SPOP and Prostate Cancer

Terrence P McGarty
White Paper No 130
November, 2015

SPOP is one of many genes involved in the normal growth and maintenance of normal prostate tissue. We examine herein some recent observations regarding SPOP and the genes it controls that putatively lead to PCa.

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

There is a continual promotion of genes as causative for PCa. The list continues to grow and the hope one believes is that they will lead to therapeutic targets to control PCa. As has been discussed elsewhere it is well known that PCa is a highly heterogeneous cancer in terms of gene expression. Unlike some of the recent success in cancer treatment where major targets allow for therapeutic targeting PCa is often much to complex.

As Berger et al have noted regarding the genetic complexity of PCa:

Genome sequencing data indicate that complex rearrangements may enact pivotal gain- and loss-of-function driver events in primary prostate carcinogenesis. Moreover, many rearrangements may occur preferentially in genes that are spatially localized together with transcriptional or chromatin compartments, perhaps initiated by DNA strand breaks and erroneous repair. The complexity of 'closed chain' and other rearrangements suggests that complete genome sequencing—as opposed to approaches focused on exons or gene fusions—may be required to elaborate the spectrum of mechanisms directing prostate cancer genesis and progression.

More recently Gundem et al have noted even more complex structures in genome expressions and changes:

Cancers emerge from an ongoing Darwinian evolutionary process, often leading to multiple competing subclones within a single primary tumour. This evolutionary process culminates in the formation of metastases, which is the cause of 90% of cancer-related deaths5. However, despite its clinical importance, little is known about the principles governing the dissemination of cancer cells to distant organs. Although the hypothesis that each metastasis originates from a single tumour cell is generally supported, recent studies using mouse models of cancer demonstrated the existence of polyclonal seeding from and interclonal cooperation between multiple subclones.

Here we sought definitive evidence for the existence of polyclonal seeding in human malignancy and to establish the clonal relationship among different metastases in the context of androgen-deprived metastatic prostate cancer. Using whole-genome sequencing, we characterized multiple metastases arising from prostate tumours in ten patients.

Integrated analyses of subclonal architecture revealed the patterns of metastatic spread in unprecedented detail. Metastasis-to-metastasis spread was found to be common, either through de novo monoclonal seeding of daughter metastases or, in five cases, through the transfer of multiple tumour clones between metastatic sites. Lesions affecting tumour suppressor genes usually occur as single events, whereas mutations in genes involved in androgen receptor signalling commonly involve multiple, convergent events in different metastases.

Our results elucidate in detail the complex patterns of metastatic spread and further our understanding of the development of resistance to androgen-deprivation therapy in prostate cancer.

In a recent study it is reported[1]:

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."

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:

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

The SPOP E3 ubiquitin ligase gene is frequently mutated in human prostate cancers. Here, we demonstrate that SPOP recognizes a Ser/Thr-rich degron 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.

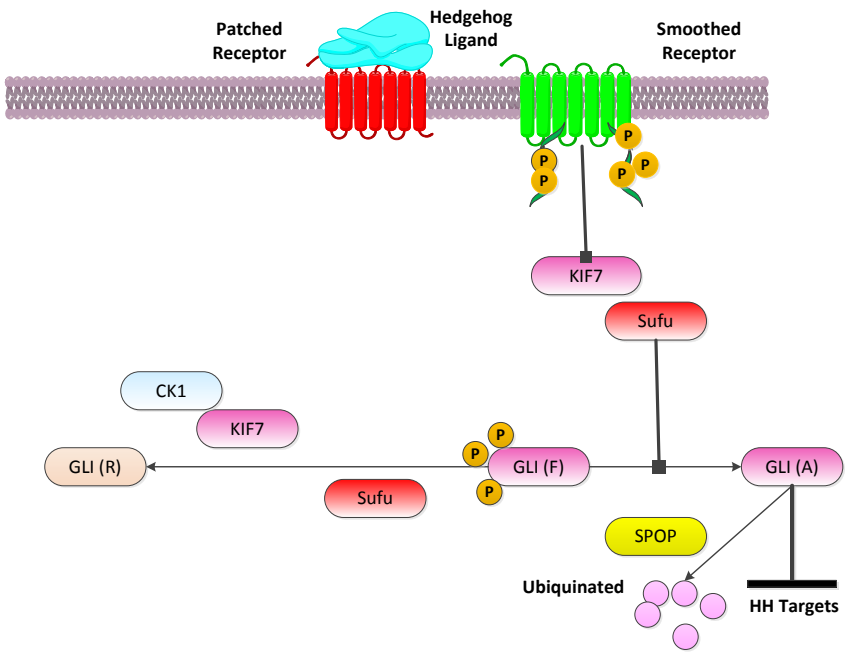
Thus is appears that SPOP seems to play a significant role in the control of certain ubiquitins and their resulting degradation of proteins.

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.

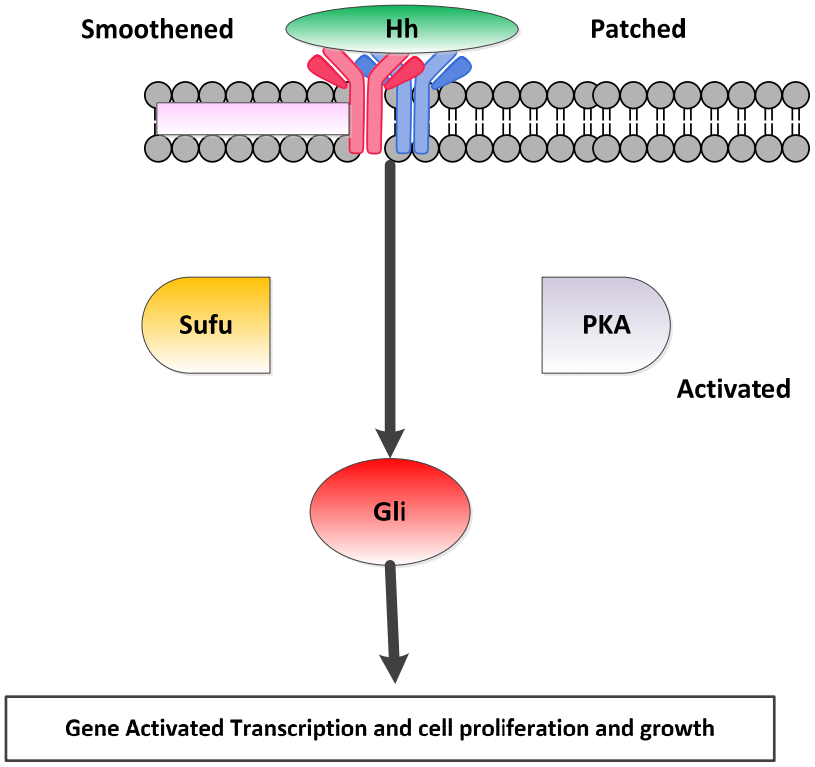
We can see this actually implemented as shown below from Chen and Jiang[2]:

[2] 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.


