7 Types of Prostate Cancer

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White Paper No 135
April, 2016

There is an ongoing study of genes involved in Prostate Cancer. A recent paper discusses seven specific gene changes that occurs in early stage PCa. We discuss those results herein.

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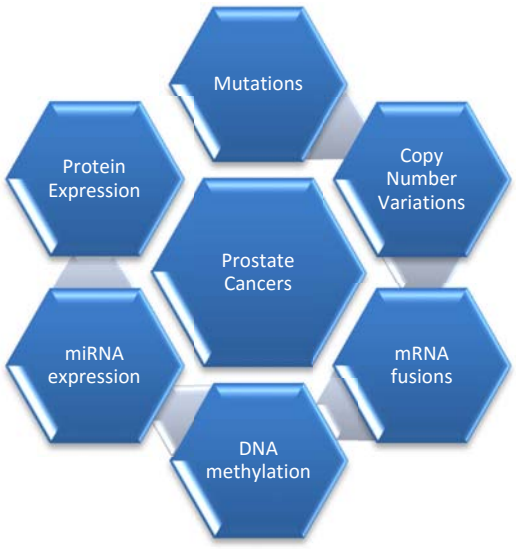
1 INTRODUCTION

Prostate Cancer has been examined with a multiplicity of genes and each time we often see a new combination of the same set. In a recent paper by The Cancer Genome Atlas Research Network we again see another proposed set of genes. Simply in this case they argue for the association of the following genes with early stages of PCa. Note that this is not for aggressive PCa but the early confined stages. They are:

1. SPOP
2. FOXA1
3. IDH1
4. ERG
5. ETV1
6. ETV4
7. FLI1

Now we have seen many of these in one combination or another over the past decade. In fact, we have discussed at length SPOP, FOXA1, and the ETS genes which are found in merging. We examine them again herein because this seems to be an interesting new development.

Overall they will argue for the following collection of related genetic factors:



Each of the above elements has been identified as part of PCa development and progression. The paper states:

There is substantial heterogeneity among primary prostate cancers, evident in the spectrum of molecular abnormalities and its variable clinical course. As part of The Cancer Genome Atlas (TCGA), we present a comprehensive molecular analysis of 333 primary prostate carcinomas. Our results revealed a molecular taxonomy in which 74% of these tumors fell into one of seven

subtypes defined by specific gene fusions (ERG, ETV1/4, and FLI1) or mutations (SPOP, FOXA1, and IDH1).

Epigenetic profiles showed substantial heterogeneity, including an IDH1mutant subset with a methylator phenotype.

Androgen receptor (AR) activity varied widely and in a subtype-specific manner, with SPOP and FOXA1 mutant tumors having the highest levels of AR-induced transcripts. 25% of the prostate cancers had a presumed actionable lesion in the PI3K or MAPK signaling pathways, and DNA repair genes were inactivated in 19%. Our analysis reveals molecular heterogeneity among primary prostate cancers, as well as potentially actionable molecular defects.

In effect they argue that primary PCa lesions for the most part (they argue 74%, with a sample base of only 333 lesions, fall into the seven groups) can be characterized by seven specific gene fusions (four) or mutations (three).

It is essential to note that this paper focuses on the genetic factors in localized PCa. They do discuss metastatic disease and the characteristics of that. However, the issue here is the almost three fourths which possess the genetic mis-expressions discussed herein.

The two effects we note are:

First: Fusion, resulting in a 3' and 5' fusion via a translocation resulting in an oncogene. We graphically show this below.

Second: Mutation where we lose the controlling element necessary. We show that graphically below:

On the mutation side we see loss of function in two AR control genes, SPOP and FOXA1, and one metabolic control gene, IDH1. The latter, albeit discussed by others previously, seems to be a first in this context.

2 GENERAL PRINCIPLES

We are aware of the ERG fusions and have discussed them at length and we are also aware of the mutations in SPOP and FOXA1. The introduction of IDH1 adds another dimension and one which focuses on cell metabolism.

We can examine some of the analysis already done regarding this work by the TCGA group. From the Cancer Letter we have[1]:

Researchers from The Cancer Genome Atlas Network recently published two studies—one identifying seven distinct molecular subtypes of prostate cancer, and one exploring the genetic drivers of papillary renal cell carcinoma.

A comprehensive analysis of 333 prostate cancers identified key genetic alterations that may help improve classification and treatment of the disease, revealing seven new molecular subtypes of prostate cancer based on known and novel genetic drivers of the disease. These subtypes may therefore have prognostic and therapeutic implications, according to researchers.

Of the seven subtypes, four are characterized by gene fusions (in which parts of two separate genes are linked to form a hybrid gene) involving members of the ETS family of transcription factors (ERG, ETV1, ETV4, and FLI1), and the other three are defined by mutations of the SPOP, FOXA1, and IDH1 genes.

Notably, the IDH1 mutation was identified as a driver of prostate cancers that occur at younger ages. Although 74 percent of the analyzed tumors could be categorized into one of the seven molecular subtypes, the remaining 26 percent of prostate tumors in this analysis could not be categorized because molecular alterations driving their growth were not identified.

Another finding from the analysis was that gene expression profiles differed based on whether the tumors were driven by gene fusions or by mutations.

Within the mutation-driven tumors, the SPOP and FOXA1 gene subtypes shared similar patterns of DNA methylation, a chemical modification of DNA that inhibits gene expression; somatic copy-number alteration and messenger RNA expression. These genomic commonalities suggest that mutations in SPOP and FOXA1 genes cause similar disruptions in the cell to bring about cancer.

Additionally, the SPOP and FOXA1 subtypes showed the highest levels of androgen receptor-mediated gene expression, suggesting potential preventive and therapeutic possibilities targeting androgens, which are male sex hormones that can stimulate the growth of prostate cancer.

[1] http://www.cancerletter.com/articles/20151106_6

In the second study, a comprehensive genomic analysis of 161 tumors from people with papillary renal cell carcinoma provided insights into the molecular basis of this cancer and may inform its classification and treatment.

PRCCs are divided into two main subtypes, Type 1 and Type 2, which are traditionally defined by how the tumor tissue appears under a microscope. Findings from this genomic analysis, carried out by investigators from The Cancer Genome Atlas Research Network, have confirmed that these subtypes are distinct diseases distinguished by certain genomic characteristics.

Researchers found that Type 1 PRCC is characterized by alterations in cell signaling involving the MET gene that are known to drive cancer cell growth, the growth of tumor blood vessels, and cancer metastasis or spread. MET gene mutations or other alterations that affect its activity were identified in 81 percent of Type 1 PRCCs examined. This finding suggests that it may be possible to treat Type 1 PRCCs with specific inhibitors of the MET cell signaling pathway, including the MET/VEGFR inhibitor foretinib, which is currently being tested in phase II clinical trials in PRCC and other cancer types.

Type 2 PRCC was found to be more genomically heterogeneous. A specific characteristic, referred to as the CpG island methylation phenotype, was found almost exclusively in Type 2 PRCC and defined a distinct Type 2 subgroup that was associated with the least favorable outcome.

CIMP is marked by increased DNA methylation, which is a chemical modification of DNA that inhibits gene expression. Across all Type 2 PRCCs examined, 25 percent demonstrated decreased expression of CDKN2A, a tumor suppressor gene that helps regulate the cell cycle. Loss of CDKN2A expression was also associated with a less favorable outcome.

The researchers in this study were led by Paul Spellman, of Oregon Health and Science University, and Marston Linehan, of NCI. Their findings were published in the New England Journal of Medicine. TCGA is a collaboration jointly supported and managed by NCI and the National Human Genome Research Institute.

