ATF2 and Melanoma

Terrence P McGarty
White Paper No 97
July, 2013

There has been recent work on the transcription factor ATF2 and melanoma. It is an interesting expressed protein in that when in the nucleus it forces proliferation and when in the cytoplasm it induces apoptosis. We provides a short summary of the literature since this may become a putative therapeutic target.

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. This document represents a work in progress and may be changed without notice. There is no intent to provide any medical advice and the document reflects opinions which may be subject to correction and change at any time.

Contents

1 Introduction 3
2 ATF2 5
2.1 Nuclear Functions 8
2.2 Cytoplasmic Functions 9
2.3 Transcriptional Functions 9
3 ATF2 and Melanoma 11
4 Observations 13
5 References 14

ATF2 and Melanoma

1 INTRODUCTION

There have been a multiplicity of genes linked to melanoma and current therapeutics attempts to start inhibiting those overactive genes and their expressions. We consider here a transcription factor recently considered; in fact it has been linked for quite a while. Its actions are typical of transcription factors but this one is sensitive to stressed conditions, especially UV radiation. It thus provides some interesting insight to melanomas and UV activation.

As reported in 1999 by Ivanoav and Ronai:

To identify mechanisms whereby activating transcription factor 2 (ATF2) alters the radiation resistance of human melanoma cells, we examined the possible role of ATF2 in UVC-induced apoptosis. Forced expression of full-length or truncated (D1–195 amino acids) forms of ATF2 in LU1205, a late-stage human melanoma cell line, elevated the levels of UVC-induced apoptosis….

Programmed cell death (apoptosis) is a common cellular response to stress caused by environmental challenges. Altered expression of apoptosis-related proteins, which coincides with decreased or absent apoptosis, is commonly observed in various tumor types and is of fundamental importance in tumor resistance to host defenses as well as to clinical therapy. Indeed, reduced ability of tumors to undergo apoptosis is often associated with elevated drug resistance and poor clinical outcomes.

Malignant melanoma is a primary example of a cancer that responds poorly to various treatments, including chemotherapy and g-irradiation. Despite the alarming increase in the incidence of this tumor in the past decade, the molecular mechanisms of its progression as well as the regulation of apoptosis in human melanoma remain largely unknown.

This 1999 article clearly demonstrates the initial understanding and interest and yet the inability to address genetic control of the melanoma cells.

Why is ATF2 of interest? As stated in Hearing and Leong, ATF2 is highly expressed in the nucleus in metastatic melanoma sites[1]. The question is why? ATF2 is also known to move from the nucleus to the cytoplasm in cells which become damaged. The continual location and over expression in the nucleus fails to activate normal apoptosis. The question is; is ATF2 a cause or a result that leads to metastatic loss of control? Clearly ATF2 plays some role but the question is what role and can that be used as a therapeutic target.

[1] Hearing and Leong, From Melanocytes to Melanoma, Humana, 2006, pp 132-134.

As Lau et al state:

A number of transcription factors, including Myc, Notch, and b-catenin, exhibit both oncogenic and tumor suppressor activities, yet the mechanism(s) controlling their opposing functions are largely associated with a given tissue/cell type. Here, we demonstrate that the tumor suppressor or oncogenic activities of ATF2 result from its cytosolic or nuclear function, respectively. Cytosolic localization of ATF2 has been seen in nonmalignant skin tumors where ATF2 exhibits tumor suppressor activity.

The Lau et al paper does discuss this dichotomy of function of ATF2. In the cytoplasm ATF2 allows for apoptosis. Left alone in the nucleus the ATF2 transcription protein appears to enhance metastatic growth. The specific recognition that these transcription factors are tissue type dependent for certain actions is of key importance. ATF2 as we shall indicate has particular interest for melanocytes and UV damage. It may also have specific interest for X-ray damage as we have argued for backscatter X-ray systems.

2 ATF2

Let us now briefly review the ATF2 protein and its functions. It is a transcription factor protein and thus is often characterized by what it does in the nuclear space and not in the cytoplasm. However it also functions in key apoptotic states especially when the cell, in this case a melanocyte, is under stress.

To begin we take the definition of ATF2from NCBI[2]:

ATF2, activating transcription factor (2q32), encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds to the cAMP-responsive element (CRE), an octameric palindrome. It forms a homodimer or a heterodimer with c-Jun and stimulates CRE-dependent transcription.

