PROSTATE CANCER: METASTATIC PATHWAY IDENTIFICATION

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Telmarc White Paper 82 This paper examines the study done at Dana Farber regarding the detection of gene products, specifically 4 gene products (Pten, Spp1, Smad4 and CyclinD1), which if over or under expressed in histologically known prostate cancer cells, that the over or under expression is highly prognostic of metastatic potential. We examine the four elements, their pathways and determine what potential this approach may have and then suggest several additional avenues for examination. The major concern with any test of this type is that without including the pathway dynamics one has a result that may lack true prognostic value in a timely manner. We also express concern regarding the issue of cancer stem cells and their place in prostate cancer.

TELMARC GROUP

WHITE PAPER

NO 82

FEBRUARY, 2011

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1 INTRODUCTION

One of the questions one frequently asks is how do we determine from a biopsy the eventual aggressiveness of PCa. This is a difficult question since we know for example that the loss of PTEN is often an ominous sign. It often pretends an already metastasized PCa, albeit without any way of determining where it has metastasized. In this section we look at a recent suggested test which would augment the histological analysis of Gleason scoring. The test proposed by the team at Dana Farber, of "Farber", entails looking at gene profiles and then using them in a prognostic manner[1]. We discuss this approach, which we have argued for in general before, and discuss its implications and present an alternative manner in which such tests in general may be analyzed.

The gene and gene products which were targeted are shown in the following Table:

Gene/Gene Product	Function	Location
Pten	Pten controls the Akt pathway which if not controlled will lead to excessive cell growth.	10q23.3
Smad4	SMAD4 controls the G1 to S transition.	18q21.1
SPP1	SPP1 is involved in immune cell activation, wound healing, and bone morphogenesis and plays a major role in regulating mineralization processes in various tissues. Increased SPP1 expression is often associated with pathological calcification.	4q21.1
CyclinD1	Cyclin D is a control with CDK4 and CDK6 of the transitions in the G1 to S stage of mitosis. Lack of control of Cyclin D will allow for uncontrolled cell growth.	11q13

We examine some of the issues related to this study and then discuss some new questions arising from it.

Basically what this study has done is looked at the genomic content of a cell, a malignant cell, and it has tried to ascertain what the degree of potency for metastasis the cell may have. If it is an indolent cell then perhaps a wait and see attitude may prevail. If, however the profile indicates the potential for aggressive growth then surgery should be the option, or some other form of treatment eradicating the cells which hopefully are localized.

[1] http://www.dana-farber.org/abo/news/press/2011/dana-farber-researchers-identify-molecular-predictor-of-metastatic-prostate-cancer.html

The approach by the researchers at the Farber seem to be to examine large samples and then using standard statistical techniques focus on a small targeted gene product set and if the expression of those genes is significantly over or under expressed then one can say with reasonable confidence that the aggressive treatment is warranted.

However this study does not seem to approach this study from a dynamical approach or an approach which relies on the essential pathways relating genes in the homeostasis of the cell.

2 DANA FARBER STUDY

In the aforementioned recent Dana Farber research study the results state[2]:

"In the current study, researchers began with the well-established fact that prostate cancers without (sic) a working copy of the *Pten* gene tend to remain fairly idle and don't trespass beyond the prostate gland itself[3]. Researchers theorized that the loss of *Pten* in turn activates a collection of genes — a pathway — functioning to constrain the tumor's growth and invasion. If that pathway was shut down, they reasoned, the tumor would begin to break loose from the prostate and spread insidiously through the body.

Using computational biology techniques to analyze gene activity in mouse prostate cancer cells with inactive *Pten*, the investigators found a few pathways that seemed to play a constraining role. One, known as TGFβ-SMAD4 (for some of the genes that comprise it), was particularly intriguing as this pathway had been implicated in the metastasis of other tumor types in the past. When researchers conducted confirmatory molecular signaling studies to see what happens when *Pten* is knocked out of commission, signaling in the TGFβ-SMAD4 pathway "shot through the roof," DePinho says, suggesting that the pathway had sprung into action.

When researchers generated mice whose prostate cells lacked both *Pten* and the *Smad4* gene, the animals developed large, fast-growing tumors that spread to their lymph nodes and beyond. Guided by these insights, they then examined whether something similar was happening in human prostate cancers.

Comparing the gene expression profiles of indolent versus aggressive mouse prostate cancers, they found about 300 genes that distinguished the two groups. "We then categorized them for known functions," DePinho says. "We were encouraged to see that the top functional category were genes playing that have roles in cell division and movement" — actions that are needed for cancer cells to grow and spread with lethal consequences.

The researchers conducted an elaborate series of experiments to identify the genes most closely linked to the aggressive biology of prostate cancer. Among the hundreds of genes analyzed, two

[2] http://www.dana-farber.org/abo/news/press/2011/dana-farber-researchers-identify-molecular-predictor-of-metastatic-prostate-cancer.html also see Nature paper http://www.nature.com/nature/journal/vaop/ncurrent/full/nature09677.html

[3] We believe that this is a mis-statement. PTEN inactivation is known in metastatic PCa and thus we suspect that they are misquoted. The remainder of the article enforces this belief.

such genes stood out: *SPP1* and *CyclinD1*, both of which, intriguingly, are close working partners of *Smad4*.

The four-gene signature — *Pten*, *Smad4*, *SPP1*, and *CyclinD1* — showed its effectiveness as a predictive tool for survival when researchers drew on data from the Physicians' Health Study, which has been tracking the health of thousands of U.S. physicians for nearly 30 years. When the investigators screened prostate cancer samples from study participants for the four-gene/protein signature, it was more accurate in predicting the ultimate course of the illness than conventional methods were.

"By integrating a variety of techniques — computational biology, genetically engineered model systems, molecular and cellular biology, and human tissue microarrays — we've identified a signature that has proven effective in distinguishing which men with prostate cancer are likely to progress and die from their disease and those who are not," DePinho remarks. "Efforts are already underway to use this knowledge to develop a clinical test — which we hope will occur within a year or so — that will enable doctors and patients to make more accurate treatment decisions and avoid unnecessary aggressive interventions which adversely impact on quality of life and deplete over-extended healthcare resources. This science holds potential to illuminate a long-sought answer for optimal management of this complex disease."

