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THE RAS/RAF/MEK/ERK AND PI3K/AKT SIGNALING PATHWAYS PRESENT MOLECULAR TARGETS FOR THE EFFECTIVE TREATMENT OF ADVANCED MELANOMA

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1. ABSTRACT

Malignant melanoma is a highly aggressive tumor of the pigment-producing cells in the skin with a rapidly increasing incidence and a poor prognosis for patients with advanced disease that is resistant to current therapeutic concepts. Therefore, the development of novel strategies for treating melanoma are of utmost importance. In melanoma, both the Ras-Raf-MEK-ERK (MAPK) and the PI3K-AKT (AKT) signaling pathways are constitutively activated through multiple mechanisms, and thus exert several key functions in melanoma development and progression. Conversely, several molecules known to play key roles in melanoma development and progression such as the adhesion molecules E-/N-cadherin, MelCAM and alphavbeta3 integrin are regulated by these pathways and/or activate the same. The results of the research to date indicate that in melanoma both the MAPK and the AKT signaling pathways may represent promising therapeutic targets.

2. INTRODUCTION

Malignant melanoma, a highly malignant tumor of the pigment-producing cells in the skin, is among the human cancers whose incidence has increased most rapidly in the last few decades (1, 2). Mortality rates have also increased, though at a slower rate (1). Malignant melanoma presents a therapeutic challenge. When diagnosed early, melanoma is highly curable by surgical excision with adequate safety margins. Patients with distant metastases, however, have a median survival time of only 6-9 months (3). Therefore, one of the major goals of melanoma research has been to identify molecular targets for the development of novel treatment strategies. This article reviews selected recent advances in the understanding of melanoma biology with an emphasis on describing molecular targets for which there is hope to allow rapid clinical translation.

3. RAS-RAF-MEK-ERK (MAPK) SIGNALING PATHWAY IN MELANOMA

3.1. The Ras-Raf-MEK-ERK (MAPK) signaling pathway

A variety of extracellular factors such as growth factors, adhesion molecules and differentiation factors as