This protein is also a histone acetyltransferase (HAT) that specifically acetylates histones H2B and H4 in vitro; thus it may represent a class of sequence-specific factors that activate transcription by direct effects on chromatin components. Several alternatively spliced transcript variants have been found for this gene

As Lau et al state regarding ATF2:

Activating transcription factor 2 (ATF2) is one of 16 Atf/Creb family transcription factors and an integral component of the activator protein-1 (AP-1) transcriptional complex, which regulates normal cellular growth and development, as well as cellular response to stress. The diverse transcriptional functions of ATF2 are attributed to its homo- or heterodimerization with other AP-1 transcription factors via a basic leucine zipper (bZIP) domain, in concert with its phosphorylation by stress kinases, JNK or p38, on residues 69/71.

As a stress-inducible transcription factor, ATF2 regulates gene expression programs implicated in cellcycle control, cytokine expression, and cell death. In addition to its transcriptional role, ATF2 functions in the DNA damage response, which requires ATM-dependent phosphorylation on residues 490/498. Mice harboring mutations at these sites are more radiosensitive and genetically unstable when crossed with p53 mutant mice or when subjected to a skin carcinogenesis protocol.

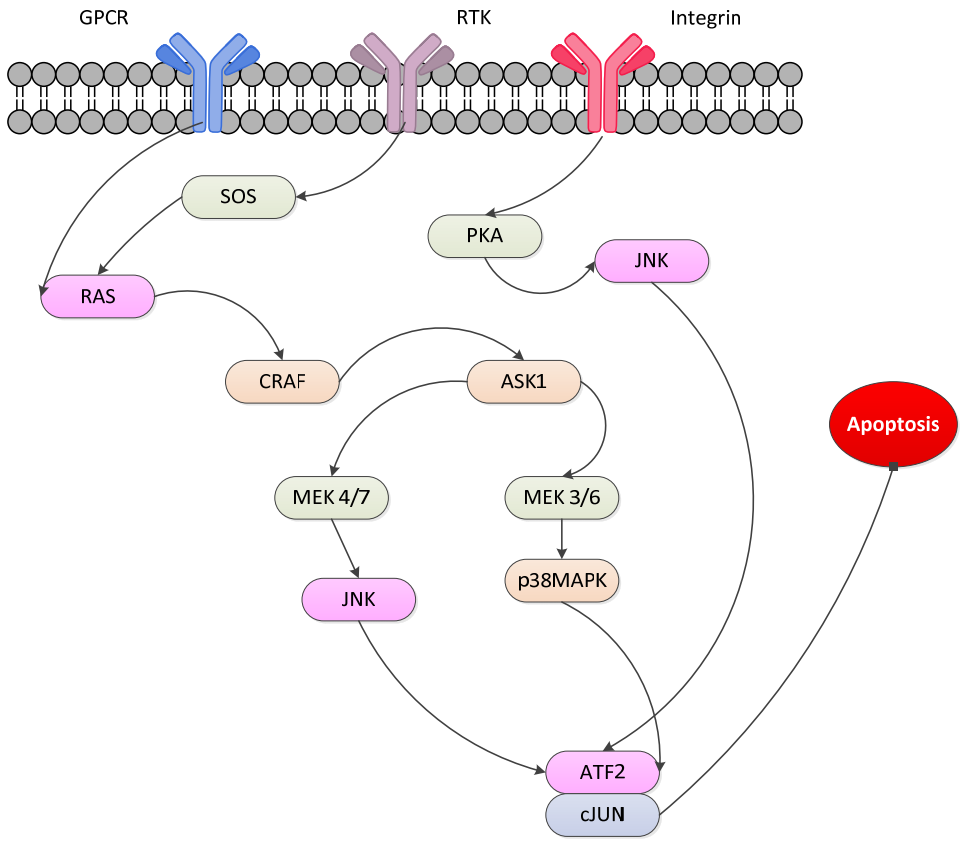
As yet as Shah et al state regarding the malignant character of ATF2:

The transcription factor ATF2 has been shown to attenuate melanoma susceptibility to apoptosis and to promote its ability to form tumors in xenograft models.

Thus there is and should be a significant interest in ATF2 as regards to melanoma.