Thus we look again at the pathways. Our interest is in those pathways which effect:

1. Pten,
2. Smad4,
3. SPP1, *and*
4. CyclinD1

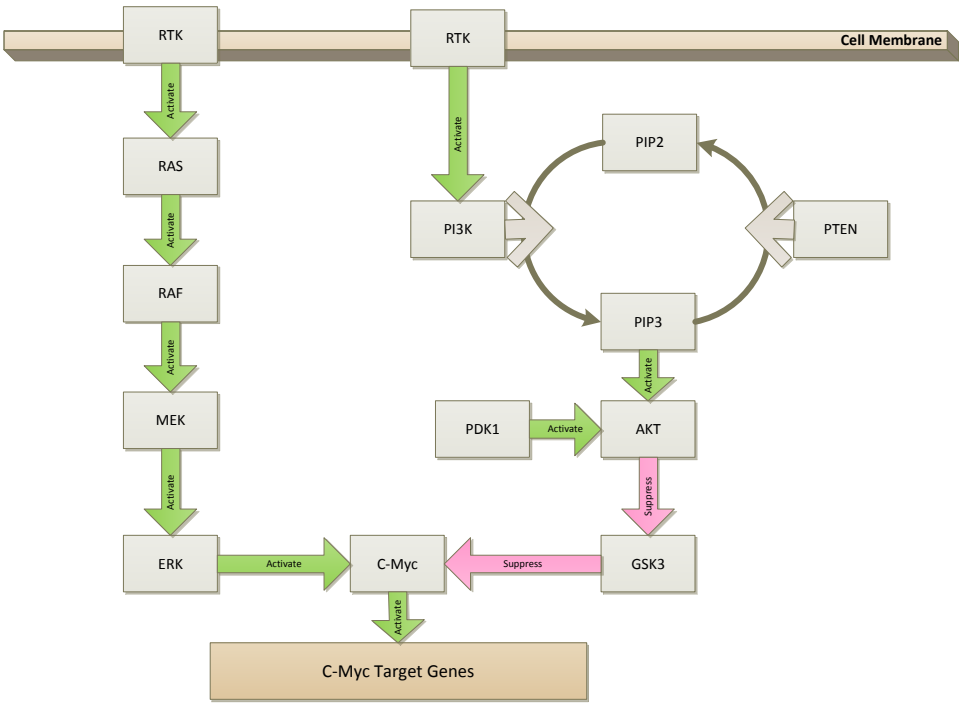
3 PTEN SUPPRESSION

PTEN is a significant gene which controls the Akt pathway which in turn controls the replication of cells. Loss of PTEN is often seen in metastatic prostate cancer. In many ways it is the hallmark of this change. As stated in NCBI[4]:

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating AKT/PKB signaling pathway.

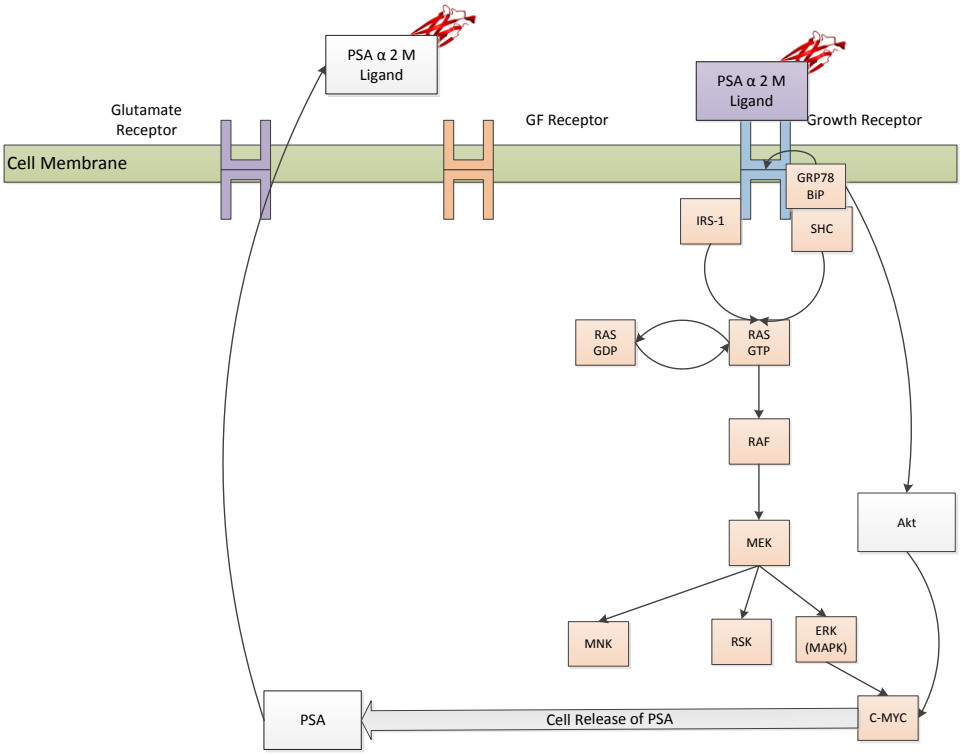
First the PTEN pathway as shown below:

[4] http://www.ncbi.nlm.nih.gov/gene/5728



Note PTEN modulates the production of Akt which in turn modulates c-Myc which in turn controls cell reproduction. Any effect which causes PTEN to not be expressed will in turn result in unfetterd cell growth.

We can amend this with the details on the Ras pathway as shown below:



4 CYCLIN D

Cyclin D is one of the key regulators of the cell cycle. As Bunz states (Bunz, pp 218-221) the cell cycle goes through several well-known phases. There are phase specific kinases which are cyclins which are called that because they were found to increase or decrease in a cyclical manner as the cell cycle phase progressed.

In the cycles the cyclin binds with a cyclin-dependent kinas or CDK. The activated cyclin-CDK complex phosphoralates phase specific substrates. Cyclin D along with CDK4 and CDK6 facilitate the transition through G1 to the start of S for example. Cyclin E with CDK2 facilitates the transition from G1 to S. Cyclin A with CDK2 moves through S. Cyclin A/B with CDK1 moves through G2. Thus activation of Cyclin D is a sign that cell replication has commenced.