Patched Receptor
Hedgehog Ligand
Smoothed Receptor
P
P
P
P
P
KIF7
Sufu
CK1
KIF7
GLI (R)
P
P
P
GLI (F)
GLI (A)
Sufu
SPOP
Ubiquinated
HH Targets

2 SPOP

SPOP is part of the Hedgehog signalling pathway[3]. The Hedgehog signalling pathway controls amongst other factors the formation of body segments in insects and in vertebrates the development of the neural tube, limbs and left-right asymmetry. In adult tissues Hedgehog is responsible for homeostasis, equilibrium between cells loss and gain while maintaining total mass and function. With an overactive Hedgehog pathway one sees excess cell proliferation and tumor growth[4]. Thus SPOP has a controlling mechanism for cell replication. Here Hedgehog attaches to Patched and the Patched inhibition of Smothered is eliminated allowing Smothered to start a transcription process enabling replication.

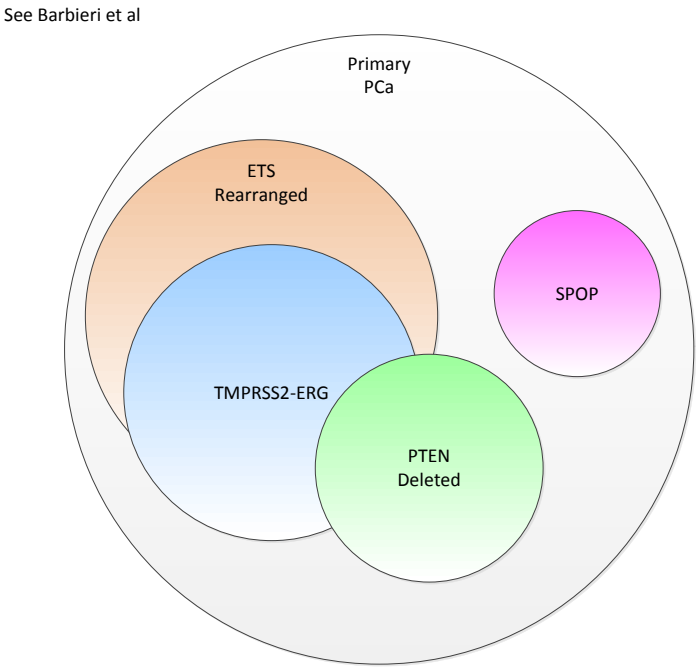


Now upon the activation of Smothered a set of processes are activated and one product is a protein called the zinc finger transcription factor Gli, which when mutually supported by SPOP

[3] http://pid.nci.nih.gov/search/MoleculePage?molid=203488 and http://pid.nci.nih.gov/search/search_landing.shtml?atom_id=208460,208462&what=graphic&jpg=on and pathway at http://pid.nci.nih.gov/search/advanced_landing.shtml?what=graphic&svg=&jpg=true&xml=&biopax=&complex_uses=on&family_uses=on&degree=1&molecule=&pathway=hedgehog&macro_process=&source_id=5&evidence_code=NIL&evidence_code=IAE&evidence_code=IC&evidence_code=IDA&evidence_code=IFC&evidence_code=IGI&evidence_code=IMP&evidence_code=IOS&evidence_code=IPI&evidence_code=RCA&evidence_code=RGE&evidence_code=TAS&output-format=graphic&Submit=Go

[4] See Marks et al p 210-212.

allows movement to the nucleus as a transcription factor activating the DNA to transcribe[5]. From Barbieri et al we have the following putative relationships:



The authors argue that SPOP is a separate and significant marker for PCa. The pathway involved is somewhat understood and is a transcription driven pathway initiated by Hedgehog activation and Patched suppression with Smothered activation. From the NCI pathway databases we have a putative requirement that SPOP is needed to activate GLI for subsequent transcription and cell reproduction.

Specifically Barbieri et al state:

As demonstrated by a subsequent analysis of significantly more genomes, there are only a few truly recurrent non-synonymous mutations in PCa (Barbieri, Rubin, Garraway and Chinnaiyan, submitted). The most common recurrent non-synonymous mutation in PCa involves SPOP. The SPOP gene encodes for the substrate-recognition component of a Cullin3-based E3-ubiquitin ligase. Mutations in SPOP in PCa were reported originally in two systematic sequencing studies.[12, 13] We have now identified the presence of recurrent mutations in SPOP in 6–13% of human PCas in multiple independent patient cohorts (C Barbieri and MA Rubin, unpublished data).

Recurrent missense mutations are found exclusively in the structurally defined substrate-binding cleft of SPOP, and structural analysis suggests that these mutations will inactivate SPOP function by disrupting SPOP–substrate interaction.[75] Further, we found that loss of SPOP function in prostate cell lines resulted in increased invasion and altered gene expression;

[5] See Pecorino, p. 168-170.

evidence of this expression signature was identified in primary tumours harbouring SPOP mutation. Importantly, all SPOP mutations occurred in tumours that were negative for ERG rearrangement; these tumours displayed characteristic somatic copy number aberrations. Taken together, these findings support a distinct molecular class of PCa.

In a recent Nature Medicine article the same authors relate[6]:

Prostate cancer is the second most common cancer in men worldwide and causes over 250,000 deaths each year. Overtreatment of indolent disease also results in significant morbidity. Common genetic alterations in prostate cancer include losses of NKX3.1 (8p21) and PTEN (10q23), gains of AR (the androgen receptor gene) and fusion of ETS family transcription factor genes with androgen-responsive promoters.

Recurrent somatic base-pair substitutions are believed to be less contributory in prostate tumorigenesis but have not been systematically analyzed in large cohorts. Here, we sequenced the exomes of 112 prostate tumor and normal tissue pairs. New recurrent mutations were identified in multiple genes, including MED12 and FOXA1. SPOP was the most frequently mutated gene, with mutations involving the SPOP substrate-binding cleft in 6–15% of tumors across multiple independent cohorts.

Prostate cancers with mutant SPOP lacked ETS family gene rearrangements and showed a distinct pattern of genomic alterations. Thus, SPOP mutations may define a new molecular subtype of prostate cancer.

This just adds another gene in the mix for PCa. Namely they authors argue that it is a different type. We would still ask the same questions:

1. What is the issue regarding the presence or absence of a CSC stem cell in PCa.

2. When does this mutation occur?

3. What causes the mutation?

4. SPOP is not a true kinase so what type of blocking would be possible to mitigate the presence of a mutant.

The following also is noted from a Cell Reports article[7]:

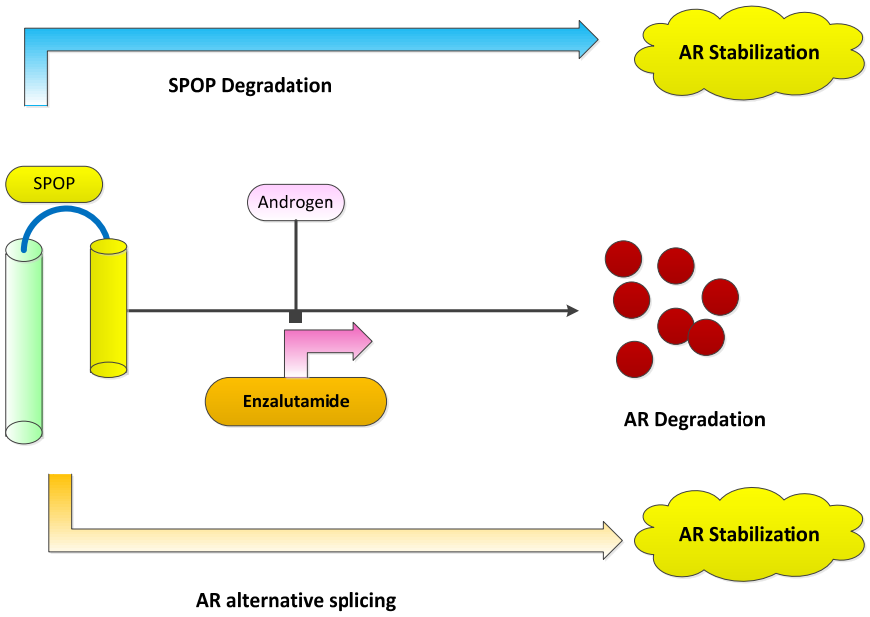
The SPOP E3 ubiquitin ligase gene is frequently mutated in human prostate cancers. Here, we demonstrate that SPOP recognizes a Ser/Thr-rich degron in the hinge domain of androgen receptor (AR) and induces degradation of full-length AR and inhibition of AR-mediated gene

