

# SPDEF, ETS, PROSTATE CANCER

Recent work examining the relationship between SPDEF, an ETS transcription factor, and Foxm1, an oncogene, has shed some light on the complexity of paths in prostate cancer. We examine some of these complex pathways to add to the collection we have examined before. Copyright 2014 Terrence P. McGarty, all rights reserved.

*Terrence P McGarty  
White Paper No 117  
November, 2014*

Notice

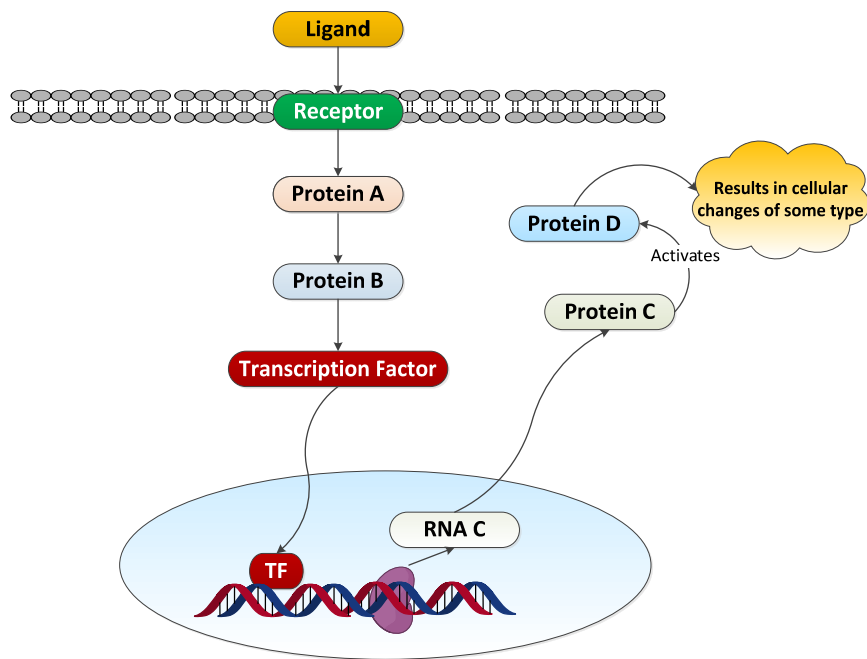
This document represents the personal opinion of the author and is not meant to be in any way the offering of medical advice or otherwise. It represents solely an analysis by the author of certain data which is generally available. The author furthermore makes no representations that the data available in the referenced papers is free from error. The Author also does not represent in any manner or fashion that the documents and information contained herein can be used other than for expressing the opinions of the Author. Any use made and actions resulting directly or otherwise from any of the documents, information, analyses, or data or otherwise is the sole responsibility of the user and The Author expressly takes no liability for any direct or indirect losses, harm, damage or otherwise resulting from the use or reliance upon any of the Author's opinions as herein expressed. There is no representation by The Author, express or otherwise, that the materials contained herein are investment advice, business advice, legal advice, medical advice or in any way should be relied upon by anyone for any purpose. The Author does not provide any financial, investment, medical, legal or similar advice in this document or in its publications on any related Internet sites.

## Contents

1	Introduction.....	3
2	ETS Family .....	5
3	Current Investigations.....	8
4	The Genes and Proteins .....	10
4.1	SPDEF .....	10
4.2	Foxm1.....	12
4.3	MMP.....	13
5	SPDEF Previous Analyses.....	16
6	Observations .....	19
7	References.....	20

## 1 INTRODUCTION

SPDEF is one of the 30-40 transcription factors found in the ETS family. Recall that transcription factors can be promoters (activators) or repressors of gene expression and that depending where they act than can dramatically change the expression of genes in the cell. The general paradigm is shown below:

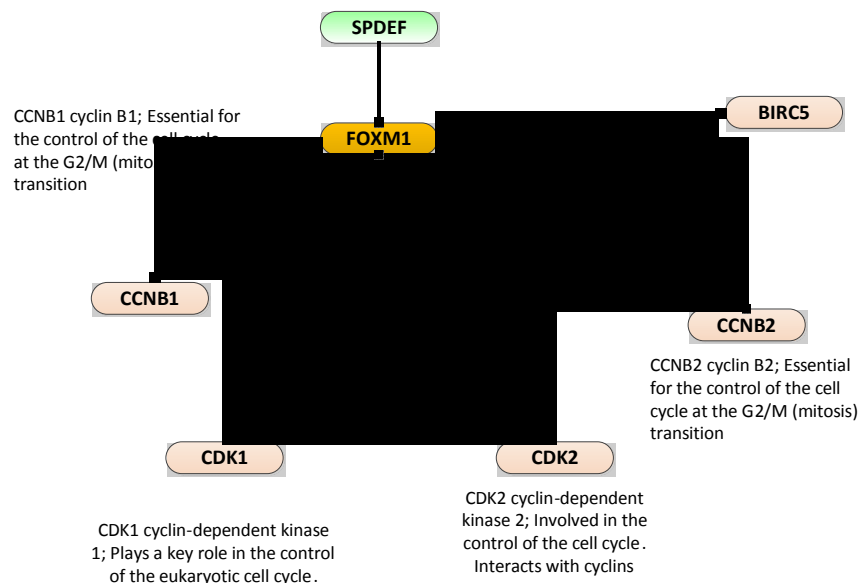


The ETS family is a powerful family of transcription factors and they are often found altered in prostate cancer. In this section we examine a specific subset of these transcription factors.

The chart below displays the specific factors we discuss herein. The driver for this discussion is a recent paper by Cheng et al which discusses SPDEF and the regulation of FOXM1 oncogene. The paper is interesting in that it examines a transcription factor and the specific influence on an oncogene expression. SPDEF is in the ETS family and thus the interest in ETS. SPDEF is the SAM pointed domain containing ETS transcription factor. Thus, the acronym was formed. It is distinct from another ETS gene the PDEF which the prostate derived epithelial factor. As they indicate it is not clear what the role of SPDEF is in PCa and it is not clear whether its expression suppresses or enhances PCa development. Yet the analysis of this process does present an alternative view of a complex PCa development mechanism.

