STAT3 and PCa: Of Mice and Men

Terrence P McGarty
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STAT3 is a gene expression that can be activated by IL-6 and previously was considered a driver in metastatic growth. A recent paper presents an alternative picture which we consider herein. This presentation demonstrates the complexity of genetic dynamic pathways as well as the issue of mouse models versus human reality.

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1 INTRODUCTION

Signal transducer and activator of transcription (STAT) proteins are powerful controllers of gene expression. Recent work has involved them in Prostate Cancer along with the many other targets which have been identified. We examine this specific gene and its recently identified significance. The specific STAT is STAT3. Previously it has been linked to aggressive cancers. In fact attempts have been made to therapeutically target this pathway. The authors in a recent paper however contend that it is just the opposite. Namely STAT3 actually prevent metastatic behavior.

This discussion is a critical one as we examine further the targeting of genes and their behavior. The STAT3 issue seems to state that on one hand over-expression is bad, yet then on the other hand over-expression is good. This highlights the issue of cross talk between paths as well as the yet to be fully understood dynamics of pathways. Add to this is the fact that STAT3 is driven by IL-6 and this links in the immune system as well.

We begin the discussion with information in a Press Release in Science Daily which reports[1]:

A gene that is responsible for cancer growth plays a totally unexpected role in prostate cancer. The gene Stat3 is controlled by the immune modulator interleukin 6 and normally supports the growth of cancer cells. The international research team led by Prof. Lukas Kenner from the Medical University of Vienna, the Veterinary University of Vienna, and the Ludwig Boltzmann Institute for Cancer Research (LBI-CR) discovered a missing link for an essential role of Stat3 and IL-6 signalling in prostate cancer progression.

Interleukin 6 (IL-6) is an important cytokine that controls the cell survival and tumor growth. Hyperactive IL-6 may support cancer growth, particularly as it controls STAT3, which was shown to have an oncogenic role in most tumours. Many therapies are therefore designed to suppress IL-6 or STAT3. But the situation is different in prostate cancer. Lukas Kenner's research group has shown that, contrary to expectations; active STAT3 suppresses cell growth in prostate tumours. It activates the gene $p14^{ARF}$, which blocks cell division and thus inhibits tumour growth.

IL-6 is one of many interleukin cytokines, activating immune cells and leading to their proliferation. In a classic model for STAT3, it is activated by IL-6 and then it progresses via phosphorlyation to act as a promoter or enhancer for a multiplicity of genes whose expression leads to cancerous growth. However there is an alternative pathway, the ARF-MDM2-p53 pathway the controls and may mitigate some of these processes. This paper focuses on this crossover effect.

The article continues:

[1] http://www.sciencedaily.com/releases/2015/07/150722081410.htm

For this reason, STAT3 and $p14^{ARF}$ are ideally suited to act as biomarkers for the prognosis of this disease. If these two factors are missing in tissue samples, the risk is massively increased that the tumour grows and forms metastases.

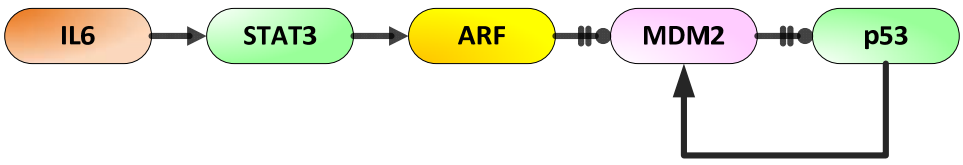
According to Lukas Kenner, this is important, as the predictive power of these proteins as biomarkers is twice as good as the previous gold standard. As only about 10 % of patients with prostate cancer die from the disease, this can help to prevent unnecessary therapeutic interventions with severe side effects such as incontinence and impotence. A non-invasive nuclear medical test based on these findings might soon be able to replace the painful removal of tissue samples to be examined.

The reversed role of interleukin 6 as an inhibitor of prostate cancer has an additional significance. Blockade of interleukin 6 is used to treat other diseases, such as rheumatoid arthritis. According to Kenner, this means that therapies that block the IL-6 pathway may enhance the growth of prostate cancer.

Thus, the drug that is used to treat inflammatory disease may exacerbate malignancies. "Applying IL-6/Stat3 blockers to clinical practice might be dangerous for patients with cancerous lesions, further studies are mandatory to assess the possibility of increased cancer risk right now," says coauthor of this study, Helmut Dolznig, also from the Medical University of Vienna. The study was financed mainly by the LBI-CR and the FWF. These results have just been published in the distinguished scientific journal Nature Communications.

The following is a generalized paradigmatic summary of Pencik et al. Namely; they observed that IL6 controls STAT3 which in turn controls the ARF-MDM2-p53 pathway, which is critical in the overall control of PCa metastasis.

1. IL6 drives increase in STAT3
2. STAT3 drives increase in ARF
3. ARF blocks MDM2
4. Low MDM2 allows increase in p53
5. p53 inhibits metastasis



Now it should also be noted that the above is not the complete presentation. For example in this pathway p53 actually drives MDM2. There are other linkages that should be considered as well. We shall discuss some of these later.