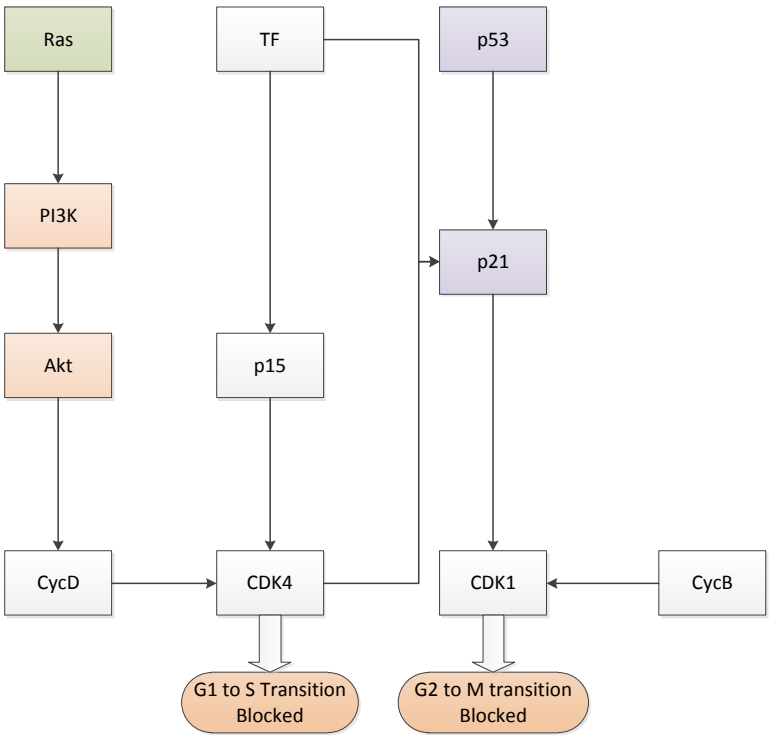
As stated in NCBI[5]:

The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance throughout the cell cycle. Cyclins function as regulators of CDK kinases. Different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event. This cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition. This protein has been shown to interact with tumor suppressor protein Rb and the expression of this gene is regulated positively by Rb.

[5] http://www.ncbi.nlm.nih.gov/gene/595

Mutations, amplification and overexpression of this gene, which alters cell cycle progression, are observed frequently in a variety of tumors and may contribute to tumorigenesis

Now we can look more closely at Cyclin D, CycD, as we show below. This we show as follows:

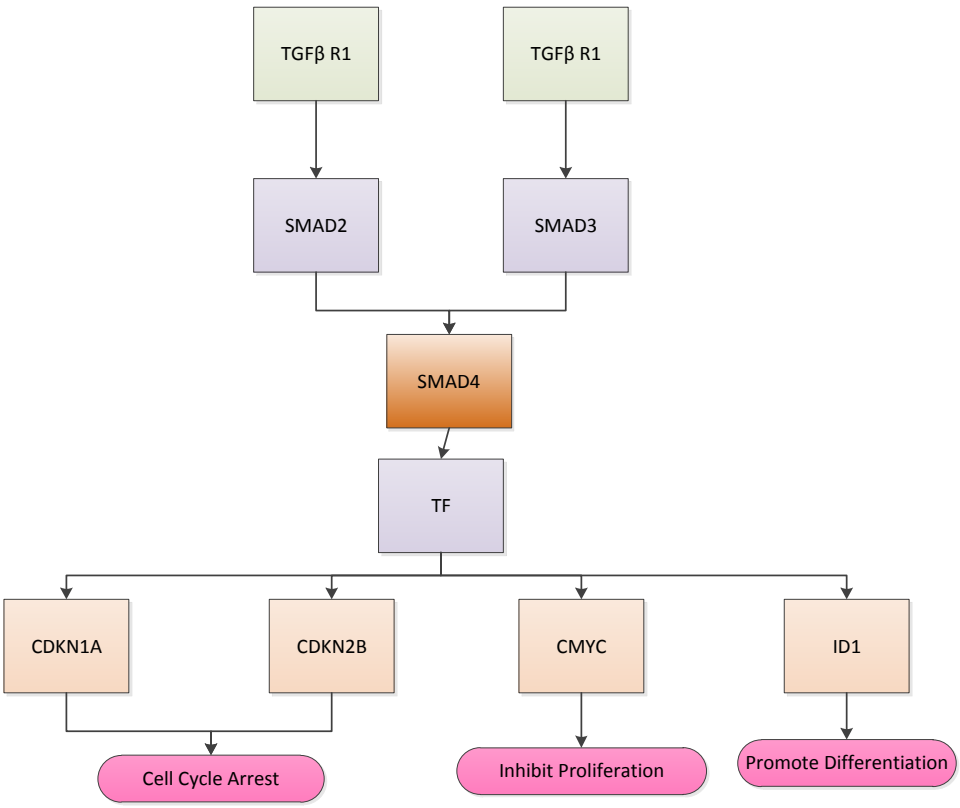


Note that Cyc D if not regulated will in turn fail to regulate the blocking of the G1 to S transition.

5 SMAD4

SMAD4 is an element in the TGF-β signalling chain. TGF is a cytokine, specifically a transforming growth factor cytokine. Like the Wnt-Apc pathway, the TGF pathway links defective development to cancer. The pathway is shown in part below (from Bunz p 199). Normal TGF signalling down-regulates the growth of most normal cells. Several of the genes in the TGF/SMAD pathway activation suppress growth. Specifically the genes CDKN1A and CDKN2B encode the cyclin dependent kinase inhibitors which suppress growth. Activated SMAD pathways also appear to suppress the transcription of other genes including c-Myc.

We show some of the TGF SMAD signalling below. We will elaborate this later.