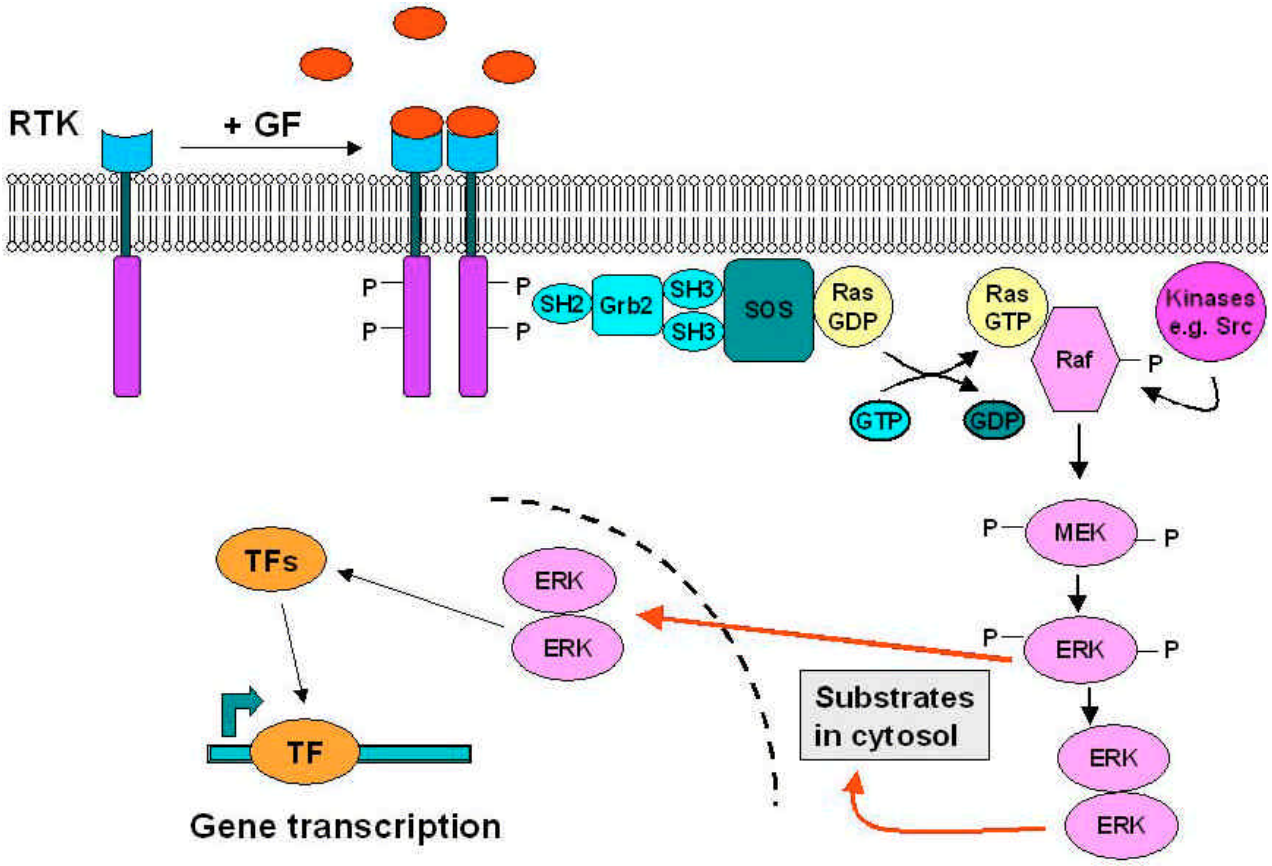

Figure 1. Schematic representation of the Ras-Raf-MEK-ERK (MAPK) signaling pathway. An extracellular factor such as a growth factor (GF) interacts with its receptor tyrosine kinase (RTK) and induces receptor dimerisation and autophosphorylation on tyrosine residues. The phosphotyrosines function as docking sites for the growth-factor-receptor-bound protein 2 adapter protein (Grb2). Grb2 pulls the GDP/GTP exchange factor son of sevenless (SOS) to the cell membrane. SOS induces switching of the Ras-family GTPases from the inactive GDP-bound state to the active GTP-bound state. Activated Ras binds to the Raf serine/threonine kinases (A-Raf, B-Raf, C-Raf/Raf-1) and recruits them to the cell membrane. Activation of B-Raf is obtained after binding to Ras alone whereas for activation of A-Raf and Raf-1 additional signals are required. Raf-1 activation is a multi-step process that requires the phosphorylation of activating sites by other kinases (e.g. Src) as well as the dephosphorylation of inhibitory sites by protein phosphatase 2A (not shown). Activated Raf phosphorylates and activates MEK which phosphorylates and activates ERK. The Raf-MEK-ERK cascade is scaffolded by the kinase suppressor of Ras (not shown). Activated ERK has many substrates in the cytosol and can also enter the nucleus to regulate gene expression by phosphorylating transcription factors (TFs).

well as tumor-promoting factors utilize the Ras/Raf/mitogen-activated protein kinase kinase /extracellular signal-regulated kinase (Ras/Raf/MEK/ERK) (MAPK) signaling pathway (figure 1) that represents an ubiquitous signaling module for the linkage of extracellular signals to the cytoplasmic and nuclear effectors and regulates cell behavior such as cellular proliferation, differentiation, survival and apoptosis (4, 5, 6). An extracellular factor such as a growth factor interacts with its respective receptor tyrosin kinase. Grb2 (growth-factor-receptor-bound protein 2 adapter protein) recognizes tyrosine phosphate docking sites located on the receptors or on receptor substrate proteins and pulls the GDP/GTP exchange factor SOS (son of sevenless) to the cell membrane. SOS induces switching of the Ras-family GTPases from the inactive GDP-bound state to the active GTP-bound state. One of these Ras-activated pathways is a family of serine/threonine kinases called the mitogen-activated protein kinases (MAPKs). Activated Ras binds to the Raf serine/threonine kinases with high affinity and causes their translocation to the cell membrane and activation. In mammals, the Raf family of serine/threonine kinases comprises three members: A-Raf, B-Raf and C-Raf (Raf-1). Activation of B-Raf is obtained after binding to Ras alone, whereas additional signals are required for activation of A-Raf and C-Raf. All three Raf isoforms share Ras as upstream activator and MEK as the only downstream effector. Activated Raf can activate both serine/threonine kinases MEK1 and MEK2 by phosphorylation of two serine residues. Activated MEK activates the serine/threonine kinases ERK1 and ERK2 via phosphorylation of a Thr-Glu-Tyr motif in the activation loop. Both MEK and ERK isoforms are usually co-expressed, and ERK1 and ERK2 appear to be functionally

