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Recent advances in melanoma research

Jon A. Reed [1,2], and Estela E. Medrano [2,3,4]

[1] Department of Pathology, [2] Department of Dermatology, [3] Huffington Center on Aging, [4] Departments of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

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1. ABSTRACT

At the end of the last decade, sporadic melanomas were still considered a genetic black box. Fortunately, in the last few years the box has been opened bringing to light melanoma-relevant oncogenes, aberrant signal transduction pathways, critical alterations in the melanoma cell cycle that go beyond $p16^{INK4a}$, and melanoma- microenvironment interactions that are essential for tumor progression. This review will discuss some of the latest findings in melanoma research including the critical role of the MAPK pathway in the genesis of melanoma and senescence of nevi, the paradoxical tumor suppressor and oncogenic activities of the transcription factor MITF, and the unexpected oncogenic activities of the low molecular weight forms of cyclin E.

2. INTRODUCTION

The incidence of cutaneous malignant melanoma has been increasing steadily over the last 30 years at an annual rate of approximately 6%. It is believed that 1 in 75 Americans will develop melanoma during their lifetime (1). The American Cancer Society estimates that 59,580 new cases of melanoma will have been diagnosed in 2005 with 7,700 deaths related to the disease (http://www.cancer.org/docroot/MED/content/downloads/MED_1_1x_CFF2005_Estimated_New_Cases_Deaths_by_Sex_US.asp).

While early-stage melanoma is curable by simple surgical excision, late-stage disease has a poor rate of clinical response to currently available therapies. As such,

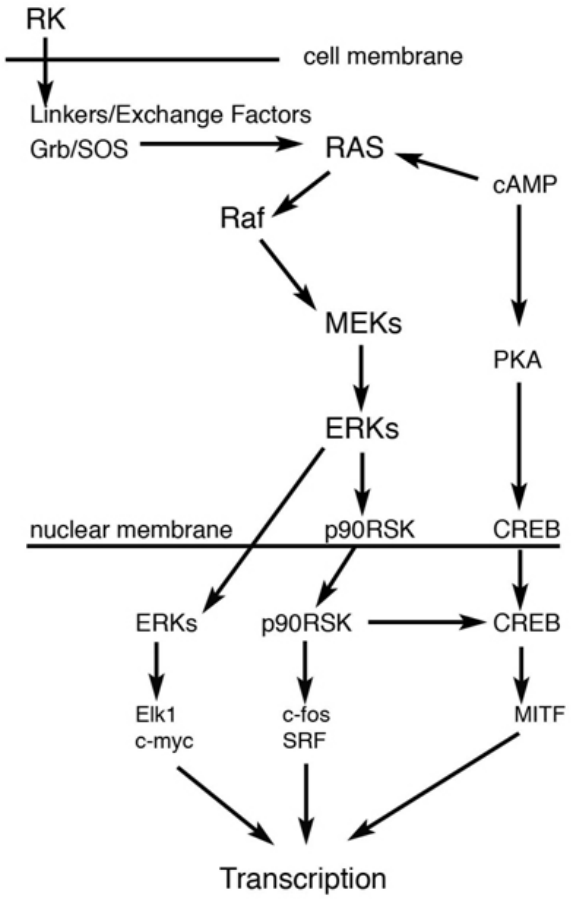

Figure 1. The MAPK/ERK Pathway.

considerable clinical effort has been placed on early diagnosis and treatment of melanoma. Developing new forms of therapy for late stage disease has been confounded by the complexities involved in the process of tumor progression despite several clear advances in our understanding of melanoma biology during the past decade (Reviewed in (2)). Critical to this understanding was the recognition of genetic instability and the resultant cell cycle dysregulation driven by loss of tumor suppressor functions (Reviewed in (3,4)). Among the tumor suppressors that have been implicated in melanomagenesis, $p16^{INK4a}$/CDKN2 has been studied most extensively (5,4). While aberrations of tumor suppressor function are common in melanoma, they do not by themselves, provide a universal target for therapy.