SMAD4 controls the G1 to S transition. As stated in NCBI[6]:

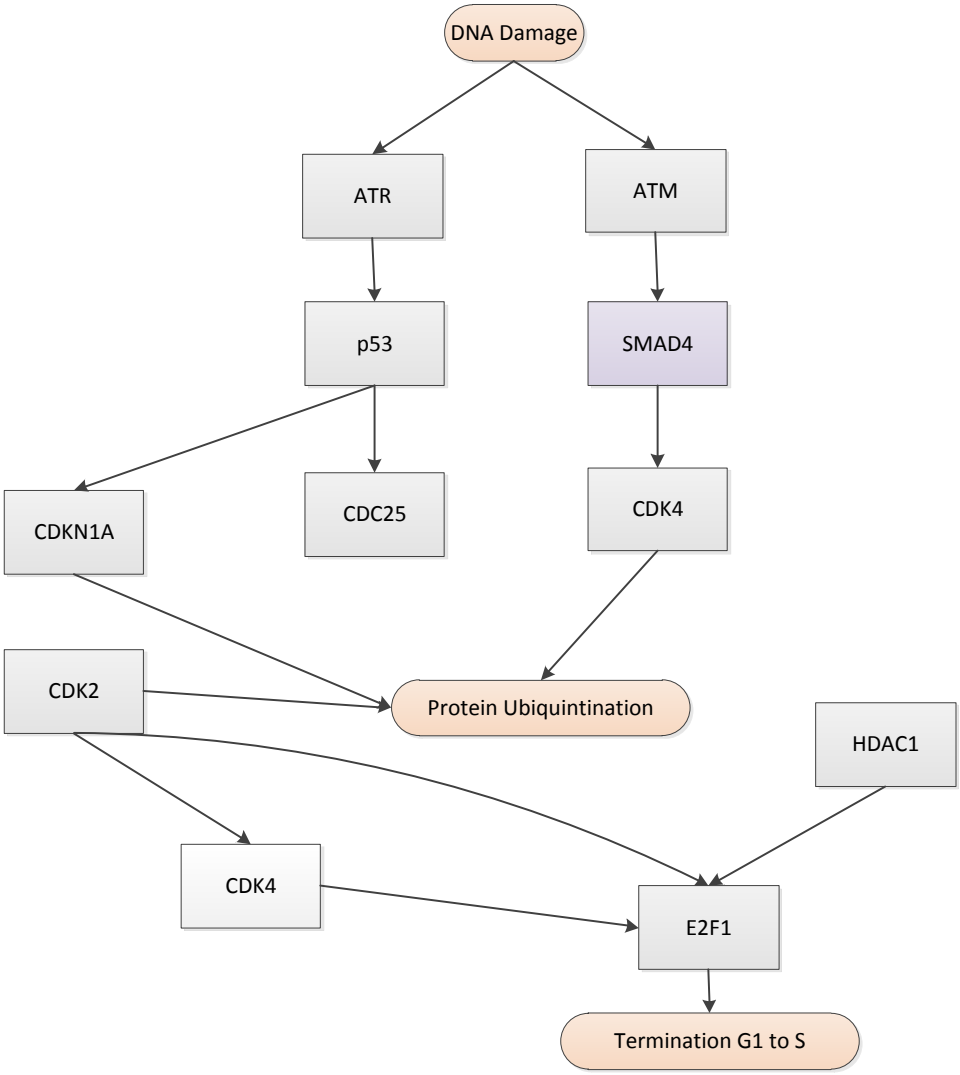
This gene encodes a member of the Smad family of signal transduction proteins. Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to TGF-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes.

This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome.

We use the NCI data set for its pathway[7]:

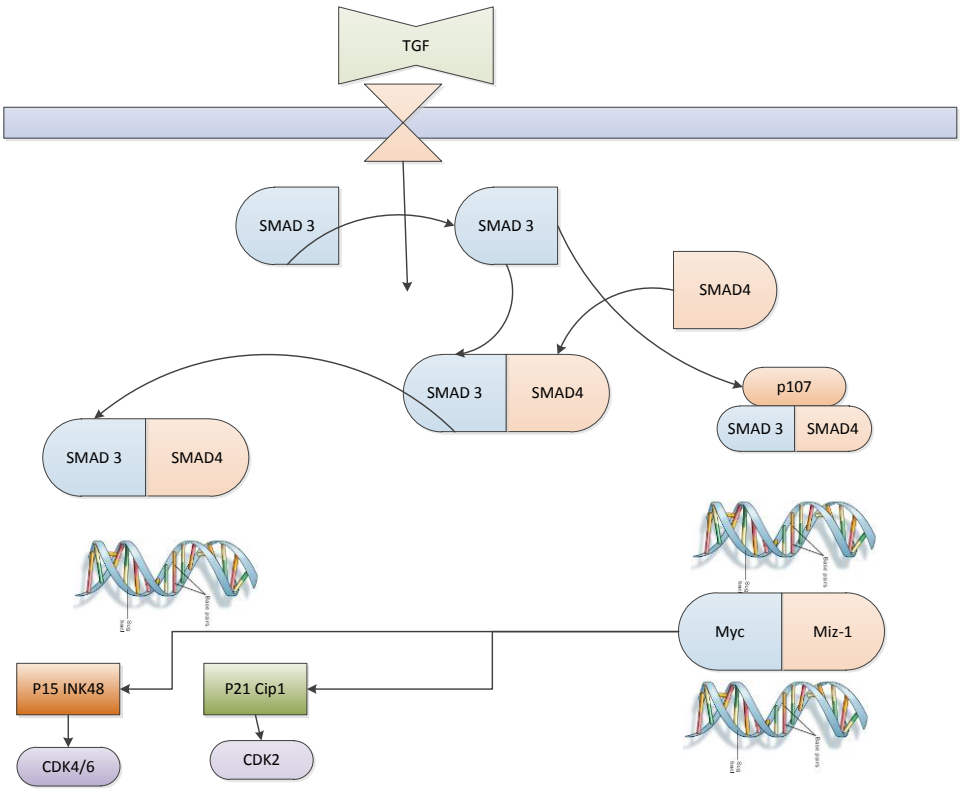
[6] http://www.ncbi.nlm.nih.gov/gene/4089

[7] http://pid.nci.nih.gov/search/pathway_landing.shtml?pathway_id=100160&source=BioCarta&genes_a=4089&genes_b=&what=graphic&jpg=on&ppage=1



The SMAD pathway is also detailed by NCI and one is referred to that source for further detail.

From Weinberg (p 291) we also have the SMAD4 pathway showing its immediate control of the DNA transcription.



As Weinberg states (p 292):

"… Half of all pancreatic carcinomas and more than a quarter of all colon carcinomas carry mutant inactivated Smad4 proteins. Without the presence of Smad4 neither Smad2-Smad4 norr Smad3-Smad4 complexes can form. These two complexes are the chief agents dispatched by the TGF-β receptor to the nucleus with the important assignment to shut down proliferation."

This control mechanism is shown above.

6 SPP1

SSPI is secreted phosphoprotein 1, also commonly known as Osteopontin (OPN), also known as bone sialoprotein I (BSP-1 or BNSP), early T-lymphocyte activation (ETA-1), 2ar and Rickettsia resistance (Ric), is a human gene product which is also conserved in other species[8].