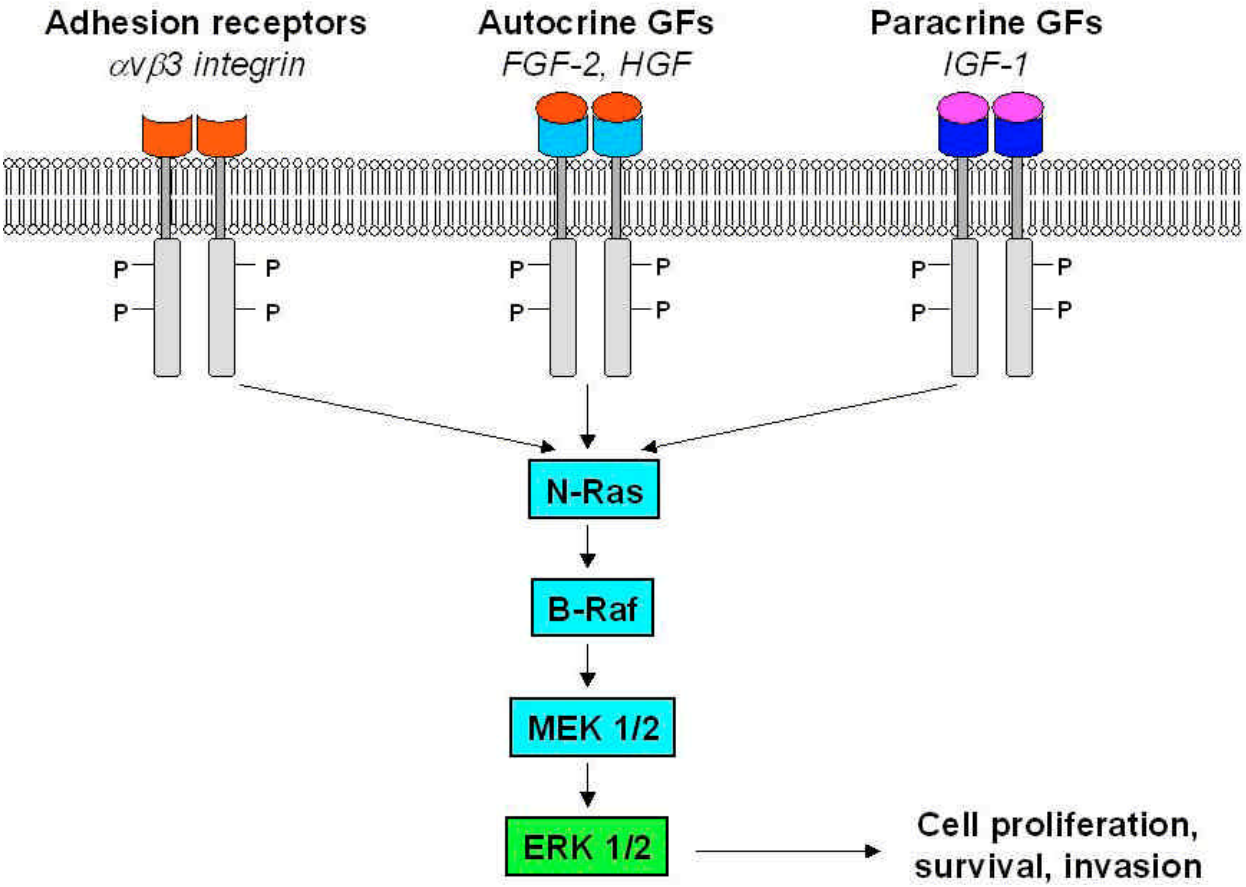

Figure 2. Activation of Ras-Raf-MEK-ERK (MAPK) signaling pathway in melanoma occurs through multiple mechanisms: activating mutations of N-Ras or B-Raf, autocrine and paracrine growth factors, and adhesion receptor signaling.

equivalent. ERK represents the endpoint of the Ras-Raf-MEK-ERK signaling pathway and has more than 50 substrates. Phosphorylated ERK is able to translocate into the nucleus and regulate gene expression via phosphorylation and activation of a variety of transcription factors.

3.2. Activation of MAPK signaling pathway in melanoma

There is growing evidence suggesting that activation of the Ras-Raf-MEK-ERK signaling pathway is important in the pathogenesis of melanoma. Activation of the Ras-Raf-MEK-ERK signaling pathway in melanoma occurs through multiple mechanisms (figure 2).

In 9-15% of melanomas activating mutations of Ras have been found with most of them being in the N-Ras gene (7, 8). ***Mutations in N-Ras*** stabilise the protein when it is bound by GTP maintaining N-Ras in the activated state. Activated N-Ras phosphorylates B-Raf, C-Raf and PI3 kinase.

A recent discovery has excited the melanoma research community (9). Davies *et al* detected B-Raf somatic missense mutations in 66% of melanomas. All mutations were within the kinase domain with a single substitution (T→A) of glutamate for valine (V599E) accounting for 80%. Mutated B-Raf proteins had elevated kinase activity, stimulated activity of endogenous ERK, transformed NIH3T3 cells and made cells independent of Ras function. Wan *et al.* analysed the activation mechanism of the MAPK pathway by ***oncogenic mutations of B-Raf*** (10). B-Raf residues G595-V599 of the activation loop have hydrophobic interactions with residues G463-V470 of the P-loop. In this conformation, the catalytic residues are not capable of ATP binding. B-Raf mutations destabilise this interaction and disrupt the inactive conformation. There is a high frequency of activating B-Raf mutations in melanomas arising on intermittently sun-exposed skin whereas melanomas arising in chronically sun-damaged skin and melanomas on skin relatively or completely unexposed to sun rarely harbor activating B-Raf mutations (11, 12). These data suggest that UV exposure plays a role in the etiology of B-Raf mutations in melanoma despite the absence of the classical UV-radiation-induced C>T or CC>TT mutation signature, and propose that mechanisms other than pyrimidine dimer formation may be important. Surprisingly, in subsequent studies B-Raf mutations were detected in 82% histologically diverse melanocytic nevi including congenital, compound, dermal and dysplastic

