Multiple alternatively spliced transcript variants encoding different isoforms have been identified.

ERG has a cytogenetic Location: 21q22.3[9] as shown in the Figure below. It is very close to TMPRSS2 and thus upon mitotic change we can see how a cross over and linkage could occur. However as we shall discuss later this crossover resulting in the translocation seems consistent in all cases in which it occurs and has an effect upon PCa. One might expect a more random set of changes but perhaps it is just this specific one which survives mitosis and goes on to incite and enhance a PCa.

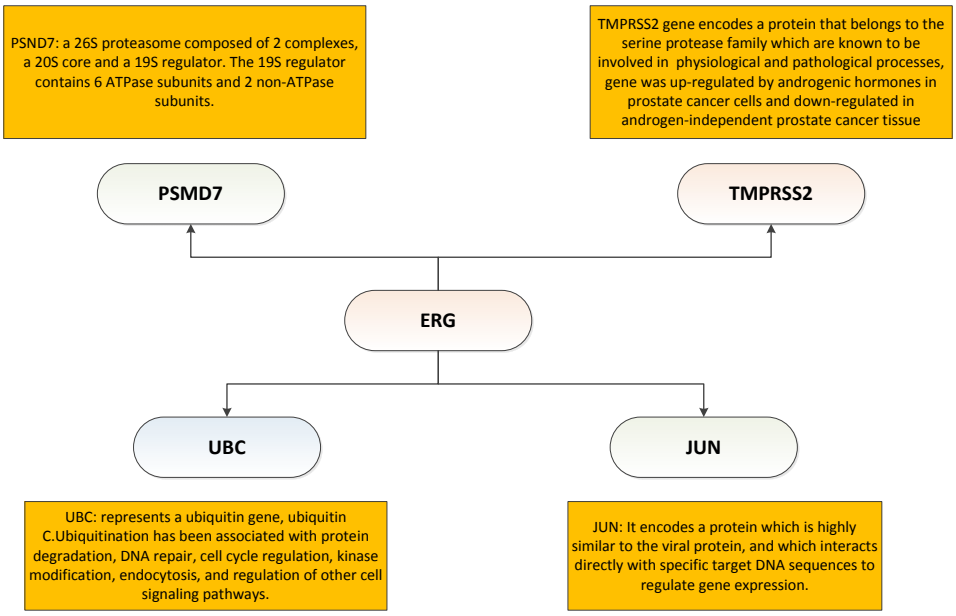


The following is a pathway connection between ERG and four other key players[10]. One should remember the MAP kinase activators of ERG and that ERG is general located in the nucleus. Thus from ERG downward we have the relationships as shown below.

[8] http://www.ncbi.nlm.nih.gov/gene/2078

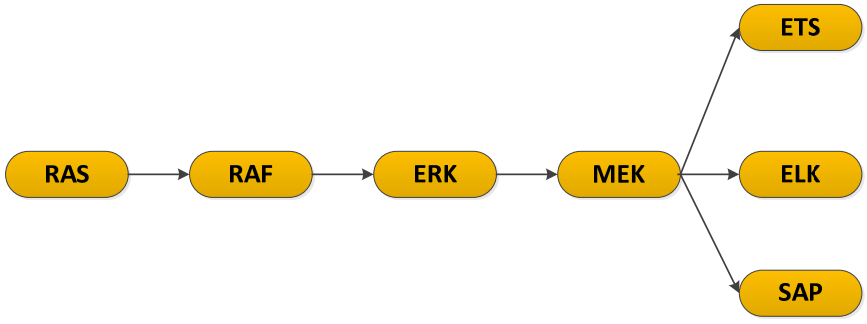
[9] http://ghr.nlm.nih.gov/gene/ERG

[10] http://string-db.org/newstring_cgi/show_network_section.pl?taskId=6sWF4IVXYYY1&allnodes=1



http://string-db.org/newstring_cgi/show_network_section.pl?taskId=6sWF4IVXYYY1&allnodes=1

For the relationships above we have the following Figure from Weinberg of upward activation paths. This is as we had discussed is a kinase activated path. Also ERG generally is retained as a promoter in the nucleus.



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

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

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

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

Further descriptions of ERG and its functioning is contained in Yuan et al who state:

ETS-related gene (ERG) is a member of the ETS transcription factor family. Our previous studies have shown that ERG expression is highly enriched in endothelial cells (EC) both in vitro and in vivo. ERG expression is markedly repressed in response to inflammatory stimuli. It has been shown that ERG is a positive regulator of several EC-restricted genes including VE-cadherin, endoglin, and von Willebrand factor, and a negative regulator of other genes such as interleukin (IL)-8 and intercellular adhesion molecule (ICAM)-1.

In this study we have identified a novel role for ERG in the regulation of EC barrier function. ERG knockdown results in marked increases in EC permeability. This is associated with a significant increase of stress fiber and gap formation in EC.

Furthermore, we identify CLDN5 as a downstream target of ERG in EC. Thus, our results suggest that ERG plays a pivotal role in regulating EC barrier function and that this effect is mediated in part through its regulation of CLDN5 gene expression.

Additional discussion of ERG is in Flajollet et al who state:

Osteopontin (OPN) is an extracellular matrix glycophosphoprotein that plays a key role in the metastasis of a wide variety of cancers. The high level of OPN expression in prostate cells is associated with malignancy and reduced survival of the patient. Recent studies on prostate cancer (PCa) tissue have revealed recurrent genomic rearrangements involving the fusion of the 50 untranslated region of a prostate-specific androgen-responsive gene with a gene coding for transcription factors from the ETS family.

The most frequently identified fusion gene is TMPRSS2:ERG, which causes ERG protein overexpression in PCa cells. ERG is a transcription factor linked to skeletogenesis.

This study was designed to test whether ERG and the product of the TMPRSS2:ERG fusion gene modulate OPN gene expression in PCa cells. To characterize ERG and TMPRSS2:ERG transcriptional activity of OPN, we focused on ETS binding sites (EBS) localized in conserved regions of the promoter. Using in vitro and in vivo molecular assays, we showed that ERG increases OPN expression and binds to an EBS (nt 115 to 118) in the OPN promoter. Moreover, stable transfection of prostate tumor cell lines by TMPRSS2:ERG upregulates endogenous OPN expression. Finally, in human prostate tumor samples, detection of the TMPRSS2:ERG fusion gene was significantly associated with OPN overexpression.