Other studies of melanoma progression have been focused on the level of gene expression. During the past ten years, it has been shown that several genes essential to a melanoma cell's ability to migrate, invade, and to escape apoptosis are regulated by critical transcription factors and/or transcriptional regulators including CREB (6,7), AP-2 (8,9,10), and ATF2 (11). Furthermore, aberrations of the TGF-β growth inhibitory pathway as effected by Smad signaling have identified a critical role for the oncogenic protein SKI in melanoma progression (Reviewed in (12,13)). Each of these lines of investigation has opened avenues for the development of novel therapeutic interventions.

This review will now focus on the most recent developments in our understanding of critical events in the early stages of melanoma formation and in tumor progression. Particular emphasis will be placed on signaling pathways known to be important in normal melanocyte biology. This will involve examination not only of aberrations of cell cycle regulatory proteins, but also key elements of upstream signaling pathways that link the melanocyte to its microenvironment ultimately leading to events significant to differentiation, proliferation, and survival. To this end, we shall discuss the myriad roles of: the mitogen-activated protein kinase (MAPK) / extracellular signal regulated kinase (ERK) pathway including its individual members such as RAS and B-RAF; the transcription factor Microphthalmia (MITF); Cyclin E/CDK2 interactions; and the recently emerging field of epigenetics in melanoma.

3. THE MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) / EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) PATHWAY: UPSTREAM DETERMINANTS OF NORMAL CELL BEHAVIOR ARE ABERRANT IN MALIGNANT MELANOMA

Although genetic instability can lead to dysregulation of the cell cycle through oncogenic stimuli, or by loss of tumor suppressor functions, the pathogenesis and progression of melanoma also must be considered in the context of the cellular microenvironment. Signaling from the cell surface to the nucleus is a major determinant of normal cellular function and homeostasis. In normal cells, external stimuli can influence morphology, motility, proliferation, and even survival, terminal differentiation, senescence, and induction of apoptosis. One of the most important mechanisms for relaying environmental signals to the nucleus involves the mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) pathway (Figure 1). The relevance of this pathway in the biology of human melanocytes is underscored by the loss of ERK2 activity and consequent failure of its nuclear translocation as melanocytes become senescent (14). It is now clear that involvement of the MAPK/ERK pathway is a critical event in the pathobiology of malignant melanoma. As such, identifying aberrations of this pathway *in vivo* would potentially identify targets for specific biological or pharmacological therapies in patients with advanced disease.

The MAPK/ERK pathway transmits an extracellular signal to the nucleus through a series of membrane-associated and cytosolic protein kinases (for review, see (15)). The extracellular signal is coupled to the downstream membrane and cytoplasmic protein kinase cascade by SH2-containing adapter proteins and exchange proteins such as Grb2 and Son of Sevenless (SOS). In melanocytes, certain receptor kinases (RKs) are key regulators of downstream MAPK/ERK signaling and

include: the Fibroblast Growth Factor Receptors, Insulin-like Growth Factor Receptors, Hepatocyte Growth Factor Receptor, the neurotrophin receptors, CD117/c-kit, and erbB/HER/neu family of receptors (Reviewed in (3,16). In normal melanocytes, these proteins function as receptors for major ligands necessary for cell function and survival. Among these, the erbB family of RKs has been studied extensively in melanoma cells (Reviewed in (17)).