nevi (13) suggesting that B-Raf mutation is an early but not sufficient event in malignant melanocyte transformation. Interestingly, activation of ERK is low in atypical nevi which are supposed to be precursors of melanoma but becomes readily detectable in early radial growth phase and advanced melanomas (14). An inhibitory molecule that down-regulates the effects of the MAPK signaling pathway is the Raf kinase inhibitor protein (RKIP). Immunostaining of RKIP in melanocytic tumors revealed strong cytoplasmic RKIP staining in benign melanocytic nevi, whereas RKIP expression was diminished or completely lost in primary malignant melanomas and metastases (15). In line with the *in vivo* fndings, overexpression of RKIP in the highly invasive Mel Im cell line resulted in significant inhibition of invasiveness *in vitro* (15). Maldonado *et al.* analysed Spitz nevi. Biologically, Spiz nevi are benign melanocytic nevi (16). Histopathologically, Spitz nevi overlap with melanoma. Immunohistochemistry yielded high levels of phospho-ERK and cyclin D1 expression suggesting MAPK pathway activation. In contrast to the MAPK pathway activation, the proliferation rate was low. An analysis of cell cycle inhibitory proteins revealed that the majority of Spitz nevus cells expressed high levels of the cell cycle inhibitory protein p16. Maldonado *et al.* propose that in benign melanocytic nevi with constitutive activation of the MAPK pathway the cell cycle inhibitory protein p16 mediates oncogene-induced cell-cycle arrest preventing progession to melanoma. Arbiser proposes the enzyme MAP kinase phosphatase as melanoma tumor suppressor (17). Atypical nevi are supposed to be precursors of melanoma and harbor two genetic mutations, hemizygosity of p16ink4a and activating mutations of B-Raf. Arbiser suggests that loss of the defensive enzyme MAP kinase phosphatase is necessary to convert atypical nevi into melanoma.

A recent study provided evidence that one of the mechanisms underlying constitutive activation of the MAPK pathway may be overexpression of wild type B-Raf as a consequence of gene amplification (18). Comparative genomic hybridization using 40 melanoma cell lines showed frequent ***amplification at 7q33-q34 containing B-Raf gene***. Both downregulation of the endogenously overexpressed wild type B-Raf by antisense oligonucleotide and treatment with a MEK inhibitor reduced phosphorylated ERK and melanoma cell growth.

For survival, proliferation and migration of normal melanocytes, paracrine growth factors such as fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF) and insulin-like growth factor are essential. Melanoma cells, in contrast, can survive in the absence of exogenous growth stimulation through several ***autocrine mechanisms***. One of the hallmarks of growth factors is stimulation of their respective receptor tyrosine kinase followed by activation of MAPK signaling pathway. Melanoma cells express FGF-2 and FGF receptor 1 (19). Inhibition of this autocrine loop inhibits phosphorylation of ERK (20). Also, melanoma cells secrete HGF and express its receptor c-Met (21). Activation of c-Met by HGF induces ERK phosphorylation. Phosphorylation of ERK can be inhibited by neutralizing antibody against HGF (21). In line with these findings are data of a recent study (22). Metastatic melanoma cells were treated with adenoviruses expressing antisense FGF-2 and neutralizing antibodies to HGF, respectively. Significant inhibition of ERK phosphorylation was observed when cell surface signaling from FGF receptor or HGF receptor was neutralized. Altogether, these data suggest that FGF-2 and HGF act in addition to activating B-Raf mutations to stimulate ERK phosphorylation.

However, melanoma in a tissue context may have additional support from exogenous stimulation such as ***paracrine mechanisms*** and ***adhesion receptor signaling***. For example, melanoma cells produce platelet-derived growth factor (PDGF) as either PDGF-AA or PDGF-BB but do not express the receptor for PDGF-B (23). PDGF-B appears to be produced solely for paracrine stimulation of fibroblasts in the tumor stroma which in turn secrete insulin-like growth factor-1 (IGF-1). ***IGF-1*** or its substitute insulin is one of the most critical growth factors that is required for growth of melanocytes, nevus cells, radial growth phase (RGP) and early vertical growth phase (VGP) melanoma cells in chemically defined media (24). The receptor for IGF-1, IGF-1R, is expressed by all melanocytic cells (25). Satyamoorthy *et al.* studied the feedback from fibroblasts to melanoma cells by using an adenoviral vector for overexpression of IGF-1 in melanoma cells (26). IGF-1 induced survival, growth and migration of RGP melanoma cells through activation of two signaling pathways, the proliferation MAPK and the survival AKT signaling pathways. The adhesion molecule ***alphavbeta3 integrin*** assumes several key functions in melanoma progression (27). Integrin signaling occurs through both the MAPK and AKT signaling pathways (28) whereas signaling of the other major adhesion molecules in melanoma, MelCAM and N-cadherin, occurs through the AKT signaling pathway (29, 30). Thus, adhesion molecules appear to co-operate with receptor tyrosine kinases for activation of the major proliferation and survival pathways. Using a three-dimensional dermal collagen model mimicking the pathophysiological environment of malignant melanoma in the dermis, Bao *et al.* found that alphav integrin controls melanoma cell survival through a pathway involving p53 regulation of MAPK signaling pathway (31).