From Hendig et al, they state that SPP1 is a secreted, highly acidic phosphoprotein that is involved in immune cell activation, wound healing, and bone morphogenesisand plays a major role in regulating mineralization processes in various tissues. Increased SPP1 expression is often associated with pathological calcification. Furthermore, SPP1 is a constitutive component of human skin and aorta, where it is localized to the elastic fiber and hypothesized to prevent calcification in the fibers.

[8] Also see http://www.ncbi.nlm.nih.gov/gene/6696 also see http://www.wikigenes.org/e/gene/e/6696.html

SPP1 is a predominantly transcriptional regulated gene, and the *SPP1* promoter is highly conserved among different species *(22)*. Several polymorphisms in the *SPP1* gene affect SPP1 expression and have been associated with various disorders, e.g., systemic lupus erythematosus and arteriosclerosis.

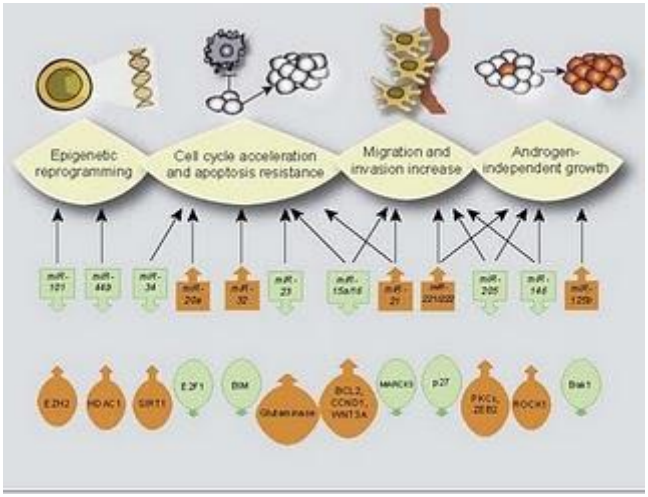
SPP1 is a SIBLING glycoprotein that was first identified in osteoblasts. OPN is an important anti-apoptotic factor in many circumstances. OPN blocks the activation-induced cell death of macrophages and T cells as well as fibroblasts and endothelial cells exposed to harmful stimuli. OPN prevents non-programmed cell death in inflammatory colitis. It has been shown that OPN drives IL-17 production; OPN is overexpressed in a variety of cancers, including lung cancer, breast cancer, colorectal cancer, stomach cancer, ovarian cancer, melanoma and mesothelioma; OPN contributes both glomerulonephritis and tubulointerstitial nephritis; and OPN is found in atheromatous plaques within arteries. Thus, manipulation of plasma OPN levels may be useful in the treatment of autoimmune diseases, cancer metastasis, osteoporosis and some forms of stress. Research has implicated osteopontin in excessive scar-forming and a gel has been developed to inhibit its effect.

7 ANALYSIS

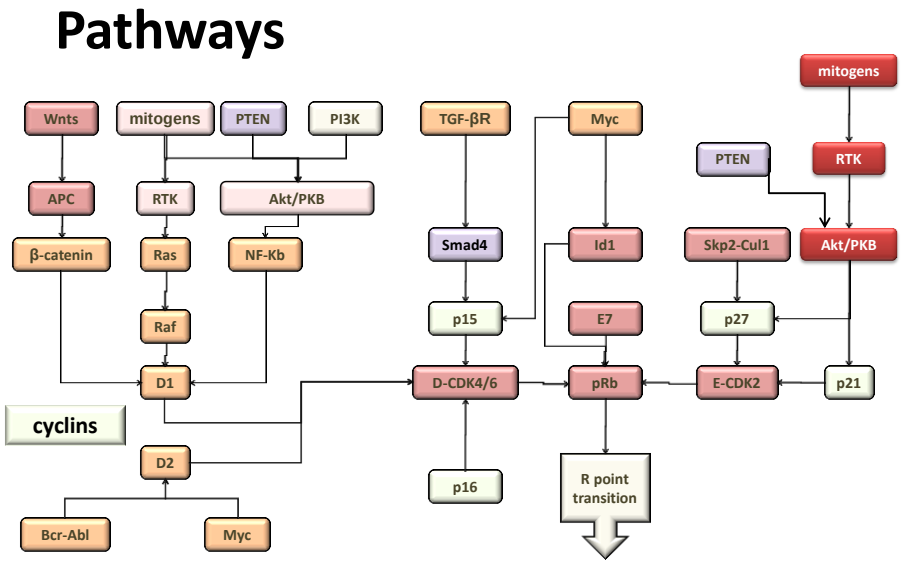
In a recent announcement from Dana Farber in Boston, a paper has been prepared that indicates that testing for four gene products significantly improves the ability to determine an indolent Prostate Cancer from an aggressive form. Thr results also hit the news including a WSJ release.

The release from DF states:

The four-gene signature — Pten, Smad4, SPP1, and CyclinD1 — showed its effectiveness as a predictive tool for survival when researchers drew on data from the Physicians' Health Study, which has been tracking the health of thousands of U.S. physicians for nearly 30 years. When the investigators screened prostate cancer samples from study participants for the four-gene/protein signature, it was more accurate in predicting the ultimate course of the illness than conventional methods were.



We show some of the pathway elements above. A more detailed version is below.



In the above we show the PTEN control, the Smad4 control and the presence of the cyclins. The loss of PTEN has been known for years to be a sign that metastasis may very well already started. SMAD4, SPP1 and the Cyclin D1 are in pathways that also control the growth of the cell. PTEN is most often the one pathway element of most concern.

The driving factor in the result is:

The standard measure of prostate cancer's aggressiveness, known as the Gleason score (which is based on cancer cells' appearance under a microscope), is accurate about 60 to 70 percent of the time depending on the skill of the pathologist. The four-gene signature method alone was accurate 83 percent of the time. Combining the markers and Gleason methods produced an accuracy of approximately 90 percent.

Now the above result need some clarification.

1. Gleason scores are score based upon the histological presentation of the cells. Gleason 1 for example is low grade and shows the cells as small gland like structures but lacking gland architecture. Gleason 5 is a mass of undifferentiated cells clustered about the stroma, internal part of the prostate, with no structure. Gleason scores are the sum of the most prevalent cell type plus the next most prevalent so a 7 is a 4+3 or a 3+4. Clearly a 4+3 is more sever than a 3+4. But Gleason alone tells one little about the metastatic potential.