Now from the paper in question, namely Pencik et al, they conclude:

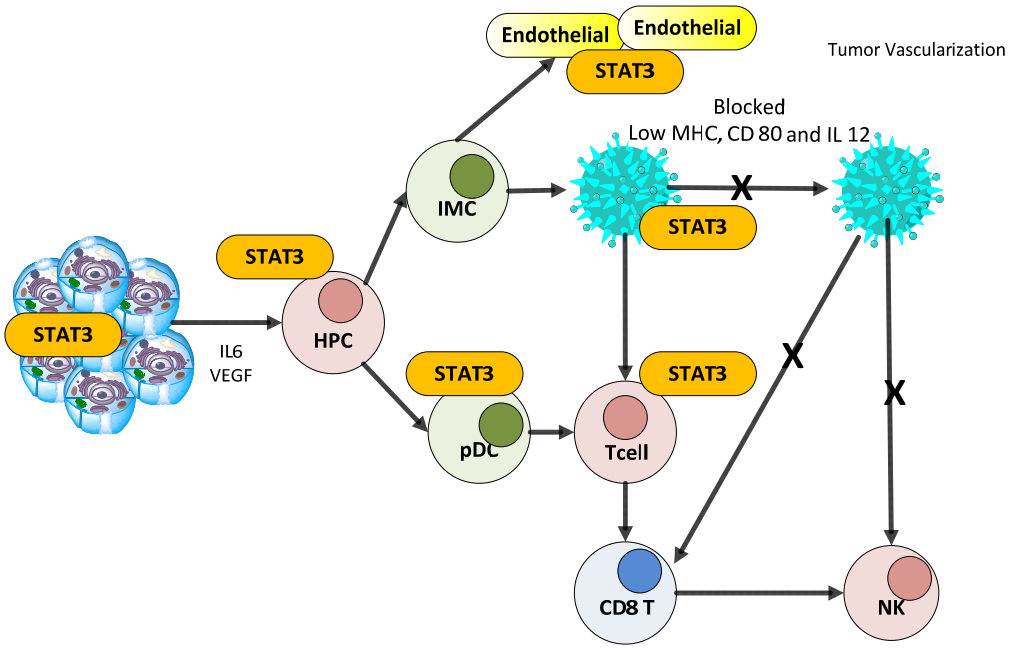
We have uncovered a paradigm shift in understanding the key function of STAT3 in tumorigenicity and metastatic progression in PCa. Therefore, our results call for cautious use of anti-IL-6- STAT3 signalling blockers in the treatment of PCa as this may turn low-grade tumours into highly malignant cancers by loss of senescence controlled by the STAT3–ARF axis. As IL-6/STAT3 signalling blockers are successful in the treatment of chronic inflammatory or autoimmune diseases, their influence on PCa development needs to be carefully evaluated in future studies.

Reactivating the IL-6/STAT3/ARF-dependent senescence pathway57 might be a promising strategy for PCa therapy via downregulation of Mdm2 (ref. 58) or p53 induction59. Alternatively, triggering ARF–p53-independent cellular senescence by a small molecule inhibitor could be beneficial for PCa patients in whom other therapies have failed.

Namely, they argue that the STAT3 control of the ARF-MDM2-p53 pathway should not be interfered with. That pathway actually enables control over metastatic behavior. We will discuss each element in some detail in what follows.

2 CLASSIC STAT3 ACTION

The classic understanding of STAT3 is that is acts to promote cancers. The figure below is a modification from Yu et al:



STAT3 signalling allows crosstalk between tumour cells and dendritic cells, forming an immunosuppressive network. *Tumour-associated factors such as vascular endothelial growth factor (VEGF), IL-10 and IL-6 can not only be upregulated by signal transducer and activator of transcription 3 (STAT3), but are also STAT3 activators. Increased STAT3 activity in haematopoietic progenitor cells (HPCs) promotes the generation of immature myeloid cells (iMCs) and increases the numbers of both immature dendritic cells and plasmacytoid dendritic cells (pDCs), each of which promotes the accumulation of regulatory T (TReg) cells in the tumour microenvironment. ...preventing their maturation and compromising their ability to stimulate the anti-tumour effects of CD8+ T cells and natural killer (NK) cells.*

As Yu et al state:

Immune cells in the tumour microenvironment not only fail to mount an effective anti-tumour immune response, but also interact intimately with the transformed cells to promote oncogenesis actively. Signal transducer and activator of transcription 3 (STAT3), which is a point of convergence for numerous oncogenic signalling pathways, is constitutively activated both in tumour cells and in immune cells in the tumour microenvironment.

Constitutively activated STAT3 inhibits the expression of mediators necessary for immune activation against tumour cells. Furthermore, STAT3 activity promotes the production of immunosuppressive factors that activate STAT3 in diverse immune-cell subsets, altering gene-expression programmes and, thereby, restraining anti-tumour immune responses. As such, STAT3 propagates several levels of crosstalk between tumour cells and their immunological microenvironment, leading to tumour-induced immunosuppression. Consequently, STAT3 has emerged as a promising target for cancer immunotherapy.

Thus the classic view is that STAT3 is an essential element in the pathology of tumorogenesis which as we indicated earlier is in contrast to the recent results. Thus do we block it or allow it? That is the question. Yu et al conclude:

The ability of STAT3 to broadly and profoundly affect tumour immunity strongly indicates that constitutively activated STAT3 both in tumour cells and in tumour stromal immune cells is an attractive target for cancer immunotherapy. Another unique and appealing aspect of targeting STAT3 for cancer immunotherapy is due to the crucial role of STAT3 in tumour-cell survival and tumour angiogenesis. Many experiments have shown that tumour rejection mediated by CD8+ T cells is always preceded by the inhibition of tumour-induced angiogenesis.

Because targeting STAT3 is expected to decrease the survival and angiogenic potential both of tumour cells and of the tumour stroma, targeting STAT3 could facilitate immune-cell-mediated anti-tumour effects at several levels. Although STAT3 is the first oncogenic target for cancer immunotherapy, other important onco proteins, such as MAPKs, might have similar roles. With the emergence of targeted delivery systems, and small molecule inhibitors or RNAi technology to block STAT3 and other relevant oncogenic pathways, a new era of molecular targeting for cancer immunotherapy is on the horizon.

Yu et al are focusing on hematopoietic cells not prostate cells. There is no reason why one should expect the same effect in different cells. Yet from a therapeutic perspective if such a drastically different model is functioning, the results would be problematic at best.

As Niu et al have stated:

Loss of p53 function by mutation is common in cancer. However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations.

It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53.