[2] http://www.ncbi.nlm.nih.gov/gene/1386

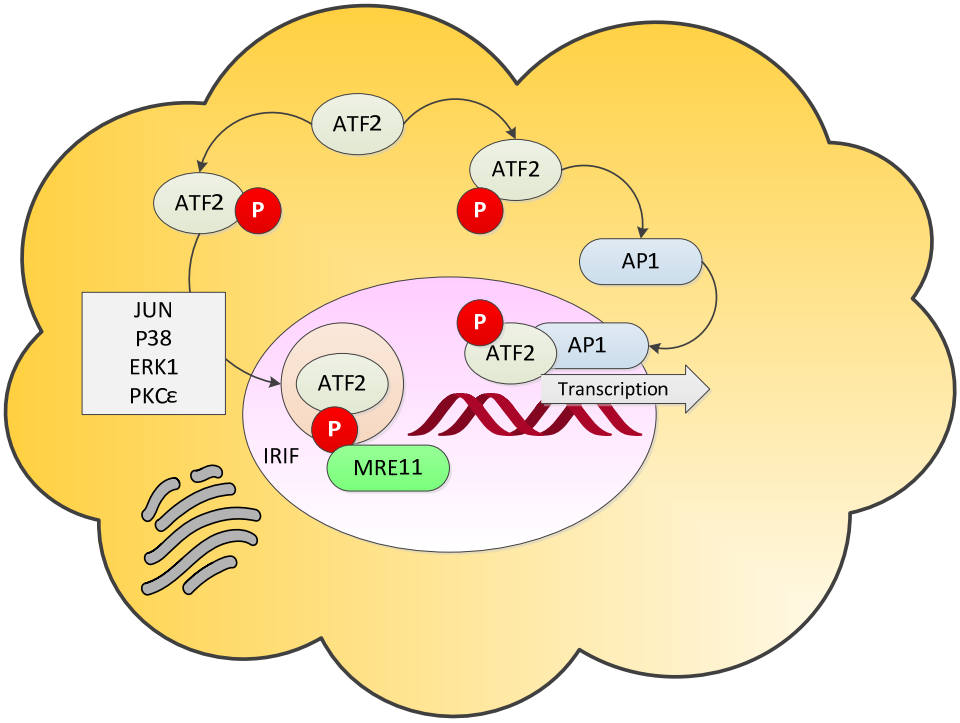
From MMMP[3] we have the following description of the pathway interactions driving ATF2 into the nucleus which we show below (as modified):



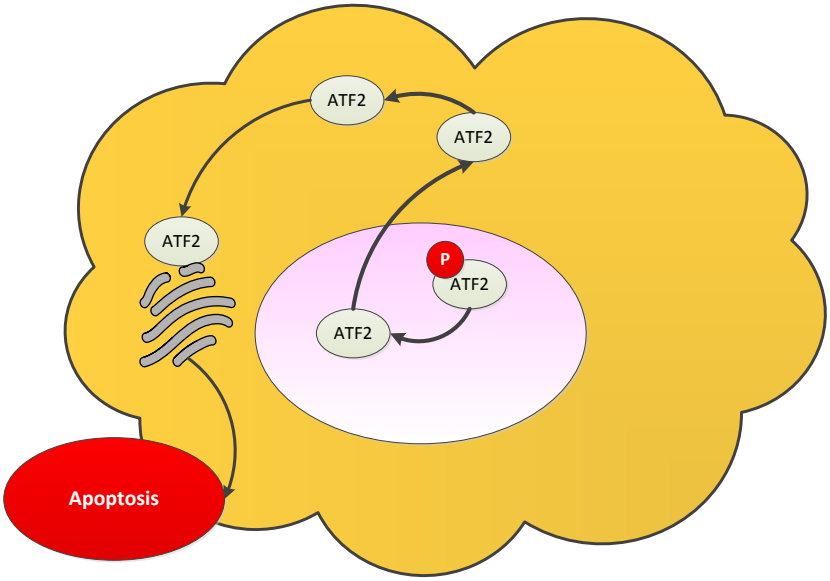
Note that it is activated by several receptors and the functionality above relates to cytoplasm functionality leading to apoptosis. Note the RAS to C-RAF pathway and also not the MEK and p38 (MAPK) connection. Unlike the BRAF connection we see CRAF. Yet we do have MEK involvement as well.

From Lau and Ronai we have the following set of cytoplasm and nuclear interactions. The first description below is the nuclear reaction without stress. Note the flow from the cytoplasm of ATF2 into the nucleus and then the activation of transcription via an ATF2 attachment.