Taken together, these data suggest that OPN is an ERG-target gene in PCa where the abnormal expression of the transcription factor ERG, due to the TMPRSS2: ERG fusion, disturbs the expression of genes that play an important role in PCa cells and associated metastases.

Finally there is a discussion of how ERG can regulate the Wnt pathway genes (See Appendix 1 for a discussion of the Wnt pathway as relates to PCa). In a recent paper by Wu et al the authors state:

ERG regulates Wnt pathway genes in prostate cancer. .. heatmap of ERG-binding sites around Wnt pathway gene promoters. ERG and AR ChIP-Seq was conducted in VCaP cells as previously described... Heatmap shows ChIP-Seq read intensity around the transcription start site of Wnt pathway genes. Genes were ranked by the height of ChIP-Seq peaks. ... ERG occupancy near the promoters of representative Wnt pathway genes. ERG ChIP-Seq was conducted in VCaP cells as in A. Chromosomal positions are shown at the top and the gene structure at the bottom. Black arrow indicates transcription start site and direction. ... ERG directly binds Wnt ligand gene promoters.

Also summarized from Wu at el[11]

[11] http://cancerres.aacrjournals.org/content/73/19/6068.figures-only

Gene	*Regulation*
Wnt	*ERG regulates Wnt pathway genes in prostate cancer. ERG directly binds Wnt ligand gene promoters*
	ERG directly induces Wnt ligand gene expression.
AR	*AR is a control gene that has been previously shown inhibited by ERG*
PSA ***TMPRSS2*** ***PLAT*** ***PLAU***	*PSA and TMPRSS2 have been previously shown inhibited by ERG, whereas PLAT and PLAU were induced by ERG. D, WNT3A protein is increased by ectopic ERG.*
β-catenin	*ERG increases active β-catenin in prostate cancer.*
LEF1	*ERG directly regulates LEF1 expression and function*
WNT/LEF1	*WNT/LEF1 signaling mediates oncogenic properties of ERG*
EMT	*ERG and LEF1 induces EMT and are dysregulated in prostate cancer*

As Cai et al state:

Fusion of the androgen receptor-regulated (AR-regulated) TMPRSS2 gene with ERG in prostate cancer (PCa) causes androgen-stimulated overexpression of ERG, an ETS transcription factor, but critical downstream effectors of ERG-mediating PCa development remain to be established. Expression of the SOX9 transcription factor correlated with TMPRSS2:ERG fusion in 3 independent PCa cohorts, and ERG-dependent expression of SOX9 was confirmed by RNAi in the fusion-positive VCaP cell line. SOX9 has been shown to mediate ductal morphogenesis in fetal prostate and maintain stem/progenitor cell pools in multiple adult tissues, and has also been linked to PCa and other cancers.

SOX9 overexpression resulted in neoplasia in murine prostate and stimulated tumor invasion, similarly to ERG. Moreover, SOX9 depletion in VCaP cells markedly impaired invasion and growth in vitro and in vivo, establishing SOX9 as a critical downstream effector of ERG. Finally, we found that ERG regulated SOX9 indirectly by opening a cryptic AR-regulated enhancer in the SOX9 gene. Together, these results demonstrate that ERG redirects AR to a set of genes including SOX9 that are not normally androgen stimulated, and identify SOX9 as a critical downstream effector of ERG in TMPRSS2:ERG fusion–positive PCa

4 TMPRSS2

TMPRSS2 is a gene which is activated by androgens and which produces transmembrane serine proteases. Serine proteases are a class of enzymes which cleave proteins.

We briefly discuss TMPRSS2 as a gene and gene product as a standalone. Unfused it has regulatory properties but fused it become an aggressive assist in activating the remaining portion of ERG. As Chen et al state:

TMPRSS2 is an androgen responsive gene that encodes a type II transmembrane serine protease (TTSP). The members of the TTSP family share common protein structures including a transmembrane domain at the N terminus, linker regions with a variety of protein–protein interaction domains, and a canonical serine protease domain at the C terminus.

TTSPs have been found to play important roles in the development and homeostasis of mammals, and the aberrant expression of TTSP genes are reported to contribute to the etiology of several human disorders, including cancer.

The importance of TMPRSS2 in vivo remains unclear because homozygous TMPRSS2-null mice are essentially phenotypically normal.

However, TMPRSS2 was reported to regulate epithelial sodium channel (ENaC) activity in vitro, implying a possible role in epithelial sodium homeostasis. TMPRSS2 may play a role in angiogenesis and tubulogenesis in microvesicular endothelial cells, potentially modulating several aspects of prostate tumor biology. In addition to its proteolytic activity, TMPRSS2 may also serve as a cell receptor, conducting external signaling or interacting with the extracellular matrix through its extracellular protein binding domains.

Overexpression of TMPRSS2 has been demonstrated in poorly differentiated prostate cancer with significant increase in the mRNA level.

The discussion above demonstrates that TMPRSS2 has multiple functions in cell regulation. The ultimate fusion of TMPRSS2 with ERG would thus inhibit the function of the gene product by itself. It is not clear that the loss of the functionality is a key factor in developing aggressive PCa. Clearly the androgen activation is a key factor in normal operation and the question is why does the gene lose this functionality?

5 TMPRSS2:ERG FUSION

The fusion of TMPRSS2 and ERG is common in PCa. In fact it is common in HGPIN as has been discussed earlier. For example as discussed in Cai et al state:

The androgen receptor (AR) plays a central role in prostate cancer (PCa) development, and its transcriptional functions are partially or fully restored in the tumors that relapse after androgen deprivation therapy (castration-resistant prostate cancer, CRPC). The role of AR in PCa was further strengthened by the discovery of recurrent genomic rearrangements that result in AR-driven overexpression of ETS family transcription factor proto-oncogenes, and in particular the v-ets erythroblastosis virus E26 oncogene homolog, ERG.