In a similar vein and in a presentation from HealthCanal we also have[2]:

Investigators published the in-depth analysis of 333 prostate cancer tumors online Nov. 5 in Cell. TCGA is jointly supported and managed by the National Human Genome Research Institute and the National Cancer Institute, both parts of the National Institutes of Health.

While 90 percent of prostate cancers are now identified as clinically localized tumors, once diagnosed, these cancers tend to have a heterogeneous and unpredictable course of progression, ranging from slow-growing to fatal disease.

[2] http://www.healthcanal.com/cancers/prostate-cancer/68419-genome-study-identifies-seven-genetic-subtypes-of-prostate-cancer.html

"We have identified seven clearly defined subtypes of prostate cancer based on genetic alterations," said Massimo Loda, MD, director of the Center for Molecular Oncologic Pathology at Dana-Farber/Brigham and Women's Cancer Center, and a co-principal director of the study.

Loda added, "Interestingly, it is clear that there is substantial diversity within each of these subtypes. We can now put this critical information into clinical and pathological context and, by developing biomarkers for the different genetic variants, use them to guide therapeutic options."

"Until now, we haven't had a reliable way of predicting the way a primary prostate cancer will act by looking at the genome," said Chris Sander, PhD, principal investigator and chair of the computational biology program at Memorial Sloan Kettering Cancer Center. "The TCGA study gives us much more information about the spectrum of alterations in tumors and can help us predict the development of the disease. This will also inform the design of new clinical trials."

According to the American Cancer Society, prostate cancer will be newly diagnosed in more than 220,000 men in the United States in 2015, making it the second most common cancer affecting men and the second leading cause of death from cancer in men. Most prostate cancers are detected early while still confined to the prostate, a walnut-sized gland located below the bladder. While most cases remain harmless - benign - for decades, other subtypes of prostate cancers can be aggressive, and spread to other parts of the body (metastasize), making them extremely difficult to treat. It is currently difficult for healthcare providers to distinguish which cancers will remain harmless and which will metastasize.

The scientists studied five aspects of the prostate tumors:

The number and kinds of genetic mutations.
Gene fusions (when genes attach to each other or otherwise combine).
The number of copies of DNA segments (abnormal differences in the cell's number of copies of DNA segments can contribute to cancer).
Gene activity, including when genes are turned on or off, and how much activity is seen.
DNA methylation (methyl chemical groups are added to many places on a cell's DNA and act like on/off switches for a gene). Mistakes in DNA methylation can turn genes on or off at the wrong time and contribute to cancer.

Of the seven subtypes, the investigators found that four are characterized by gene fusions, while the other three are defined by mutations in the SPOP, FOXA1 and IDH1 genes. The subtypes with SPOP and FOXA1 mutations share several genomic characteristics, suggesting that mutations in these genes cause similar disruptions in the cell to bring about cancer.

Investigators discovered that mutations in the IDH1 gene are similar to those found in leukemia and brain cancer. Such a cancer subtype could be a candidate for a "basket" clinical trial, which tests for similar mutations across cancer types. The goal of this type of trial would be to personalize a patient's treatment based on the mutations, not on the anatomical location of the cancer.

… said that while previous studies have examined gene copy number or the number and kinds of genetic mutations or the level of gene activity in prostate tumors, the TCGA study is the first to comprehensively and systematically examine many different types of data together on a large scale.

As we shall note, many of these gene mis-expressions and fusions have been well known especially in the area of advanced PCa. One must ask; therefore, what is essentially new in this study. First we do have a group of putatively early stage PCa. The fusions have often been seen in later stages but at the same time fusions have been alleged in even HGPIN. Secondly, the size of the sample is still relatively small. Third, the question is; do these insights reflect in some therapeutic effect. We know with imatinib and the Philadelphia chromosome story that perhaps there may be a way to mitigate against this. However, we also know that a prostate stem cell issue may also be present. This may or may not result in a clear therapeutic path.

The authors summarize their findings as follows:

In total, 53% of tumors were found to have ETS family gene fusions (ERG, ETV1, ETV4, and FLI1) after analysis with two complementary algorithms.

While TMPRSS2 was the most frequent fusion partner in all ETS fusions, we identified fusions with other previously described androgen-regulated 50 partner genes, including SLC45A3 and NDRG.

We also identified several tumors that overexpressed full-length ETS transcripts that were mutually exclusive with ETS fusions (12 ETV1 high tumors, 6 ETV4, and 2 FLI1). ETS overexpression in these cases could possibly be mediated via epigenetic mechanisms or cryptic translocations of the entire gene locus to a transcriptionally active neighborhood. In the one case with elevated ETV1 full-length expression studied by whole-genome sequencing, we identified a cryptic genomic rearrangement 30 of the ETV1 locus with a region on chromosome 14 near the MIPOL1 gene adjacent to FOXA1. This event is similar to previously described ETV1 translocations in LNCaP and MDA-PCa2b cell lines and in patient samples.

Overall, while fusions in the four genes were mostly mutually exclusive, three tumors showed evidence for fusions involving more than one of these genes. Given that histologically defined single tumor foci have been shown to be rarely composed of different ETS fusion-positive clones, it is likely these cases reflect convergent phenotypic evolution in clonally heterogeneous tumors.

Tumors defined by SPOP mutations were mutually exclusive with all ETS fusion positive cases, though four of the SPOP mutant tumors also possessed FOXA1 mutations. In all four of these tumors, both the SPOP and FOXA1 mutations were clonal, indicating that they are present in the same tumor cells.

Details regarding the mutations and the effect that the mutation has had is open to question. Namely is the gene made over-expressed, under-expressed or mis-expressed.

3 DETAILS ON THE SPECIFIC GENES

We now examine the genes in some detail. The following Table is a summary from NCBI. We shall expand this for each class.

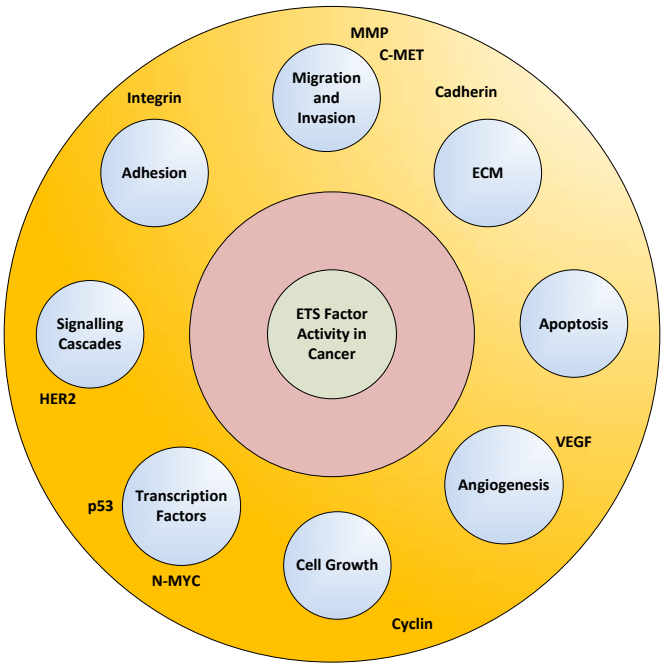
Gene	***Description (From NCBI)***
SPOP	*This gene encodes a protein that may **modulate the transcriptional repression** activities of death-associated protein 6 (DAXX), which interacts with histone deacetylase, core histones, and other histone-associated proteins. In mouse, the encoded protein binds to the putative leucine zipper domain of macroH2A1.2, a variant H2A histone that is enriched on inactivated X chromosomes. The BTB/POZ domain of this protein has been shown in other proteins to mediate transcriptional repression and to interact with components of histone deacetylase co-repressor complexes. Alternative splicing of this gene results in multiple transcript variants encoding the same protein.*
FOXA1	*This gene encodes a member of the **forkhead** class of DNA-binding proteins. These hepatocyte nuclear factors are **transcriptional activators** for liver-specific transcripts such as albumin and transthyretin, and they also interact with chromatin. Similar family members in mice have roles in the regulation of metabolism and in the differentiation of the pancreas and liver.*
IDH1	*Isocitrate dehydrogenases catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate. These **enzymes belong to two distinct subclasses, one of which utilizes NAD (+) as the electron acceptor and the other NADP (+)**. Five isocitrate dehydrogenases have been reported: three NAD (+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP (+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP (+)-dependent isozyme is a homodimer. The protein encoded by this gene is the NADP (+)-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. It contains the PTS-1 peroxisomal targeting signal sequence. The presence of this enzyme in peroxisomes suggests roles in the regeneration of NADPH for intraperoxisomal reductions, such as the conversion of 2, 4-dienoyl-CoAs to 3-enoyl-CoAs, as well as in peroxisomal reactions that consume 2-oxoglutarate, namely the alpha-hydroxylation of phytanic acid. The cytoplasmic enzyme serves a significant role in cytoplasmic NADPH production. Alternatively, spliced transcript variants encoding the same protein have been found for this gene*