2. Genetic pathway changes are often the sine qua non for determination. But what genes and in what cells. The problem is the existence of the cancer stem cell idea, namely that one of the many cancer like cells is pluripotent and if this is true in prostate cancer then it is that cell we want. So perhaps in a biopsy we missed the killer cell, or it may have already escaped.

3. This procedure statistically takes us a step forward, now we can test to see if we do have some very bad cells. However one may ask if we are finding out when the cow has already left the barn. Namely what we may have is a test which conforms the fact that the patient's cancer is one of those very bad types so nothing much can be done. On the other hand we may have a patient with an aggressive form which is just a threshold below the bad level. Thus one need significant clinical tests.

4. The question then is; are there other pathway constituents that are prognostic enough to have a meaningful result in mortality, namely how well do we know the PCa pathways. What are the pathway dynamics between these four, we know some, and many are available through NCI.

5. The results appear to have quite extensive, but causality and dynamics still need some filling in. What caused the change. In addition one may look at the HGPIN cases and see that we have HGPIN which all too often is considered as pre-malignant, but we know that HGPIN also regresses to a fully benign prostate. What happened.

The questions that seem to remain all relate to pathway dynamics. They are:

1. What are the pathway dynamics of each of these markers. Is there some causal relationship or are they independent events?

2. What caused the change in expression? Is there a change in the gene or are there other factors. Are there epigenetic issues such as miRNA or methylation. Are there activator or repressor genes related to the transcription of these genes which result in the change. If so how are these genes related in the overall gene network.

3. What are the temporal issues of this gene network? Which changes are causal and which are simultaneous.

4. What are the spatial issues, namely do the modified cells communicates with others to allow for the development of PCa clusters and if so how do these genes function in such an environment.

5. What of the stem cell issue? If there are PCa stem cells and these cells are drivers of the overall metastatic process, does sampling for these markers reflect the stem cell or the tumor mass in general? The CSC is a significant question and if one must select that cell how does one identify the cell? Are there CSC markers for CSC PCa cells.

These are but a few of the questions. The lingering one however is when these markers are detected is it really one where the cow is out of the barn or is it just time to bar the door?

8 GENE TABLES

The following Tables are taken from the paper by Chandran et al and represent a significant amount of detail on all genes which have been identified as either over or under expressed in PCa. Causality is not evident.

Table 3: Transcripts with median values with at least 3 fold difference between metastatic and primary tumor samples

Gene Symbol	**Probe_ID**	P1	P2	P3	P4
HBB	32052_at	22.37	5.78	13.25	56.28
SPP1	34342_s_at	24.16	26.78	4.75	5.39
HBA1///HBA2	31525_s_at	15.14	4.47	13.65	108.11
LGR4	43585_at	7.39	7.43	20.89	24.82
AR	1577_at	14.35	12.97	12.24	14.78
PRO1073	49666_s_at	4.56	13.25	10.01	13.50
UTRN	42646_at	10.11	6.02	12.11	16.31
HNT	59070_at	5.37	9.69	12.08	13.67
SDCCAG3	43014_at	7.99	8.57	11.24	17.32
LOC64744	42739_at	7.30	9.64	9.57	14.51
---	1089_i_at	5.06	4.14	12.12	22.01
SPP1	2092_s_at	14.05	12.94	3.35	4.07
UBE2H	58777_at	9.50	7.45	6.55	15.13
SRPK1	63687_at	6.06	4.36	10.61	12.82
NCK2	33003_at	5.00	9.14	8.50	7.34
HIST1H3H	36757_at	7.26	17.07	8.47	5.61
PPP4R2	48663_at	5.09	6.97	8.59	16.16
C8orf16	47339_at	6.54	8.15	9.53	7.39
---	55943_at	3.41	7.47	15.31	8.01
---	64642_s_at	8.25	6.42	7.04	10.60
EP400	47518_at	5.94	9.27	4.51	9.32
GOLT1A	45144_at	3.90	6.17	8.37	12.32
---	52853_g_at	9.83	7.10	6.25	7.03
LOC284058	44791_at	8.25	10.17	4.55	5.86
DAPK1	51580_at	3.42	6.04	8.03	11.32
NFATC2IP	38864_at	3.26	4.83	9.58	9.19
SEL1L	40689_at	4.71	7.84	6.13	10.94
TM4SF9	47746_at	3.43	6.26	8.92	7.52
MLLT2	65205_at	3.43	7.13	6.57	13.01
SC4MOL	46802_at	22.91	7.35	5.62	6.17
---	62671_at	6.38	7.13	5.74	11.03
BIRC6	46558_at	5.67	8.59	7.50	5.92
MAP4K4	51474_at	4.86	4.32	8.70	8.52
MLLT2	53300_at	4.65	3.99	9.39	8.10
---	52851_at	8.71	5.94	6.35	6.25
MRRF	51635_at	4.23	4.87	7.39	8.23
ACAS2	62783_at	4.29	6.14	7.02	5.90
---	60658_at	3.40	6.84	5.19	9.10
SUMO1	49551_at	4.05	7.20	4.77	7.75
AR	1578_g_at	7.56	4.86	5.32	6.37
GALNT7	59101_at	8.41	4.12	5.11	6.54
GPR75	44203_at	5.14	8.32	3.90	6.31
TBL1XR1	65001_r_at	3.53	12.30	4.06	7.19