In approximately half of primary PCa cases, a gene fusion between the 5' untranslated region of the androgen-regulated TMPRSS2 gene and an exon in the ERG gene results in androgen-regulated high-level expression of a transcriptionally active, N-terminal–truncated ERG protein (amino acids 1–44 being deleted in the most common fusion).

This fusion is an early event, as it is found in precursor prostatic intraepithelial neoplasia (PIN) lesions located adjacent to TMPRSS2:ERG fusion–positive cancers.

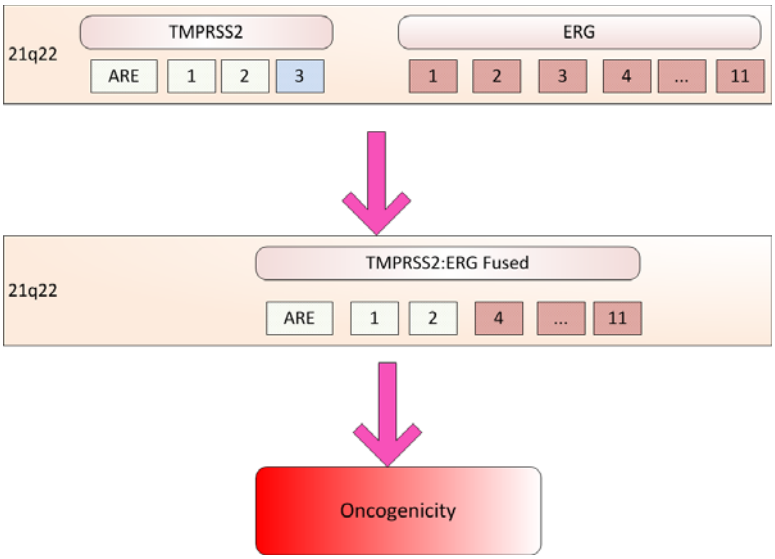
The fusion gene is also highly expressed in CRPC, indicating that overexpressed ERG contributes to PCa development and progression

Note that the authors indicate that the fusion is quite evident in HGPIN and thus the paper in question seems to further analyze that factor, not necessarily provide some initial discovery.

5.1 FUSION

We now discuss the fusion of the genes. Note also that they state that the ERG gene still functions but in a truncated manner. Namely, that the amino acids 1 to 44, the lead amino acids of normal ERG are truncated and the remaining amino acids are present. The following demonstrates that effect. The fusion of TMPRSS and ERG is another genetic promoter of PCa and it is the primary translocation gene seen. We repeat a Figure presented earlier to demonstrate this effect[12].

[12] See Rubin and Chinnaiyan



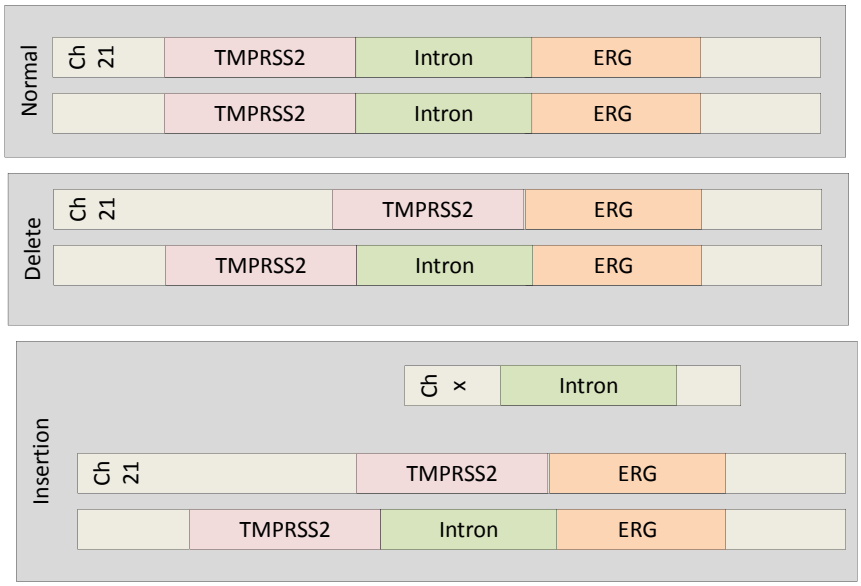
The following Table depicts the locations of the two genes on chromosome 21.

Gene	Start (000)	Stop (000)	Length KBp
ERG[13]	39,695	40,078	383
TMPRSS2[14]	42,829	42,886	57

The fusion takes the above and truncates some of the ERG exons and thus amino acids, as it also does to the trailing legs of the TMPRSS2 gene. The original genes are still expressed in the fusion albeit with truncated amino acid sequences.

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

[13] http://www.ncbi.nlm.nih.gov/gene/2078
[14] http://www.ncbi.nlm.nih.gov/gene/7113



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

As the work of King et al state:

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

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

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

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

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

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

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

5. ERG rearranged PCa have highly negative outcomes.

6. ERG rearranged response to abiraterone is different.

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

5.2 PRESENCE IN HGPIN

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

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

The authors then continue regarding HGPIN:

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

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

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

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

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

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

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

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

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

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

5.3 PATHWAYS AND CONTROL

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

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

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

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

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

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

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

6 OBSERVATIONS

The key question here is: is there anything substantially new in the fusion presence in HGPIN that was not already known? Secondly one may ask if this is a better marker than some of the genetic profiles that have been developed. Third, we may also inquire as to the cause of this HGPIN and especially the conversion to PCa and just as importantly the regression from HGPIN to a benign state.

6.1 SIMILAR EFFORTS

Lippolis et al have remarked:

We did not detect any co-expression of ERG and TATI in the same cancer cells, which confirms previous suggestions from in silico studies. ERG was associated with Gleason score (GS), surgical margins and pathological stage, but had no prognostic value in this cohort. TATI was weakly associated with pathological stage but had no significant association with outcome.

They like many others we have mentioned herein have examined the TMPRSS2:ERG translocation and its impact on both HGPIN and PCa. We believe that this is well known and further that is may be a useful marker. However significant clinical effort should be expended in this area to ascertain the clinical prognostic value.