Gene	*Description (From NCBI)*
ERG	*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. More than two dozens of transcript variants generated from combinatorial usage of three alternative promoters and multiple alternative splicing events have been reported, but the full-length nature of many of these variants has not been determined.*
ETV1	*This gene encodes a member of the **ETS (E twenty-six) family of transcription factors.** The ETS proteins regulate many target genes that modulate biological processes like cell growth, angiogenesis, migration, proliferation and differentiation. All ETS proteins contain an ETS DNA-binding domain that binds to DNA sequences containing the consensus 5'-CGGA[AT]-3'. The protein encoded by this gene contains a conserved short acidic transactivation domain (TAD) in the N-terminal region, in addition to the ETS DNA-binding domain in the C-terminal region. This gene is involved in chromosomal translocations, which result in multiple fusion proteins including EWS-ETV1 in Erwing sarcoma and at least 10 ETV1 partners (see PMID: 19657377, Table 1) in prostate cancer. In addition to chromosomal rearrangement, this gene is overexpressed in prostate cancer, melanoma and gastrointestinal stromal tumor. Multiple alternatively spliced transcript variants encoding different isoforms have been identified*
ETV4 (E1AF)	*E1AF is associated with **malignant aggressiveness via regulation of matrix metalloproteinases** (MMPs), which play pivotal roles in invasion through the degradation of extracellular matrix of tissues surrounding tumors. However, the clinical significance of E1AF and MMPs in patients with prostate cancer is not fully understood. Our results demonstrated that increased expression of E1AF is involved in tumor aggression of prostate cancer. This finding may be influenced by regulation of MMP-7. We speculate that E1AF is a possible target in treatment and prevention of tumor growth in prostate cancer.* *http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0463.2009.02534.x/abstract;jsessionid=8867FC127A0B3BB25FA89F96B6E7175A.f04t03*
FLI1	*This gene encodes a **transcription factor** containing an ETS DNA-binding domain. The gene can undergo a t (11;22) (q24; q12) translocation with the Ewing sarcoma gene on chromosome 22, which results in a fusion gene that is present in the majority of Ewing sarcoma cases. An acute lymphoblastic leukemia-associated t (4;11) (q21; q23) translocation involving this gene has also been identified. Alternative splicing results in multiple transcript variants*

We will now review some of the details of each gene and gene fusion product.

3.1 ERG

ERG is one of the ETS genes. We have shown previously that ETS fusions play a significant role in PCa. We show below some of these factors:



In a recent paper by Wu et al discussing ERG in colon cancer the authors note:

Chromosomal translocations juxtaposing the androgen-responsive TMPRSS2 promoter with the ETS family transcription factor ERG result in aberrant ERG upregulation in approximately 50% of prostate cancers. Studies to date have shown important roles of ERG in inducing oncogenic properties of prostate cancer.

Its molecular mechanisms of action, however, are yet to be fully understood. Here, we report that ERG activates Wnt/LEF1 signaling cascade through multiple mechanisms. ERG bound to the promoters of various Wnt genes to directly increase ligand expression.

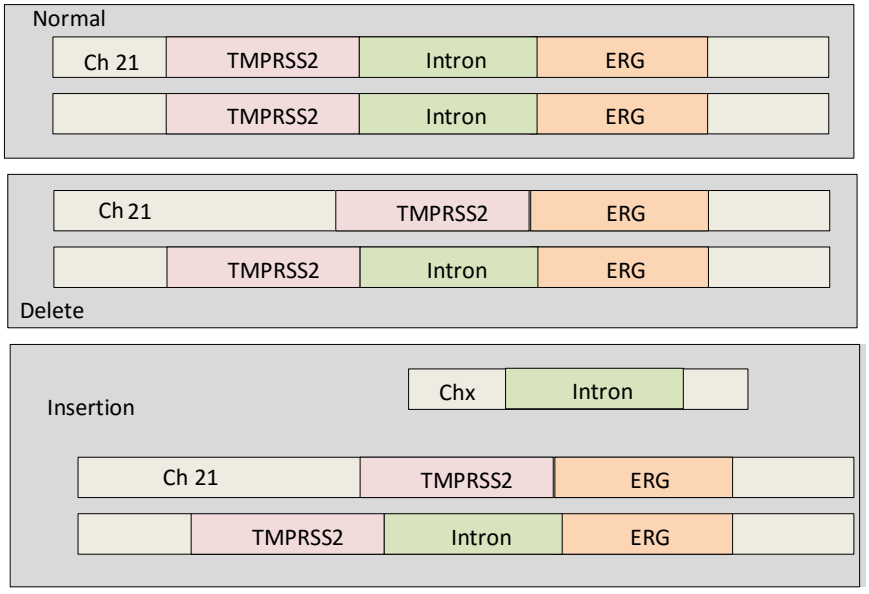
Consequently, ERG overexpression increased active b-catenin level in the cells and enhanced TCF/LEF1 luciferase reporter activity, which could be partially blocked by WNT-3A inhibitor IWP-2. Most importantly, our data defined LEF1 as a direct target of ERG and that LEF1 inhibition fully abolished ERG-induced Wnt signaling and target gene expression. Furthermore, functional assays showed that Wnt/LEF1 activation phenocopied that of ERG in inducing cell growth, epithelial-to-mesenchymal transition, and cell invasion, whereas blockade of Wnt signaling attenuated these effects.

Concordantly, LEF1 expression is significantly upregulated in ERG-high human prostate cancers. Overall, this study provides an important mechanism of activation of Wnt signaling in prostate cancer and nominates LEF1 as a critical mediator of ERG-induced tumorigenesis.

Wnt/LEF1 pathway might provide novel targets for therapeutic management of patients with fusion-positive prostate cancer

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[3]. 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[4][5]. 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.



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

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

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

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

HGPIN resolves itself, especially after extensive high density core biopsies. The answer to why such a resolution occurs is open to speculation.

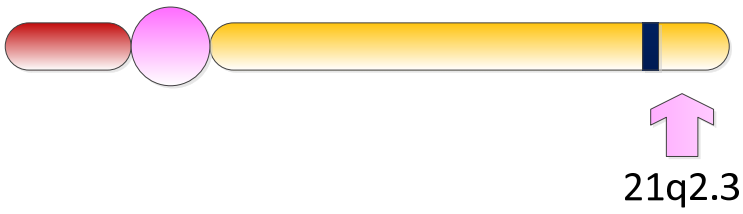
What has instigated this study was the Press Release ascribing to ERG in an HGPIN environment a significant prognostic value. Namely the release states[6]:

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.