We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

Thus, Niu et al also present a model for Stat3 inhibiting p53, again in contrast to the paper in question. Niu et al conclude:

1. Stat3 protein interacts with the p53 promoter.
2. Stat3 inhibits p53 expression at the transcription level.
3. Stat3 binds to the p53 promoter in vitro as determined by EMSA.
4. Interaction between Stat3 protein and the p53 promoter contributes to Stat3-mediated inhibition.
5. Stat3 activity inhibits the p53-responsive element and UV-induced p53-mediated growth arrest.
6. Blocking Stat3 activates p53 expression in human cancer cells.
7. Blocking Stat3 induces p53-mediated tumor cell apoptosis and facilitates UV-induced tumor cell growth inhibition.

The results of these two studies seem fairly conclusive regarding Stat3. Namely it is oncogenic. But despite the study in question here seems to reverse that position. We will examine that in some detail.

3 ARF-MDM2-P53

Let us now review what is understood about the ARF-MDM2-p53 pathway. This will be necessary before linking this pathway to STAT3 and its functions.

Now this is a classic pathway whose ultimate control mechanism is p53 expression. p53 is generally understood to be a control gene, keeping the cell in some homeostasis and preventing malignancy. As we will not later this may not always be the case but that will not apply to the current discussion.

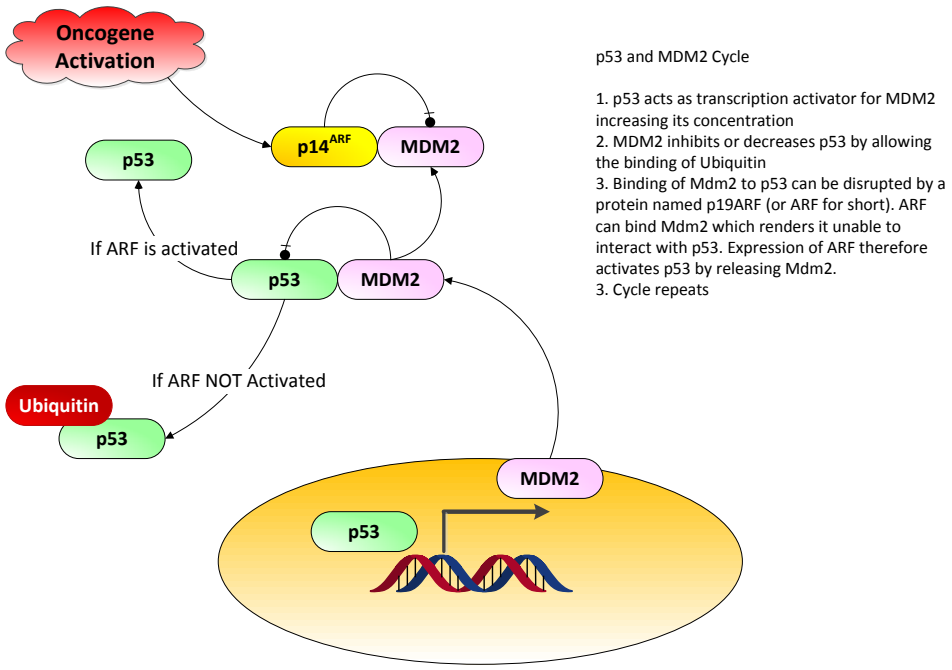
The following Figure depicts the process of the three gene control mechanism. Simply:

1. p53 activates the production of MDM2

2. MDM2 can bind to p53 and result in its dissolution via an Ubiquination

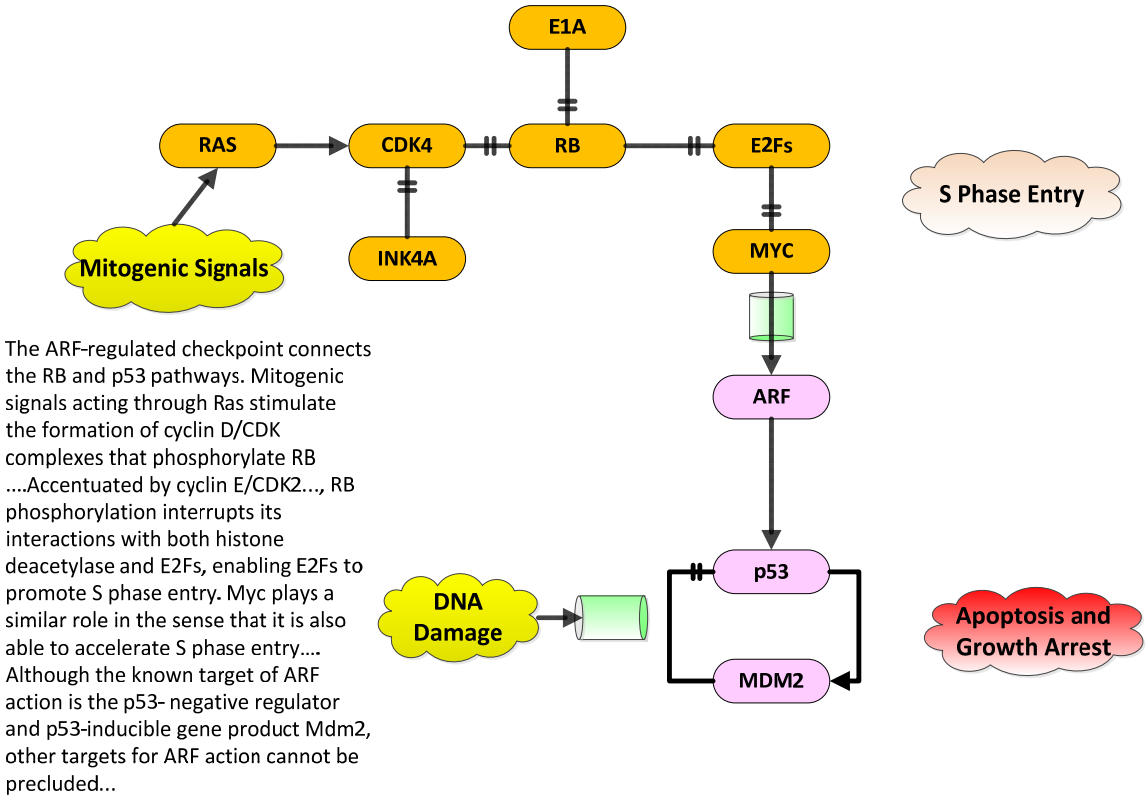
3. ARF can bind to MDM2 and allow the p53 to survive.