6.2 OTHER MARKERS

As we have examined before there is a plethora of markers for PCa and there is now a growing set for HGPIN and PCa. For example a recent study correlated elevated HgA1b with HGPIN. In the study by Protopsaltis et al:

Benign prostatic hyperplasia (BPH) represents a pattern of non-malignant growth of prostatic fibromuscular stroma. Metabolic disturbances such us pre-diabetes and metabolic syndrome may have a role in BPH pathophysiology. A potential explanation for the above relationship involves the insulin-like growth factor (IGF) axis as well as IGF binding proteins, (IGFBPs) of which the most abundant form is IGFBP-3.

Therefore, the aim of the present study was to investigate the association between intra-prostatic levels of IGF-1, IGF-2 as well as to evaluate the role of locally expressed IGFBP-3 in BPH development in pre-diabetes. … In addition, total prostate (TP) volume or transitional zone (TZ) volume were estimated by transrectal ultrasonography. ..

The results of the multivariate analysis regarding TP volume showed that higher PSA, larger waist circumference and higher IGFBP-3 expression levels independently predicted higher TP volume. The results regarding the volume of the TZ showed that higher PSA ($p<0.001$), larger waist circumference ($p<0.001$) and higher IGFBP-3 expression levels ($p=0.024$) were independently associated with higher TZ volume.

Our findings show that intraprostatic levels of IGFBP-3, PSA and waist circumference, but not overall obesity, are positively associated with prostate volume. IGFBP-3 seems to be a multifunctional protein, which can potentiate or inhibit IGF activity

We have examined several cases of this type where inflammatory factors tend to be highly correlated with PCa as well as HGPIN. Namely one could see this in Type 2 Diabetes, which is mostly an inflammatory related disease. In Type 2 Diabetes we often see the combination of obesity which itself sets off inflammatory actions as well as increased

6.3 CAUSES

There is always the question of what causes the fusion and where? Specifically:

1. Mitotic activity often results in some linkage crossing which in turn may result in a translocation. In this case it is a movement of the gene by apparently a cutting out of a section of the chromosome. What makes this a favorable cut? What causes the cut?

2. Which cell has the aberrant mitotic activity. We have discussed the issue of cell source, basal versus luminal, and this is the major question as well.

3. If mitotic activity is the driver then which cells have the activity the most. Translocations are common in hematopoietic cancers but those specific cells are under constant mitotic growth. Is this the case with prostate cells and why? Do the prostate cells go through mitotic growth more in certain environments, such as those of an inflammatory nature? We examine this in a bit with the implications of translocations with Type 2 Diabetes and the inflammatory effects of high blood sugar.

As DeNunzio et al state:

PIA, typically associated with prostatic inflammation, is considered a possible precursor of high-grade prostatic intraepithelial neoplasia (HGPIN) and PCa. PIA lesions tend to occur in the periphery of the prostate and arise as a consequence of prostate epithelial cells' regenerative proliferation as a response to an injury caused by infection, cell trauma resulting from oxidant damage, hypoxia, and autoimmunity.

PIA lesions are often observed adjacent to HGPIN or early cancer, and there is an identifiable genetic pathway between PIA, HGPIN, and cancer, with progressively frequent TP53 mutations as well as gains in centrometric DNA sequences of chromosome 8 and glutathione S-transferase P1 (GSTP1) CpG island hypermethylation . Epithelial cells in PIA also show different molecular signs of stress, such as high levels of GSTP1, GSTA1, and COX-2 [50].

GSTP1 encodes a glutathione S-transferase, an antioxidant enzyme that is involved in the detoxification of carcinogens and inflammatory oxidants in prostate cells. GSTP1, generally considered a signal of cellular stress, is overexpressed in PIA and increases in chronic prostatic inflammation. GSTP1 inactivation, mostly by hyper- methylation, is associated with HGPIN and

PCa and may increase prostate cells' susceptibility to additional genome damage caused by inflammatory oxidant or nutritional carcinogens, with a consequent selective growth advantage.

6.4 REGRESSION

The major question is not progression but regression. Why do some HGPIN regress? Most researchers, for example Goldstein et al, argue that HGPIN evolution is possible in only one direction. Yet there are examples demonstrating the fact that regression occurs. The question is; what causes the regression and how does the regression fit the profile of the discussions herein. We believe that a study of regression, albeit limited, may provide substantial insight into the process. If inflammation is a driver of HGPIN and in turn PCa then what happened in an individual to reduce that state? Again we emphasize that all dimensions should be considered.

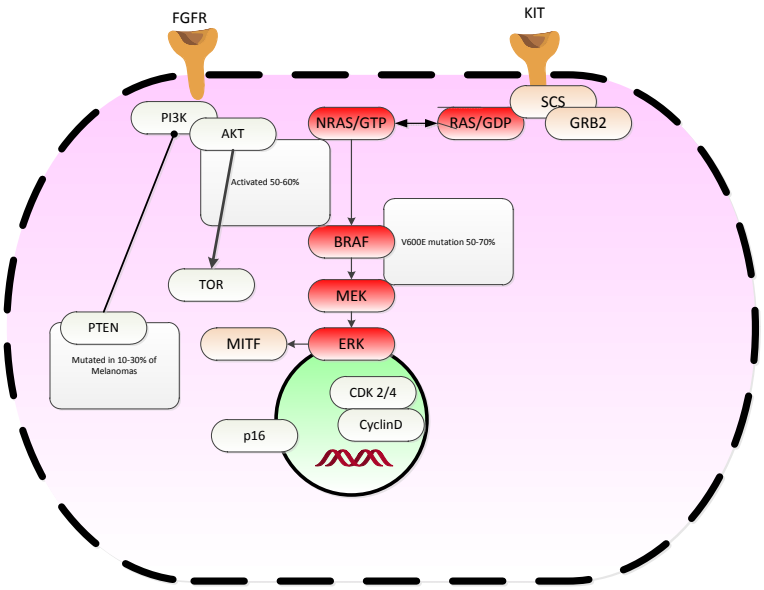
7 APPENDIX 1: WNT PATHWAY

In this appendix we consider the Wnt pathway, the effects of extracellular signals and the resultant pathway activation. As we indicated earlier ERG is part of the ETS family which is activated by MAP kinase pathways. We show herein the extent of such activation.