SPDEF	<ul style="list-style-type: none"> <li>•Member of the ETS family</li> <li>•SAM-pointed domain-containing ETS transcription factor</li> </ul>
ETS	<ul style="list-style-type: none"> <li>•Large family of 29 genes in humans</li> <li>•Regulates transcription as activator or repressor</li> </ul>
MMP9/MMP13	<ul style="list-style-type: none"> <li>•Matrix metallopeptidase</li> <li>•manages breakdown of ECM and enables angiogenesis, ETS transcription factor SRDEF activates their transcription</li> </ul>
FOXM1	<ul style="list-style-type: none"> <li>•member of FOX family of transcription factors</li> <li>•may have role in upregulated in cell proliferation</li> </ul>
E cadherin	<ul style="list-style-type: none"> <li>•cell binding and stabilization and localization</li> <li>•loss allows for movement of cells</li> </ul>

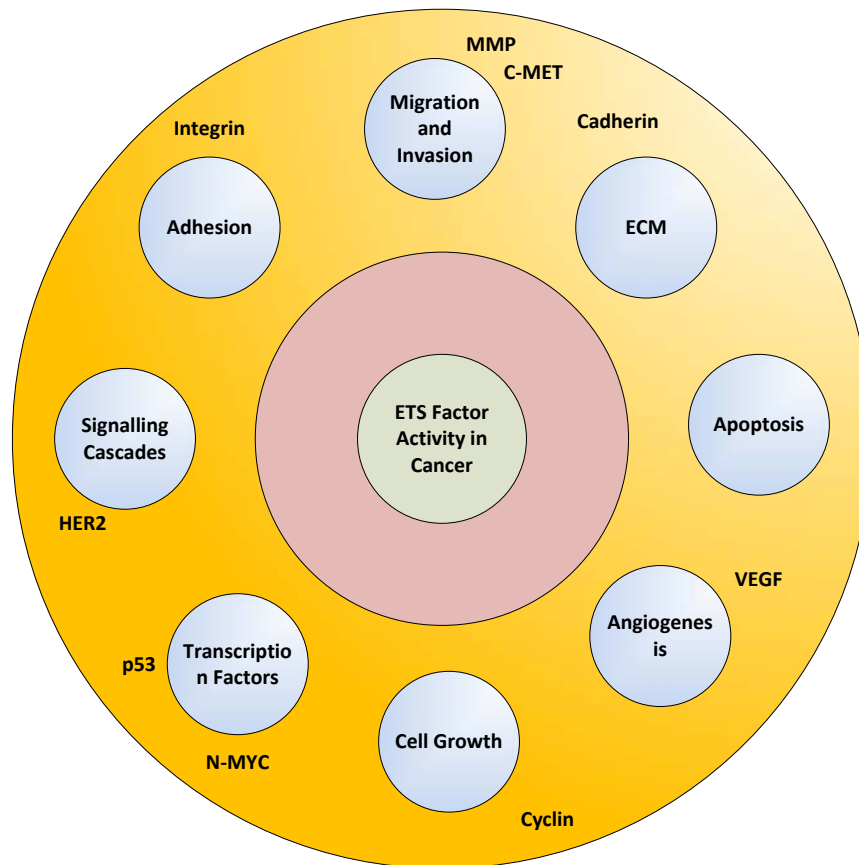
As Cheng et al have postulated the increase in SPDEF results in a suppression of FOXM1 which is a known oncogene, especially for PCa. As in Gellmann et al (pp 328-333) FOXM1 is a known oncogene. It drives the cell cycle and thus leads to uncontrolled cell proliferation. We show this below:



## 2 ETS FAMILY

The ETS (“E26 transformation specific”) family has some 30-40 genes and many relate to prostate cancer. For example the ERG (the “ETS related gene”) gene is often found translocated with TPRSS in a fused state and this translocation is a clear indication of an aggressive form of PCa.

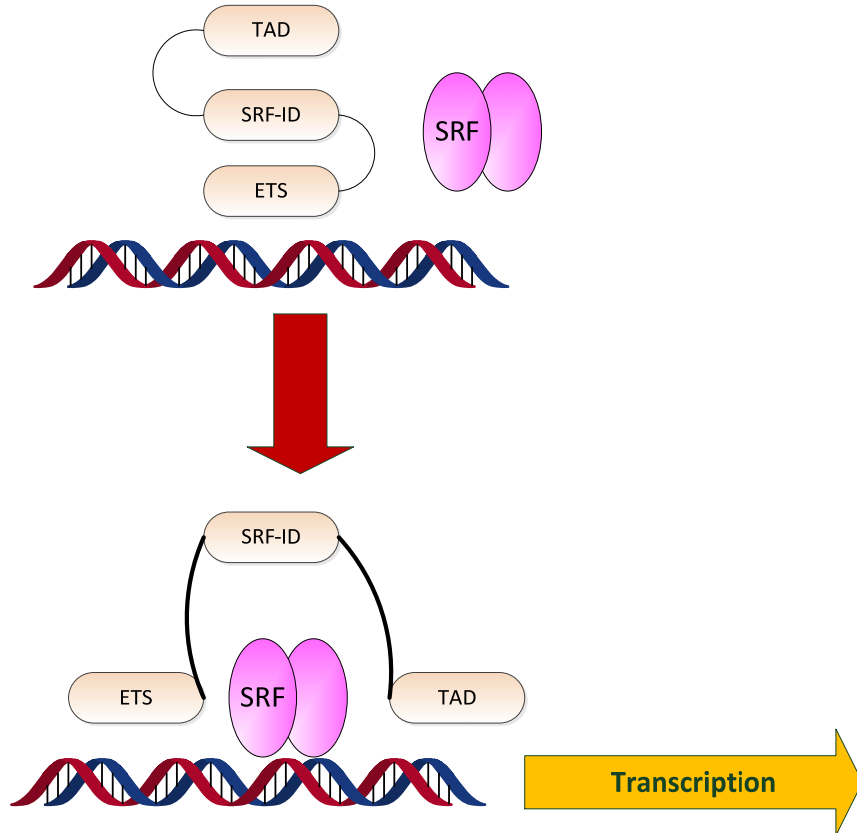
From Watson et al we have the following breath of structure for the ETS family:



Let us begin with a brief overview of ETS family and specifically the inclusion of SPDEF. As Wasyluk et al state:

*The Ets family of transcription factors includes nuclear phosphoproteins that are involved in cell proliferation, differentiation and oncogenic transformation. The family is defined by a conserved DNA-binding domain (the ETS-DBD), which forms a highly conserved, winged, helix-turn-helix structural motif. As targets of the Ras-MAPK signaling pathway, Ets proteins function as critical nuclear integrators of ubiquitous signaling cascades. To direct signals to specific target genes, Ets proteins interact with (other) transcription factors that promote the binding of Ets proteins to composite Ras-responsive elements.*

We demonstrate the winged or “butterfly” operation of ETS transcription factors as shown below<sup>1</sup>:



In a 2012 report in Science Daily they state<sup>2</sup>:

*Prostate cancer doesn't kill in the prostate -- it's the disease's metastasis to other tissues that can be fatal. A University of Colorado Cancer Center study published this week in the Journal of Biological Chemistry shows that prostate cancer cells containing the protein SPDEF continue to grow at the same pace as their SPDEF- cousins, but that these SPDEF+ cells are unable to survive at possible sites of metastasis.*

*"It's as if these cancer cells with SPDEF can't chew into distant tissue and so are unable to make new homes," says Hari Koul, PhD, investigator at the CU Cancer Center and director of urology research at the University of Colorado School of Medicine, the study's senior author.*

*Koul and his group discovered the homesteading power of cancer cells that have lost SPDEF by introducing a gene into cells that makes them glow in the presence of a dye, and then introducing them into the bloodstream of animal models. Cells without SPDEF traveled through the blood and successfully attached to tissue, surviving and so fluorescing many weeks later when dye was*

<sup>1</sup> See Marks et al p 405. As adapted.

<sup>2</sup> <http://www.sciencedaily.com/releases/2012/07/120706164422.htm>

*introduced. However, cells with SPDEF flowed through the blood but were unable to successfully establish new colonies and so soon died out.*

***In fact, the protein SPDEF doesn't act directly to allow cells to attach at possible metastasis sites, but is a transcription factor that controls the production (or lack thereof) of two other proteins MMP9 and MMP13. These two downstream proteins work to break down tissue, like a dissolving agent -- they are the cleaning crew that clears space for new and different growth, and in the case of prostate cancer metastasis they chip the tissue footholds that cancer cells need to create micrometastases.***

There has been a great deal of work on MMPs especially MMP9<sup>3</sup>. We will expand this discussion later.

*"Given that MMP9 and perhaps MMP13 are also involved in metastasis of several other cancers including lung, ovarian, breast and colon to name a few, our findings could potentially have far-reaching consequences outside prostate cancer," adds Koul*

*The group's continuing work points in two directions.*

*"First, we hope that the presence of SPDEF could help doctors recognize prostate cancers that don't require treatment." If future studies confirm the group's initial findings, the presence of SPDEF could predict prostate cancers that are unable to metastasize and so unable to kill. These cancers could be left to run their course without the use of treatments that sometimes carry difficult side effects.*

*"And second," Koul says, "we hope to regulate expression of this protein to remove prostate cancers' ability to metastasize."*

*Koul points to small molecules, gene therapy or nanodelivery as possible mechanisms for introducing SPDEF into cells that lack the protein.*

*"With this discovery we have opened a hopeful door into a future in which prostate and potentially other cancers are unable to metastasize," Koul says.*

However it appears that this work has been withdrawn in several venues. It is not clear where the problem was that caused the withdrawal.

---

<sup>3</sup> See Jamaspishvili et al, *Matrix metalloproteinases (MMPs) have been implicated in invasion and metastasis of human malignancies. Moses et al. used substrate gel electrophoresis (zymography) to determine MMPs in the urine of patients with a variety of cancers. MMP9 yielded better sensitivity (64%) than MMP2 (39%) for CaP whereas specificities (84 and 98%, respectively) were calculated from controls of both sexes. The same group also detected several unidentified urinary gelatinase activities with molecular weights 4125 kDa and recently used chromatography, zymography and mass spectrometry for their identification. The approximately 140, 4220 and approximately 190 kDa gelatinase species were identified as MMP9/TIMP1 complex, MMP9 dimer and ADAMTS7, respectively. MMP9 dimer and MMP9 were independent predictors for distinguishing between patients with prostate and bladder cancer.*



### 3 CURRENT INVESTIGATIONS

We will now consider a recent paper by Cheng et al which we referred to in the Introduction. The interest here is the collecting together of multiple elements in this SPDEF chain and the effects of ETS transcription factors.

In the recent paper by Cheng et al the authors state<sup>4</sup>:

*SAM-pointed domain-containing ETS transcription factor (SPDEF) is expressed in normal prostate epithelium. While its expression changes during prostate carcinogenesis (PCa), the role of SPDEF in prostate cancer remains controversial due to the lack of genetic mouse models. In present study, we generated transgenic mice with the loss- or gain-of-function of SPDEF in prostate epithelium to demonstrate that SPDEF functions as tumor suppressor in prostate cancer. Loss of SPDEF increased cancer progression and tumor cell proliferation, whereas over-expression of SPDEF in prostate epithelium inhibited carcinogenesis and reduced tumor cell proliferation in vivo and in vitro.*

*Transgenic over-expression of SPDEF inhibited mRNA and protein levels of Foxm1, a transcription factor critical for tumor cell proliferation, and reduced expression of Foxm1 target genes, including Cdc25b, Cyclin B1, Cyclin A2, Plk-1, AuroraB, CKS1 and Topo2alpha.*