ERG has a cytogenetic Location: 21q22.3[7] 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.

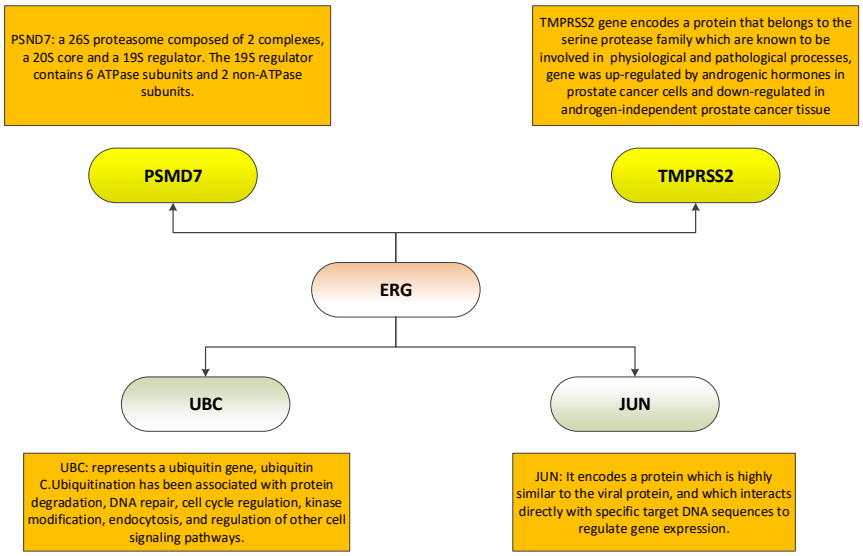


The following is a pathway connection between ERG and four other key players[8]. One should remember the MAP kinase activators of ERG and that ERG is general located in the nucleus. Thus from ERG downward we have the relationships as shown below.

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

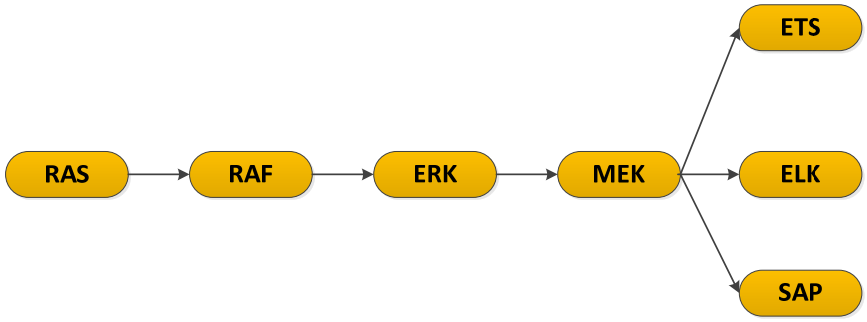
[7] http://ghr.nlm.nih.gov/gene/ERG

[8] 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

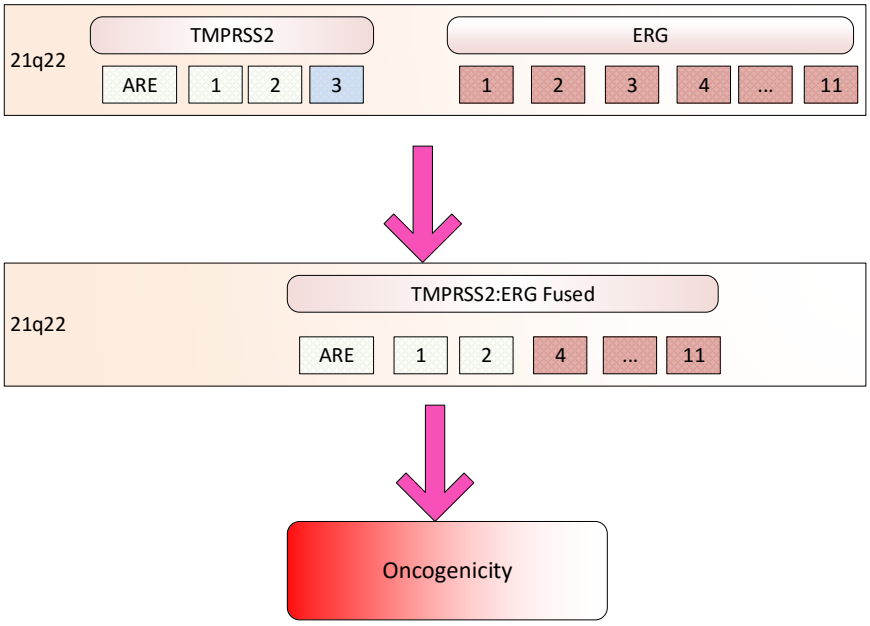
For the relationships above we have the following Figure from Weinberg of upward activation paths. This is as we had discussed is a kinase activated path. Also ERG generally is retained as a promoter in the nucleus.



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.

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[9].



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.

3.2 ETV1 AND ETV4

ETV1 and ETV4 are two of the ETS family of genes.

As Barros-Silva et al have noted:

Gene fusions involving the erythroblast transformation-specific (ETS) transcription factors ERG, ETV1, ETV4, ETV5, and FLI1 are a common feature of prostate carcinomas (PCas). The most common upstream fusion partner described is the androgen regulated prostate-specific gene TMPRSS2, most frequently with ERG, but additional 5' fusion partners have been described. We performed 5' rapid amplification of cDNA ends in 18 PCas with ETV1, ETV4, or ETV5 outlier expression to identify the 5' fusion partners. We also evaluated the exon-level expression profile of these ETS genes in 14 cases.

[9] See Rubin and Chinnaiyan

We identified and confirmed by fluorescent in situ hybridization (FISH) and reverse transcription-polymerase chain reaction the two novel chimeric genesOR51E2-ETV1 andUBTF-ETV4 in two PCas.OR51E2 encodes a G-protein–coupled receptor that is overexpressed in PCas, whereas UBTF is a ubiquitously expressed gene encoding an HMG-box DNA-binding protein involved in ribosome biogenesis. We additionally describe two novel gene fusion combinations of previously described genes, namely, SLC45A3- ETV4 and HERVK17-ETV4. Finally, we found one PCa with TMPRSS2-ETV1, one with C15orf21-ETV1, one with EST14-ETV1, and two with 14q133-q21.1–ETV1. In nine PCas (eight ETV1 and one ETV5), exhibiting ETS outlier expression and genomic rearrangement detected by FISH, no 5' fusion partner was found.

Our findings contribute significantly to characterize the heterogeneous group of ETS gene fusions and indicate that all genes described as 5' fusion partners with one ETS gene can most likely be rearranged with any of the other ETS genes involved in prostate carcinogenesis.

These findings and reinforced by the paper under discussion.

3.3 FLI1

FLI1 or Friend leukemia virus integration, is a gene which has been linked to many cancers and is also known to be part of the ETS fusion process acting as ERG does and in this case in PCa.