7.1 EXTRACELLULAR FACTORS AND MELANOMA: WNT AND E-CADHERIN

Cell growth and cell movement are two characteristics of cancer. We examine this from two perspectives, one for melanoma and another for the benign condition called Dupuytren's disease which is a genetically related disease of excess growth of the fascia in the hands. Both are controlled by the Wnt gene product and both have a relationship with E cadherin which is a protein on the surface of the cell which causes adhesion of cells to cells. Also both Wnt and cadherin are extracellular. Namely Wnt flows between the cells and attaches to certain ligands and then if the intracellular elements are properly aligned the cell starts to proliferate. E cadherin is a surface protein which binds the cell to a certain location. When E cadherin fails then we see the cell start to move from where it is supposed to stay. Thus these two factors present a small picture on the loss of control which we observe in cancers.

The key issue is to understand pathways and points at which they break and where they can be controlled as shown below:



Although the above diagram deals not with Wnt or E Cadherin but the more classic sets of pathway elements we can see how progress can be made. PTEN for example is common in many cancers. We will discuss this at length later. It is seen somewhat in melanoma as well. BRAF is a major control point for melanoma and most of the recent work in pathway control has been focused here. It is possible to block this point which when the mutation occurs and it fails to control the cell reproduction we see uncontrolled growth. This type of model we will use over and over again.

Thus in the section we look briefly at two key characteristics of cancer, uncontrolled replication and uncontrolled movement, and examine two related mechanisms which control both actions. There will be many others we will introduce. These are useful as examples.

7.2 WNT

Wnt is a gene product which acts by adhering to surface proteins on the outside of a cell. By so adhering, the Wnt can then influence the internal pathways in the cell often blocking apoptosis and initiating a cell growth and proliferation. In simple terms Wnt activates a pathway that allows β-catenin to enter the nucleus and activate a set of transcription promoters which in turn start the process of cell growth and proliferation.

The following is a typical Wnt pathway action:

1. Wnt is generated in a cell and is then secreted and in turn moves extra-cellularly to bind as a ligand on other cell surface receptors.

2. Wnt binds to the cell surface receptor Frizzled and it results in the activation of that receptor.

3. Activated Frizzled inhibits GSK-3 by means of the Disheveled protein. GSK-3 (Glycogen synthase kinase) normally inhibits β-catenin. This is a critical step because once activated β-catenin will result in a cascade of other actions resulting in cell growth. GSK normally activated phosphorylates β-catenin to keep it inactivated..

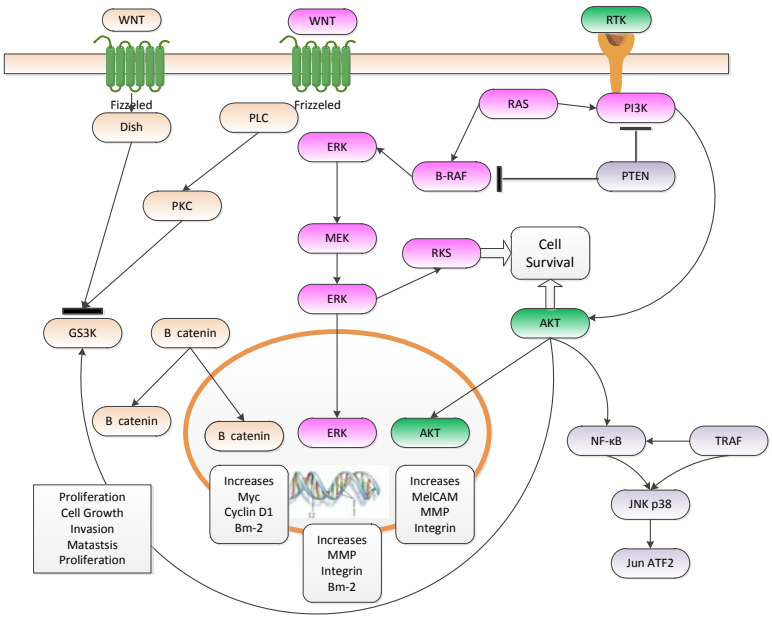
4. β-catenin now accumulates in the cytoplasm and at a certain concentration level β-catenin is transported into the nucleus.

5 When β-catenin is in the nucleus it activates TCF/LEF, a protein which is a transcription factor, and combined these results in the transcription of MYC, a strong proto-oncogene as well as CCDN1.

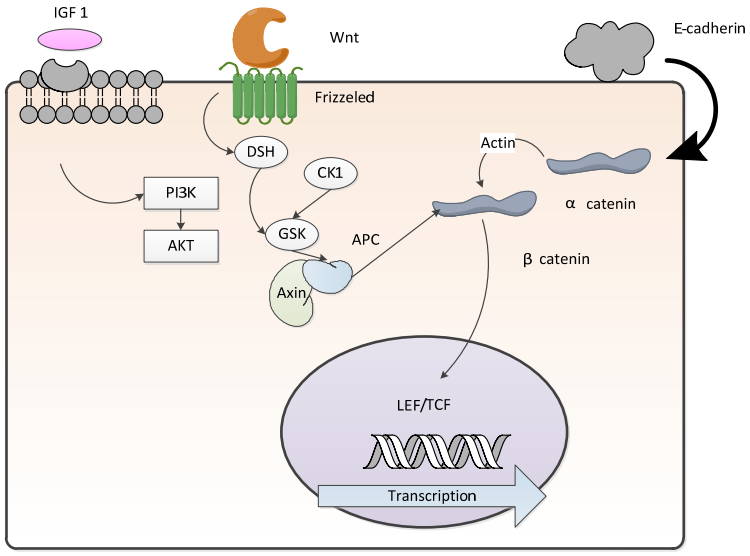
6. This results in uncontrolled cell growth and proliferation.

This is but one of many such pathways but one which is surface activated.

In the graphic below we depict some of the complex pathway processes and their effects. We show the catenin, ERK and Akt effects as each of their control mechanisms are affected. These are three of the major pathway challenges to normal cell homeostasis.