[3] http://www.mmmp.org/MMMP/public/biomap/viewBioMapImage.mmmp



However, when under sustained stress, the ATF2 leaves the nucleus and via the mitochondria results in apoptosis. This "normal" reaction is a control mechanism that stops transcription, recognizing aberrant cells, and then results in natural cell death.



Sustained nuclear localization of ATF2 functions together with lack of its export into the cytoplasm. In response to severe and/or sustained stress (shown on the right), including genotoxic stress chemicals, UV irradiation and ionizing radiation that culminate in cell death, a portion of nuclear ATF2 is exported from the nucleus and accumulates in the cytoplasm. There, ATF2 interacts with and perturbs HK1- and VDAC1-containing complexes at the mitochondrial outer membrane, thereby impairing mitochondrial membrane potential, inducing mitochondrial leakage (e.g. cytochrome c leakage, which is shown on the right as pink circles exiting from mitochondria through VDAC1), and promoting cell death. From Lau and Romai

The above is a critical characteristic of ATF2. One may wonder how to reactivate its transition from the nucleus to the cytoplasm and in turn create an apoptotic state. That perhaps is a therapeutic approach.

Some insight into therapeutic targeting may be obtained from Lau and Ronai:

An increasing number of transcription factors have been shown to elicit oncogenic and tumor suppressor activities, depending on the tissue and cell context. Activating transcription factor 2 (ATF2; also known as cAMP-dependent transcription factor ATF-2) has oncogenic activities in melanoma and tumor suppressor activities in non-malignant skin tumors and breast cancer.

Recent work has shown that the opposing functions of ATF2 are associated with its subcellular localization. In the nucleus, ATF2 contributes to global transcription and the DNA damage response, in addition to specific transcriptional activities that are related to cell development, proliferation and death. ATF2 can also translocate to the cytosol, primarily following exposure to severe genotoxic stress, where it impairs mitochondrial membrane potential and promotes mitochondrial-based cell death.

Notably, phosphorylation of ATF2 by the epsilon isoform of protein kinase C (PKCε) is the master switch that controls its subcellular localization and function. Here, we summarize our current understanding of the regulation and function of ATF2 in both subcellular compartments. This mechanism of control of a non-genetically modified transcription factor represents a novel paradigm for 'oncogene addiction'.

We will return to this discussion later after we have the opportunity to address the issue of specific operations. The authors continue to discuss the specifics of nuclear and cytoplasmic functions.

2.1 NUCLEAR FUNCTIONS

Let us first consider the nucleus functions of ATP2. This generally is how ATF2 operates in a benign and unstressed environment.

From Lau and Ronai:

1. *Binding of ATF2–AP-1 dimers to DNA alters the local structure of DNA and facilitates the recruitment and directional orientation of other regulatory transcriptional complexes, which either enhance (i.e. enhanceosomes) or repress (i.e. repressosomes) transcription.*

2. *ATF2 can also affect transcription of target genes in trans through its interaction with other transcription factors. In hypoxia, for example, ATF2 binds and stabilizes hypoxia-inducible factor 1a (HIF1a), thereby promoting its transcriptional activity*

3. *The transcriptional function of ATF2 is also modulated by its interaction with transcriptional coactivators or corepressors. For example, p300/CBP and C/EBPa bind to the bZIP domain of ATF2, disrupting its intrinsic autoinhibition and augmenting its transcriptional activity*

4. *ATF2 also affects more global transcriptional programs through its association with histone modifying enzymes.*

The above is a simple and yet detailed description of functioning in the nucleus. We have also demonstrated this with the Figure previously.

2.2 CYTOPLASMIC FUNCTIONS

Localization of ATF2 to the cytoplasm has been observed under conditions of cellular stress and in disease states.

As noted in Lau and Ronai:

ATF2 recruitment to the mitochondria is also associated with reduced membrane potential, activation of the pro-apoptotic Bcl-2 family protein BAX, leakage of cytochrome c and sensitization of cells to genotoxic-stress-induced cell death. Collectively, our recent studies reveal a new function for cytoplasmic ATF2 in promoting mitochondrial-based cell death following exposure to genotoxic stress. The nuclear localization and mitochondrial function of ATF2 are dependent on PKCe.

The nuclear export of ATF2, which enables its localization and function at the mitochondria, has been observed following genotoxic stimuli in both non-malignant (keratinocytes, melanocytes and fibroblasts) and malignant (BCC and early-phase melanoma) cells. Significantly, an exclusion of ATF2 from the nucleus is not observed in the more aggressive melanoma cells, which prevents ATF2 from functioning at the mitochondria in these cells

Thus the free flow of ATF2 to the cytoplasm and its interaction with mitochondria will result in apoptosis.