As Schwentner et al note:

A major hallmark of oncogenesis is the deregulation of cell cycle genes in order to promote proliferation of cancer cells (27). In ES, EWS-FLI1 binds to and activates the promoters of several cell cycle regulators, in particular of E2F transcription factor genes including E2F3, and silencing of EWS-FLI1 induces cell cycle arrest. Our previous data suggested a feed-forward loop activation of E2F3 and of at least 50% of E2F3 target genes by combinatorial binding of EWS-FLI1 and E2F3

As Paulo et al have noted:

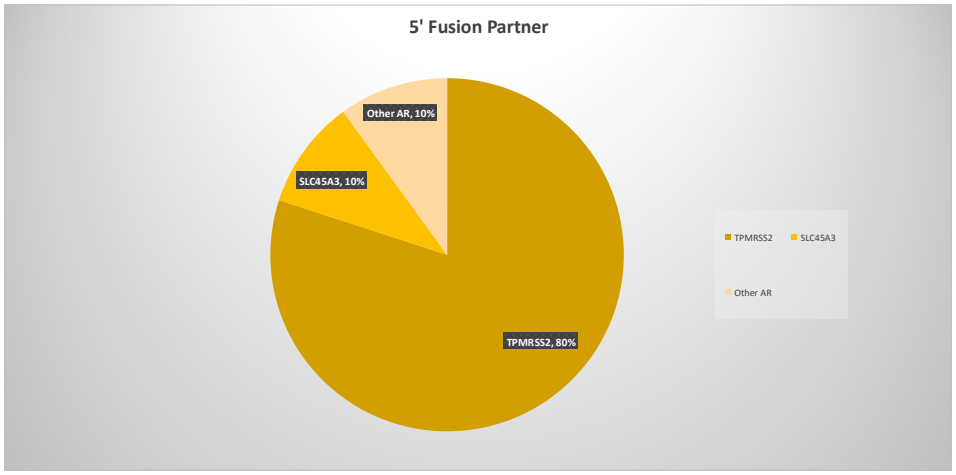
Besides confirming the recurrent presence of ERG, ETV1, ETV4, and ETV5 rearrangements, we here report FLI1 as the fifth ETS transcription factor involved in fusion genes in prostate cancer. Outlier expression of the FLI1 gene was detected by TLDAs in one PCa that showed relative overexpression of FLI1 exons 4:5 as compared with FLI1 exons 2:3.

Their work clearly demonstrates a substantial number of fusions of ETS genes that are found in PCa. The issue is at what point do these occur, what causes them and what therapeutic targets are there?

From Feng et al we have the following data regarding the participation of FLI1 and other ETS genes in fusions of PCa.

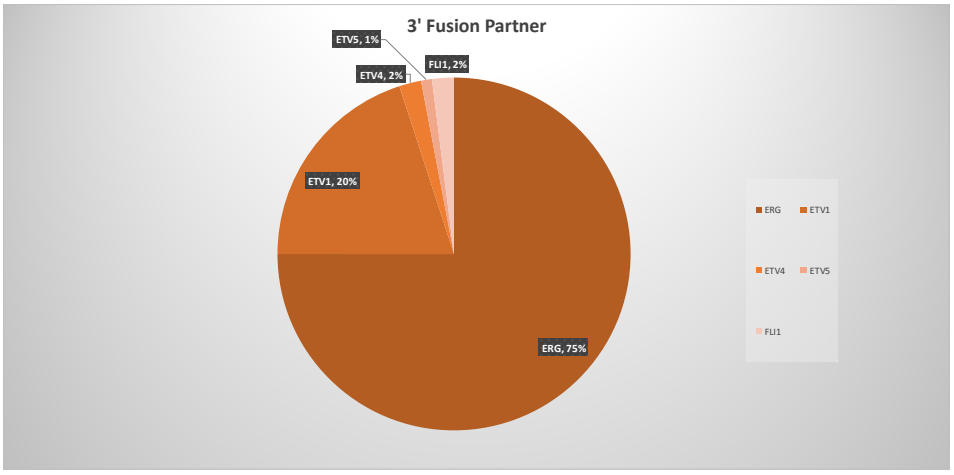
First we show the 5’ fusion end dominated by TPMRRSS1:

5' Fusion Partner	
ON	TMPRSS2

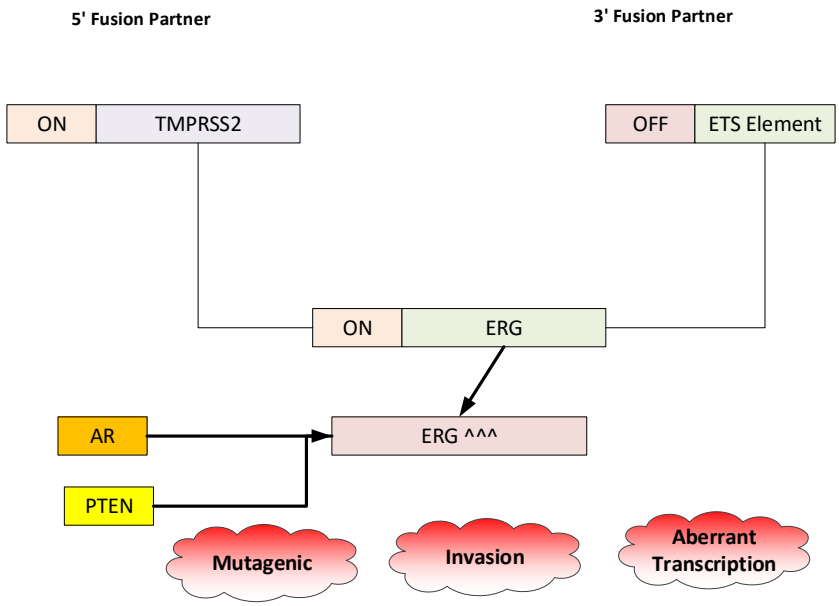


Then we depict the 3’ Fusion partner also dominated by ERG.

3' Fusion Partner	
OFF	ETS Element



The details of this fusion process are shown below. The result of these fusions is as we have discussed the generation of an oncogene, the gene fusion product, leading to proliferation and metastatic growth.



As with ERG we also have a similar effect with FLI1.

3.4 SPOP

We have examined SPOP extensively in prior papers. We summarize some of these issues herein as well as bring them up to date as best as possible.

In a recent study it is reported[10]:

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

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

"Since this mutation appears so frequently in prostate cancer, understanding how it functions as a tumor suppressor when it operates normally helps us determine why the mutated version causes cancer… Our study shows how SPOP is not only able to induce senescence but how mutated SPOP is able to bypass senescence."

[10] http://www.medicalnewstoday.com/releases/301792.php

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

In this study, the research team directly linked this behavior of SPOP to an enzyme called SENP7. The function of SENP7 is not entirely clear, but this study showed just how important it is with regard to SPOP. When SPOP is not mutated, SENP7 remains in check and senescent cells are able to keep cancer activity at bay. To test what happens when SPOP is not functioning properly, the researchers inactivated the gene and observed the effect this had on SENP7.

They found that the levels of SENP7 increase enough that cells are able to overcome senescence and become cancerous. Notably, when SENP7 activity was inhibited, prostate cancer cells showed senescent behavior and stopped growing, suggesting that SENP7 might be an important therapeutic target.

SPOP has become a focus for PCa control in many prior studies and here we see SENP7 as an added but related target.

Recently An et al have noted:

The SPOP E3 ubiquitin ligase gene is frequently mutated in human prostate cancers. Here, we demonstrate that SPOP recognizes a Ser/Thr-rich region in the hinge domain of androgen receptor (AR) and induces degradation of full-length AR and inhibition of AR-mediated gene transcription and prostate cancer cell growth. AR splicing variants, most of which lack the hinge domain, escape SPOP-mediated degradation. Prostate-cancer-associated mutants of SPOP cannot bind to and promote AR destruction. Furthermore, androgens antagonize SPOP-mediated degradation of AR, whereas antiandrogens promote this process. This study identifies AR as a bona fide substrate of SPOP and elucidates a role of SPOP mutations in prostate cancer, thus implying the importance of this pathway in resistance to antiandrogen therapy of prostate cancer.

In a recent study by Theurillat et al the authors noted:

Cancer genome characterization has revealed driver mutations in genes that govern ubiquitylation; however, the mechanisms by which these alterations promote tumorigenesis remain incompletely characterized. Here, we analyzed changes in the ubiquitin landscape induced by prostate cancer–associated mutations of SPOP, an E3 ubiquitin ligase substrate-binding protein. SPOP mutants impaired ubiquitylation of a subset of proteins in a dominant-negative fashion. Of these, DEK and TRIM24 emerged as effector substrates consistently up-regulated by SPOP mutants.