E cadherin is a set of molecules which are attached to the surface of cells and act in a manner to affect cell to cell adhesion. For example a melanocyte adheres to a keratinocyte in the basement membrane of the skin. If this adhesion fails for some reason then the melanocyte can start to wander off. When that happens and the melanocyte moves upward to the epidermis away from the basement layer we call that a melanoma in situ. The cells may not have yet gained the ability to reproduce in excess but they have lost a key element of a health melanocyte, namely the ability to stay fixed. In fact as we shall discuss the E cadherin is replaced by an N cadherin which often allows proliferating melanocytes to cluster together in groups and not have the simple keratinocyte structure.



As noted by Cavallaro and Christofori:

As well as their crucial role in assembling the E-cadherin- mediated cell-adhesion complex, β-catenin and γ-catenin also have important functions in the canonical WNT signalling pathway. Non-sequestered, free β-catenin and γ-catenin are rapidly phosphorylated by glycogen synthase kinase 3β (GSK-3β) in the adenomatous polyposis coli (APC)–axin–GSK-3β complex and are subsequently degraded by the ubiquitin–proteasome pathway.

If the tumour suppressor APC is non-functional, as in many colon cancer cells, or if GSK-3β activity is blocked by the activated WNT-signalling pathway, β-catenin accumulates at high levels in the cytoplasm.

Subsequently, it translocates to the nucleus, where it binds to members of the TCF/LEF1 family of transcription factors and modulates the expression of their target genes, including c-MYC, cyclin D1, fibronectin, MMP7, ID2, CD44, NrCAM, axin-2 (conductin), TCF1 and others, which are mostly genes implicated in cell proliferation and tumour progression. This dual function of β-catenin has motivated several experiments to address whether the loss of E-cadherin function would subsequently lead to the activation of the WNT signalling pathway.

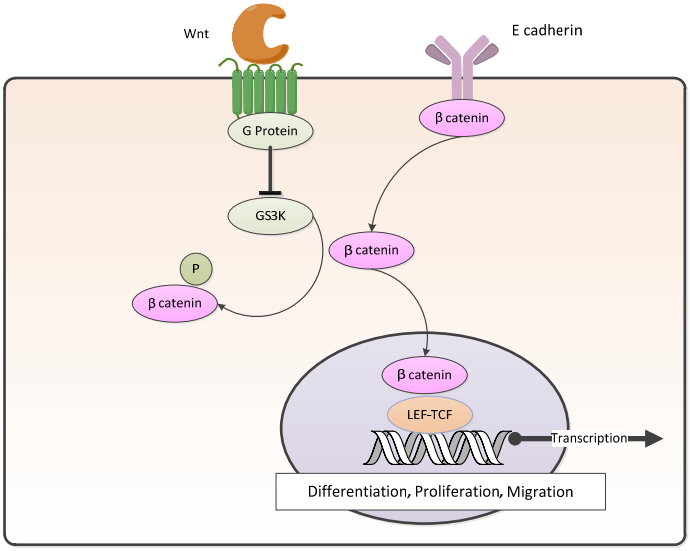
We demonstrate some of this detail below. The catenin is attached to the E-cadherin which is released and if Wnt is activated then the GSK3 is blocked and it migrates to the nucleus where it induces cell proliferation.

Now Wnt is a major component in many cells and especially those that are required for reproduction like bone marrow, colon cells, and skin cells. The control of the Wnt process is essential for homeostasis. Clearly one would want to have cells requiring proliferation to have a well regulated Wnt path. Thus keratinocytes need continual proliferation since the move from the basement membrane to the skin surface where they are sloughed away. However this would not be the case for melanocytes, where we need limited control. Melanocytes generally remain fixed at the basement membrane and movement or proliferation is inhibited.

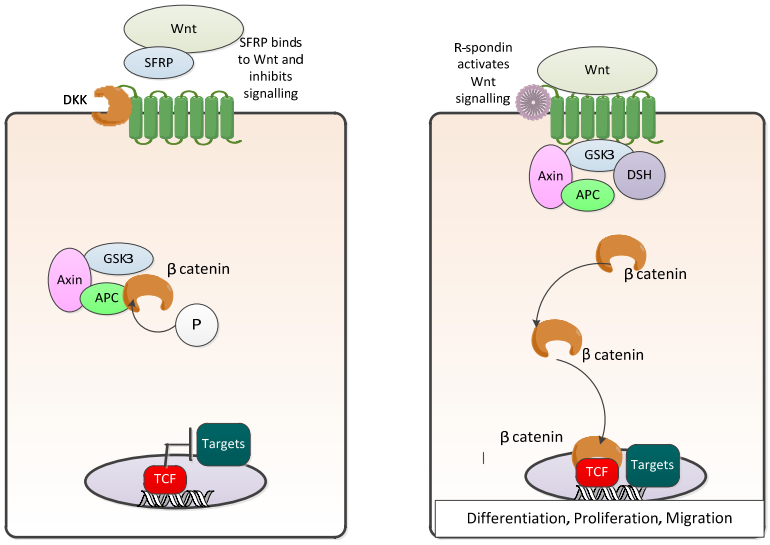
The authors continue:

*In several human cancer types, including **melanoma, prostate and breast cancer**, loss of E-cadherin function is accompanied by de novo expression of mesenchymal cadherins, such as N-cadherin and cadherin-11 (OB-cadherin. Cadherin-11 is expressed in invasive breast cancer and in breast cancer cell lines, and a carboxy-terminally truncated, alternatively spliced form of cadherin-11 can induce an invasive phenotype even in E-cadherin-positive breast cancer cell line. Upregulated expression of P-cadherin in breast cancers and of cadherin-6 in renal cell carcinoma also correlates with poor prognosis.*

By contrast, T-cadherin (also known as H-cadherin) behaves more like E-cadherin: it is downregulated in basal and squamous-cell carcinomas of the skin, correlating with an invasive phenotype. N-cadherin has been shown to promote cell motility and migration — an opposite effect to that of E-cadherin.