3.3. Role of MAPK signaling pathway in melanoma proliferation

Regulation of cell proliferation takes place during the G1 phase. The G1 phase is the first pause in the cell cycle where converging signals determine wether the cell stays in the G1 phase or progresses into the DNA synthesising S-phase. Physiologically, regulation at the G1 phase is controlled by growth promoting cyclins and cyclin-dependent kinases (CDKs) and by CDK inhibitors. In cancer, breakdown in the regulation at G1, through overstimulation of cyclins and their kinases, loss of CDK inhibitors, or inactivation of the retinoblastoma protein, leads to dysregulated cell growth. The MAPK signaling pathway plays a relevant role in the control of progression through G1 into S-phase. ERK regulates the expression of cyclin D1 (32), and sustained ERK activation is required to pass the G1-restriction point (33).

ERK activity is involved in the proliferation of human melanoma cell lines which can be blocked by MEK inhibitors (34, 35, 36). Inhibition of constitutive ERK activity in melanoma cells leads to cell cyle arrest at the G1 phase through downregulation of CDK2 activity and retinoblastoma protein phosphorylation and through upregulation of the CDK inhibitor p27/Kip1 (36). Data of several recent studies indicate that in melanoma ^{V599E}B-Raf stimulates constitutive ERK activity and induces proliferation (37, 38, 39, 40). *In vitro*, both B-Raf depletion by siRNA and Raf inhibitor BAY 43-9006 blocked ERK activity and inhibited DNA synthesis in melanoma cell lines (37). *In vivo*, Raf inhibitor BAY 43-9006 induced reduction in MEK activity and growth delay in melanoma tumor xenografts (37). RNA interference (RNAi) with HIV lentiviral vectors specific for either wild type B-Raf or ^{V599E}B-Raf inhibited the growth of most melanoma cell lines *in vitro* and *in vivo* accompanied by decrease of B-Raf protein and ERK phosphorylation (38). Interestingly, the ^{V599E}B-Raf-specific siRNA exclusively inhibited MAPK activity and growth of melanoma cells harboring the ^{V599E}B-Raf mutation and did not affect the normal fibroblast culture. Experimental studies by Goodall *et al.* provided evidence that the Brn-2 transcription factor links activated B-Raf to melanoma proliferation (41). Goodall *et al*. found that 1) Brn-2 is not expressed in melanocytes but is highly expressed in melanoma cells, 2) overexpression of Brn-2 in melanocytes results in increased proliferation, 3) the Brn-2 promoter is stimulated by activating B-Raf mutants, and 4) siRNA-mediated depletion of Brn-2 in melanoma cells expressing activated B-Raf leads to decreased proliferation.

3.4. Role of MAPK signaling pathway in melanoma survival/apotosis

Inhibition of MAPK signaling pathway induces apoptosis in melanoma cells *in vitro* (37, 39). Hingorani *et al.* reported that suppression of ^{V599E}B-Raf expression by RNA interference in cultured melanoma cells inhibited the MAPK cascade, caused growth arrest and promoted apoptosis (39). In a study by Karasarides *et al*., both B-Raf depletion by siRNA and Raf inhibitor BAY 43-9006 blocked ERK activity, inhibited DNA synthesis and induced apoptosis in melanoma cell lines (37). The mechanisms by which ERK inhibits apoptosis are not well established.

ERK is able to modulate the activity of Microphthalmia-associated transcription factor (MITF) (6). ***MITF*** is discussed as the master gene for melanocytic survival. In a study by King *et al*., MITF was expressed in all melanoma samples tested (42). MITF regulates the expression of the antiapoptotic protein Bcl-2 and thereby modulates cell survival (43). Using a dominant negative MITF protein, melanoma cells underwent apoptosis; after overexpression of Bcl-2, melanoma cells were protected from apoptosis.

Eisenmann *et al*. suggest that the MAPK pathway mediates melanoma-specific survival signaling by differentially regulating RSK-mediated phosphorylation of the proapoptotic protein Bad. The 90-kDa serine/threonine ribosomal S6 kinase (***RSK***) is a downstream effector in the MAPK signaling pathway. RSK phosphorylates the proapoptotic Bcl-2 family protein Bad at Ser^{75}, thereby facilitating its inactivation through binding to the chaperone protein 14-3-3 and sequestration in the cytosol. In melanoma cells, the MAPK/RSK signaling module is constitutively hyperactivated, and Bad is maintained in its inactive state, thereby promoting melanoma cell survival. Inhibiting MAPK/RSK signaling by a MEK inhibitor effectively induces apoptotic cell death. Taken together, these findings indicate that constitutive activation of MAPK/RSK signaling and sustained Bad inactivation provide a tumor-specific survival mechanism for melanoma cells. (44).