3.1. Oncogenic Ras: recruitment of the MAPK/ERK cascade and a potential target for constitutive pathway activation

The RAS family of oncogenes that includes *N-RAS, K-RAS, and H-RAS* are small guanine-nucleotide binding proteins that localize to the inner plasma membrane. These proteins are activated by a variety of stimuli including receptor tyrosine kinases and adaptor proteins (Reviewed in (18). Activated, GTP-bound RAS recruits the next member of the MAPK/ERK cascade, RAF to the plasma membrane where it is activated by phosphorylation. Activating mutations in codon 61 of *N-RAS* occur in approximately 20-30% of melanomas, a phenomenon not reported for benign melanocytic nevi (19,20). The most common mutation results in a glutamine to arginine substitution, a missense mutation that impairs GTP hydrolysis and thus maintains RAS in a state of constitutive activation. Early studies suggested that the activation of RAS in melanoma may not have significant biological effects because alterations of $p21^{waf1}$ often are not detected in cell lines harboring such mutations (19). However, silencing mutant N-RAS expression in cell lines harboring this mutation leads to apoptosis, as well as decreased levels of activated ERK and cyclin D1 (20). Looking at global gene expression, it is not surprising that many of the genes upregulated in melanoma lines harboring N-RAS mutations are related to cell survival and MAPK activation (21).

RAS also can be activated by cAMP, a feature independent of Protein Kinase A activation, an observation that suggests the presence of a novel melanocyte-specific RAS exchange factor (22). Indeed, high levels of activated RAS have been reported in melanoma lines lacking *RAS* mutations further supporting a role for receptor-mediated or autocrine signaling in melanoma (23).

Interestingly, mutations of N-RAS (and of B-RAF) occur at much lower frequency in non-cutaneous melanomas (24). Activating mutations of N-RAS (and B-RAF) also are rare occurrences in Spitz nevi; however, H-RAS mutations are common especially in those lesions with increases in copy number of chromosome 11p (25). These observations in sum identify N-RAS as a possible target for therapy in human cutaneous melanoma.

3.2. RAF kinases: a target for pathway activation responsible for carrying the signal downstream

Upon activation, RAS recruits members of the serine-threonine kinase RAF family to the plasma membrane. In mammals, RAF family members include: A-RAF, B-RAF, and C-RAF (RAF-1). Activated RAS activates the RAF kinases which then dissociate from the membrane complex and enter the cytosolic compartment to in turn activate members of the MEK family of proteins (26,27).

Much interest has been generated by the recent observation that activating mutations of B-RAF occur in approximately 60% of melanomas (28,29). These mutations occur with greatest frequency in codon 15 ^{V600E}B-RAF (originally reported to NCBI as ^{V599E}B-RAF) (30). This missense mutation results in a valine to leucine substitution leading to constitutive activation of the kinase with a multi-fold increase in its basal activity resulting in downstream activation of MEK1/2 and ERK1/2 without effects on MKK4 and subsequent involvement of the JNK/p38MAPK pathway (26).

Interestingly, activating mutations of B-RAF occur almost exclusively in melanomas lacking activating mutations of N-RAS (31). As such, approximately 90% of all melanomas will have upstream activating mutations within the MAPK pathway involving either N-RAS or B-RAF. Although B-RAF mutations occur in a 60% of melanomas, it must be remembered that similar aberrations affect greater than 80% of melanocytic nevi and are not observed in most Spitz nevi, a lesion histologically similar to melanoma (32). Another study found B-RAF mutations in only 20-30% of melanomas and nevi (33), a finding that may be related to differences in the methods of mutation analysis used in the various laboratories (34). Melanomas harboring B-RAF mutations are more likely to produce liver and multi-organ metastases, but have not been shown to have any effect on patient survival (35). The complexity of B-RAF signaling in melanocyte biology is underscored by recent observations that this component of the MAPK pathway also has a role in cellular senescence.

3.3. The B-RAF^{v6003} oncogene triggers cellular senescence in nevi

Normal human cells respond to potentially oncogenic events such as short telomeres, DNA damage and activating oncogenes by irreversibly arresting growth with a characteristic phenotype termed cellular senescence (Reviewed in (36,37,38,39). Senescent cells exhibit multiple changes in gene expression, including down regulation of cell cycle regulatory and stress-response genes and up-regulation of matrix-remodeling proteins (Reviewed in (40)). Activating oncogenes have been known for some time to induce cellular senescence in cultured cells. These studies suggested but did not prove that cellular senescence is a tumor suppressive mechanism that also contributes to aging (40). In fact, based on the high frequency of chromosomal aberrations in melanomas, and their relative absence in nevi, it was proposed that replicative senescence is a tumor-suppressive mechanism in melanocytic neoplasia (41).