4. The process, albeit a bit complex, reaches a steady state for all three proteins.



From Sherr and Weber (as modified) we have the following details as well shown graphically:

From Sherr and Weber



The ARF-regulated checkpoint connects the RB and p53 pathways. Mitogenic signals acting through Ras stimulate the formation of cyclin D/CDK complexes that phosphorylate RB ….Accentuated by cyclin E/CDK2…, RB phosphorylation interrupts its interactions with both histone deacetylase and E2Fs, enabling E2Fs to promote S phase entry. Myc plays a similar role in the sense that it is also able to accelerate S phase entry…. Although the known target of ARF action is the p53- negative regulator and p53-inducible gene product Mdm2, other targets for ARF action cannot be precluded…

Note in the above we have the cyclic MDM2 and p53 control as well as the cell instigators.

Now Van Maerken, T., et al notes the following regarding the details of this feedback loop:

The p53-MDM2 autoregulatory feedback loop.

(a) The p53 protein induces expression of MDM2, which negatively regulates the stability and activity of p53, providing a means to keep p53 levels and activity low in unstressed cells and to switch off p53 at the end of a stress response.

(b) The p53-mediated expression of MDM2 results from binding of p53 to response elements in the MDM2 gene and subsequent transactivation of MDM2. The domain structure of p53 is shown schematically:

i. *TAD, transactivation domain, amino acids;*
ii. *PRD, proline-rich domain, amino acids; DBD, DNA-binding domain, amino acids;*
iii. *TD, tetramerization domain, amino acids;*
iv. *CTD, C-terminal regulatory domain, amino acids.*

(c) The p53-inhibitory activity of MDM2 relies on multiple mechanisms. Binding of MDM2 to p53 conceals the TAD and consequently blocks the transcriptional activity of p53. MDM2 also recruits several corepressor proteins to p53, including HDAC1, CTBP2, YY1, and KAP1.

The E3 ubiquitin ligase activity of MDM2 results in ubiquitination of lysine residues in the CTD of p53, preventing acetylation of p53, favoring nuclear export, and promoting proteasomal degradation (see text for details). Some of these lysine residues can also be neddylated by MDM2, resulting in inhibition of the transcriptional activity of p53. Finally, MDM2 may also serve as a p53-specific transcriptional silencer by binding and monoubiquitinating histone proteins in the proximity of p53-responsive promoters. Nd, NEDD8; Ub, ubiquitin. …

They continue the discussion as follows:

The $p14^{ARF}$ protein is predominantly localized to the nucleolus, in which it is stabilized by binding to nucleophosmin within maturing pre-ribosomal particles, pointing to a function in the regulation of ribosome biogenesis.

Nucleophosmin promotes the processing of ribosomal RNA precursors and the nuclear export of ribosomal subunits, whereas overexpression of $p14^{ARF}$ or its murine homolog $p19^{ARF}$ interferes with transcription and processing of ribosomal RNA, impedes nucleocytoplasmic shuttling of nucleophosmin, and inhibits ribosome nuclear export. However, the precise biological function of the nucleophosmin– $p14^{ARF}$ complexes remains a subject of debate. Stress signals trigger the disruption of the interaction between $p14^{ARF}$ and nucleophosmin, and induce translocation of p14ARF to the nucleoplasm.

This redistribution enables $p14^{ARF}$ to interact with p53-bound MDM2 and to antagonize MDM2 function by inhibiting its E3 ubiquitin ligase activity and by blocking nucleocytoplasmic shuttling of MDM2 and p53, resulting in p53 stabilization. The p53-inhibitory activity of MDM2 may also be neutralized by $p14^{ARF}$ -mediated mobilization of MDM2 into the nucleolus, although this mechanism is not strictly required for the p53-dependent functions of $p14^{ARF}$.

This is clearly a highly complex mechanism. They continue:

Furthermore, the $p14^{ARF}$ protein is capable of inhibiting the activity of another E3 ubiquitin ligase that targets p53 for degradation, ARF-BP1/Mule, and of counteracting the p53-antagonizing NF-kappaB pathway. It should be noted that p14ARF also exerts a potent tumor suppressor activity independently of p53.

Various researchers have tried to model these systems using different techniques. One technique is the use of Petri Nets[2]. From CSML we have a Petri Net models describing the details of such a network and they state[3]:

[2] See Reisig

[3] http://www.csml.org/models/csml-models/p53-arf-dependent-stabilization-pathway/

Proteins p53, MDM2, and $p19^{ARF}$ are proteins closely related to cancer. The protein p53 is a protein which suppresses the formation of tumors, and the protein MDM2 promotes the formation of tumors by decreasing the activity of the protein p53.

Understanding of control mechanism of these proteins connects to development of an effective medicine for suppressing the tumor. It is known that protein p53 works as a transcription factor for many genes and its transcriptional activity is controlled by a complex formed with proteins MDM2 and $p19^{ARF}$.

However, it is still unclear whether protein p53 keeps its transcriptional activity in the form of the trimer with proteins p53, MDM2 and $p19^{ARF}$. ... a hybrid functional Petri net (HFPN) model which has been constructed by compiling and interpreting the information of p53-MDM2 interactions... With our HFPN model, we have simulated mutual behaviors between genes p53, MDM2, $p19^{ARF}$, and their products. Through simulation, we discussed whether the complex p53-MDM2-$p19^{ARF}$ has transcriptional activity for genes Bax and MDM2 or not.