*Deletion of SPDEF in transgenic mice and cultures prostate tumor cells increased expression of Foxm1 and its target genes. Furthermore, an inverse correlation between SPDEF and Foxm1 levels was found in human prostate cancers. The two-gene signature of low SPDEF and high Foxm1 predicted poor survival in prostate cancer patients. Mechanistically, SPDEF bound to, and inhibited transcriptional activity of Foxm1 promoter by interfering with the ability of Foxm1 to activate its own promoter through auto-regulatory site located in the 2745/2660 bp Foxm1 promoter region. Re-expression of Foxm1 restored cellular proliferation in the SPDEF-positive cancer cells and rescued progression of SPDEF-positive tumors in mouse prostates. Altogether, SPDEF inhibits prostate carcinogenesis by preventing Foxm1-regulated proliferation of prostate tumor cells.*

*The present study identified novel crosstalk between SPDEF tumor suppressor and Foxm1 oncogene and demonstrated that this crosstalk is required for tumor cell proliferation during progression of prostate cancer in vivo.*

The relationship between SPDEF and Foxm1 are significant and could become a possible therapeutic target. They continue:

*Development of prostate cancer is a multistep process that involves the loss of tumor suppressor functions and activation of oncogenes. SPDEF transcription factor is expressed in normal prostate epithelium and its expression changes during prostate carcinogenesis (PCa). Since the role of SPDEF in PCa remains controversial, we generated transgenic mice with loss- and gain-of-function of SPDEF to demonstrate that SPDEF functions as a tumor suppressor in PCa. In*

---

<sup>4</sup> <http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1004656>

*animal models, the loss of SPDEF promoted PCa and increased the levels of Foxm1, a well-known oncogenic protein.*

*Overexpression of SPDEF in prostate epithelium decreased PCa and reduced Foxm1 levels. Proliferation defects in SPDEF-containing tumor cells were corrected by re-expression of Foxm1, providing direct evidence that SPDEF inhibits tumor cell proliferation through Foxm1. We further showed that SPDEF directly bound to Foxm1 promoter and prevented its autoregulatory activation. In prostate cancer patients, the low SPDEF and high Foxm1 were found in most aggressive prostate tumors that were associated with poor prognosis. The combined two-gene signature of low SPDEF and high Foxm1 was a strong predictor of survival in prostate cancer patients. The present study identified novel molecular mechanism of prostate cancer progression, providing a crosstalk between SPDEF tumor suppressor and Foxm1 oncogene.*

## 4 THE GENES AND PROTEINS

We now return to a consideration of some of the specific proteins we have seen in the above and which we have seen in the original overview. We start with SPDEF, one of the many ETS transcription factors, and then detail a bit on ETS in general. Secondly we discuss the Foxm1 gene which also seems integral to these operations. Also there is a significant yet peripheral role for MMD proteins which help metastatic process by degrading the ECM fabric and allowing movement.

### 4.1 SPDEF

From NCBI we have the following regarding SPDEF<sup>5</sup>:

*The protein encoded by this gene, SPDEF, belongs to the ETS family of transcription factors. It is highly expressed in the prostate epithelial cells, and functions as an androgen-independent transactivator of prostate-specific antigen (PSA) promoter. Higher expression of this protein has also been reported in brain, breast, lung and ovarian tumors, compared to the corresponding normal tissues, and it shows better tumor-association than other cancer-associated molecules, making it a more suitable target for developing specific cancer therapies. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.*

The following is a short list of some of these factors<sup>6</sup>:

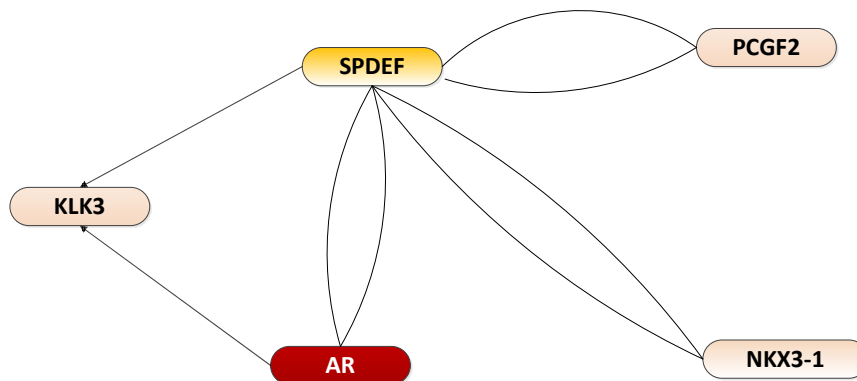
<i>Regulates</i>	<i>Regulated by</i>	<i>Binds</i>	<i>Role in cell</i>	<i>Disease</i>
PLAU	SERPINB5	NKX3-1	migration	breast cancer
ITGA5	Crf	TICAM1	invasion	
ITGA6	FOXA1	MYD88	expression in	
SNAI2	Human rhinovirus 16	LRP6	abnormal morphology	
DNA promoter	ERBB2	EMX1	activation	

The Figure below details a putative pathway element<sup>7</sup>:

<sup>5</sup> <http://www.ncbi.nlm.nih.gov/gene/25803>

<sup>6</sup> <https://targetexplorer.ingenuity.com/gene/EG/25803/pathways>

<sup>7</sup> See also: <https://targetexplorer.ingenuity.com/gene/EG/25803/pathways>



<http://www.pathwaycommons.org/pc/record2.do?id=64346>

From NCBI we have the following descriptions of these elements:

<i>Gene</i>	<i>Function</i>
AR	The androgen receptor gene is more than 90 kb long and codes for a protein that has 3 major functional domains: the N-terminal domain, DNA-binding domain, and androgen-binding domain. The protein functions as a steroid-hormone activated transcription factor. Upon binding the hormone ligand, the receptor dissociates from accessory proteins, translocates into the nucleus, dimerizes, and then stimulates transcription of androgen responsive genes. This gene contains 2 polymorphic trinucleotide repeat segments that encode polyglutamine and polyglycine tracts in the N-terminal transactivation domain of its protein.
SPDEF	The protein encoded by this gene belongs to the ETS family of transcription factors. It is highly expressed in the prostate epithelial cells, and functions as an androgen-independent transactivator of prostate-specific antigen (PSA) promoter. Higher expression of this protein has also been reported in brain, breast, lung and ovarian tumors, compared to the corresponding normal tissues, and it shows better tumor-association than other cancer-associated molecules, making it a more suitable target for developing specific cancer therapies.
KLK3	Kallikreins are a subgroup of serine proteases having diverse physiological functions. Growing evidence suggests that many kallikreins are implicated in carcinogenesis and some have potential as novel cancer and other disease biomarkers. This gene is one of the fifteen kallikrein subfamily members located in a cluster on chromosome 19. Its protein product is a protease present in seminal plasma. It is thought to function normally in the liquefaction of seminal coagulum, presumably by hydrolysis of the high molecular mass seminal vesicle protein. Serum level of this protein, called PSA in the clinical setting, is useful in the diagnosis and monitoring of prostatic carcinoma.
NKX3-1	This gene encodes a homeobox-containing transcription factor. This transcription factor functions as a negative regulator of epithelial cell growth in prostate tissue. Aberrant expression of this gene is associated with prostate

<i>Gene</i>	<i>Function</i>
<b>PCGF2</b>	tumor progression. Alternate splicing results in multiple transcript variants of this gene. The protein encoded by this gene contains a RING finger motif and is similar to the polycomb group (PcG) gene products. PcG gene products form complexes via protein-protein interaction and maintain the transcription repression of genes involved in embryogenesis, cell cycles, and tumorigenesis. This protein was shown to act as a negative regulator of transcription and has tumor suppressor activity. The expression of this gene was detected in various tumor cells, but is limited in neural organs in normal tissues. Knockout studies in mice suggested that this protein may negatively regulate the expression of different cytokines, chemokines, and chemokine receptors, and thus plays an important role in lymphocyte differentiation and migration, as well as in immune responses.

Furthermore, Pal et al in a paper, that has been subsequently withdrawn, had stated:

*Loss of E-cadherin is one of the key steps in tumor progression. Our previous studies demonstrate that SAM pointed domain-containing ETS transcription factor (SPDEF) inhibited prostate cancer metastasis in vitro and in vivo. In the present study, we evaluated the relationship between SPDEF and E-cadherin expression in an effort to better understand the mechanism of action of SPDEF in prostate tumor cell invasion and metastasis.*

*The results presented here demonstrate a direct correlation between expression of E-cadherin and SPDEF in prostate cancer cells. Additional data demonstrate that modulation of E-cadherin and SPDEF had similar effects on cell migration/invasion. In addition, siRNA-mediated knockdown of E-cadherin was sufficient to block the effects of SPDEF on cell migration and invasion. We also show that stable forced expression of SPDEF results in increased expression of E-cadherin, whereas down-regulation of SPDEF decreased E-cadherin expression.*

*In addition, we demonstrate that SPDEF expression is not regulated by E-cadherin. Moreover, our chromatin immunoprecipitation and luciferase reporter assay revealed that SPDEF occupies E-cadherin promoter site and acts as a direct transcriptional inducer of E-cadherin in prostate cancer cells. Taken together, to the best of our knowledge, these studies are the first demonstrating requirement of SPDEF for expression of E-cadherin, an essential epithelial cell junction protein. Given that loss of E-cadherin is a central tenant in tumor metastasis, the results of our studies, by providing a new mechanism for regulation of E-cadherin expression, could have far reaching impact.*

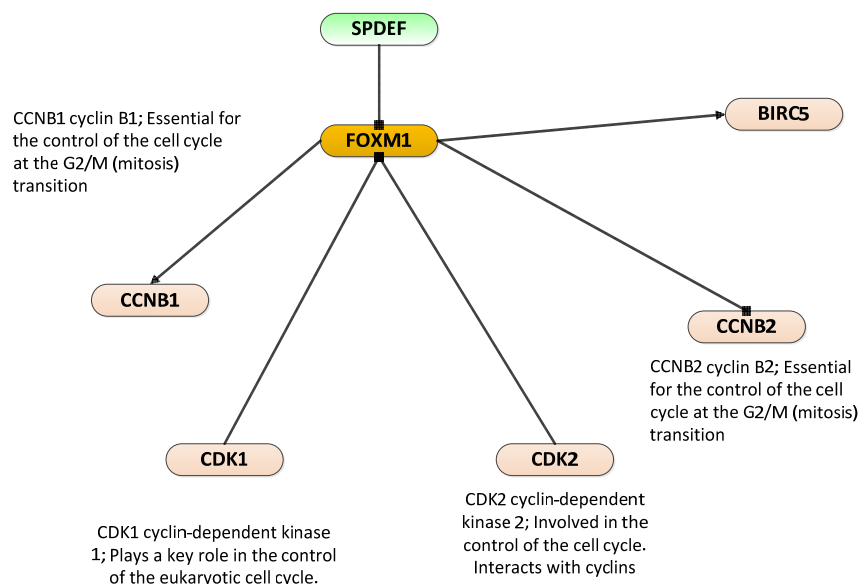
The SPDEF capability to deal with adhesion via the paths shown is a significant factor in its overall importance in metastasis.

## **4.2 FoxM1**

Foxm1 is a transcription activator and can be silenced by SPDEF. However when SPDEF is deficient then Foxom1 can act as an aggressive oncogene and can press metastatic growth.

From NCBI we have<sup>8</sup>:

*The protein encoded by this gene is a transcriptional activator involved in cell proliferation. The encoded protein is phosphorylated in M phase and regulates the expression of several cell cycle genes, such as cyclin B1 and cyclin D1. Several transcript variants encoding different isoforms have been found for this gene.*



The Foxm1 gene may be a therapeutic target. It pushes the cell through the cell cycle and can kick off aggressive metastatic growth. This simple connection between the regulatory role of SPDEF and the aggressive cell cycle capabilities of Foxm1 is an important observation.