Common nevi are benign pigmented lesions characterized by nested proliferations of melanocytes that usually appear at an early age, remaining quiescent for several decades, losing pigmentation with increased donor age and occasionally disappearing from the skin. Confirming the aforementioned hypotheses, four different laboratories have mechanistically demonstrated that

cellular senescence functions as a protective guard against cancer *in vivo* (42,43,44,45). The study of Michaloglou *et al.* (45) is particularly important for understanding how the common nevus is virtually a senescent entity that never progresses to melanoma. First, it is important to remember that 82% of nevi display the B-RAF^{V600E} mutation also present in melanomas (32), which indicates that activation of the RAS/RAF/MAPK pathway alone is insufficient for human melanoma tumor progression; 2) even large congenital nevi are positive for the senescent-associated acidic β galactosidase (SA-β-gal) marker (46). It is now evident that what triggers senescence in these melanocytes is the oncogenic B-RAF^{V600E} mutation. Thus, critical, regulated levels of activated ERK may be required for maintaining limited proliferation, as both excess activation by oncogenic signals (46), or loss of activation at the end of the proliferative lifespan (14), correlate with melanocyte senescence.

Intriguingly, Michaloglou *et al.* (45) found that the growth arrested state of nevi correlated with positive SA-β-gal staining rather than with positive staining for the tumor suppressor p16^{INK4a}. This is certainly unexpected, as p16^{INK4a} is induced in senescent melanocytes in culture and epigenetically silenced or deleted in melanoma cells (47,48). The B-RAF study confirmed in nevi previous results showing that both p53 and p21^{Waf-1} (initially identified in senescent fibroblasts) do not play any evident role in melanocyte senescence (Reviewed in (49)). A potentially interesting candidate for maintaining the permanent state of growth arrest in nevi is p27^{Kip1}. Although not studied in the context of the B-RAF mutation, p27^{Kip1} is highly expressed in benign nevi (50,51) and primary melanomas (52), and is required by the RB pathway to mediate senescence (53,54).

It is important to mention, though, that the senescent role of CDK-Is is not yet fully understood. For example, fibroblasts up-regulate and down-regulate p21^{Waf-1} and p16^{INK4a} at different stages of senescence. Adding to this complexity, CDK-I expression and function appear to be cell- and tissue-type dependent (55).

Clearly, the relationship between the MAPK cascade and cell cycle regulation needs to be further clarified. At this point in time it is safe to say that the activation of the MAPK pathway by B-RAF alone is insufficient to induce melanoma genesis in humans. In the future, considerable effort may be directed toward discovery of new pharmacologic agents that target specific points in the MAPK pathway such as N-RAS and B-RAF (56,27).

3.4. MEK and ERK: kinases that transmit signals to the nucleus and mediate cross talk with other signaling pathways

Whether activation of the MAPK/ERK pathway occurs via stimuli from plasma membrane receptors, or by RAS / RAF signaling, the ultimate cellular effects are mediated by the downstream serine-threonine kinases in the MAPK pathway (26,57). These downstream kinases include MEK1/2 and ERK1/2. Cytoplasmic, activated B-RAF is capable of propagating a signal downstream by phosphorylation of MEK1/2. MEK1/2 in turn activates ERK1/2. Activated ERK1/2 may interact with cytosolic proteins serving as a point of cross-talk with other signaling pathways or it can translocate to the nucleus and activate several transcription factors thereby influencing expression of myriad genes including: cell cycle regulatory proteins, matrix metalloproteinases, angiogenic factors, survival factors, and inhibitors of apoptosis (Figure 1). Many of these downstream proteins have been implicated in the pathogenesis and the progression of human malignant melanoma (57).