It is worth examining these structures, namely the Petri Nets. We leave the examination to the reference. From Moll and Petrenko we have the following result:

Activation of the p53 protein protects the organism against the propagation of cells that carry damaged DNA with potentially oncogenic mutations. MDM2, a p53- specific E3 ubiquitin ligase, is the principal cellular antagonist of p53, acting to limit the p53 growthsuppressive function in unstressed cells. In unstressed cells, MDM2 constantly monoubiquitinates p53 and thus is the critical step in mediating its degradation by nuclear and cytoplasmic proteasomes.

The interaction between p53 and MDM2 is conformation-based and is tightly regulated on multiple levels. Disruption of the p53-MDM2 complex by multiple routes is the pivotal event for p53 activation, leading to p53 induction and its biological response. Because the p53-MDM2 interaction is structurally and biologically well understood, the design of small lipophilic molecules that disrupt or prevent it has become an important target for cancer therapy.

4 STAT3

Let us go back and re-examine the functions of STAT3 and this time in the context of the paper in study. As NCBI states[4]:

The protein encoded by this gene is a member of the STAT protein family. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators.

This protein is activated through phosphorylation in response to various cytokines and growth factors including IFNs, EGF, IL5, IL6, HGF, LIF and BMP2. This protein mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis. The small GTPase Rac1 has been shown to bind and regulate the activity of this protein. PIAS3 protein is a specific inhibitor of this protein.

As Niu et al have noted:

Loss of p53 function by mutation is common in cancer.

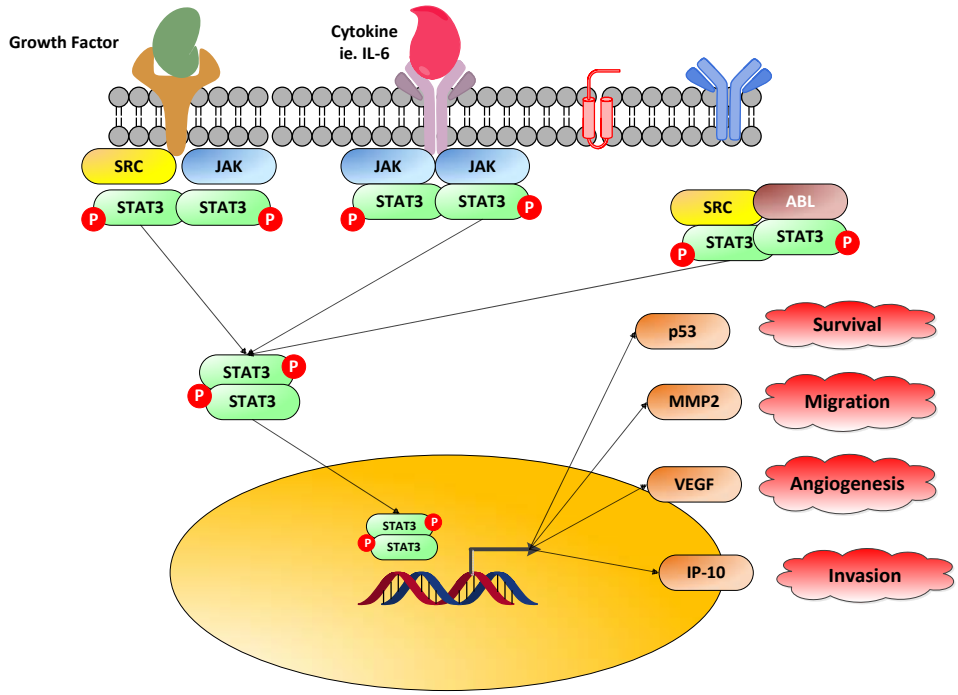
However, most natural p53 mutations occur at a late stage in tumor development, and many clinically detectable cancers have reduced p53 expression but no p53 mutations. It remains to be fully determined what mechanisms disable p53 during malignant initiation and in cancers without mutations that directly affect p53. We show here that oncogenic signaling pathways inhibit the p53 gene transcription rate through a mechanism involving Stat3, which binds to the p53 promoter in vitro and in vivo.

Site-specific mutation of a Stat3 DNA-binding site in the p53 promoter partially abrogates Stat3-induced inhibition. Stat3 activity also influences p53 response genes and affects UV-induced cell growth arrest in normal cells. Furthermore, blocking Stat3 in cancer cells up-regulates expression of p53, leading to p53-mediated tumor cell apoptosis. As a point of convergence for many oncogenic signaling pathways, Stat3 is constitutively activated at high frequency in a wide diversity of cancers and is a promising molecular target for cancer therapy.

Thus, repression of p53 expression by Stat3 is likely to have an important role in development of tumors, and targeting Stat3 represents a novel therapeutic approach for p53 reactivation in many cancers lacking p53 mutations.

Namely in many cancers the excess expression of STAT3 leads to an inactivation of p53 and thus an oncogenic state. The figure below is a depiction of this process.

[4] http://www.ncbi.nlm.nih.gov/gene/6774



However, Pencik, J., have recently noted the following as regards to PCa.

Prostate cancer (PCa) is the most prevalent cancer in men. Hyperactive STAT3 is thought to be oncogenic in PCa. However, targeting of the IL-6/STAT3 axis in PCa patients has failed to provide therapeutic benefit. Here we show that genetic inactivation of Stat3 or IL-6 signalling in a Pten-deficient PCa mouse model accelerates cancer progression leading to metastasis. Mechanistically, we identify p19ARF as a direct Stat3 target.