### 4.3 MMP

MMP genes have been found to assist metastatic growth by degrading the ECM structures. As Chiang et al state:

*Various members of the matrix metalloproteinase (MMP) family (e.g., MMP-2 and MMP-9) are also implicated in cancer cell invasion. Independent screens for genes that mediate bone or lung metastasis in breast cancer have identified MMP-1 as being necessary for spread to the bone and lungs.*

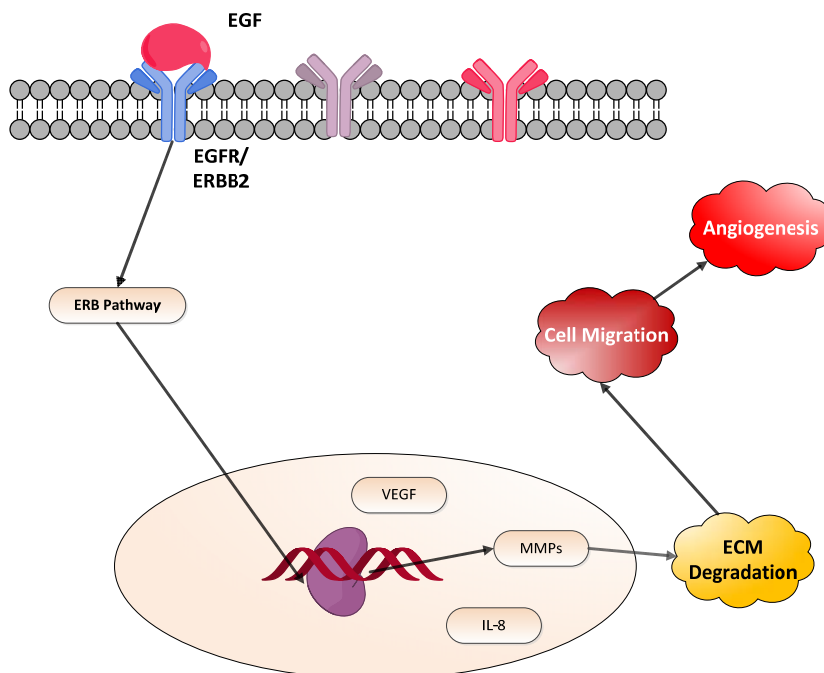
As noted in NCBI:

*Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. Most MMP's are secreted as inactive proproteins which are activated when cleaved*

<sup>8</sup> <http://www.ncbi.nlm.nih.gov/gene/2305>

by extracellular proteinases. The enzyme encoded by this gene degrades type IV and V collagens. Studies in rhesus monkeys suggest that the enzyme is involved in IL-8-induced mobilization of hematopoietic progenitor cells from bone marrow, and murine studies suggest a role in tumor-associated tissue remodeling.

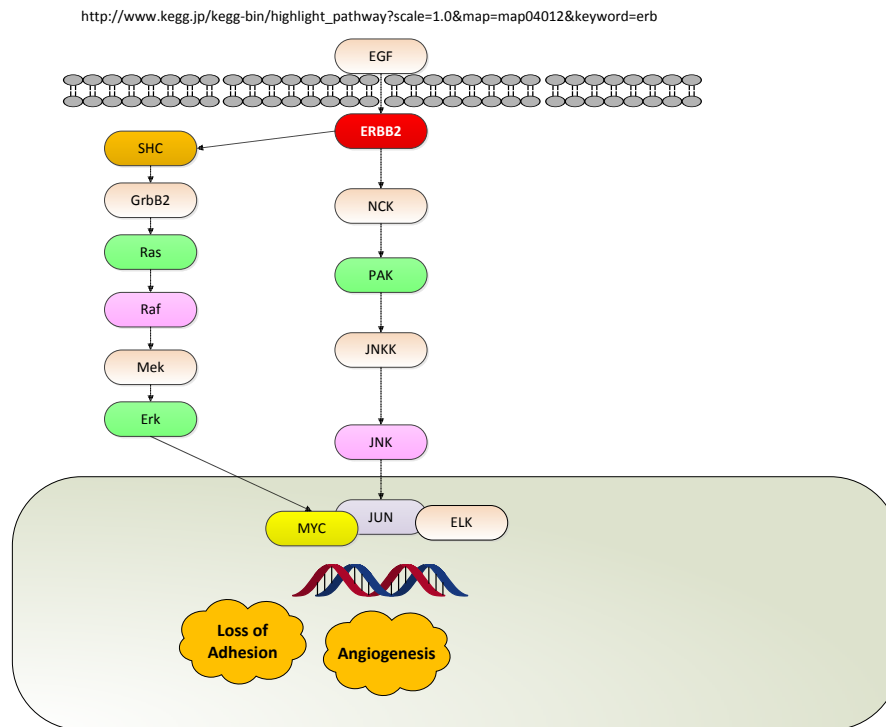
MMP actions are shown below in general terms depicting the activation via the ERB pathway:



[http://www.kegg.jp/kegg-bin/highlight\\_pathway?scale=1.0&map=map05219&keyword=mmp](http://www.kegg.jp/kegg-bin/highlight_pathway?scale=1.0&map=map05219&keyword=mmp)

MMPs initiate their actions via ECM degradation first and then enable cell migration and sustainability via angiogenesis. Thus the evidence of MMP-9 and MMP-14 are significant. As Marks et al note (p 242) there is no known ligand for ErbB2 but it does form an active heterodimer with either ErbB1 or ErbB4. We examine that pathway shortly. Also Marks et al note (p 243) that the organization of the ErbB network is quite complex and demands a systems based approach. This “systems based approach” is essential as we consider the interaction of all of these elements.

The details of the ErbB2 pathway are shown below:



Although the paper in question regarding SPDEF does read onto the MMPs directly the discussing surrounding it does.