As would be predicted, increased levels of phospho-p42/p44 ERK1/2 have been demonstrated in melanomas *in vivo,* including the early radial growth phase of tumor progression (58). Furthermore, increased levels of phosphorylated ERK in melanoma correlates with tumor thickness and cyclin D levels, but does not predict overall survival (59). Neither of these studies found significant phosphorylated ERK levels in melanocytic nevi.

Activation of ERK also has been shown in uveal (60) and in acral (61) melanomas, in which N-RAS and B-RAF mutations are rare further stressing the importance of downstream elements of the MAPK/ERK pathway in melanoma. It is also important to note the role of ERK in establishing cross talks with other signaling pathways such as the p38MAPK/JNK, p90RSK, CREB, and MITF (62,57).

3.5. Oncogenic signaling through erbB family receptors: another way of activating the MAPK/ERK pathway in melanomas?

The identification of ligand-independent activating mutations in erbB family receptors in the melanoma-prone swordtail fish *Xiphophorus* first implicated the MAPK/ERK pathway in melanoma pathogenesis (63). These mutations result in constitutive activation of the receptor and the downstream MAPK/ERK pathway and are sufficient to induce tumorigenesis in *Xiphophorus*. The mutant oncogenic receptor has been termed Xmrk (*Xiphophorus* melanoma receptor kinase). Although the *Xmrk* oncogene has not been described in human melanomas, functional erbB receptors are present in human melanocytes and melanoma cell lines (64). In addition, melanoma lines over-express and secrete heregulins, a ligand for erbB receptor family members. Interestingly, erbB receptors can mediate migration and/or proliferation in melanocytes and melanoma cells (65). Thus, erbB receptor ligands may have some as yet unidentified autocrine function in melanomas *in vivo* allowing for growth factor independence.

4. THE MANY LIVES OF MITF: A MASTER PIGMENT REGULATOR, A SURVIVAL ONCOGENE, A GROWTH INHIBITORY/ PRO-APOPTOTIC PROTEIN, OR ALL OF THE ABOVE?

The micropthlamia-associated transcription factor (MITF) promotes the differentiation of neural crest cells into the melanocyte differentiation pathway (Reviewed in

(66,67)). Although MITF is considered a master transcriptional activator and lineage-specific regulator of major pigment genes that include *tyrosinase*, the *tyrosinase-related protein 1 (TRP-1)* and *p-Mel-17* (68), its transcriptional activity can be very puzzling. For example, the human *dopachrome tautomerase gene* (*DCT)*, also a major pigment gene, is repressed by MITF in human normal melanocytes cultured with phorbol esters (69). This is quite surprising, as the *DCT* promoter also contains the MITF canonical binding sequence TCATGTG (M-box) (70). Similar studies showed that MITF had either no effect on *DCT* in human melanomas or it activated the *dct* promoter in B16 mouse melanomas (71). These contradictory results suggest that that the promoter context is also critical for MITF activity. Supporting this hypothesis, promoter-reporter assays demonstrated that the transcriptional co-activator p300 switches MITF from a repressor to an activator of *DCT* whereas MITF alone can induce robust activation of the *tyrosinase* and *TRP-1* promoters (69,72). But the paradoxical activities of MITF do not end with the pigment genes.

MITF expression is regulated by several transcription factors including Sox 10, Pax3, CREB, OC, and Lef-1 (reviewed in (73)). So, what does MITF do in melanomas? 1) MITF is amplified in ~ 10% on human melanomas, 2) overexpression of both MITF and B-RAF^{V600E} can transform telomerase-(hTERT) immortalized human melanocytes in culture, 3) overexpression of a dominant negative MITF (dnMITF) can inhibit growth and increase the susceptibility of melanoma cell lines to the chemotherapeutic agents cysplatin and docetaxel (74). Thus, MITF functions as a survival oncogene. Contradicting these results, MITF can also function as an anti-proliferative protein by associating with the retinoblastoma protein RB and activating expression of the cyclin-dependent kinase inhibitor p21^{Waf-1} (75).