In a study by Zhang *et al*., the relation between MAPK signaling pathway and TRAIL-induced apoptosis was examined (45). TNF-related apoptosis-inducing ligand (***TRAIL***) induces apoptosis of melanoma by release of Smac/DIABLO from mitochondria into the cytosol. Activation of ERK protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. Inhibition of MAPK signaling by the MEK inhibitor U0126 sensitized melanoma cells to TRAIL-induced apoptosis. In the presence of U0126 increased translocation of the multidomain BH3 proapoptotic protein Bax from the cytosol to the mitochondria was observed leading to changes in mitochondrial membrane permeability and increased release of Smac/DIABLO into the cytosol. These results suggest that MAPK signaling may protect melanoma cells against TRAIL-induced apoptosis by inhibiting the relocation of Bax from the cytosol to mitochondria and that this may reduce TRAIL-mediated release of Smac/DIABLO and induction of apoptosis.

There is evidence that the sustained activation of the MAPK signaling pathway is involved in the expression of ***beta3 integrin*** (46). Petitclerc *et al*. demonstrated that the survival and proliferation of melanoma cells in the dermis is regulated by the interaction of beta3 integrin with denatured collagen (47). Consistent with this observation, Hsu *et al.* found that the overexpression of alphavbeta3 integrin in radial growth phase melanoma cells promotes both anchorage-dependent and -independent growth, protects the invading tumor cells from apoptosis, and promotes tumor growth *in vivo* (27).

3.5. Role of MAPK signaling pathway in melanoma invasion

In normal skin, melanocytes interact with keratinocytes through the adhesion molecule ***E-cadherin*** (48). During melanoma development, downregulation of E-cadherin with upregulation of N-cadherin has been observed (48). The switching of cadherin subtypes frees melanoma cells from keratinocyte-mediated control and enables melanoma cells to interact directly with other N-cadherin-expressing cells such as surrounding melanoma cells, fibroblasts and endothelial cells, thus affecting tumor-host cell adhesion, tumor cell invasion and migration, and gene expression (49). Downregulation of E-cadherin expression appears to be mediated by autocrine hepatocyte