## 5 SPDEF PREVIOUS ANALYSES

In a 2012 paper by Stefan et al<sup>9</sup>:

*The role of SPDEF in tumor biology remains hotly debated. SPDEF suppressed tumor metastasis in-part by modulating MMP9 and MMP13. SPDEF is a modifiable therapeutic target in prostate tumors. This is the first study directly implicating SPDEF as a tumor metastasis suppressor in any system in vivo. Emerging evidence suggests that SAM Pointed Domain Containing ETS Transcription Factor (SPD*

*EF), plays a significant role in tumorigenesis in prostate, breast, colon, and ovarian cancer. However, there are no in vivo studies with respect to the role of SPDEF in tumor metastasis. The present study examined the effects of SPDEF on tumor cell metastasis using prostate tumor cells as a model. Utilizing two experimental metastasis models, we demonstrate that SPDEF inhibits cell migration and invasion in vitro and acts a tumor metastasis suppressor in vivo.*

*Using stable expression of SPDEF in PC3-Luc cells and shRNA-mediated knockdown of SPDEF in LNCaP-Luc cells, we demonstrate for the first time that SPDEF diminished the ability of disseminated tumors cells to survive at secondary sites and establish micrometastases. These effects on tumor metastasis were not a result of the effect of SPDEF on cell growth as SPDEF expression had no effect on cell growth in vitro, or subcutaneous tumor xenograft-growth in vivo. Transcriptional analysis of several genes associated with tumor metastasis, invasion, and the epithelial-mesenchymal transition demonstrated that SPDEF overexpression selectively down-regulated MMP9 and MMP13 in prostate cancer cells.*

*Further analysis indicated that forced MMP9 or MMP13 expression rescued the invasive phenotype in SPDEF expressing PC3 cells in vitro, suggesting that the effects of SPDEF on tumor invasion are mediated, in part, through the suppression of MMP9 and MMP13 expression. These results demonstrate for the first time, in any system, that SPDEF functions as a tumor metastasis suppressor in vivo.*

From Science Daily they state<sup>10</sup>:

*Prostate cancer doesn't kill in the prostate -- it's the disease's metastasis to other tissues that can be fatal. A University of Colorado Cancer Center study published this week in the Journal of Biological Chemistry shows that prostate cancer cells containing the protein SPDEF continue to grow at the same pace as their SPDEF- cousins, but that these SPDEF+ cells are unable to survive at possible sites of metastasis.*

---

<sup>9</sup> <http://www.jbc.org/content/early/2012/07/02/jbc.M112.379396.abstract?sid=d84a3600-887d-4147-88e8-debf7bc61fd8>

<sup>10</sup> <http://www.sciencedaily.com/releases/2012/07/120706164422.htm>

*"It's as if these cancer cells with SPDEF can't chew into distant tissue and so are unable to make new homes," says Hari Koul, PhD, investigator at the CU Cancer Center and director of urology research at the University of Colorado School of Medicine, the study's senior author.*

*Koul and his group discovered the homesteading power of cancer cells that have lost SPDEF by introducing a gene into cells that makes them glow in the presence of a dye, and then introducing them into the bloodstream of animal models. Cells without SPDEF traveled through the blood and successfully attached to tissue, surviving and so fluorescing many weeks later when dye was introduced. However, cells with SPDEF flowed through the blood but were unable to successfully establish new colonies and so soon died out.*

*In fact, the protein SPDEF doesn't act directly to allow cells to attach at possible metastasis sites, but is a transcription factor that controls the production (or lack thereof) of two other proteins MMP9 and MMP13.*

*These two downstream proteins work to break down tissue, like a dissolving agent -- they are the cleaning crew that clears space for new and different growth, and in the case of prostate cancer metastasis they chip the tissue footholds that cancer cells need to create micrometastases.*

*"Given that MMP9 and perhaps MMP13 are also involved in metastasis of several other cancers including lung, ovarian, breast and colon to name a few, our findings could potentially have far-reaching consequences outside prostate cancer," adds Koul*

*The group's continuing work points in two directions. "First, we hope that the presence of SPDEF could help doctors recognize prostate cancers that don't require treatment." If future studies confirm the group's initial findings, the presence of SPDEF could predict prostate cancers that are unable to metastasize and so unable to kill.*

*These cancers could be left to run their course without the use of treatments that sometimes carry difficult side effects.*

*"And second," Koul says, "we hope to regulate expression of this protein to remove prostate cancers' ability to metastasize." Koul points to small molecules, gene therapy or nanodelivery as possible mechanisms for introducing SPDEF into cells that lack the protein.*

*"With this discovery we have opened a hopeful door into a future in which prostate and potentially other cancers are unable to metastasize," Koul says.*

From Stefan et al (2011) the authors had stated the following about another ETS transcription factor, PDEF, not to be confused with SPDEF. :

*The prostate-derived ETS factor (PDEF) is the latest family member of the ETS transcription factor family, although it is unique in many aspects. PDEF was first described as an mRNA transcript highly expressed in prostate tumor cells where it regulates prostate-specific antigen gene expression and is an androgen receptor co-regulator.*

*PDEF expression is highly restricted to epithelial cells and has only been found in prostate, breast, colon, ovary, gastric, and airway epithelium. Strong preclinical evidence is emerging that PDEF is a negative regulator of tumor progression and metastasis. PDEF expression is often lost in late-stage, advanced tumors.*

*The induction of tumor aggressiveness in response to the loss of PDEF is thought to be due to the plethora of PDEF-regulated gene targets, many of which are known players in tumor progression including tumor cell invasion and metastasis. These data have led to the hypothesis that PDEF may function as a tumor metastasis suppressor.*

*In this review, we summarize what is known about PDEF since its discovery over a decade ago and give a detailed overview of PDEF-regulated gene products and the expression profiles of PDEF in clinical tumor samples.*

Thus many other ETS transcription factors have similar roles. The therapeutic targeting of these factors may be of significant merit.