To this end, SKI and mutated B-RAF show opposing roles in MITF expression. Overexpression of B-RAF^{V600E} can suppress MITF expression via ERK-induced degradation, whereas its reintroduction inhibits proliferation of human melanocytes (76). Conversely, overexpression of SKI induces MITF transcription in melanoma (77) and non-melanoma cells (E. Stavnezer, personal communication). We propose that as human melanomas display considerable levels of MITF protein (78), SKI-mediated MITF transcription (78) may counteract MITF protein degradation (79,80) induced by the MAPK signaling pathway.

New data also suggests that MITF may also participate in apoptotic pathways. In this scenario, a low molecular weight, caspase-cleaved MITF can function as a proapoptotic protein in melanocytes and melanoma cells (81). It is presently unclear, though, whether such fragment truly exists in the notoriously apoptosis-resistant human melanoma tissues. One can speculate that enhancing caspase activation may be one way to switch MITF's survival activities to pro-apoptotic functions in a melanoma tumor-specific manner.

In conclusion, additional studies are needed to place the current MITF data in an appropriate context. In the interim, we suggest that protein levels, oncogenic load of the melanoma tumor and promoter context may all contribute to the ultimate role of MITF in melanoma tumor progression.

5. MORE THAN JUST p16INK4A: THE (VERY) ALTERED MELANOMA CELL CYCLE

Progression of normal cells through the cell cycle is tightly regulated by the rapid synthesis and degradation of a family of proteins known as cyclins. Cyclins constitute the regulatory subunit of proteins known as the cyclin-dependent kinases (CDKs), which can be negatively regulated by proteins generically known as the cyclin-dependent kinase inhibitors (CDK-Is) of which several have been identified. Among several substrates, the CDKs phosphorylate members of the retinoblastoma (RB) protein family and abrogate their cell cycle inhibitory activity allowing E2F transcription factors to activate genes necessary for DNA synthesis.

Cancer cell cycles exhibit, in a tumor-type dependent manner, high levels and activity of cyclins and/or CDKs and abnormal expression of CDK-Is, Such abnormal cell cycles favor accelerated proliferation, chromosomal instability, resistance to apoptosis and immortality (resistance to the senescence response). For example, cyclin D1 may function as a melanoma oncogene (82). This cyclin is frequently amplified in acral melanoma (44.4%), a particular melanoma sub-type, whereas occasional amplifications have been observed in lentigo maligna melanoma (10.5%) and superficial spreading melanoma (5.6%). However, cyclin E is the only cyclin consistently overexpressed during the progression of human malignant melanoma (83,84). The high number of cyclin E-positive cells within the melanoma lesions suggests that cyclin E has lost the cell cycle-regulated periodicity.

5.1. Oncogenic activity of the low molecular weight forms of cyclin E

Cyclin E is perhaps the most interesting of the known cyclins; in association with CDK2 controls three major S phase events, DNA replication, centrosome duplication, and histone gene expression (85). Overexpression of cyclin E alters cell cycle dynamics: it promotes progression into S and G2-M phases and increases the frequency of centrosome duplication which results in genetic instability. In breast and melanoma tumors, cyclin E displays and intriguing pattern; in addition to the full length protein, several low molecular weight forms (LMW) of cyclin E are detected by a variety of cyclin E antibodies. Proteolysis of cyclin E results from the activation of the elastase class of serine proteases (86). The LMW forms of cyclin E show significant biochemical and biological activities compared to full length cyclin E. Intriguingly, such activities are tumor-dependent. In melanoma cell lines, CDK2 complexes containing endogenous or transfected LMW forms of cyclin E display decreased affinity for the cyclin-dependent kinase inhibitor

$p21^{Waf-1}$ (87). In contrast, the LMW forms of cyclin E/CDK2 complexes exhibit increased binding to $p21^{Waf-1}$ in breast tumor cell lines (88).