We highlight DEK as a SPOP substrate that exhibited decreases in ubiquitylation and proteasomal degradation resulting from heteromeric complexes of wild-type and mutant SPOP

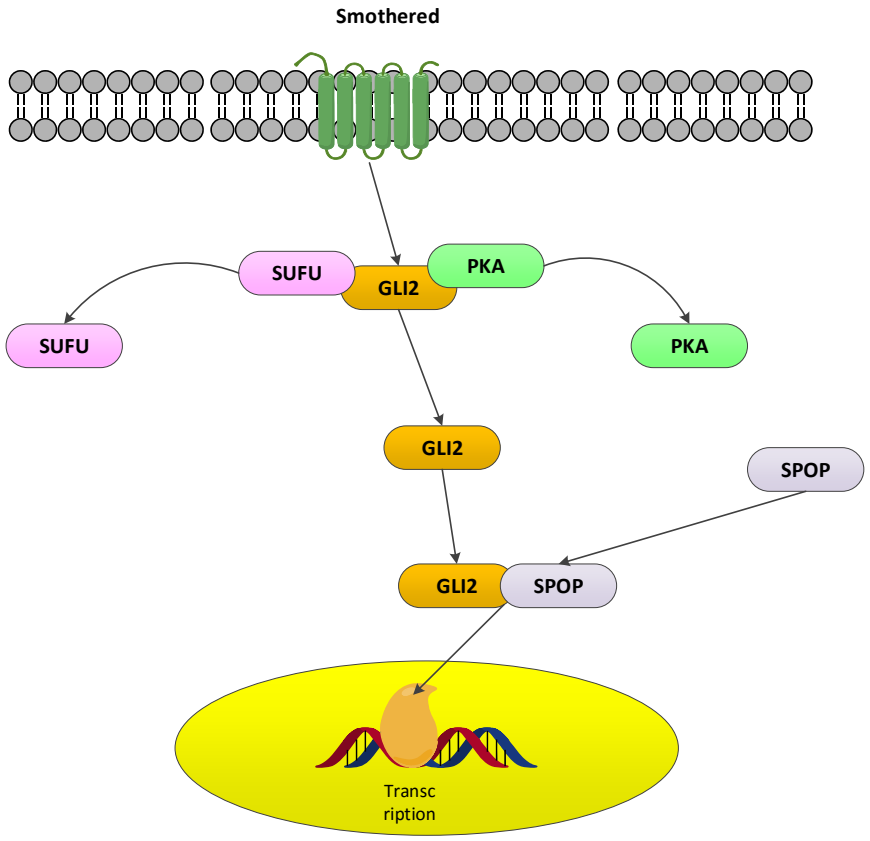
protein. DEK stabilization promoted prostate epithelial cell invasion, which implicated DEK as an oncogenic effector. More generally, these results provide a framework to decipher tumorigenic mechanisms linked to dysregulated ubiquitylation.

Thus is appears that SPOP seems to play a significant role in the control of certain ubiquitins and their resulting degradation of proteins.

From Zeng et al:

A working model. Gli2 is normally kept in the cytoplasm. We propose that SPOP may directly combine with Gli2 and the complex is recognized by ubiquitin. Then the ubiquitinated Gli2 encounters a proteasome-dependent degradation. Or SPOP acts to inhibit Gli2-mediated transcriptional activation and thereby block the effect of Gli2 on the activation of target genes, thus further impact on initiation of cancer cell proliferation, migration, invasion, and apoptosis.

We can see this actually implemented as shown below from Chen and Jiang[11]:



[11] Note that: Models Depicting SPOP-Mediated Degradation of AR in Physiological and Pathological Conditions in Prostate Cancer. (A) Unmutated SPOP promotes degradation of full-length wild-type AR (AR-WT). (B) Prostate-cancer-associated SPOP mutants lose the capacity to promote AR degradation. (C) Prostate-cancer-derived hinge domain-deficient AR splice variants escape from SPOP-mediated degradation. (D) Androgens attenuate SPOP-mediated degradation of AR, whereas the antiandrogen enzalutamide accelerates this process.

3.5 FOXA1

From Jin et al we have:

FoxA1 (FOXA1), also named HNF-3a, is a winged-helix transcription factor of the forkhead family. It plays essential roles in the epithelial differentiation and development of a number of organs including the pancreas, prostate, and breast (1–6). For example, while FoxA1-knockout mice are developmentally lethal, conditional FoxA1 knockout in the mouse prostate results in severely altered ductal development that contains immature epithelial cells surrounded by abnormally thick stromal layers (7).

Concordantly, in the adult prostate, FoxA1 has also been tightly linked to the maintenance of the prostate epithelial phenotype and the expression of prostatespecific genes. This is mediated through its regulation of the androgen receptor (AR) transcriptional activities (8, 9). As a pioneering factor, FoxA1 opens up compact chromatin to facilitate subsequent AR recruitment (4, 10–13).

Genome-wide location analysis of prostate cancer cells have shown that FoxA1 preoccupies lineage-specific enhancers even before androgen stimulation and cooccupies a majority of AR binding sites in androgen-treated cells. FoxA1 is thus indispensable for defining a prostatic AR program and is critical to prostate development, function, as well as malignant transformation.
From Gerhardt et al we have:

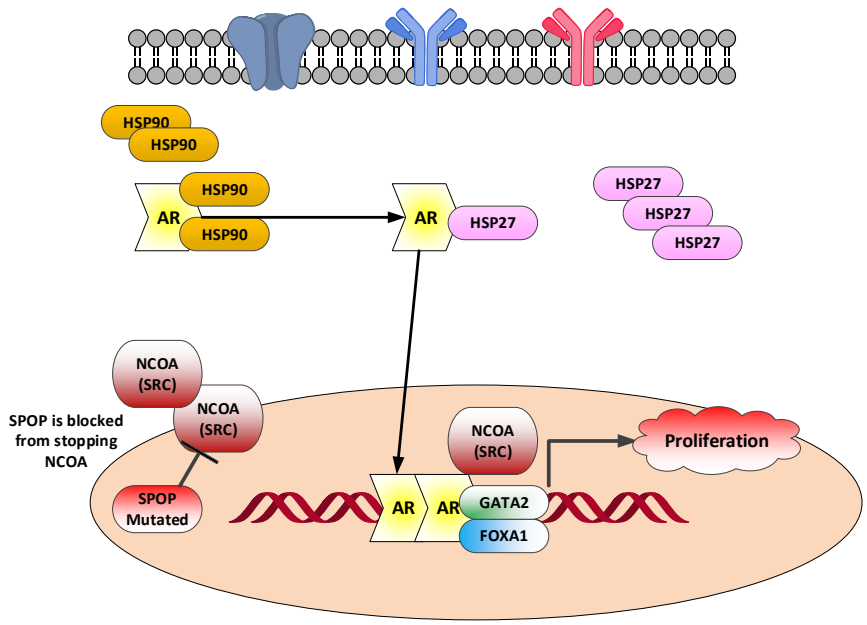
Forkhead box protein A1 (FOXA1) modulates the transactivation of steroid hormone receptors and thus may influence tumor growth and hormone responsiveness in prostate cancer. We therefore investigated the correlation of FOXA1 expression with clinical parameters, prostate-specific antigen (PSA) relapse-free survival, and hormone receptor expression in a large cohort of prostate cancer patients at different disease stages. FOXA1 expression did not differ significantly between benign glands from the peripheral zone and primary peripheral zone prostate carcinomas.