HSD17B12	43292_at	4.74	8.88	3.64	6.28
MRPS28	43095_at	5.79	5.39	5.58	5.14
FN1	64719_at	27.07	6.02	4.05	4.93
GPR158	44214_at	7.21	3.33	4.32	6.62
---	48069_at	6.27	9.88	3.38	4.55
FLJ21657	58778_at	4.34	5.60	6.17	5.18
MLL5	43301_at	4.76	3.61	5.87	10.34
---	55761_at	3.78	4.88	5.65	6.93
DLG1	47231_at	3.40	4.77	6.22	5.70
MYO5B	63281_r_at	3.29	6.17	4.29	6.84
---	49268_at	3.55	19.86	3.61	6.75
FUS	43501_at	3.93	3.78	6.42	8.97
CCDC35	54684_at	4.90	8.14	3.55	5.43
---	43435_at	6.85	4.83	4.82	5.49
SMA4	32921_at	4.68	5.53	5.74	4.26
NCOA1	45953_at	6.53	4.13	3.58	6.06
S100A8	41096_at	4.22	5.89	3.80	22.58
PRKCBP1	53493_at	4.65	7.37	4.50	5.35
RNPC2	65083_at	3.18	3.96	6.01	9.19
CAMSAP1	62630_at	4.45	5.80	3.36	5.36
EEF1G	41903_at	5.19	4.58	4.31	5.34
EIF5	51379_at	3.44	4.08	5.62	11.07
MAML3	49879_at	3.39	3.22	10.27	5.87
C21orf106	59651_at	3.19	4.02	5.23	6.44
VCIP135	42715_at	3.37	3.61	5.52	8.55
FOXO3A	55502_at	3.48	4.37	6.97	4.74
C7orf20	49143_s_at	4.23	4.62	4.41	5.78
GNMT	46482_at	3.59	4.84	4.24	4.64
DONSON	48549_at	4.10	3.58	4.66	5.28
---	43436_g_at	4.98	3.75	3.58	5.09
PKP4	66327_at	3.31	3.88	4.56	6.20
PCBP2	55393_at	3.73	3.19	4.36	6.29
CPEB4	57169_at	3.70	3.92	4.14	4.48
CUGBP1	34683_at	4.26	3.76	3.13	4.78
FALZ	47458_at	4.21	3.65	3.82	4.09
---	51586_at	3.51	4.00	4.99	3.89
RALA	39253_s_at	3.92	4.30	3.29	3.85
MLL5	45092_at	4.36	3.21	4.48	3.39
PABPC1	44806_at	3.74	3.98	4.20	3.07
EIF1AX	34278_at	3.99	3.47	3.84	3.19
C7orf2	42173_at	3.15	3.27	5.07	4.04
---	63147_at	3.25	5.40	3.12	4.04
RAD23B	41157_at	3.20	3.46	3.64	4.45
---	61037_at	3.44	3.56	3.47	3.73
NFATC1	39143_at	3.13	3.21	9.06	3.78
JARID1A	50532_at	3.22	3.32	3.54	4.12
PDLIM5	37366_at	3.02	3.58	3.42	3.16

Table 3: Transcripts with median values with at least 3 fold difference between metastatic and primary tumor samples

Gene Symbol	**Probe_ID**	P1	P2	P3	P4
NEFH	33767_at	(117.15)	(147.36)	(9.90)	(17.18)
C10orf116	32527_at	(35.49)	(29.63)	(46.85)	(66.50)
KLK11	40035_at	(23.65)	(19.24)	(39.73)	(62.15)
FAM3B	59657_at	(15.81)	(27.92)	(26.09)	(25.97)
PGM5	52140_at	(23.87)	(26.50)	(44.27)	(17.72)
MRGPRF	52946_at	(15.61)	(18.57)	(30.59)	(70.95)
KRT15	37582_at	(21.85)	(20.74)	(19.22)	(33.68)
PTN	34820_at	(11.62)	(31.95)	(10.24)	(27.11)
SELM	64449_at	(6.36)	(8.40)	(29.23)	(39.36)
MYLK	46276_at	(5.87)	(15.22)	(22.57)	(20.86)
SYNPO2	50361_at	(15.14)	(15.77)	(20.15)	(84.14)
KRT5	613_at	(13.21)	(11.12)	(22.66)	(32.96)
FOS	2094_s_at	(10.72)	(25.75)	(13.72)	(16.45)
PKP1	51214_at	(11.57)	(16.34)	(11.83)	(17.85)
---	42921_at	(9.96)	(11.67)	(15.61)	(16.50)
RAB34	45269_at	(14.36)	(11.54)	(17.49)	(10.35)
---	48927_at	(10.61)	(14.93)	(8.77)	(21.91)
ALOX15B	37430_at	(12.47)	(12.41)	(14.17)	(9.10)
FOS	1915_s_at	(7.59)	(26.38)	(11.03)	(12.11)
TMEM16G	62387_at	(9.63)	(13.32)	(12.59)	(9.93)
---	64676_at	(17.30)	(9.39)	(6.32)	(13.05)
SFRP1	32521_at	(13.10)	(5.73)	(8.29)	(16.73)
NDFIP2	60510_at	(7.20)	(9.23)	(11.72)	(15.15)
FHOD3	50298_at	(9.96)	(12.84)	(5.59)	(10.96)
WNT5B	61292_s_at	(8.72)	(11.85)	(5.42)	(13.92)
SYNPO2	48039_at	(11.04)	(8.80)	(12.64)	(9.34)
BOC	64423_s_at	(3.63)	(8.16)	(11.80)	(54.66)
SLC20A2	1137_at	(9.27)	(5.08)	(10.51)	(12.61)
COL8A2	52652_g_at	(7.95)	(9.99)	(11.56)	(9.75)
---	52678_at	(9.69)	(9.99)	(3.76)	(17.93)
FOS	1916_s_at	(7.58)	(21.81)	(6.93)	(11.58)
ARGBP2	51939_at	(7.77)	(13.86)	(10.40)	(8.71)
CTGF	64342_at	(4.21)	(4.15)	(20.44)	(14.87)
EPHB6	39930_at	(8.61)	(9.66)	(8.32)	(19.41)
SYNPO2	60532_at	(9.77)	(5.54)	(8.77)	(9.03)
NR4A1	280_g_at	(8.68)	(13.49)	(5.82)	(8.58)
DKFZP564O0823	54033_at	(4.67)	(3.72)	(11.83)	(20.00)
GSTO2	45609_at	(4.73)	(6.81)	(9.60)	(16.18)
---	49321_at	(7.91)	(8.41)	(9.24)	(3.88)
EGR3	40375_at	(9.89)	(7.71)	(8.49)	(6.44)
SYNPO2	61681_at	(7.85)	(8.33)	(4.56)	(18.57)