The LMW forms of cyclin E are oncogenic *in vivo*. Melanoma xenografts expressing the LMW forms of cyclin E can generate tumors in mice that exhibit prominent perineural invasion and increased vasculogenesis compared to full length cyclin E (87) . In addition, overexpression of the LMW forms of cyclin E can dramatically augment the incidence and number of metastases in an experimental lung metastasis assay (87). Thus, the LMW forms of cyclin E not only contribute to the total cyclin E protein dosage; they also augment and expand cyclin E function by their poor affinity for $p21^{Waf-1}$. Further studies are expected to determine whether the increased tumorigenicity and metastatic potential of the LMW forms results from CDK2-dependent and/or independent activities including phosphorylation of novel substrates and/or association with alternative protein partners. In summary, sufficient experimental evidence indicates that overexpression of total levels of cyclin E may be considered a relevant marker for highly invasive melanoma tumors. It is less certain, though, that the LMW forms of cyclin E can be used as prognostic markers for aggressive melanoma; the small size of the primary melanoma tissues will likely prevent its analysis by conventional techniques such as western blotting or immunoprecipitation.

5.2 CDK2 is required for melanoma proliferation

CDK2, the catalytic partner of cyclins E and A is overexpressed in approximately 50% of primary invasive and in 50-70% of the metastatic melanoma tumors (87,84). However, absence of CDK2 activating mutations in melanomas (89), indicates that increased levels of CDK2 may result from transcriptional and/or translational events. Recent findings support the transcriptional hypothesis (90). *Cdk2* localizes less than 800 nucleotides apart from the *SILVER* gene, and is regulated by the same enhancer. Unexpectedly, and due to the close vicinity of *SILVER*, MITF also functions as a CDK2 transcriptional regulator (90). Correlation studies using microarray expression profiling from ten primary melanomas demonstrated that whereas CDK2 is significantly associated with MITF, expression of CDK1, CDK4 and CDK5 do not show such correlation. Functional studies showed that CDK2 is essential for melanoma clonogenicity and proliferation (90). In contrast, *Cdk2* ablation in mice demonstrated that the CDK2 protein is dispensable for proliferation and survival of most cell types (91), and for cell cycle inhibition and tumor suppression mediated by the cyclin-dependent kinase inhibitors $p21^{Cip-1}$ and $p27^{Kip1}$ (92). The fact that both cyclin E and CDK2 are disregulated in melanoma tumors suggests a cancer-specific role of these cell cycle regulators.

Yet it is still unclear why high levels of MITF (69) do not up-regulate CDK2 in normal human melanocytes (55). Possible explanations for these intriguing results include activation of normal melanocyte checkpoints that prevent abnormal cell cycles, and/or alterations in MITF protein levels or activity resulting from post-translational modifications. For example, phosphorylation of MITF in serine 73 by the MAP kinases triggers its degradation via the ubiquitin-proteasome pathway (80), whereas the function of sumoylated MITF depends on the promoter context (93,94). Alternatively, MITF and yet to be defined transcription factors may synergistically activate the *CDK2* gene in melanoma tumors. A TESS (Transcription Element Search Software, URL: http://www.cbil.upenn.edu/tess/) search of the human *CDK2* promoter identified a number of candidate transcription factor binding sites that are relevant for melanoma including AP-1, TCF-1, E2F-1 and two TCATGgG sequences containing the critical 5' flanking T residue of the core CATGTG E-box required for MITF binding (95). It remains to be seen whether *CDK2* expression in melanomas can be directly regulated by any of the proteins mentioned above.