Adapted from Miller and Mihm NEJM July 2006

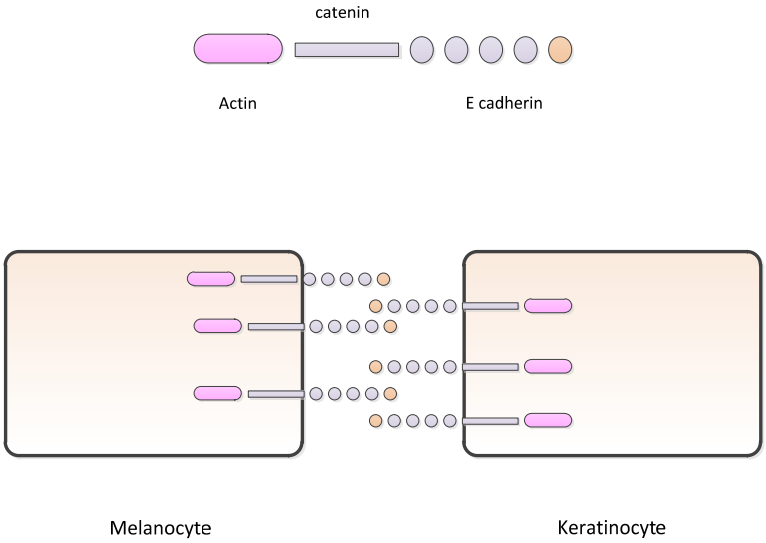


Modified from http://www.nejm.org/doi/full/10.1056/NEJMoa1101029#t=article

7.3 E CADHERIN

We now examine the cadherin structure. This we depict below as the bonding of two cells via the E cadherin elements which themselves are attached to a catenin and actin proteins within the cell

wall. The E cadherin is a bonding protein which finds other specific bonding proteins and then attaches itself within a specified framework. Thus in the basement layer of the skin, at the bottom of the epidermis, the melanocyte attaches uniquely to a keratinocyte and fixes its position in the basement layer so as not to migrate.



E-cadherin is generated at 16q21.1. As is stated in MMMP[15]:

E-cadherin is one of the most important molecules of cell-cell adhesion in non-neural epithelial tissues. This 120 kDa transmembrane glycoprotein is generally localized on the surface of epithelial cells in a region of cell-cell contact that is known as the adherens junction. Cadherins are calcium-dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells: cadherins may thus contribute to the sorting of heterogeneous cell types. CDH1 is involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells. It is a ligand for integrin alpha-E/beta-7. Acts as a disulfide-linked homodimer. Interacts directly (via the cytoplasmic domain) with CTNNB1 or JUP to form the PSEN1/cadherin/catenin adhesion complex which connects to the actin skeleton through the actin binding of alpha-catenin.

Interaction with PSEN1 cleaves CDH1 resulting in the disassociation of cadherin-based adherens junctions (CAJ). Anchored to actin microfilaments through association with alpha-catenin, beta-catenin and gamma-catenin. Sequential proteolysis induced by apoptosis or calcium influx, results in translocation from sites of cell-cell contact to the cytoplasm.

During apoptosis or with calcium influx, cleaved by a membrane-bound metalloproteinase (ADAM10), PS1/gamma-secretase and caspase-3 to produce fragments of about 38 kDa (E-CAD/CTF1), 33 kDa (E-CAD/CTF2) and 29 kDa (E-CAD/CTF3), respectively. Processing by the metalloproteinase, induced by calcium influx, causes disruption of cell-cell adhesion and the subsequent release of beta-catenin into the cytoplasm. The residual membrane-tethered cleavage product is rapidly degraded via an intracellular proteolytic pathway. Cleavage by caspase-3

[15] http://www.mmmp.org/MMMP/public/biocard/viewBiocard.mmmp?id=1301

releases the cytoplasmic tail resulting in disintegration of the actin microfilament system. The gamma-secretase-mediated cleavage promotes disassembly of adherens junctions.

In fact the β catenin is bound to the tail of the E cadherin complex and it is released when Wnt is activated. It is this release and movement to the nucleus which gives rise to proliferation.

7.4 WNT, E-CADHERIN AND DUPUTRYEN'S DISEASE

Duputryen's disease is a benign disorder of excess clustered nodular growth of the fibrin, and is a benign fibromatosis of the hand (see Dolmans et al and see Tubiana et al) . What seems to happen is the cells of fibroblasts are overly activated and bind together in clumps creating nodule like structures which then impinge on nerves and muscles impeding hand motion.

As Dolmans et al state:

The WNT gene family consists of structurally related genes that encode glycoproteins, extracellular signaling molecules. Abnormal Wnt signaling is linked to a range of diseases, especially cancer. The best-understood Wnt-signaling pathway is the canonical pathway, which activates the nuclear functions of β-catenin, leading to changes in gene expression that influence cell proliferation and survival.[18] Abnormal proliferation of fibroblasts is a key feature in the early development of Dupuytren's disease. The disease can be divided into three histologic stages:

stage 1, proliferation of fibroblasts;

stage 2, differentiation of fibroblasts into myofibroblasts; and

stage 3, formation of mature type 1 collagen.

Wnt signaling is known to regulate the proliferation and differentiation of fibroblasts in both cancer and fibromatosis. Most of our knowledge of Wnt signaling is derived from studies of cancer. In colon cancer, up-regulation of Wnt signaling causes intestinal crypt cells to proliferate for longer than usual before they migrate and differentiate. This prolonged proliferation phase results in the formation of polyps and confers a predisposition to cancer.

There is substantial evidence to show that this up regulation leading to proliferation is common in prostate cancer and melanoma as well.

They continue:

The Wnt proteins Wnt2, Wnt4, and Wnt7B, which were identified on GRAIL analysis, bind to frizzled receptors, leading to a cascade of events that eventually result in a decrease in the rate of β-catenin degradation. Secreted frizzled-related proteins, such as SFRP4, antagonize the Wnt-signaling pathway by binding to either Wnts or frizzled receptors, thereby affecting receptor occupancy. In the absence of active Wnt, β-catenin is degraded, and potential target genes will not be activated.

We depict the signalling below. Note in the inhibited case we have an extracellular SFRP binding to Wnt and preventing it from activating the pathway as a ligand. Also not that what is driving this is Wnt 2,4 and 7B. Melanoma id driven by Wnt 5.

7.5 BRAF AND WNT

Recently we have seen targeted drugs to control BRAF.