[6] http://www.nature.com/ng/journal/vaop/ncurrent/full/ng.2279.html

[7] http://download.cell.com/cell-reports/pdf/PIIS2211124714000308.pdf?intermediate=true

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 anti-androgens 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 discussion of some prior SPOP research it is noted[8]:

… researchers have shed light on a new mechanism by which prostate cancer develops in men. Central to development of nearly all prostate cancer cases are malfunctions in the androgen receptor — the cellular component that binds to male hormones.

The research team has shown that SPOP, a protein that is most frequently mutated in human prostate cancers, is a key regulator of androgen receptor activity that prevents uncontrolled growth of cells in the prostate and thus helps prevent cancer. The findings appear in the journal Cell Reports.

"By uncovering this new and important pathway of androgen receptor destruction, we may one day be able to develop more effective treatments for a substantial proportion of prostate cancer patients who have developed resistance to standard antiandrogen therapy,"

[8] http://www.healthcanal.com/cancers/prostate-cancer/47500-mayo-clinic-identifies-a-key-cellular-pathway-in-prostate-cancer.html

SPOP mutations have been detected in approximately 15 percent of prostate cancer cases. In addition, it has been shown that in about 35 percent of prostate cancers, the SPOP protein is expressed at abnormally low levels. Despite its prevalence in prostate cancer, it was not known whether or how SPOP defects contributed to tumor development. What the research team discovered is that SPOP is an enzyme that selectively destroys androgen receptor protein. Failure to do so due to alterations in SPOP results in overabundance of androgen receptor, a master regulator of prostate cancer cell growth.

The above mentioned Mayo Clinic research team made four major discoveries:

1. *The antiandrogen receptor is a bona fide degradation substrate of SPOP.*
2. *Androgen receptor splicing variants are resistant to SPOP-mediated degradation.*
3. *Prostate cancer-associated SPOP mutants cannot bind to and promote androgen receptor degradation.*
4. *Androgens antagonize, but antiandrogens promote SPOP-mediated degradation of androgen receptor.*

It is noted and well known that the Androgen receptor (AR) is essential for normal prostate cell growth and survival. It is also important for initiation and progression of prostate cancer. Androgen deprivation therapy, including chemical castration and/or antiandrogen therapy, is the mainstay for treating advanced/disseminated prostate cancer. However, tumors almost always reoccur two to three years after initial response and relapse into a disease called castration-resistant prostate cancer. Development of this therapy-resistant symptom is related to a persistent activation of androgen receptor.

As Medical Express states concerning the most recent work on SPOP[9]:

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," said Hengrui Zhu, Ph.D., first author of the study and a postdoctoral fellow in the laboratory of Rugang Zhang, Ph.D., associate professor in Wistar's Gene Expression and Regulation program. "Our study shows how SPOP is not only able to induce senescence but how mutated SPOP is able to bypass senescence."

[9] http://medicalxpress.com/news/2015-10-scientists-frequently-mutated-prostate-cancer.html

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.

As Zhu et al note[10]:

The SPOP gene, which encodes an E3 ubiquitin ligase adaptor, is frequently mutated in a number of cancer types. However, the mechanisms by which SPOP functions as a tumor suppressor remain poorly understood. Here, we show that SPOP promotes senescence, an important tumor suppression mechanism, by targeting the SENP7 deSUMOylase for degradation. SPOP is upregulated during senescence.

This correlates with ubiquitin-mediated degradation of SENP7, which promotes senescence by increasing HP1α sumoylation and the associated epigenetic gene silencing. Ectopic wild-type SPOP, but not its cancer-associated mutants, drives senescence. Conversely, SPOP knockdown overcomes senescence. These phenotypes correlate with ubiquitination and degradation of SENP7 and HP1α sumoylation, subcellular re-localization, and its associated gene silencing.

From NCBI we note regarding SENP7:

The reversible posttranslational modification of proteins by the addition of small ubiquitin-like SUMO proteins is required for many cellular processes. SUMO-specific proteases, such as SENP7, process SUMO precursors to generate a C-terminal diglycine motif required for the conjugation reaction. They also display isopeptidase activity for deconjugation of SUMO-conjugated substrates.

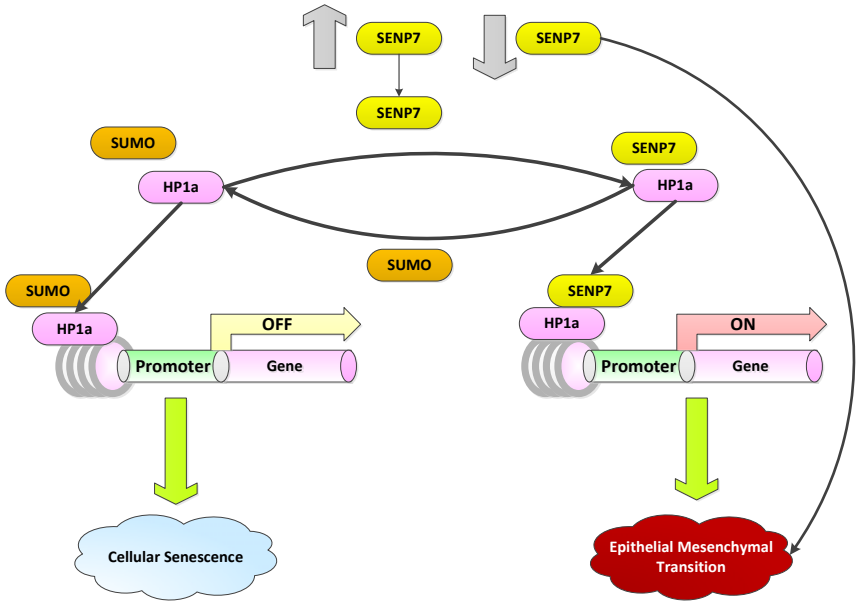
As we have shown before SUMO and SPOP all play a role in degrading via ubiquidation. The degrading process is a part of normal homeostasis. The loss of such functionality is often noted in PCa. However it is not at all clear that these can or should be therapeutic targets.