There clearly is a back and forth here. One the one hand we would seek to reduce ATF2 in the nucleus while on the other hand introducing it into the cytoplasm. Perhaps is therapeutic approach is exogenous ATF2 introduction directly into the cytoplasm.

2.3 TRANSCRIPTIONAL FUNCTIONS

There is also research with more details regarding the transcriptional functions. We find this of use to also understand the nucleus functionality. As Kohler et al state:

Transcriptional activation often requires the rapid assembly of complexes between dimeric transcription factors and specific DNA sites. Here we show that members of the basic region leucine zipper and basic region helix–loop–helix zipper transcription factor families follow an assembly pathway in which two protein monomers bind DNA sequentially and form their dimerization interface while bound to DNA.

Nonspecific protein or DNA competitors have little effect on the rate of assembly along this pathway, but slow a competing pathway in which preformed dimers bind DNA. The sequential monomer-binding pathway allows the protein to search for and locate a specific DNA site more quickly, resulting in greater specificity prior to equilibrium.

We now move to melanoma specific issues.

3 ATF2 AND MELANOMA

ATF2 has become of particular interest in melanoma. It is just one further gene and gene product that may be a target for later therapeutic control.

From Lau and Ronai:

Analyses of tumor microarrays have revealed the principal differences between melanoma and non-malignant skin cancers. Whereas the nuclear enrichment of ATF2 correlates with poor prognosis in melanoma, cytoplasmic ATF2 is associated with a more favorable clinical outcome. Notably, cytosolic localization of ATF2 is also seen in non-malignant skin tumors, e.g. squamous and basal cell carcinomas (SCCs and BCCs, respectively).

Hence, the nuclear accumulation of ATF2 appears to be associated with its oncogenic activities, because this localization is observed in melanoma, whereas the cytosolic localization, as are observed in non-malignant skin tumors, is associated with its tumor suppressor activities. Consistent with this, the cytoplasmic accumulation of ATF2 that is observed in prostate cancer cells following ionizing irradiation has been associated with a transient latency of tumor proliferation and a more 'differentiated' state

The observation of nuclear accumulation allows for putatively a prognostic tool. There has been no clinical work reported on such at this time however. As Lau et al state:

The transcription factor ATF2 elicits oncogenic activities in melanoma and tumor suppressor activities in nonmalignant skin cancer. Here, we identify that ATF2 tumor suppressor function is determined by its ability to localize at the mitochondria, where it alters membrane permeability following genotoxic stress. The ability of ATF2 to reach the mitochondria is determined by PKCε, which directs ATF2 nuclear localization.

Genotoxic stress attenuates PKCε effect on ATF2; enables ATF2 nuclear export and localization at the mitochondria, where it perturbs the HK1-VDAC1 complex; increases mitochondrial permeability; and promotes apoptosis.

Significantly, high levels of PKCε, as seen in melanoma cells, block ATF2 nuclear export and function at the mitochondria, thereby attenuating apoptosis following exposure to genotoxic stress. In melanoma tumor samples, high PKCε levels associate with poor prognosis. Overall, our findings provide the framework for understanding how subcellular localization enables ATF2 oncogenic or tumor suppressor functions.

As noted above, there is on the other hand a tumor suppressor function in ATF2 as well. Namely if one could arguably get it into the cytoplasm one could then have it activate apoptosis. Again there is not reported clinical evidence at this time.

From Shah et al we have:

Activating transcription factor 2 (ATF2), a member of the bZIP family, is activated by stress kinases including JNK and p38 and is implicated in transcriptional regulation of immediate early genes regulating stress and DNA damage responses and expression of cell cycle control proteins . To activate transcription, ATF2 heterodimerizes with bZIP proteins, including C-JUN and CREB, both of which are constitutively upregulated in melanomas. ATF2 is also implicated in the DNA damage response through phosphorylation by ATM/ATR. Knock-in mice expressing a form of ATF2 that cannot be phosphorylated by ATM are more susceptible to tumor development.

Nuclear localization of ATF2 in melanoma tumor cells is associated with poor prognosis, likely due to transcriptional activity of constitutively active ATF2. Indeed, expression of transcriptionally inactive ATF2 or peptides that attenuate endogenous ATF2 activity inhibits melanoma development and progression in xenograft models. These studies suggest that ATF2 is required for melanoma development and progression.