In the paper by Biechele et al we have:

Because the Wnt/β-catenin signaling pathway is linked to melanoma pathogenesis and to patient survival, we conducted a kinome small interfering RNA (siRNA) screen in melanoma cells to expand our understanding of the kinases that regulate this pathway. We found that BRAF signaling, which is constitutively activated in many melanomas by the $BRAF^{V600E}$ mutation, inhibits Wnt/β-catenin signaling in human melanoma cells.

Because inhibitors of $BRAF^{V600E}$ show promise in ongoing clinical trials, we investigated whether altering Wnt/β-catenin signaling might enhance the efficacy of the $BRAF^{V600E}$ inhibitor PLX4720. We found that endogenous β-catenin was required for PLX4720-induced apoptosis of melanoma cells and that activation of Wnt/β-catenin signaling synergized with PLX4720 to decrease tumor growth in vivo and to increase apoptosis in vitro. This synergistic enhancement of apoptosis correlated with reduced abundance of an endogenous negative regulator of β-catenin, AXIN1. In support of the hypothesis that AXIN1 is a mediator rather than a marker of apoptosis, siRNA directed against AXIN1 rendered resistant melanoma cell lines susceptible to apoptosis in response to treatment with a $BRAF^{V600E}$ inhibitor.

Thus, Wnt/β-catenin signaling and AXIN1 may regulate the efficacy of inhibitors of $BRAF^{V600E}$, suggesting that manipulation of the Wnt/β-catenin pathway could be combined with BRAF inhibitors to treat melanoma.

We now know that if a person has the BRAF V600E presence that use of xxx will manage the melanoma for a period.

8 APPENDIX 2: SUMMARY OF PCA GENES

The following is a summary of some common PCa genes[16].

Genes and alterations	*Description*	*Alterations*	*Frequency in primary versus metastatic (when known)*	*PATHWAY*
AR	Androgen receptor	Amplification and Mutations Variant splicing	Only CRPC, in majority of tumors together with cofactors	Androgen receptor signaling
AR cofactors and regulators NCOA1,2,3; NCOR1, NCOR2, TNK2 and more	Regulation of the AR activity	Amplification Mutations	About 50% in localized; >80% in CRPC	
Androgen synthesis enzymes: CYP17 etc	Steroidogenic/androgen synthesis	Overexpression, activating mutations, copy gains	Observed in CRPC	
FOXA1	AR licensing Factor	Mutations	5% of localized	
TMPRSS2:ERG, other ETS	Gene fusion involving ERG; rarely other ETS family members		40-60 % of localized	Transcription, most likely controlled by AR
NKX3.1	Homeobox, prostate specific, androgen regulated	Deletions, mutations, decreased expression	3-5% mutations, 10-20% deletions, and frequent decreased expression in localized cancers	Developmental lineage specific, transcription, AR pathway
PTEN	Phosphatase suppressor of PI3K	Deletions, rare mutations	40-50% of primary, >80% CRPC; PTEN loss is the most frequent alterations in PI3K pathway	PI3K signal transduction Co-operates with AR pathway in pathogenesis of PCa
MAGI2	PTEN interactor	Rearrangement		
PIK3CA1 catalytic subunit	PIP2 kinase	Overexpression, mutations		
PHLPP1/2	Phosphatase, inhibits AKT	Deletion, down-regulation		
Akt1	Central kinase in PI3K pathway	Point mutations (rare)		
SPOP	Speckle-type POZ domain ubiquitin ligase	Mutations	5-10% primary, same in metastatic, mutually exclusive with ERG rearrangements	Responsible for the degradation of AR cofactor NCOA3/SRC-3. AR pathway connected?
SPINK1	Serine peptidase inhibitor	Overexpression	5-10%, mutually exclusive with ERG rearrangements	Unknown

[16] http://www.cancercommons.org/researchers-clinicians/prostate-cancer/prostate-cancer-model/

Genes and alterations	*Description*	*Alterations*	*Frequency in primary versus metastatic (when known)*	*PATHWAY*
MYC	Master of transcription regulation; opposes NKX3.1	Overexpressed in primary, genetic gain in metastatic	20-30% with gain in metastatic disease	Transcription
NMYC	Transcriptional regulation	Overexpression, amplification	40% of neuroendocrine PCa; 5% overall	
MED12	Regulatory component of mediator complex	Mutations	2-5%	
EZH2	Polycomb group	Elevated expression	Localized (poor prognosis) and CRPC	Transcriptional suppression
BMI	Polycomb group, transcriptional suppression	Elevated expression	Localized and metastatic	
Aurora A kinase	Mitotic kinase	Overexpression, amplification	40% of neuroendocrine PCa; 5% overall	Cell Cycle
BRAF	Serine-threonine kinase at the top of MAPK cascade	Rearrangements	1%, all	MAPK
CADM2	Cell adhesion molecule	Rearrangements	Primary and metastatic	Cell polarity, potential tumor suppressor
CHD1	Nucleosome positioning	Mutations	8%, mostly with SPOP mutations, in ETS normal	Chromatin remodeling
MLL complex (MLL2, ASH2L and more)	Epigenetic transcriptional activation	Mutations	9% CRPC	
TP53	Controls many aspects of cell cycle, apoptosis, metabolism	Loss, LOF*, GOF* mutations	30-100%, mostly in metastatic	Tumor suppressors
RB1	Cell cycle	Loss, LOF	50% metastatic	
ERCC2,4,5; ATM, XRCC4, PRKDC and more	Various genes involved in DNA repair	Losses, mutations	Mostly in metastatic	DNA damage repair
APC, BMP7, WNT family, CTNNB1 and more	WNT pathway involved on proliferation, differentiation, EMT	Losses, mutations	Metastatic	Developmental signaling pathways: WNT
EGFR, IGF1R, FGFR	Growth factor receptors	Activation	NA	Growth factor induced signaling, activation of PI3K and MAPK pathways, and AR signaling

Genes and alterations	*Description*	*Alterations*	*Frequency in primary versus metastatic (when known)*	*PATHWAY*
IL6-IL6R	Cytokine receptor	Activation by IL6	NA	JAK-STAT3 pathway; activates AR
SRC	Tyrosine kinase	Activation	NA	Many signaling pathways
HSP90, HSP27Clusterin/TRPM2	Maintain stability of various signaling proteins including AR and many others		NA	Protein Chaperons

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