[10] http://www.cell.com/cell-reports/abstract/S2211-1247%2815%2901137-7

As Bawa-Khalfe et al state:

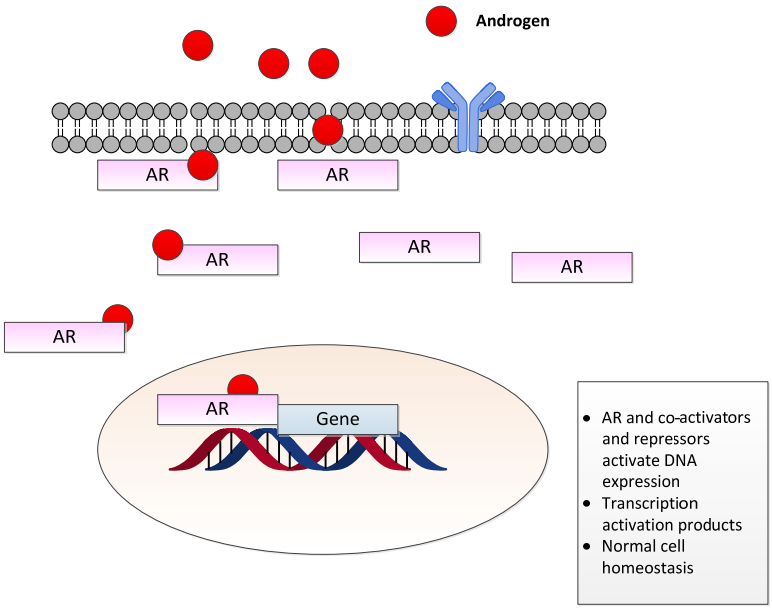
SENP7L levels dictate PCa cells' choice between senescence and EMT. Onset of cancer in breast epithelia decreases the SENP7S splice variant and increases SENP7L, which expresses an HP1α-interaction motif. Loss of SENP7LHP1α interaction causes HP1α hyper-SUMOylation, an enrichment of HP1α at E2F-responsive and mesenchymal gene promoters, silences transcription of these genes, and elicits cellular senescence. Induction of SENP7Lmaintains hypo-SUMOylated HP1α, which relieves HP1α-mediated repression of proliferation promoting E2F-responsive genes as well as mesenchymal genes. SENP7L decreases epithelial gene expression via an unidentified HP1α-independent pathway (dashed line), and concurrently with the HP1α-dependent pathway promotes dedifferentiation.

We demonstrate this below:

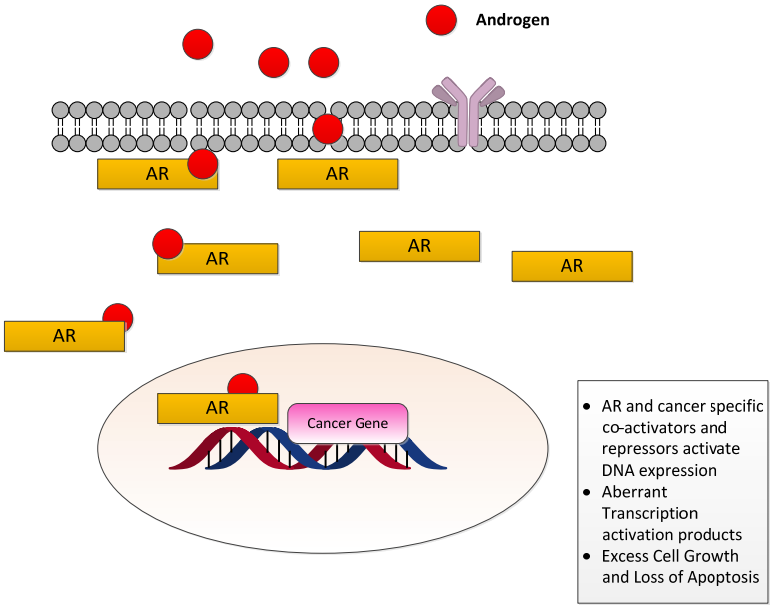


3 ANDROGEN RECEPTOR

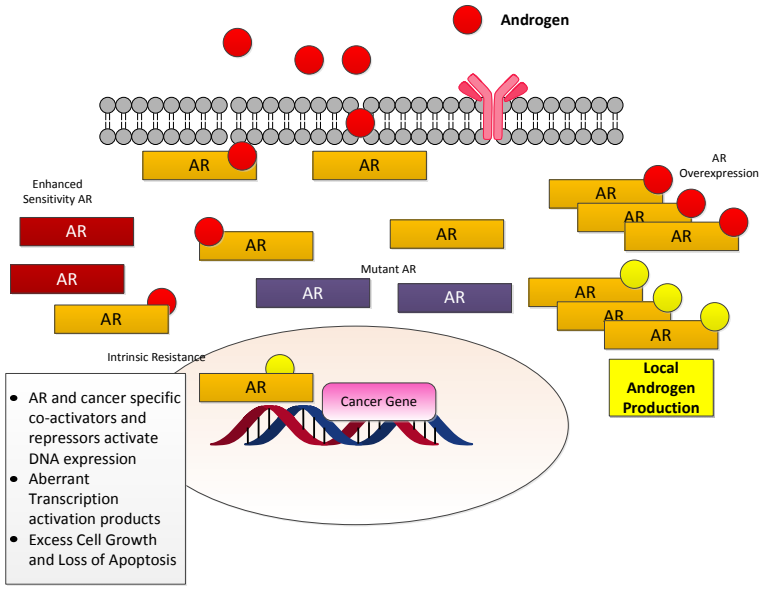
A brief review of the androgen receptor is in order. We consider three steps. First, normal homeostasis does use androgens. Androgens are moved into the cell, attach to the AR and enable normal gene expression.



Second, when an aberrant gene is expressed or over expressed, the androgens still come into the cell and are attached to the AR and enhance the production of that aberrant gene.



It is the understanding of this second step that leads to effective use of androgen deprivation therapy for a while. But further changes occur and intracellular generation of androgen occurs so the process begins anew as shown below:



Note that in the above we have the exogenous androgen in red and the self-produce androgen in yellow.

As Geng et al note:

The androgen receptor (AR) is a critical driver of prostate adenocarcinoma (PC) pathophysiology, regulating proliferation, metabolism and migration, and is also a validated therapeutic target. The importance of the AR axis in PC is further illustrated by the frequent overexpression, especially in the state of castration-resistant PC (CRPC), of steroidogenic enzymes that lead to persistence of intratumoral androgens, as well as AR itself, and its coactivators.

Additional mechanisms of non-canonical AR activation, including AR mutations, ligand-independent AR splice variants and cytokine-induced ligand-independent activation, as well as epigenetic dysregulation of miRNAs that control AR homeostasis, contribute to CRPC progression and further highlight the critical importance of the AR axis in PC. There is compelling evidence that even taxanes, the only family of cytotoxic chemotherapeutics that has ever demonstrated an overall survival benefit in PC, exert anti-cancer activity in CRPC by inhibiting the AR axis.

Also they note:

Somatic missense mutations in the substrate-binding pocket of the E3 ubiquitin ligase adaptor SPOP are present in up to 15% of human prostate adenocarcinomas (PC), but are rare in other malignancies suggesting a prostate-specific mechanism of action. SPOP promotes ubiquitination and degradation of several protein substrates, including the androgen receptor (AR) coactivator

factor SRC-3. However, the relative contributions that SPOP substrates may contribute to the pathophysiology of SPOP-mutant (mt) PC is unknown.