Understanding mechanisms underlying early stages in melanoma development is of major interest and importance. Recent studies indicate a role for MITF, a master regulator of melanocyte development and biogenesis, in melanoma progression. Here we demonstrate that the transcription factor ATF2 negatively regulates MITF transcription in melanocytes and in about 50% of melanoma cell lines. Increased MITF expression, seen upon inhibition of ATF2, effectively attenuated the ability of BRAFV600E- expressing melanocytes to exhibit a transformed phenotype, an effect partially rescued when MITF expression was also blocked.

Significantly, the development of melanoma in mice carrying genetic changes seen in human tumors was inhibited upon inactivation of ATF2 in melanocytes. Melanocytes from mice lacking active ATF2 expressed increased levels of MITF, confirming that ATF2 negatively regulates MITF and implicating this newly discovered regulatory link in melanoma development.

Primary melanoma specimens that exhibit a high nuclear ATF2-to-MITF ratio were found to be associated with metastatic disease and poor prognosis, further substantiating the significance of MITF control by ATF2. In all, these findings provide genetic evidence for the role of ATF2 in melanoma development and indicate an ATF2 function in fine-tuning MITF expression, which is central to understanding MITF control at the early phases of melanocyte transformation.

This is a significant set of observations.

4 OBSERVATIONS

ATF2 is an interesting protein or gene product perhaps for prognostic purposes and also perhaps for therapeutic purposes. Clearly its presence in the nucleus of malignant cells portends an aggressive form of metastatic melanoma. On the other hand its presence in the cytoplasm may result in apoptosis.

As Yemelyanov et al state:

Further, we showed that among numerous TFs whose activity was altered by GR in LNCaP cells, more than 85% were downregulated upon GR activation. Many of those, including AP-1, SRF, Ets-1, Elk-1, STAT1/ISRE, ATF2, C/EBPa, GATA4, EGR1 and PAX6 are recognized MAPK targets

The evidence of the effects of a transcriptional protein such as ATF2 expands our understanding of pathway interactions as well. Summarizing from Shah et al we have:

Malignant melanoma is one of the most highly invasive and metastatic tumors, and its incidence has been increasing at a higher rate than other cancers in recent years. Significant advances in understanding melanoma biology have been made over the past few years, thanks to identification of genetic changes along the MAPK signaling pathway. Those include mutations in BRAF, NRAS, KIT and GNAQ, all of which result in a constitutively active MAPK pathway.

Consequently, corresponding transcription factor targets such as microphthalmia-associated transcription factor (MITF), AP2, and C-JUN and its heterodimeric partner ATF2 are activated and induce changes in cellular growth, motility and resistance to external stress. In addition, constitutively active MAPK/ERK causes rewiring of other signaling pathways.

Among examples of rewired signaling is upregulation of C-JUN expression and activity, which potentiates other pathways, including PDK1, AKT and PKC, and plays a critical role in melanoma development.

Thus we have added another target of interest to the puzzle of metastatic melanoma.

5 REFERENCES

1. Ivanov, V., Z. Ronai, Down-regulation of Tumor Necrosis Factor an Expression by Activating Transcription Factor 2 Increases UVC-induced Apoptosis of Late-stage Melanoma Cells, THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 274, No. 20, Issue of May 14, pp. 14079–14089, 1999.

2. Kohler, J., et al, DNA specificity enhanced by sequential binding of protein monomers, PNAS, October 12, 1999, vol. 96, no. 21, pp. 11735–11739.

3. Lau E., Z., Ronai, ATF2 - at the crossroad of nuclear and cytosolic functions, J Cell Sci. 2012 Jun 15; Vol. 125 (Pt 12):28, pp15-24.

4. Lau, E., et al, PKCε Promotes Oncogenic Functions of ATF2 in the Nucleus while Blocking Its Apoptotic Function at Mitochondria, Cell 148, 543–555, February 3, 2012.

5. Pardo, M. et al, An Expanded Oct4 Interaction Network: Implications for Stem Cell Biology, Development, and Disease, Cell Stem Cell 6, 382–395, April 2, 2010.

6. Shah, M., et al, A Role for ATF2 in Regulating MITF and Melanoma Development, PLoS Genetics, 2 December 2010, Volume 6, Issue 12.

7. Yemelyanov, A., et al, Tumor suppressor activity of glucocorticoid receptor in the prostate, Oncogene (2006), 1–12.