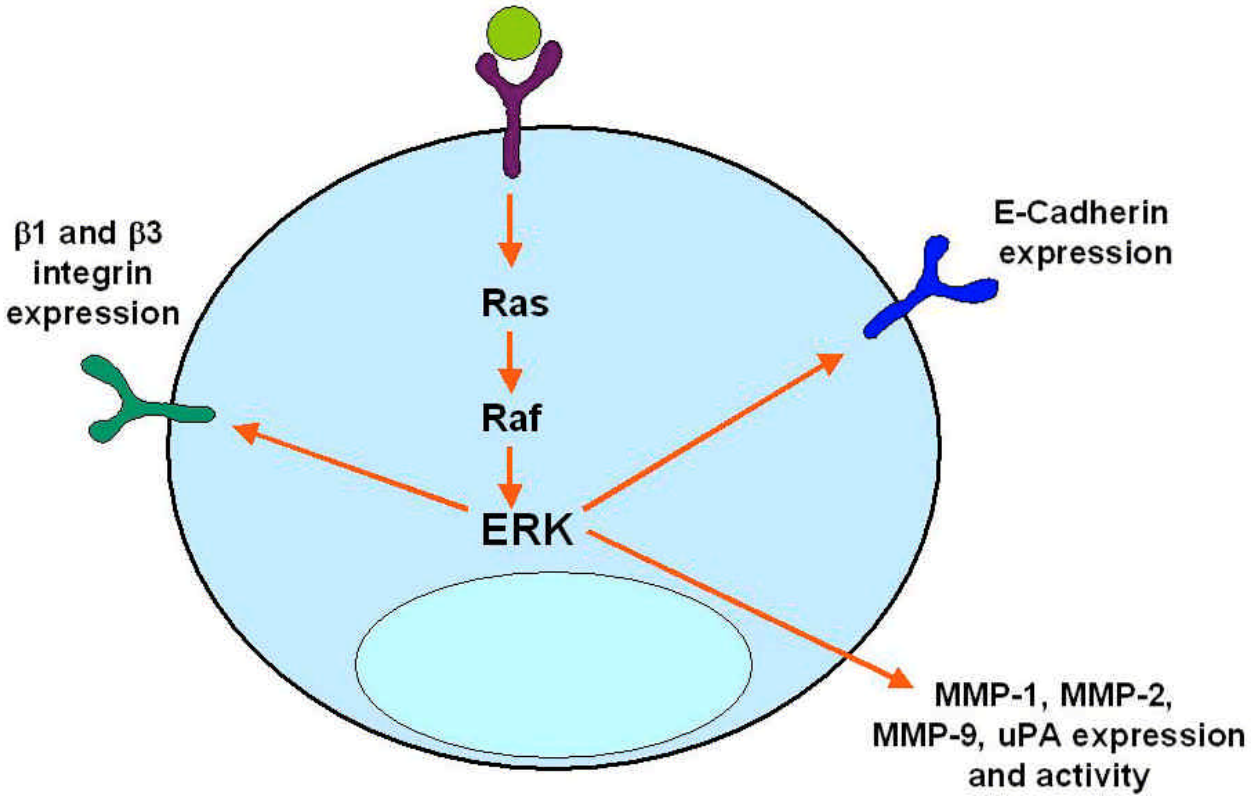

Figure 3. Role of Ras-Raf-MEK-ERK (MAPK) signaling pathway in melanoma invasion. Constitutive activation of MAPK signaling pathway appears to be associated with (1) downregulated E-cadherin expression freeing melanoma cells from keratinocyte-mediated control, (2) upregulated integrin expression promoting survival and invasive growth of melanoma cells in ECM and (3) increased expression and activity of proteolytic enzymes facilitating degradation of ECM.

growth factor (HGF) during melanoma development (21). In contrast to melanocytes, melanoma cells express HGF. The HGF receptor c-Met is expressed by all melanocytic cells. In melanoma cells, autocrine HGF caused constitutive activation of both the MAPK and phosphatidyl-3 kinase (PI3K)-AKT signaling pathways. When autocrine activation was induced with HGF-expressing adenoviruses in melanocytes, E-cadherin expression was decreased (figure 3). Inhibition of MAPK and PI3K-AKT signaling pathways by the MEK inhibitor PD98059 and the PI3K inhibitor wortmannin, respectively, partially blocked the downregulation of E-cadherin suggesting that both signaling pathways are involved in this process.

Sustained activation of the MAPK signaling pathway is involved in the expression of the adhesion molecule *beta3 integrin* (figure 3) (46). The pronounced expression of beta3 integrin in all advanced vertical growth phase melanomas indicates that beta3 integrin is decisively involved in melanoma invasion (50). Overexpression of alphavbeta3 integrin in radial growth phase melanoma cells initiated invasive tumor growth into the dermis of human skin reconstructs, and promoted tumor growth *in vivo* (27). Results of both *in vitro* and *in vivo* studies indicate that beta3 integrin-mediated signal processes induce the expression and activation of MT-MMP-1 (membrane-type matrix metalloproteinase-1) and matrix metalloproteinase-2 (MMP-2) and thus facilitate the degradation of the matrix and invasion of the tumor cells (51).

Furthermore, the MAPK signaling pathway appears to be involved in the expression of ***beta1 integrin*** (figure 3) (38). RNA interference (RNAi) with HIV lentiviral vectors specific for ^{V599E}B-Raf inhibited matrigel invasion of melanoma cells associated with a decrease of beta1 integrin expression and matrix metalloproteinase-2 activity.

Apparently, not all melanoma cell lines utilize the same signaling pathways to regulate the expression of ***proteases*** (figure 3). In VMM5 melanoma cells, both ERK and p38 MAP kinase regulate the expression of matrix metalloproteinase-1 (MMP-1), whereas in A2085 melanoma cells only ERK regulates MMP-1 expression (52). Treatment of A375 melanoma cells with the MEK inhibitors U0126 and PD98059 reduced matrigel invasion (53) associated with decreased expression of urokinase plasminogen activator (uPA) and matrix metalloproteinase-9 (MMP-9) (53) and decreased transcription of the MMP-1 promoter (54). When 1984-1 melanoma cells were treated with the MEK inhibitor U0126, matrigel invasion as well as expression and activity of MMP-2 were significantly decreased (55). In a study by Sumimoto *et al*., A375mel