Using an unbiased bioinformatics approach, we determined that the gene expression profile of PC cells engineered to express mt-SPOP overlaps greatly with the gene signature of both SRC-3 and AR transcriptional output, with a stronger effect on AR than SRC-3. This finding suggests that in addition to its SRC-3-mediated effects, SPOP also exerts SRC-3-independent effects that are AR mediated. Indeed, we found that wild-type (wt) but not PC-associated mutants of SPOP promoted AR ubiquitination and degradation, acting directly through a SPOP-binding motif in the hinge region of AR. In support of these results, tumor xenografts composed of PC cells expressing mt-SPOP expressed higher AR protein levels and grew faster than tumors composed of PC cells expressing wt-SPOP.

Further, genetic ablation of SPOP was sufficient to increase AR protein levels in mouse prostate. Examination of public human PC datasets confirmed a strong link between transcriptomic profiles of mt-SPOP and AR. Overall, our studies highlight the AR axis as the key transcriptional output of SPOP in PC, and they provide an explanation for the prostate-specific tumor suppressor role of wt-SPOP.

4 OBSERVATIONS

Thus SPOP becomes another putative target for controlling metastatic PCa. The overall conclusion from this recent focus is the importance of understanding and controlling SPOP and SENP7. The discussions look at SENP7 as a possible therapeutic target. However there are many other factors we should examine as well.

1. Causality: The impact of SPOP and SENP7 are recognized via this focal study and previous ones. However the cause of this expression change is not at all clear. This becomes an all too frequent problem as we see new markers and promoters of aberrant cell growth. The key question is; what specifically is the initiating action resulting in the expression alterations. For example; do we have an epigenetic change? Is it perhaps a methylation effect? Is it perhaps a histone epigenetic effect?

2. Heterogeneity: As Gundem et al have indicated there is significant genetic heterogeneity in PCa cells. They vary by location in the body as well as varying by intra-location as well. Thus with such variability is this but one of a multiplicity of genes to be controlled.

3. Modelling: Understanding the behavior of these pathway aberrations will eventually entail predictable models of how PCa evolves. This is a complex and challenging issue but it is a necessity. Otherwise we will keep asking "why?" again and again. As Kirk et al states:

Deriving mathematical models in biology is rarely straightforward. Although biology is, of course, subject to the same fundamental physical laws—for example, conservation of mass, energy, and momentum—as the other sciences, these laws often do not provide a good starting point for understanding how biological organisms and systems work. Biological modeling is an example of the so-called inverse problem (1), and instead emphasizes context specific levels of abstraction and relies upon experimental observations to decide if a particular model is useful.

Indeed, the models are complex and iterative. Create a model based on current understanding, and then test it to learn what does not work. There are many assumptions that are useless, and the scale may be too large or too small. In the case we discuss here it will however be an essential requirement.

4. Conclusion: Let us examine the final conclusion of Zhu et al on SPOP:

In summary, we showed that SPOP promotes cellular senescence, an important tumor suppression mechanism, by degrading SENP7 deSUMOylase, a SPOP-binding substrate. This correlates with HP1a-associated epigenetic gene silencing during senescence through a relay of ubiquitination and sumoylation post-transcriptional modifications. These findings establish that SPOP functions as a tumor suppressor in the context of cellular senescence and the associated cell growth arrest.

Then active SPOP degrades SENP7 and thus we can either activate SPOP or suppress SENP7. This is the putative therapeutic path. The issue however is: what causes this and what other paths can be activated?

5 REFERENCES

1. An, J., et al, Destruction of Full-Length Androgen Receptor by Wild-Type SPOP, but Not Prostate-Cancer-Associated Mutants, Cell Rep. 2014 February 27; 6(4): 657–669
2. Barbieri, C. et al, Molecular genetics of prostate cancer: emerging appreciation of genetic complexity, Histopathology 2012, 60, 187–198.
3. Barbieri, C., et al, Exome Sequencing Identifies Recurrent SPOP, FOXA1 and MED12 Mutations in Prostate Cancer, Nature Genetics (2012).
4. Bawa-Khalfe, T., et al, Differential expression of SUMO-specific protease 7 variants regulates epithelial–mesenchymal transition, PNAS, October 23, 2012, vol. 109, no. 43
5. Berger, M., et al, The genomic complexity of primary human prostate cancer, Nature 470, 214–220 (10 February 2011)
6. Chen Y., J. Jiang, Decoding the phosphorylation code in Hedgehog signal transduction, Cell Research (2013) 23:186–200; doi:10.1038/cr.2013.10
7. Geng, C., et al, Androgen receptor is the key transcriptional mediator of the tumor suppressor SPOP in prostate cancer, Cancer Res. 2014 October 1; 74(19): 5631–5643
8. Gundem et al, The evolutionary history of lethal metastatic prostate cancer, Nature 520, 353–357, (16 April 2015)
9. Kirk, P., et al, Systems biology (un)certainties, Science, 23 October 2015 • VOL 350 ISSUE 6259
10. Marks, F., et al, Cellular Signal Processing, Garland (New York) 2009.
11. Pecorino, L, Molecular Biology of Cancer, Oxford (New York) 2008.
12. Theurillat, J., et al, Ubiquitylome analysis identifies dysregulation of effector substrates in SPOP-mutant prostate cancer, Science 3 October 2014: Vol. 346 no. 6205 pp. 85-89
13. Zeng, C., et al, SPOP suppresses tumorigenesis by regulating Hedgehog/Gli2 signaling pathway in gastric cancer, Journal of Experimental & Clinical Cancer Research 2014, 33:75
14. Zhu, H., et al, SPOP E3 Ubiquitin Ligase Adaptor Promotes Cellular Senescence by Degrading the SENP7 deSUMOylase, Cell Reports October 2015, http://www.cell.com/cell-reports/abstract/S2211-1247%2815%2901137-7

6 GENES AND FUNCTIONS

Element	*Type (Ligand, Receptor, Cell Surface, Pathway, Intra Nucleus, Transcription)*	*Function (See http://www.ncbi.nlm.nih.gov/gene/)*
ABL	Pathway	The *ABL1* proto-oncogene encodes a cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response. Activity of ABL1 protein is negatively regulated by its SH3 domain, and deletion of the SH3 domain turns ABL1 into an oncogene.
AKT	Pathway	The serine-threonine protein kinase encoded by the AKT1 gene is catalytically inactive in serum-starved primary and immortalized fibroblasts. AKT1 and the related AKT2 are activated by platelet-derived growth factor. The activation is rapid and specific, and it is abrogated by mutations in the pleckstrin homology domain of AKT1. It was shown that the activation occurs through phosphatidylinositol 3-kinase.
APC	Pathway	The activity of one protein in particular, beta-catenin, is controlled by the APC protein (see: Wnt signaling pathway). Regulation of beta-catenin prevents genes that stimulate cell division from being turned on too often and prevents cell overgrowth.
ARF	Pathway	p14ARF is an alternate reading frame (ARF) product of the CDKN2A locus. Both p16INK4a and p14ARF are involved in cell cycle regulation. p14ARF inhibits mdm2, thus promoting p53, which promotes p21 activation, which then binds and inactivates certain cyclin-CDK complexes, which would otherwise promote transcription of genes that would carry the cell through the G_1/S checkpoint of the cell cycle. Loss of p14ARF by a homozygous mutation in the CDKN2A (INK4A) gene will lead to elevated levels in mdm2 and, therefore, loss of p53 function and cell cycle control.
AR	Receptor	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. Expansion of the polyglutamine tract causes spinal bulbar muscular atrophy (Kennedy disease). Mutations in this gene are also associated with complete androgen insensitivity (CAIS). Two alternatively spliced variants encoding distinct isoforms have been described.
BAD	Pathway	The Bcl-2-associated death promoter (BAD) protein is a pro-apoptotic member of the Bcl-2 gene family which is involved in initiating apoptosis. BAD is a member of the BH3-only family , a subfamily of the Bcl-2 family. It does not contain a C-terminal transmembrane domain for outer mitochondrial membrane and nuclear envelope targeting, unlike most other members of the Bcl-2 family.[2] After activation, it is able to form a heterodimer with anti-apoptotic proteins and prevent them from stopping apoptosis.