In summary, exploration of the melanoma cell cycle has yielded unexpected lessons in its complexity. Future work should determine whether any of the lesions identified so far can be the target of therapeutical interventions.

6. EPIGENETICS AND MELANOMA

Epigenetics, which means outside genetics, is the study of stable alterations in gene expression that arise during development and cellular proliferation, and, importantly, by the influence of the environment (Reviewed in (96,97)). Epigenetic modifications consist of DNA-methylation, histone post-transcriptional modifications including methylation, acetylation and phosphorylation, and ATP-dependent, structural modifications of chromatin. Genome modifications resulting from epigenetic changes appear to play a critical role in the development and/or progression of cancer. Histone deacetylase (HDAC) inhibitors have proven to be potent inducers of apoptosis in human cancers including melanoma (Reviewed in (98)). For example, sodium butyrate (NaBu), a classical HDAC inhibitor, induces rapid melanoma apoptosis by increasing the levels of acetylated, transcriptionally active p53 and Bax (99). In addition, HDAC1 inhibitors may impair the senescence-suppressor activity of TBX2, a developmental transcription factor highly expressed in melanomas, pancreatic cancer and breast tumors (100). However, lethality of NaBu and other HDAC inhibitors likely results from massive histone acetylation and genome-wide transcriptional alterations. A major advantage of HDAC inhibitors compared to other therapeutic modalities is that they can kill both proliferating and non-proliferating tumor cells but spare proliferating or arrested normal cells (101). Although not yet proven, one critical HDAC target may be the slow proliferating, tumor stem cells (102,103).

The potency of several HDAC inhibitors is currently being tested in several clinical trials (Reviewed in (104)). One word of caution, however, is the fact that the killing potency of these inhibitors is mostly regulated by HDACs levels in the tumor's cell (99). Other chromatin-targets include the histone- and DNA- methyltransferases.

For instance, 5-Azacytidine, a DNA methyltransferse inhibitor was approved by the FDA for treating myelodysplastic syndrome (105), and has shown promising results in cultured melanoma cells (106,107).

Recent data also suggest that global changes in histone modifications have predictive risk value for tumor recurrence. For example, increased levels of dimethylated arginine 3 in histone H4 can predict the risk that a low grade prostate tumor will recur after surgical removal (108,109). It will be interesting to determine whether similar changes can predict tumor recurrency in other human cancers including melanoma. Such studies may be forthcoming, as antibodies specific for a variety of histone modifications are currently available for immunhistochemical analysis of surgical specimens in the pathology laboratory.

7. CONCLUSIONS

We now know that several mutated proteins including those discussed in this review but also AKT (110), AP-2 (10), ATF2 (111), β-catenin (112), NFκB (113), RB/E2F-1 (114) and SKI (Reviewed in (12,13)), participate in altering physiological events that collectively characterize the "mutator" phenotype (115) of human melanoma. Thus, in theory, some if not all should become useful targets for specific melanoma therapies. Indeed, clinical trials are in progress evaluating inhibitory agents that target specific activating mutations in the B-RAF pathway. However, the B-RAF inhibitor BAY32-9006 has shown limited potency in melanoma patients (Discussed in (30)). It is important to mention, though, that the BAY32-9006 inhibitor was originally designed as a C-RAF inhibitor. Thus, more specific B-RAF inhibitors may provide better outcomes. However, results from a variety of clinical trials have taught us that monotherapies are in general a poor choice. In fact, clinical trials including both BAY32-9006 and conventional agents are under way. In the bigger picture of the post-genomic era, one could envision future clinical trials that will be based not only the faulty protein/pathway but also on how it interacts with gene networks in regulatory circuits of the tumor cell.

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Send correspondence to: Dr Estela E. Medrano, Baylor College of Medicine, One Baylor Plaza M320, Houston, TX 77030, USA, Tel: 713-798-1569, Fax: 713-798-4161, E-mail: medrano@bcm.tmc.edu

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