PI15	58361_at	(3.59)	(4.26)	(12.77)	(11.74)
FOSB	36669_at	(8.81)	(6.27)	(7.60)	(8.39)
OGN	43507_g_at	(3.56)	(8.26)	(7.19)	(25.54)
MOXD1	36834_at	(5.40)	(11.70)	(10.00)	(3.85)
LSAMP	43930_at	(3.05)	(7.62)	(9.76)	(7.67)
EGR2	37863_at	(7.70)	(5.52)	(7.23)	(15.41)
DKFZp686D0853	49770_at	(10.18)	(7.66)	(7.16)	(4.39)
LGP1	52826_at	(13.75)	(5.94)	(3.83)	(8.11)
ME3	35216_at	(7.45)	(9.26)	(6.54)	(5.32)
PPP1R14A	58774_at	(6.68)	(6.14)	(7.31)	(7.87)
FLJ22386	50198_at	(6.80)	(3.64)	(6.98)	(6.65)
NR4A1	279_at	(5.31)	(8.04)	(5.11)	(8.48)
WFDC1	64111_at	(3.79)	(11.21)	(6.64)	(6.66)
ZFP36	40448_at	(6.39)	(6.86)	(7.25)	(3.61)
CACHD1	43554_at	(6.68)	(3.34)	(17.46)	(6.57)
RLN1	35070_at	(6.78)	(11.78)	(5.14)	(6.39)
---	49975_at	(6.43)	(6.16)	(6.74)	(10.11)
CYBRD1	65852_at	(6.43)	(4.79)	(6.70)	(7.23)
PER3	53766_at	(15.43)	(6.79)	(5.56)	(6.29)
MN1	37283_at	(4.47)	(7.36)	(5.55)	(7.48)
DNCI2	35788_at	(4.20)	(8.68)	(3.02)	(10.64)
MRVI1	43966_at	(6.76)	(5.28)	(12.19)	(6.09)
AZGP1	35834_at	(6.32)	(3.86)	(38.18)	(6.18)
MGC14839	48949_at	(8.96)	(4.19)	(8.25)	(3.61)
SMTN	64499_s_at	(5.20)	(15.22)	(7.18)	(4.42)
HSPC157	50179_at	(5.66)	(3.18)	(6.63)	(8.09)
WFDC2	33933_at	(5.30)	(6.50)	(5.73)	(6.81)
BTG2	36634_at	(6.99)	(3.13)	(9.25)	(5.22)
AXIN2	64129_at	(4.97)	(6.97)	(7.18)	(4.20)
PDGFC	45217_at	(4.32)	(7.53)	(8.81)	(3.97)
MLLT10	63345_at	(7.20)	(5.85)	(5.90)	(3.84)
BMP7	49273_g_at	(4.58)	(4.89)	(6.82)	(13.13)
MCC	49504_r_at	(5.90)	(5.71)	(5.08)	(5.84)
HEXA	39340_at	(8.15)	(5.65)	(4.18)	(5.88)
GSTT2	1099_s_at	(6.47)	(5.05)	(6.80)	(4.66)
SSPN	65647_at	(5.40)	(5.88)	(3.12)	(17.61)
UPK3A	36379_at	(5.37)	(4.71)	(5.81)	(6.91)
PDE5A	54668_at	(4.44)	(5.17)	(5.87)	(9.56)
PSD3	63832_at	(3.19)	(6.04)	(4.98)	(6.58)
ALDH7A1	61965_at	(5.85)	(5.14)	(5.88)	(3.13)
FMOD	33431_at	(7.62)	(4.30)	(4.90)	(6.04)
TSPAN2	53693_at	(6.38)	(4.49)	(4.54)	(6.77)
DKFZP586H2123	40017_at	(6.52)	(6.49)	(4.32)	(3.91)
EFS	33883_at	(5.43)	(3.58)	(6.35)	(5.18)
PODN	63953_at	(4.16)	(5.30)	(4.84)	(4.98)
DUSP1	1005_at	(6.53)	(16.66)	(3.02)	(3.19)
SLC22A17	58898_s_at	(4.93)	(5.81)	(4.66)	(4.44)

CDH10	47535_at	(4.87)	(3.19)	(8.27)	(4.65)
---	64163_at	(3.66)	(5.03)	(4.79)	(4.70)
---	42587_at	(4.68)	(4.62)	(4.90)	(3.45)
TSPAN2	57331_at	(4.44)	(8.06)	(3.42)	(4.71)
SORBS1	56409_at	(5.45)	(5.70)	(3.17)	(3.53)
C21orf63	50658_s_at	(4.54)	(3.36)	(4.15)	(5.31)
NBL1	37005_at	(3.34)	(4.27)	(4.31)	(6.36)
CIRBP	39864_at	(4.38)	(3.53)	(4.19)	(6.80)
KLF4	48587_at	(3.77)	(3.62)	(4.57)	(12.50)
ZCSL2	45320_at	(3.10)	(3.19)	(5.88)	(5.13)
C12orf10	53911_at	(3.62)	(4.44)	(3.86)	(6.46)
CERKL	60314_at	(4.68)	(3.03)	(7.37)	(3.62)
NOV	39250_at	(3.20)	(3.90)	(4.38)	(7.37)
EPB41L5	60293_at	(4.33)	(4.97)	(3.06)	(3.92)
WNT5B	66142_s_at	(3.94)	(3.87)	(4.49)	(4.16)
ACYP2	64090_s_at	(3.36)	(4.33)	(3.68)	(5.82)
C9orf103	56186_at	(3.14)	(4.62)	(4.03)	(3.73)
FBXO2	57811_at	(3.51)	(3.37)	(4.16)	(5.33)
CD38	40323_at	(3.25)	(3.37)	(4.27)	(4.27)
BCAS1	37821_at	(4.96)	(3.19)	(4.26)	(3.34)
TMSL8	36491_at	(3.03)	(4.11)	(3.45)	(7.67)
ISL1	39990_at	(3.12)	(3.78)	(3.61)	(3.91)
HSPB8	56474_at	(3.45)	(3.87)	(3.04)	(7.50)
B3GALT3	53879_at	(3.04)	(4.02)	(3.77)	(3.48)
CYBRD1	50955_at	(3.70)	(3.51)	(3.21)	(5.60)
EFEMP2	63644_at	(3.25)	(3.91)	(3.28)	(3.97)
TU3A	45260_at	(3.14)	(3.94)	(3.22)	(4.82)
LOC57228	34176_at	(3.68)	(5.30)	(3.41)	(3.16)
IER2	36097_at	(4.79)	(3.20)	(3.11)	(3.88)
DKFZP564K1964	65860_at	(3.53)	(3.11)	(3.52)	(4.62)

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