CCNA2	Cell Cycle	The protein encoded by this gene belongs to the highly conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through 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. In contrast to cyclin A1, which is present only in germ cells, this cyclin is expressed in all tissues tested. This cyclin binds and activates CDC2 or CDK2 kinases, and thus promotes both cell cycle G1/S and G2/M transitions.
CCND1	Transcription	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.
CDK4	Transcription	The protein encoded by this gene is a member of the Ser/Thr protein kinase family. This protein is highly similar to the gene products of S. cerevisiae cdc28 and S. pombe cdc2. It is a catalytic subunit of the protein kinase complex that is important for cell cycle G1 phase progression. The activity of this kinase is restricted to the G1-S phase, which is controlled by the regulatory subunits D-type cyclins and CDK inhibitor p16(INK4a).
Disheveled Dsh	Pathway	Dishevelled (Dsh) is a family of proteins involved in canonical and non-canonical Wnt signalling pathways. Dsh is a cytoplasmic phosphoprotein that acts directly downstream of frizzled receptors
E cadherin	Cell Surface	This gene is a classical cadherin from the cadherin superfamily. The encoded protein is a calcium dependent cell-cell adhesion glycoprotein comprised of five extracellular cadherin repeats, a transmembrane region and a highly conserved cytoplasmic tail. Mutations in this gene are correlated with gastric, breast, colorectal, thyroid and ovarian cancer. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis. The ectodomain of this protein mediates bacterial adhesion to mammalian cells and the cytoplasmic domain is required for internalization.
EGF	Ligand	This gene encodes a member of the epidermal growth factor superfamily. The encoded protein is synthesized as a large precursor molecule that is proteolytically cleaved to generate the 53-amino acid epidermal growth factor peptide. This protein acts a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of numerous cell types. This protein acts by binding the high affinity cell surface receptor, epidermal growth factor receptor. Defects in this gene are the cause of hypomagnesemia type 4. Dysregulation of this gene has been associated with the growth and progression of certain cancers.
ERBB4	Receptor	Receptor tyrosine-protein kinase erbB-4 is an enzyme that in humans is encoded by the *ERBB4* gene.[1][2] Alternatively spliced variants that encode different protein isoforms have been described; however, not all variants have been fully characterized.[3] Receptor tyrosine-protein kinase erbB-4 is a receptor tyrosine kinase that is a member of the epidermal growth factor receptor subfamily. ERBB4 is a single-pass type I transmembrane protein with multiple furin-like cysteine rich domains, a tyrosine kinase domain, a phosphotidylinositol-3 kinase binding site and a PDZ domain binding motif. The protein binds to and is activated by neuregulins-2 and -3, heparin-binding EGF-like growth factor and betacellulin.

ERK	Pathway	Ephrin receptors and their ligands, the ephrins, mediate numerous developmental processes, particularly in the nervous system. Based on their structures and sequence relationships, ephrins are divided into the ephrin-A (EFNA) class, which are anchored to the membrane by a glycosylphosphatidylinositol linkage, and the ephrin-B (EFNB) class, which are transmembrane proteins. The Eph family of receptors are divided into 2 groups based on the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. Ephrin receptors make up the largest subgroup of the receptor tyrosine kinase (RTK) family.
ETV1	Transcription	ETS translocation variant 1 is a protein that in humans is encoded by the *ETV1* gene. 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.
FGFR	Receptor	This gene encodes a member of the fibroblast growth factor receptor (FGFR) family, with its amino acid sequence being highly conserved between members and among divergent species. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. This particular family member binds acidic and basic fibroblast growth hormone and plays a role in bone development and maintenance.
FOS	Transcription	c-Jun is the name of a gene and protein that, in combination with c-Fos, forms the AP-1 early response transcription factor. It was first identified as the Fos-binding protein p39 and only later rediscovered as the product of the c-jun gene. It is activated through double phosphorylation by the JNK pathway but has also a phosphorylation-independent function. c-Jun knockout is lethal, but transgenic animals with a mutated c-Jun that cannot be phosphorylated (termed c-JunAA) can survive.
Frizzled	Receptor	This gene encodes a member of the SFRP family that contains a cysteine-rich domain homologous to the putative Wnt-binding site of Frizzled proteins. Members of this family act as soluble modulators of Wnt signaling; epigenetic silencing of SFRP genes leads to deregulated activation of the Wnt-pathway which is associated with cancer. This gene may also be involved in determining the polarity of photoreceptor cells in the retina. NOTE: There are several "frizzled" genes.
GAS1	Pathway	Growth arrest-specific 1 plays a role in growth suppression. GAS1 blocks entry to S phase and prevents cycling of normal and transformed cells. Gas1 is a putative tumor suppressor gene.
GLI2	Transcription	GLI2 belongs to the C2H2-type zinc finger protein subclass of the Gli family. Members of this subclass are characterized as transcription factors which bind DNA through zinc finger motifs

GOLPH3	Pathway	The Golgi complex plays a key role in the sorting and modification of proteins exported from the endoplasmic reticulum. The protein encoded by this gene is a peripheral membrane protein of the Golgi stack and may have a regulatory role in Golgi trafficking. Several alternatively spliced transcript variants of this gene have been described, but the full-length nature of these variants has not been determined.
GR	Receptor	Growth receptor
GSK-3β	Pathway	Glycogen synthase kinase-3 (GSK-3) is a proline-directed serine-threonine kinase that was initially identified as a phosphorylating and an inactivating agent of glycogen synthase. Two isoforms, alpha (GSK3A) and beta, show a high degree of amino acid homology.[1] GSK3B is involved in energy metabolism, neuronal cell development, and body pattern formation
Hedgehog (Sonic)	Ligand	This gene encodes a protein that is instrumental in patterning the early embryo. It has been implicated as the key inductive signal in patterning of the ventral neural tube, the anterior-posterior limb axis, and the ventral somites. Of three human proteins showing sequence and functional similarity to the sonic hedgehog protein of Drosophila, this protein is the most similar. The protein is made as a precursor that is autocatalytically cleaved; the N-terminal portion is soluble and contains the signalling activity while the C-terminal portion is involved in precursor processing. More importantly, the C-terminal product covalently attaches a cholesterol moiety to the N-terminal product, restricting the N-terminal product to the cell surface and preventing it from freely diffusing throughout the developing embryo. Defects in this protein or in its signalling pathway are a cause of holoprosencephaly (HPE), a disorder in which the developing forebrain fails to correctly separate into right and left hemispheres. HPE is manifested by facial deformities. It is also thought that mutations in this gene or in its signalling pathway may be responsible for VACTERL syndrome, which is characterized by vertebral defects, anal atresia, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, cardiac anomalies, and limb abnormalities.
HGF	Ligand	Hepatocyte growth factor regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic c-Met receptor. Hepatocyte growth factor is secreted by mesenchymal cells and acts as a multi-functional cytokine on cells of mainly epithelial origin. Its ability to stimulate mitogenesis, cell motility, and matrix invasion gives it a central role in angiogenesis, tumorogenesis, and tissue regeneration. It is secreted as a single inactive polypeptide and is cleaved by serine proteases into a 69-kDa alpha-chain and 34-kDa beta-chain. A disulfide bond between the alpha and beta chains produces the active, heterodimeric molecule. The protein belongs to the plasminogen subfamily of S1 peptidases but has no detectable protease activity.
IGFBP7	Ligand	This gene encodes a member of the insulin-like growth factor (IGF)-binding protein (IGFBP) family. IGFBPs bind IGFs with high affinity, and regulate IGF availability in body fluids and tissues and modulate IGF binding to its receptors. This protein binds IGF-I and IGF-II with relatively low affinity, and belongs to a subfamily of low-affinity IGFBPs. It also stimulates prostacyclin production and cell adhesion.