Loss of Stat3 signalling disrupts the ARF–Mdm2–p53 tumour suppressor axis bypassing senescence. Strikingly, we also identify STAT3 and CDKN2A mutations in primary human PCa. STAT3 and CDKN2A deletions co-occurred with high frequency in PCa metastases. In accordance, loss of STAT3 and p14ARF expression in patient tumours correlates with increased risk of disease recurrence and metastatic PCa. Thus, STAT3 and ARF may be prognostic markers to stratify high from low risk PCa patients. Our findings challenge the current discussion on therapeutic benefit or risk of IL-6/STAT3 inhibition.

But Pencik et al further note:

PTEN is one of the most frequently deleted or mutated tumour suppressors in PCa, with an estimated incidence of 70% in metastatic PCa, causing aberrant activation of the PI3K– AKT–mTOR signalling pathway

We have examined this extensively in our analyses of PCa.

Loss of Pten leads to senescence, which is critically regulated by the ARF–p53 pathway.

PTEN is a major controller of PI3K and its pathway. Loss of PTEN is common in most PCa. On the other hand we have the ARF-MDM2-p53 dynamic which we shall discuss later.

While the tumour suppressor ARF ($p14^{ARF}$ in humans; $p19^{ARF}$ in mice) is readily degraded in normal cells, it is stabilized to increase p53 function on loss of Pten. ARF was shown to augment p53 stability by promoting the degradation of Mdm2, a negative regulator of p53.

Concomitant inactivation of Pten and p53 leads to bypass of senescence and as a consequence to a malignant PCa phenotype.

Loss of PTEN and of p53 is potentially a universally catastrophic event. It is a loss of two of the most significant stabilization elements in any cell, especially the prostate.

Previous studies report PTEN–STAT3 signalling crosstalk in malignant glioblastoma, but the detailed molecular mechanisms in cancer progression and metastasis remain unresolved.

In this study, we show that loss of IL-6/Stat3 signalling in a Pten-deficient PCa model accelerates cancer progression leading to metastasis. Loss of IL-6/Stat3 signalling in PCa bypasses senescence via disrupting the ARF–Mdm2–p53 tumour suppressor axis.

We identify ARF as a novel direct Stat3 target. Notably, loss of STAT3 and p14ARF expression correlates with increased risk of recurrence in PCa patients. In addition, STAT3 and p14ARF expression was lost in metastasis compared with the primary tumours.

This is the nexus between the STAT3 pathway and the ARF-MDM2-p53 pathways. Namely the authors seem to argue that STAT3 targets ARF and it is through this "targeting" that the latter pathway becomes defective.

We identified STAT3 and CDKN2A mutations in primary PCa patients. Furthermore, PCa metastases show a high frequency of STAT3 and CDKN2A deletions.

We propose STAT3 and ARF as prognostic markers for high versus low risk PCa patient stratification.

Pencik et al also note the following inference:

Stat3 regulates the ARF–Mdm2–p53pathway. Since loss of Pten triggers senescence thereby restricting cancer progression and metastasis11, we next tested whether Stat3 exerts a tumour suppressive function by activating senescence-inducing programmes in Ptenpc-/-PCa cells at an early stage of PCa development.

Senescence is generally characterized by upregulation of p53, cyclin-dependent kinase inhibitor 1 (Cdkn1, p21), promyelocytic leukaemia protein (PML) and elevated senescence-associated-b-galactosidase activity. Of note, Ptenpc-/-Stat3-/- tumours lacked p21 expression, displayed reduced numbers of PML nuclear bodies and decreased SA-b-Gal activity compared with

Ptenpc-/- tumours, suggesting Stat3 as a novel mediator of senescence in response to loss of Pten.

Again the statement is "suggesting" and there is no definitive well defined mechanism.

Senescence associated with loss of Pten was shown to be bypassed by deletion of p53 leading to early lethality11. We show here that loss of Stat3 and Pten revealed a phenotype strikingly similar to that of p53 and Pten loss11. Intriguingly, Stat3 and Pten deletion resulted in downregulation of p53 expression in the prostate epithelium, which was accompanied by the loss of p19ARF

The authors make the following statement:

The p53 expression in the tumour stromal cells remained unchanged. Since $p19^{ARF}$ is a critical regulator of Mdm2 degradation, our results suggest that the tumour suppressive capacity of Stat3 in senescent tumour cells may rely on the p19ARF–Mdm2–p53 tumour suppressor axis.

The conclusion is still a bit tentative. Just what the mechanism is may not be well understood.

Now Yu et al state:

The Janus kinases (JAKs) and signal transducer and activator of transcription (STAT) proteins, particularly STAT3, are among the most promising new targets for cancer therapy. In addition to interleukin-6 (IL-6) and its family members, multiple pathways, including G-protein-coupled receptors (GPCRs), Toll-like receptors (TLRs) and microRNAs were recently identified to regulate JAK–STAT signalling in cancer.

Well known for its role in tumour cell proliferation, survival, invasion and immunosuppression, JAK–STAT3 signalling also promotes cancer through inflammation, obesity, stem cells and the pre-metastatic niche. In addition to its established role as a transcription factor in cancer, STAT3 regulates mitochondrion functions, as well as gene expression through epigenetic mechanisms. Newly identified regulators and functions of JAK–STAT3 in tumours are important targets for potential therapeutic strategies in the treatment of cancer.

Huang, et al state that STAT3 is a preferred target for cancer therapy. Specifically:

Numerous cytokines, growth factors, and oncogenic proteins activate signal transducer and activator of transcription 3 (Stat3), which has been recognized as one of the common pathways in cancer cells. Stat3 signaling affects the expression and function of a variety of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and immune evasion. ***Evidently, the Stat3 signaling pathway regulates cancer metastasis and constitutes a potential preventive and therapeutic target for cancer metastasis****. .*

Furthermore Huang et al outline the reasons for this:

Contribution of Stat3 signaling pathway to cancer metastasis.