However, FOXA1 was overexpressed in metastases and particularly in castration-resistant cases, but was expressed at lower levels in both normal and neoplastic transitional zone tissues. FOXA1 levels correlated with higher pT stages and Gleason scores, as well as with androgen (AR) and estrogen receptor expression. Moreover, FOXA1 overexpression was associated with faster biochemical disease progression, which was pronounced in patients with low AR levels. Finally, siRNA-based knockdown of FOXA1 induced decreased cell proliferation and migration.

Moreover, in vitro tumorigenicity was inducible by ARs only in the presence of FOXA1, substantiating a functional cooperation between FOXA1 and AR.

In conclusion, FOXA1 expression is associated with tumor progression, dedifferentiation of prostate cancer cells, and poorer prognosis, as well as with cellular proliferation and migration and with AR signaling. These findings suggest FOXA1 overexpression as a novel mechanism inducing castration resistance in prostate cancer.

From Wyatt and Gleave we have the following descriptive of the pathway blocking impact of SPOP and FOXA1. This is shown below for CRPC.



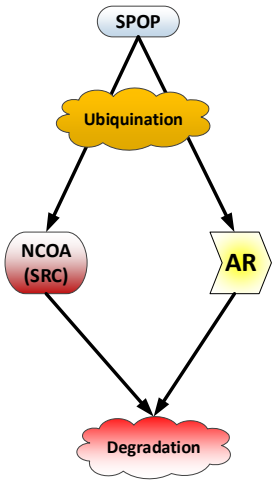
Now Wyatt and Gleave specifically note regarding FOXA1:

The forkhead protein FOXA1 is a critical interacting partner of the AR, functioning as a pioneer factor to modulate chromatin accessibility and facilitate transcription. In prostate cancer, FOXA1 is capable of specifying unique AR binding sites and has an AR-independent function as a metastasis regulator. Although it can be genomically amplified, deleted, or mutated in CRPC patients, suggesting complex context-dependent, the precedent set by the development of a FOXM1 inhibitor suggests that forkhead protein modulation in prostate cancer might hold promise.

Interestingly, FOXA1 and AR co-localize on chromatin with GATA2, a transcription factor that enhances recruitment of NCOAs to the AR complex. Additionally, at the transcriptional level, there appears to be a complex feedback balance between GATA2 and the AR itself, since GATA2 is repressed by the AR and androgen, but is necessary for optimal expression of the AR. High GATA2 expression predicts poor outcome in prostate cancer patients and further promotes the concept of therapeutically targeting the AR transcriptional complex in CRPC patients.

A promising contemporary strategy to disrupt AR in this manner is to use bromodomain inhibitors (e.g. JQ1) to inhibit the chromatin reader BRD4 that interacts with the N-terminal domain of the AR. Preclinical studies have shown that JQ1 disrupts AR- mediated gene transcription in CRPC models, significantly reducing tumour volume relative to controls.

They also depict the normal process of ubiquitination and elimination as shown below. This assumes a normal SPOP and FOXA1.



3.6 IDH1

IDH1 is a metabolically related gene. As Yang et al have noted:

Altered metabolic regulation in tumor cells was observed more than 80 years ago. Tumor cells, despite having an increased uptake of glucose, produce much less ATP than expected from complete oxidative phosphorylation and accumulate a significant amount of lactate. This phenomenon, representing arguably the first molecular phenotype characterized in cancer, is commonly known as Warburg Effect. The Warburg Effect's most notable clinical application is in 2[18F] fluoro-2-deoxy-D-glucose-positron emission tomography (FDG-PET), where it provides the theoretical basis for the detection of tumors because of their increased glucose uptake relative to surrounding normal tissues[12].

Despite its long history and broad clinical application, however, relatively little progress has been made over past 4 decades in understanding how altered metabolic regulation contributes to tumorigenesis. This is largely because of the fact that cancer research during this period has focused on genetic mutations in human cancer that, until very recently, were not known to include metabolic enzymes. The recent discovery of mutations targeting metabolic genes in cancer has renewed interest in cancer metabolism.

Eight genes: FH, SDHA, SDHB, SDHC, SDHD, SDHAF2, IDH1, and IDH2, encoding for 4 different metabolic enzymes: fumurate hydratase (FH), succinate dehydrogenase (SDH), and

[12] As van der Heiden et al note: *In contrast to normal differentiated cells, which rely primarily on mitochondrial oxidative phosphorylation to generate the energy needed for cellular processes, most cancer cells instead rely on aerobic glycolysis, a phenomenon termed "the Warburg effect." Aerobic glycolysis is an inefficient way to generate adenosine 5′-triphosphate (ATP), however, and the advantage it confers to cancer cells has been unclear. Here we propose that the metabolism of cancer cells, and indeed all proliferating cells, is adapted to facilitate the uptake and incorporation of nutrients into the biomass (e.g., nucleotides, amino acids, and lipids) needed to produce a new cell. Supporting this idea are recent studies showing that (i) several signaling pathways implicated in cell proliferation also regulate metabolic pathways that incorporate nutrients into biomass; and that (ii) certain cancer-associated mutations enable cancer cells to acquire and metabolize nutrients in a manner conducive to proliferation rather than efficient ATP production. A better understanding of the mechanistic links between cellular metabolism and growth control may ultimately lead to better treatments for human cancer.*

isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are frequently mutated. These mutations are both germinal and somatic, and occur in a wide range of human cancers (4). In this review, we will focus the discussion on the mechanisms and the translational research of IDH1 and IDH2, 2 of the most frequently mutated metabolic genes in human cancer.

The Warburg effect was a powerful observation which had not seen extensive follow-up. However, with recent understanding we see that genes like IDH1 plays a key role.

From Koseki et al:

In 2008 and 2009, two independent cancer sequencing projects reported high frequencies of mutations of the gene encoding the cytosolic enzyme IDH1, a critical component of the tricarboxylic acid (TCA) cycle, in glioblastoma multiforme (GBM) and acute myeloid leukemia (AML). Further study reported that IDH2, the gene encoding the homologous enzyme in the mitochondria was also frequently mutated in GBM patients (10). Studies indicated that >75% of grade 2 and 3 GBM and 20% of AML harbor mutations of IDH1 at R132, or IDH2 at the homologous R172 residue.

These oncogenic mutations in IDH1 and IDH2 enzymes reduce their native activity, and generate neomorphic activity that converts α-ketoglutarate (α-KG; also known as 2-oxyoglutarate) to D-2-hydroxygutarate (D2HG. D2HG is an oncometabolite that can cause changes to the epigenetic landscape by inhibiting the activities of iron (II), α-KG-dependent dioxygenases, including:

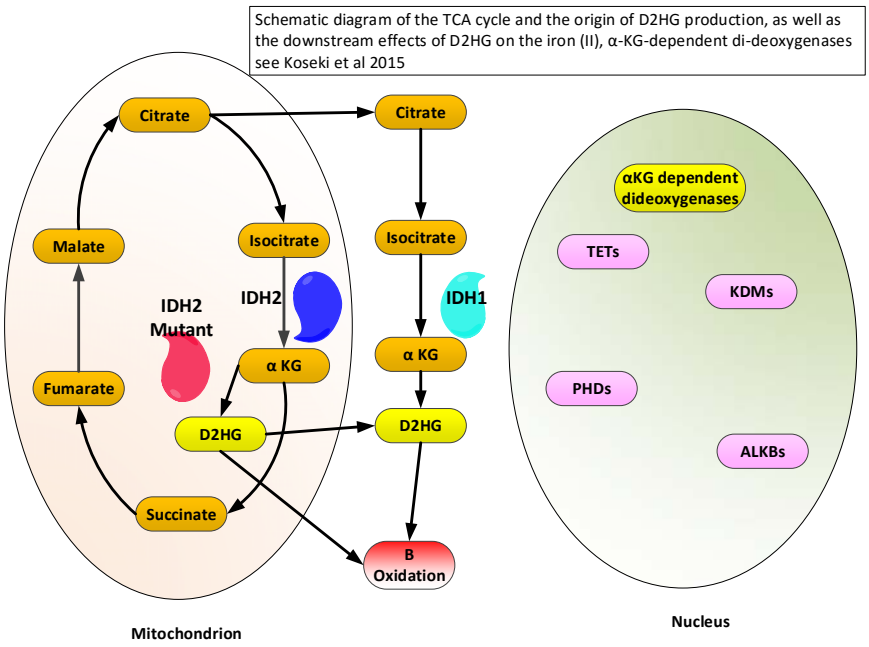
prolyl-hydroxylase-domains (PHDs) resulting in pseudo-hypoxic environment and activation of hypoxia-inducible-factor (HIF) pathway leading to aberrant cellular proliferation;

ten-eleven translocation (TET)-family demethylases,

AlkB-family dioxygenases, which protect nucleotides against methylating reactions by directly dealkylating bases (1mA, 3mC, 1mG and 3mT);

and histone lysine demethylases (KDMs).

We graphically demonstrate this factor below.



The TCA cycle this changes as we see changes in IDH1. Those changes then lead to proliferation and metastatic behavior. Initially proliferation is dominant. As Yang et al note:

The first biochemical alteration that is associated with tumor-derived IDH1 or IDH2 mutants is the loss of their normal activity in catalyzing the NADP þ -dependent oxidative decarboxylation of isocitrate into a-ketoglutarate.

They conclude as follows:

In conclusion, the discovery and subsequent investigation of IDH1/2 mutations in tumors have renewed interest into the research of tumor metabolism, identified a good biomarker for early detection and prognosis of several types of tumors, and led to the elucidation of the IDH-TET pathway in the epigenetic control of cell differentiation and tumor suppression. The extensive efforts that are currently underway should lead to a better understanding of the role of altered metabolic enzymes and metabolites in tumorigenesis, and novel strategies for therapeutic intervention in IDH1/2-mutated tumors.

4 OBSERVATIONS

We now will provide a few observations regarding this work. It does provide another significant marker for understanding PCa but it does not appear to be a significant diagnostic or prognostic marker. Yet a great deal can be drawn from the results.

4.1 GENERAL CONCLUSIONS

Let us first examine the general conclusions of the paper. The authors conclude as follows:

Primary prostate cancers exhibit a wide range of androgen receptor activity. This study demonstrates for the first time a direct association between mutations in SPOP or FOXA1 and increased AR-driven transcription in human prostate cancers. Further studies in preclinical models, as well as in clinical trial settings, will be required to understand the implications of variable AR activity in the contexts of chemoprevention and prostate cancer-directed treatment strategies.

Other, more immediately actionable opportunities for targeted therapy exist for the 19% of primary prostate cancers that have defects in DNA repair and for the nearly equal number of cancers with altered key effectors of both PI3K and MAPK pathways.

While the numbers of DNA repair defects found in organ confined prostate tumors may be lower than those found in metastatic prostate cancer, an increase in the number of such defects with disease progression suggests a possible advantage to targeting DNA repair-deficient tumors at an earlier stage of disease, perhaps at initial diagnosis.

The above argues for aggressive early stage treatment. However, it does not do so for a variation in tumor profile. Namely, not all Ca are the same in genetic makeup. Thus we must consider the complexity of the genetic profile and that with some early ostensibly confined PCa there may have already been metastatic departure from the primary tumor. Also as we have noted before there is also the issue of cancer stem cell, CSC, composition and if the cells being measured are CSC or just derivatives thereof.

Such strategies may include preventing DNA damage, as well as targeting deficient DNA repair. Alterations in the PI3K/MTOR pathway also play an important role: beyond the frequent inactivation of PTEN, we document rare activation of PIK3CA, PIK3CB, AKT1, and MTOR, and of several small GTPases, including HRAS, as well as BRAF. As DNA sequencing of tumor samples becomes more widely adopted earlier in the clinical care of cancer patients, such alterations may emerge as candidates for inclusion in clinical trials after front-line therapy.

Again these are suggestions for therapeutic approaches.

4.2 CRPC EXPRESSIONS

The paper by TCGA focuses on early stage localized PCa. In contrast and in a recent paper by Grasso et al they note regarding CRPC:

Characterization of the prostate cancer transcriptome and genome has identified chromosomal rearrangements and copy number gains and losses, including ETS gene family fusions, PTEN loss and androgen receptor (AR) amplification, which drive prostate cancer development and progression to lethal, metastatic castration resistant prostate cancer (CRPC).

Clearly the ETS fusion issues have been well known in CRPC. The presence in early stage PCa is not unexpected. Also we know that AR amplification can give rise to extensive proliferation.

However, less is known about the role of mutations. Here we sequenced the exomes of 50 lethal, heavily pre-treated metastatic CRPCs obtained at rapid autopsy (including three different foci from the same patient) and 11 treatment-naive, high-grade localized prostate cancers.

The mutation issue is critical. Just knowing what mutation exists is not the answer. We must know what the resultant mutation leads to and why it occurred. Many mutations may be a result of the other key changes as the cells divide, and they may actually be a direct result of the reformation of the DNA and the result may or may not be causative of other factors. The Gundem et al work is an example of the proliferation of changes.

We identified low overall mutation rates even in heavily treated CRPCs (2.00 per megabase) and confirmed the monoclonal origin of lethal CRPC. Integrating exome copy number analysis identified disruptions of CHD1 that define a subtype of ETS gene family fusion negative prostate cancer. Similarly, we demonstrate that ETS2, which is deleted in approximately one-third of CRPCs (commonly through TMPRSS2:ERG fusions), is also deregulated through mutation. Furthermore, we identified recurrent mutations in multiple chromatin- and histone-modifying genes, including MLL2 (mutated in 8.6% of prostate cancers), and demonstrate interaction of the MLL complex with the AR, which is required for AR-mediated signaling.

We also identified novel recurrent mutations in the AR collaborating factor FOXA1, which is mutated in 5 of 147 (3.4%) prostate cancers (both untreated localized prostate cancer and CRPC), and showed that mutated FOXA1 represses androgen signaling and increases tumour growth. Proteins that physically interact with the AR, such as the ERG gene fusion product, FOXA1, MLL2, UTX (also known as KDM6A) and ASXL1 were found to be mutated in CRPC. In summary, we describe the mutational landscape of a heavily treated metastatic cancer, identify novel mechanisms of AR signaling deregulated in prostate cancer, and prioritize candidates for future study.

Their recognition of FOXA1 is also key since it is part of the loss of control in AR proliferation.

4.3 CLASSIC ISSUES

There are a set of classic issues which would also require discussion. They are:

Cause: What causes these changes. Namely what is the cause of the fusion and translocations on ETS. This is one of the major issues faced in understanding the PCa issue.

Cancer Stem Cell: There is an ongoing discussion of CSC for PCa[13]. One issue is the CSC in either the basal or luminal cell. However, the question is; does the CSC contain the controlling driver and if so what is it. If the cells derived from the CSC contain other gene expressions are they of any merit? How does one ascertain the CSC and focus on it alone?

Epigenetic Effects: We know that methylation and also miRNAs have significant effects. Thus we ask what factors methylation may have, especially in translocation and fusion during cell proliferation.

Cell of Origin: The cell of origin issue is an adjunct to the debate in the CSC discussion. Namely is it necessary to know a cell of origin in order to perform this analysis?

Therapeutic Targets: There are suggested therapeutic targets mentioned. Just what ones are worth the focus?

[13] See Zhang et al, *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.*

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