INK4A	Transcription	This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, MDM1, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene.
INSR	Receptor	Insulin receptor. This gene encodes a member of the receptor tyrosine kinase family of proteins. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that form a heterotetrameric receptor. Binding of insulin or other ligands to this receptor activates the insulin signaling pathway, which regulates glucose uptake and release, as well as the synthesis and storage of carbohydrates, lipids and protein. Mutations in this gene underlie the inherited severe insulin resistance syndromes including type A insulin resistance syndrome, Donohue syndrome and Rabson-Mendenhall syndrome. Alternative splicing results in multiple transcript variants.
JUN	Transcription	See FOS
KIF7		This gene encodes a cilia-associated protein belonging to the kinesin family. This protein plays a role in the sonic hedgehog (SHH) signaling pathway through the regulation of GLI transcription factors. It functions as a negative regulator of the SHH pathway by preventing inappropriate activation of GLI2 in the absence of ligand, and as a positive regulator by preventing the processing of GLI3 into its repressor form. Mutations in this gene have been associated with various ciliopathies.
KIT	Receptor	This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous lukemia, and piebaldism.
LEF	Transcription	This gene encodes a transcription factor belonging to a family of proteins that share homology with the high mobility group protein-1. The protein encoded by this gene can bind to a functionally important site in the T-cell receptor-alpha enhancer, thereby conferring maximal enhancer activity. This transcription factor is involved in the Wnt signaling pathway, and it may function in hair cell differentiation and follicle morphogenesis. Mutations in this gene have been found in somatic sebaceous tumors. This gene has also been linked to other cancers, including androgen-independent prostate cancer.
LKB1	Pathway	This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and functions as a tumor suppressor. Mutations in this gene have been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms. Alternate transcriptional splice variants of this gene have been observed but have not been thoroughly characterized.

MEK	Pathway	The protein encoded by this gene is a serine/threonine kinase and is part of some signal transduction cascades, including the ERK and JNK kinase pathways as well as the NF-kappa-B pathway. The encoded protein is activated by autophosphorylation and requires magnesium as a cofactor in phosphorylating other proteins
MITF	Transcription	Microphthalmia-associated transcription factor (MITF) is a basic helix-loop-helix leucine zipper transcription factor involved in melanocyte and osteoclast development
mTOR	Pathway	The mammalian target of rapamycin (mTOR) also known as mechanistic target of rapamycin or FK506 binding protein 12-rapamycin associated protein 1 (FRAP1) is a protein which in humans is encoded by the *FRAP1* gene.[1][2] mTOR is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, and transcription.[3][4] mTOR belongs to the phosphatidylinositol 3-kinase-related kinase protein family.
MYC	Transcription	The protein encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. Mutations, overexpression, rearrangement and translocation of this gene have been associated with a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma. There is evidence to show that alternative translation initiations from an upstream, in-frame non-AUG (CUG) and a downstream AUG start site result in the production of two isoforms with distinct N-termini. The synthesis of non-AUG initiated protein is suppressed in Burkitt's lymphomas, suggesting its importance in the normal function of this gene.
NEDD9	Pathway	CRK-associated substrate-related protein; Cas scaffolding protein family member 2; Crk-associated substrate related; NEDD-9; cas-like docking; dJ49G10.2 (Enhancer of Filamentation 1 (HEF1)); dJ761I2.1 (enhancer of filamentation (HEF1)); enhancer of filamentation 1; neural precursor cell expressed developmentally down-regulated protein 9; p105; renal carcinoma antigen NY-REN-12
NF1	Pathway	NF1 encodes the protein neurofibromin, which appears to be a negative regulator of the ras signal transduction pathway. NF1 is found within the mammalian postsynapse, where it is known to bind to the NMDA receptor complex. It has been found to lead to deficits in learning, and it is suspected that this is a result of its regulation of the Ras pathway. It is known to regulate the GTPase HRAS, causing the hydrolyzation of GTP and thereby inactivating it
NF-κB	Transcription	NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens
Notched	Receptor	
NRAS	Pathway	This is an N-ras oncogene encoding a membrane protein that shuttles between the Golgi apparatus and the plasma membrane. This shuttling is regulated through palmitoylation and depalmitoylation by the ZDHHC9-GOLGA7 complex. The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein. Mutations in this gene have been associated with somatic rectal cancer, follicular thyroid cancer, autoimmune lymphoproliferative syndrome, Noonan syndrome, and juvenile myelomonocytic leukemia.

p15	Pathway	CDKN2B This gene lies adjacent to the tumor suppressor gene CDKN2A in a region that is frequently mutated and deleted in a wide variety of tumors. This gene encodes a cyclin-dependent kinase inhibitor, which forms a complex with CDK4 or CDK6, and prevents the activation of the CDK kinases, thus the encoded protein functions as a cell growth regulator that controls cell cycle G1 progression. The expression of this gene was found to be dramatically induced by TGF beta, which suggested its role in the TGF beta induced growth inhibition. Two alternatively spliced transcript variants of this gene, which encode distinct proteins, have been reported.
p16	Pathway	CDKN2A This gene generates several transcript variants which differ in their first exons. At least three alternatively spliced variants encoding distinct proteins have been reported, two of which encode structurally related isoforms known to function as inhibitors of CDK4 kinase. The remaining transcript includes an alternate first exon located 20 Kb upstream of the remainder of the gene; this transcript contains an alternate open reading frame (ARF) that specifies a protein which is structurally unrelated to the products of the other variants. This ARF product functions as a stabilizer of the tumor suppressor protein p53 as it can interact with, and sequester, MDM1, a protein responsible for the degradation of p53. In spite of the structural and functional differences, the CDK inhibitor isoforms and the ARF product encoded by this gene, through the regulatory roles of CDK4 and p53 in cell cycle G1 progression, share a common functionality in cell cycle G1 control. This gene is frequently mutated or deleted in a wide variety of tumors, and is known to be an important tumor suppressor gene
p21	Pathway	CDKN1A This gene encodes a potent cyclin-dependent kinase inhibitor. The encoded protein binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at G1. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. This protein can interact with proliferating cell nuclear antigen (PCNA), a DNA polymerase accessory factor, and plays a regulatory role in S phase DNA replication and DNA damage repair. This protein was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Multiple alternatively spliced variants have been found for this gene.
p27	Pathway	The 26S proteasome is a multicatalytic proteinase complex with a highly ordered structure composed of 2 complexes, a 20S core and a 19S regulator. The 20S core is composed of 4 rings of 28 non-identical subunits; 2 rings are composed of 7 alpha subunits and 2 rings are composed of 7 beta subunits. The 19S regulator is composed of a base, which contains 6 ATPase subunits and 2 non-ATPase subunits, and a lid, which contains up to 10 non-ATPase subunits. Proteasomes are distributed throughout eukaryotic cells at a high concentration and cleave peptides in an ATP/ubiquitin-dependent process in a non-lysosomal pathway. An essential function of a modified proteasome, the immunoproteasome, is the processing of class I MHC peptides. This gene encodes a non-ATPase subunit of the 19S regulator. Three transcript variants encoding two different isoforms have been found for this gene.