Stat3 in the cytoplasm of unstimulated cells becomes activated by recruitment to phosphotyrosine motifs within complexes of growth factor receptors (e.g., epidermal growth factor receptor), cytokine receptors (e.g., IL-6 receptor), or non-receptor tyrosine kinases (e.g., Src and BCR-ABL) through their SH2 domain. Stat3 is then phosphorylated on a tyrosine residue by activated tyrosine kinases in receptor complexes.

Phosphorylated Stat3 forms homodimers and heterodimers and translocates to the nucleus. In the nucleus, Stat3 dimers bind to specific promoter elements of target genes and regulate gene expression. The Stat3 signaling pathway regulates cancer metastasis by regulating the expression of genes that are critical to cell survival, cell proliferation, invasion, angiogenesis, and tumor immune evasion.

It would be useful if somehow these conflicting views could be brought into alignment. In addition we have the work Marcias, E., et al, who state:

Pathways associated with Stat3 activation. Stat3 is activated downstream of receptor tyrosine kinases (e.g., EGFR), cytokine receptors via associated Janus family kinases (JAKs) (e.g., IL-6 receptor), and nonreceptor-associated tyrosine kinases (e.g., c-src). Tumor promoters such as TPA and UVB activate Stat3 in keratinocytes primarily via the EGFR.

Activation of PKCs by tumor promoters leads to the processing of membrane-bound preforms of EGFR ligands such as heparin-binding EGF (HB-EGF) by matrix metalloproteinases (MMPs). In addition, PKCs associate with and phosphorylate Stat3 at Ser727, which is necessary for maximal Stat3 transcriptional activity. Furthermore, transcriptional induction of cytokines and EGF ligands can lead to autocrine stimulation and sustained Stat3 phosphorylation.

After phosphorylation, STAT3 dimerizes and translocates to the nucleus, where Stat3 dimers directly regulate gene expression of transcriptional targets including Bcl-xL, cyclin D1, c-myc, Twist and Survivin. STAT3-mediated regulation of target gene expression is involved in various cellular functions including cell differentiation, proliferation, survival, and oncogenesis. Stat3 can also act through noncanonical signaling pathways. In this regard, unphosphorylated Stat3 (U-Stat3) can drive gene expression of a subset of genes that are different from p-Stat3 dimers in an NF-κB-dependent and independent manner.

In addition, p-Stat3 Ser727 can translocate into the mitochondria and influence mitochondrial respiratory chain activity. These noncanonical Stat3 signaling pathways have protumorigenic roles in certain cell/tissue types; however their role in epithelial carcinogenesis has not been evaluated.

Thus the nature of STAT3 and its importance must be better investigated.

5 OBSERVATIONS

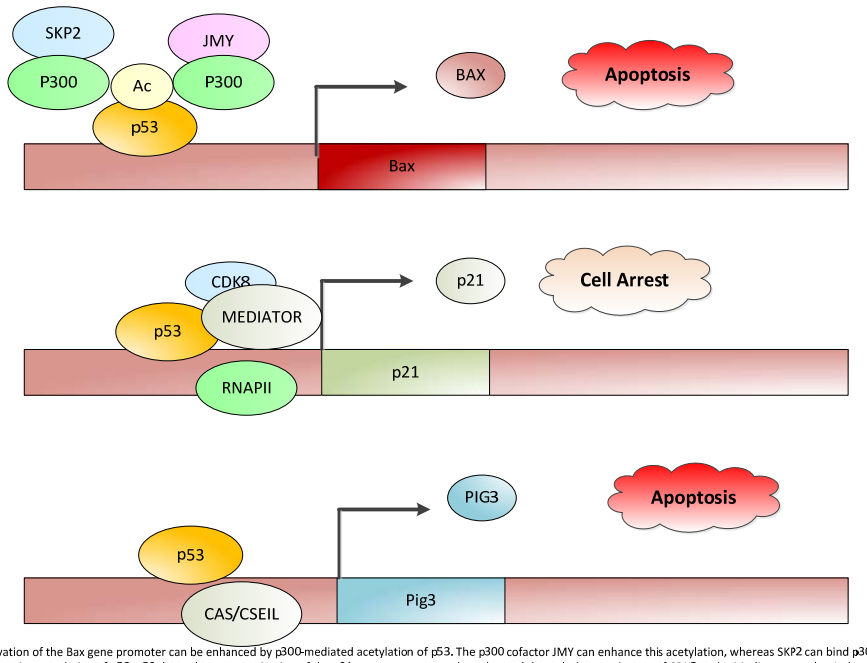
This paper by Pencik et al presents an interesting challenge to the ability to identify genetic markers for various cancers. What may at one time seem to be a problem may later be understood in a more complete fashion to be a necessary control element. To some degree we have observed this with BRAF inhibitors in melanoma, which lead to SCC and thus require a MEK inhibitor. In some sense unless a full dynamic understanding of pathways is established one may continue to see this "whack a mole" approach to therapeutics.

5.1 SUMMARY CONCLUSIONS OF KEY PAPER

To reiterate the Pencik et al observations:

1. *Co-deletion of Stat3 and Pten triggers PCa*: We know that PTEN loss is found in PCa and we also know that active Stat3 is a significant factor in many malignancies. Yet the loss of both may appear as being of significance.
2. *Stat3 regulates the ARF–Mdm2–p53pathway*: This is the key observation which they articulate and stress and the main divergence from standard thought.
3. *Loss of IL-6 and Pten leads to cancer and metastasis*: We know that IL-6 drives Stat3 and that loss of IL-6 would most likely lead to a loss of Stat3 expression. As noted above loss of both Pten and Stat3 would lead to a malignant state.
4. *Loss of STAT3 and ARF in PCa is associated with metastases*: ARF is key to the ARF-MDM2 –p53 pathway. MDM2 inhibits p53. Thus the association of Stat3 being the "driver" of the ARF process is essential.

We reiterate the p53 processes as shown below. The three lead to either apoptosis or cell arrest as one would expect. In all cases p53 plays a key role but it is also clear that other proteins are required in some cases.