## 6 OBSERVATIONS

The analysis of SPDEF is interesting especially because it raises so many other issues.

1. SPDEF deals with multiple other pathway elements from receptors to promoter factors and the resulting complex pathway interactions demonstrate the need for having a complete systems model.
2. No clear therapeutic targets seem to be evident. Although the results are compelling the complexity of the pathways and their interactions lead one to examine more specific control points, since SPDEF by itself seems to be a multiple set of paths leading to metastasis.
3. There is the question of whether SPDEF can be prognostic and/or therapeutic. Many of the prognostic tests use large banks of gene expressions to develop a single metric. Oftentimes this metric can be useful but it also does not per se reflect what process is defective and what cells are the most of concern. The problem is that all too often when one samples a section of tumor that the cells may have substantially different gene expression profiles. We have examined technologies that allows the sampling of individual cells and creating a profile of the tumor in broad profile terms, namely how many cells express what genes (mRNA or proteins) and from that ascertaining prognostic measures.
4. The complexity of the relationships between the ETS transcription factor SPDEF, the oncogene Foxm1 and the MMD metastatic facilitators is of interest. It demonstrates a “system” view of the cancer. The key questions are; when does this occur, in what percent of the cells does this occur, and what is its prognostic value?

## 7 REFERENCES

1. Aronson, B., et al, Spdef deletion rescues the crypt cell proliferation defect in conditional Gata6 null mouse small intestine, BMC Molecular Biology 2014, 15:3
2. Carver et al, ETS rearrangements and prostate cancer initiation, NATURE| Vol 457| 12 February 2009, <http://www.nature.com/nature/journal/v457/n7231/abs/nature07738.html>
3. Cheng, X, et al, SPDEF Inhibits Prostate Carcinogenesis by Disrupting a Positive Feedback Loop in Regulation of the Foxm1 Oncogene, PLOS Genetics, September 2014 | Volume 10 | Issue 9 , J. Biol. Chem. <http://www.jbc.org/content/289/32/22020.full.pdf+html> (Withdrawn)
4. Chiang, A., J. Massagué, Molecular Basis of Metastasis, NEJM, V 359 2814-2823 2008.
5. Davuluri, G., et al, WAVE3-NFkB Interplay Is Essential for the Survival and Invasion of Cancer Cells, PLOS ONE, [www.plosone.org](http://www.plosone.org), 1 October 2014, Volume 9, Issue 10, e110627.
6. Gelmann, E., Molecular Oncology, Cambridge (New York) 2014.
7. Deutsch, E., et al, Environmental, genetic, and molecular features of prostate cancer, THE LANCET Oncology Vol 5 May 2004.
8. Jamaspishvili, T., et al, Urine markers in monitoring for prostate cancer, Prostate Cancer and Prostatic Diseases (2010) 13, 12–19
9. Kalin, T., et al, Increased Levels of the FoxM1 Transcription Factor Accelerate Development and Progression of Prostate Carcinomas in both TRAMP and LADY Transgenic Mice, Cancer Res. 2006 February 1; 66(3): 1712–1720.
10. Marks, F., et al, Cellular Signal Processing, Garland (New York) 2009.
11. Mintu Pal, Sweaty Koul and Hari K. Koul, (SAM) pointed domain-containing ETS transcription factor (SPDEF) is required for E-cadherin expression in prostate cancer cells, 2014, 289:22020.
12. Pal, M., et al, The Transcription Factor Sterile Alpha Motif (SAM) Pointed Domain-containing ETS Transcription Factor (SPDEF) Is Required for E-cadherin Expression in Prostate Cancer Cells, <http://www.jbc.org/content/288/17/12222.long> April 26, 2013 The Journal of Biological Chemistry, 288, 12222-12231. (Withdrawn)
13. Pestel, R., M. Nevalainen, Prostate Cancer, Humana, 2008.
14. Penny, K., et al, Association of KLK3 (PSA) genetic variants with prostate cancer risk and PSA levels, Carcinogenesis vol.32 no.6 pp.853–859, 2011.
15. Riley, T., et al, Transcriptional control of human p53-regulated genes, Nature Reviews Molecular Cell Biology, Volume 9, May 2008.
16. Schrecengost, R., K. Knudsen, Molecular Pathogenesis and Progression of Prostate, Seminars in Oncology, Vol 40,No3, June2 013, pp244-258.
17. Stefan, J., et al, Prostate derived ETS factor (PDEF): a putative tumor metastasis suppressor, Cancer Lett. 2011 Nov 1; 310(1):109-17. Epub 2011 Jun 29.

18. Stefan, J., et al, The transcription factor SPDEF suppresses prostate tumor metastasis, First Published on July 2, 2012, doi: 10.1074/jbc.M112.379396 jbc.M112.379396. JBC.
19. Tomlins, S., et al, ETS Gene Fusions in Prostate Cancer: From Discovery to Daily Clinical Practice, *European Urology*, 2009. doi:10.1016/j.eururo.2009.04.036
20. Turner, D., The Role of ETS Transcriptional Regulation in Hormone Sensitive and Refractory Prostate Cancer, *The Open Cancer Journal*, 2010, 3, 40-48.
21. Turner, D., et al, Mechanisms and functional consequences of PDEF protein expression loss during prostate cancer progression, *Prostate*. 2011 December; 71(16): 1723–1735. doi:10.1002/pros.21389.
22. Watson, D., et al, ETS Transcription Factor Expression and Conversion During Prostate and Breast Cancer Progression, *The Open Cancer Journal*, 2010, 3, 24-39
23. Wasyluk B1, Hagman J, Gutierrez-Hartmann A., Ets transcription factors: nuclear effectors of the Ras-MAP-kinase signaling pathway. *Trends Biochem Sci*. 1998 Jun; 23(6):213-6.
24. Yordy, J. and Robin C Muise-Helmericks, Signal transduction and the Ets family of transcription factors, *Oncogene* (2000) 19, 6503  
<http://www.nature.com/onc/journal/v19/n55/pdf/1204036a.pdf>