p53	Pathway	This gene encodes tumor protein p53, which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. p53 protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. p53 is a DNA-binding protein containing transcription activation, DNA-binding, and oligomerization domains. It is postulated to bind to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mutants of p53 that frequently occur in a number of different human cancers fail to bind the consensus DNA binding site, and hence cause the loss of tumor suppressor activity. Alterations of this gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome. Multiple p53 variants due to alternative promoters and multiple alternative splicing have been found. These variants encode distinct isoforms, which can regulate p53 transcriptional activity
Patched	Receptor	This gene encodes a member of the patched gene family. The encoded protein is the receptor for sonic hedgehog, a secreted molecule implicated in the formation of embryonic structures and in tumorigenesis, as well as the desert hedgehog and indian hedgehog proteins. This gene functions as a tumor suppressor. Mutations of this gene have been associated with basal cell nevus syndrome, esophageal squamous cell carcinoma, trichoepitheliomas, transitional cell carcinomas of the bladder, as well as holoprosencephaly. Alternative splicing results in multiple transcript variants encoding different isoforms.
PI3K	Pathway	Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers.
PIP2 PIP3	Pathway	Phosphatidylinositol 4,5-bisphosphate (PIP2) is a minority phospholipid of the inner leaflet of plasma membranes. Many plasma membrane ion channels and ion transporters require PIP2 to function and can be turned off by signaling pathways that deplete PIP2. This review discusses the dependence of ion channels on phosphoinositides and considers possible mechanisms by which PIP2 and analogues regulate ion channel activity.
PREX2	Pathway	An activator of Rac, P-REX2, that is structurally related to the exchange factor PtdIns(3,4,5)-dependent Rac exchanger (P-REX1), but exhibits distinct tissue-specific expression. P-REX2 is spliced into two RNA species, approximately 3.5 and approximately 10 kb in size. The cDNA corresponding to the smaller transcript encodes a protein that exhibits strong similarity with P-REX1 within its N-terminal domains, but differs in the C-terminal region. P-REX2 promoted increased levels of GTP-bound Rac that could be further stimulated by enhancing PI-3K activity. Thus, P-REX2 may serve as a novel link between Rac activation and the PI-3 kinase pathway.

PTEN	Pathway	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.
RAF	Pathway	This gene is the cellular homolog of viral raf gene (v-raf). The encoded protein is a MAP kinase kinase kinase (MAP3K), which functions downstream of the Ras family of membrane associated GTPases to which it binds directly. Once activated, the cellular RAF1 protein can phosphorylate to activate the dual specificity protein kinases MEK1 and MEK2, which in turn phosphorylate to activate the serine/threonine specific protein kinases, ERK1 and ERK2. Activated ERKs are pleiotropic effectors of cell physiology and play an important role in the control of gene expression involved in the cell division cycle, apoptosis, cell differentiation and cell migration.
RAS	Pathway	The protein encoded by this gene is located in the cytoplasm and is part of the GAP1 family of GTPase-activating proteins. The gene product stimulates the GTPase activity of normal RAS p21 but not its oncogenic counterpart. Acting as a suppressor of RAS function, the protein enhances the weak intrinsic GTPase activity of RAS proteins resulting in the inactive GDP-bound form of RAS, thereby allowing control of cellular proliferation and differentiation. Mutations leading to changes in the binding sites of either protein are associated with basal cell carcinomas. Mutations also have been associated with hereditary capillary malformations (CM) with or without arteriovenous malformations (AVM) and Parkes Weber syndrome. Alternative splicing results in two isoforms where the shorter isoform, lacking the N-terminal hydrophobic region but retaining the same activity, appears to be abundantly expressed in placental but not adult tissues.
SENP7	Pathway	The reversible posttranslational modification of proteins by the addition of small ubiquitin-like SUMO proteins (see SUMO1; MIM 601912) is required for many cellular processes. SUMO-specific proteases, such as SENP7, process SUMO precursors to generate a C-terminal diglycine motif required for the conjugation reaction. They also display isopeptidase activity for deconjugation of SUMO-conjugated substrates.
SMAD4	Pathway	SMADs are intracellular proteins that transduce extracellular signals from transforming growth factor beta ligands to the nucleus where they activate downstream TGF-β gene transcription. The SMADs, which form a trimer of two receptor-regulated SMADs and one co-SMAD, act as transcription factors that regulate the expression of certain genes
Smoothened	Receptor	The protein encoded by this gene is a G protein-coupled receptor that interacts with the patched protein, a receptor for hedgehog proteins. The encoded protein tranduces signals to other proteins after activation by a hedgehog protein/patched protein complex.

SPOP	Pathway	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.
SRC3		The protein encoded by this gene is a nuclear receptor coactivator that interacts with nuclear hormone receptors to enhance their transcriptional activator functions. The encoded protein has histone acetyltransferase activity and recruits p300/CBP-associated factor and CREB binding protein as part of a multisubunit coactivation complex. This protein is initially found in the cytoplasm but is translocated into the nucleus upon phosphorylation. Several transcript variants encoding different isoforms have been found for this gene. In addition, a polymorphic repeat region is found in the C-terminus of the encoded protein.
SUFU		The Hedgehog signaling pathway plays an important role in early human development. The pathway is a signaling cascade that plays a role in pattern formation and cellular proliferation during development. This gene encodes a negative regulator of the hedgehog signaling pathway. Defects in this gene are a cause of medulloblastoma. Alternative splicing results in multiple transcript variant
TCF	Transcription	The protein encoded by this gene is a nuclear transcription factor which binds DNA as a homodimer. The encoded protein controls the expression of several genes, including hepatocyte nuclear factor 1 alpha, a transcription factor which regulates the expression of several hepatic genes. This gene may play a role in development of the liver, kidney, and intestines. Mutations in this gene have been associated with monogenic autosomal dominant non-insulin-dependent diabetes mellitus type I. Alternative splicing of this gene results in multiple transcript variants encoding several different isoforms.
TGF	Ligand	This gene encodes a member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth factors. The secreted protein is cleaved into a latency-associated peptide (LAP) and a mature TGFB1 peptide, and is found in either a latent form composed of a TGFB1 homodimer, a LAP homodimer, and a latent TGFB1-binding protein, or in an active form composed of a TGFB1 homodimer. The mature peptide may also form heterodimers with other TGFB family members. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease
β catenin	Pathway	Beta-catenin (or β-catenin) is a protein that in humans is encoded by the *CTNNB1* gene