Activation of the Bax gene promoter can be enhanced by p300-mediated acetylation of p53. The p300 cofactor JMY can enhance this acetylation, whereas SKP2 can bind p300 and antagonize acetylation of p53. p53-dependent transactivation of the p21 gene promoter can be enhanced through the recruitment of CDK8 to the Mediator complex. Independent binding of the long-range chromatin modifier CAS/CSE1L, which is the human orthologue of yeast Cse1, to promoters, such as the Pig3 gene promoter, can also enhance p53-dependent transcription.

Pencik et al finally note:

Interestingly, loss of PTEN expression in primary human PCa did not correlate with overall survival and could not predict PCa-specific death. Moreover, heterozygous PTEN deletions far outnumber homozygous deletions in primary human PCa and we show here that PTEN is mutated or lost only in a small subset (4.7%) of a large cohort of patients with primary PCa.

However, PTEN is lost in >50% of human PCa metastases suggesting an important role for PTEN in this process. Finally, we show in our study that STAT3 is co-deleted with PTEN in 66% of human PCa metastases in two independent data sets.

Since PTEN is mutated or lost in only a minor fraction of primary PCa, other aberrations must occur (oncogene induction or loss of tumour suppressor function) to activate STAT3 and ARF to induce senescence in human cancers. Indeed, several studies indicate that different aberrations can lead to induction of senescence in human cancers

From Soissi and Wiman:

The standard classification used to define the various cancer genes confines tumor protein p53 (TP53) to the role of a tumor suppressor gene. However, it is now an indisputable fact that many p53 mutants act as oncogenic proteins.

This statement is based on multiple arguments including the mutation signature of the TP53 gene in human cancer, the various gains-of-function (GOFs) of the different p53 mutants and the heterogeneous phenotypes developed by knock-in mouse strains modeling several human TP53 mutations.

In this review, we will shatter the classical and traditional image of tumor protein p53 (TP53) as a tumor suppressor gene by emphasizing its multiple oncogenic properties that make it a potential therapeutic target that should not be underestimated.

Analysis of the data generated by the various cancer genome projects highlights the high frequency of TP53 mutations and reveals that several p53 hotspot mutants are the most common oncoprotein variants expressed in several types of tumors.

The use of Muller's classical definition of mutations based on quantitative and qualitative consequences on the protein product, such as 'amorph', 'hypomorph', 'hypermorph' 'neomorph' or 'antimorph', allows a more meaningful assessment of the consequences of cancer gene modifications, their potential clinical significance, and clearly demonstrates that the TP53 gene is an atypical cancer gene.

5.2 GENETIC CLASSIFICATION

There is an interesting paper from CSHL on progress on cancer classification. Linnaeus some 300 years ago came up with a classification system for various species. Aristotle was driven by his desire to classify, and ever since we have people trying their best to do that task. Patients always want to know what they have, and that is a form of classification.

We classify cancers based upon organs. We may modify it based on cell types or based on cell markers such as immunological markers. I remember back in the 60s that Leukemias were simple; acute or chronic, you died now or later. Now we have a plethora of subtypes and a multiplicity of therapeutics.

But we also know genomic data. Perhaps then we should classify cancers based upon genes, not upon organs, binding proteins, or the like,

As the authors state:

Classification is an everyday instinct as well as a full-fledged scientific discipline. Throughout the history of medicine, disease classification is central to how we organize knowledge, obtain diagnosis, and assign treatment. Here we discuss the classification of cancer, the process of categorizing cancers based on their observed clinical and biological features. Traditionally, cancer nomenclature is primarily based on organ location, e.g., "lung cancer" designates a tumor originating in lung structures. Within each organ-specific major type, further subgroups can be defined based on patient age, cell type, histological grades, and sometimes molecular markers, e.g., hormonal receptor status in breast cancer, or microsatellite instability in colorectal cancer. In the past 15+ years, high-throughput technologies have generated rich new data for somatic variations in DNA, RNA, protein, or epigenomic features for many cancers. These data, representing increasingly large tumor collections, have provided not only new insights into the biological diversity of human cancers, but also exciting opportunities for discovery of new cancer subtypes.

They continue:

An ever finer classification system has many potential benefits. It is needed to capture the full spectrum of biological diversity—the "endless forms" that Darwin spoke of. It could lead to a better recognition of patient-specific disease mechanisms, and importantly, could suggest treatment options that are more accurately matched to the patient's tumor. Precision medicine, at its very foundation, relies on valid and continuously optimized disease classification that reflects the underlying mechanisms. However, a fine-grained classification system also has many potential drawbacks. The newly proposed splits may not be technically robust. Even when the finer categories are robustly supported by statistical significance and by replication, they may still lack a clear biological meaning, or have little impact on treatment options (#3 below) if it turns out that some subtypes share the same clinical endpoint, or if treatment options are limited.

Indeed, we may find it much more powerful to have a new Linnaeus type look at classification. Classifying genomically, via genes, RNA, and epigenetic factors, may help stratify and focus on therapeutics. This article raises an interesting dialog.

5.3 SUMMARY OBSERVATIONS:

Overall we can make some summary observations:

1. Perhaps one should be cautious as regards to murine and human models. All too often what we see in mouse models does not pan out in human. The reasons may very well be the complexity of the signally paths.

2. Signalling paths are complex and dynamic. What may work at one instant may not at another? The question then is: how critical are realistic repeatable and predictive models in assisting in both prognostic evaluation and therapeutic approaches?

3. Cells are not the same everywhere. Thus when we perform a prostate biopsy we may get one profile but when that cell metastasizes to other organs we get dramatically different cells. As we have discussed before the paper by Gundem et al presets a compelling picture of the complexity of gene expression in PCa. Namely each cell cluster may have complex and disparate genes expressed. If that is the case then we would also be concerned that we look at similar expression when performing biopsies.

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