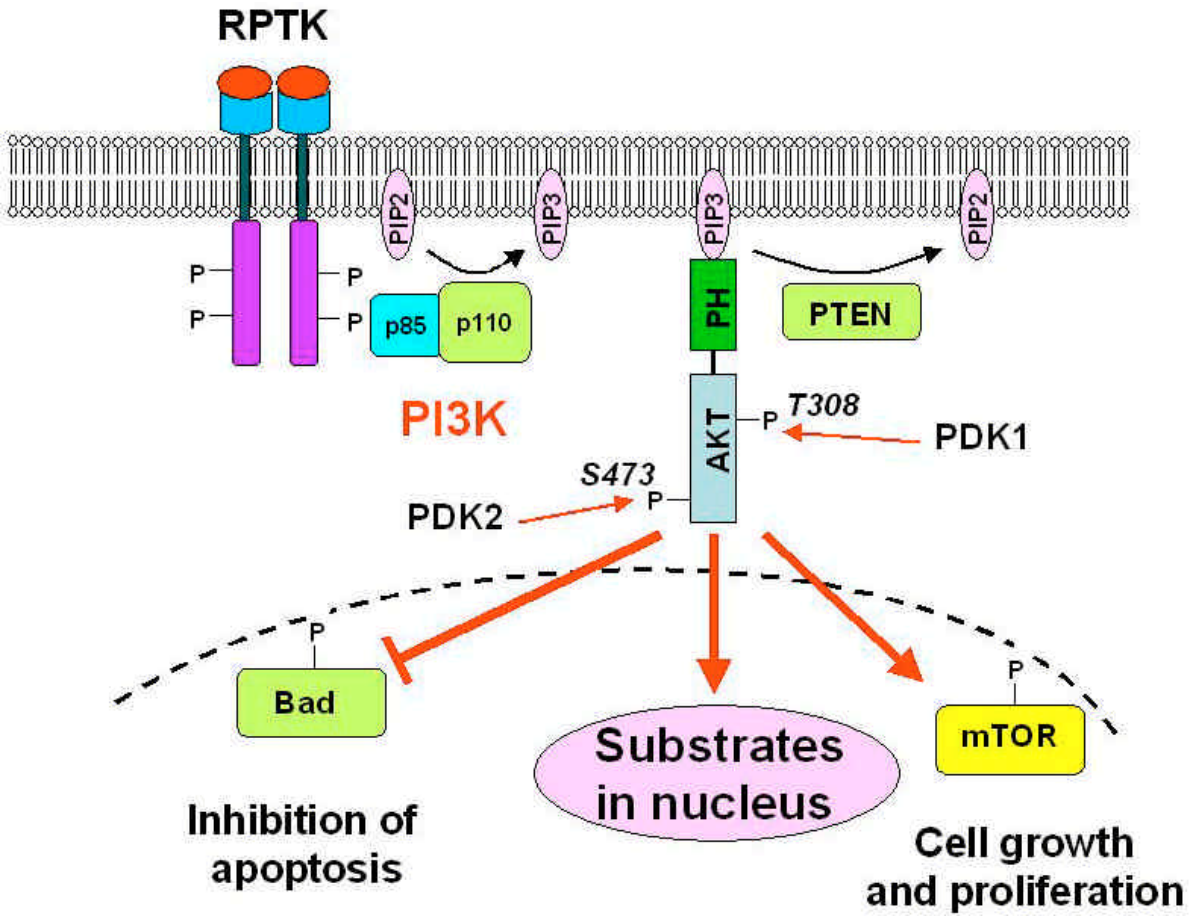

Figure 4. Schematic representation of the PI3K-AKT (AKT) signaling pathway (57)**.** An extracellular factor such as a growth factor interacts with its receptor protein tyrosine kinase (RPTK) resulting in autophosphorylation of tyrosine residues. Phosphatidylinositol-3 kinase (PI3K) consisting of an adaptor subunit p85 and a catalytic subunit p110 is translocated to the cell membrane and binds to phosphotyrosine consensus residues of the RPTK through ist its adaptor subunit. This results in allosteric activation of the catalytic subunit leading to production of phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 recruits signaling proteins with pleckstrin homolgy (PH) domains to the cell membrane including AKT. PTEN (phosphatase and tensin homologue deleted from chromosome 10) is a PIP3 phosphatase and negatively regulates the PI3K-AKT pathway. The interaction of PIP3 with the PH domain of AKT likely induces conformational changes in AKT, thereby exposing the two main phosphorylation sites at T308 and S473. T308 and S473 phosphorylation by protein serine/threonine kinase 3'-phosphoinositide-dependent kinases 1 and 2 (PDK1 and PDK2) is required for maximal AKT activation. Activated AKT translocates to the nucleus and mediates the activation and inhibition of various targets resulting in cellular survival and cell growth and proliferation.

melanoma cells transduced with lentiviruses containing siRNA against V599EB-Raf mRNA displayed significantly reduced matrigel invasion accompanied by decreased MMP-2 activity and beta1 integrin expression clarifying the important role of V599EB-Raf in melanoma invasion (38).This is in agreement with a study by Huntington et al., showing that in four melanoma cell lines tested ERK was constitutively active driving the constitutive expression of MMP-1 (40). Blocking MEK/ERK activity inhibited not only proliferation but also abrogated collagen degradation. Thus, constitutive activation of ERK not only promotes proliferation of melanoma cells but is also important for the acquisition of an invasive phenotype. In contrast, MEK inhibition had no effect on invasion of MeWo melanoma cells; instead, invasion was mediated through activation of p38 MAP kinase (56).

4. PI3K-AKT SIGNALING PATHWAY IN MELANOMA

4.1. The PI3K-AKT (AKT)signaling pathway

Phosphatidylinositol-3 kinases (PI3Ks) are a family of lipid kinases. Lipid kinases are able to phosphorylate inositol ring 3'-OH group in inositol phospholipids. Class I PI3Ks are heterodimers consisting of a catalytic subunit (p110) and an adaptor subunit (p85). Subclass IA PI3Ks are activated by receptor protein tyrosine kinases (RPTKs), subclass IB PI3Ks are activated by receptors coupled with G proteins. The substrate for class I PI3Ks is phosphatidylinositol-4,5-biphosphate (PIP2). Activation of RPTKs (figure 4) (57) leads to autophosphorylation on tyrosine residues. PI3K is

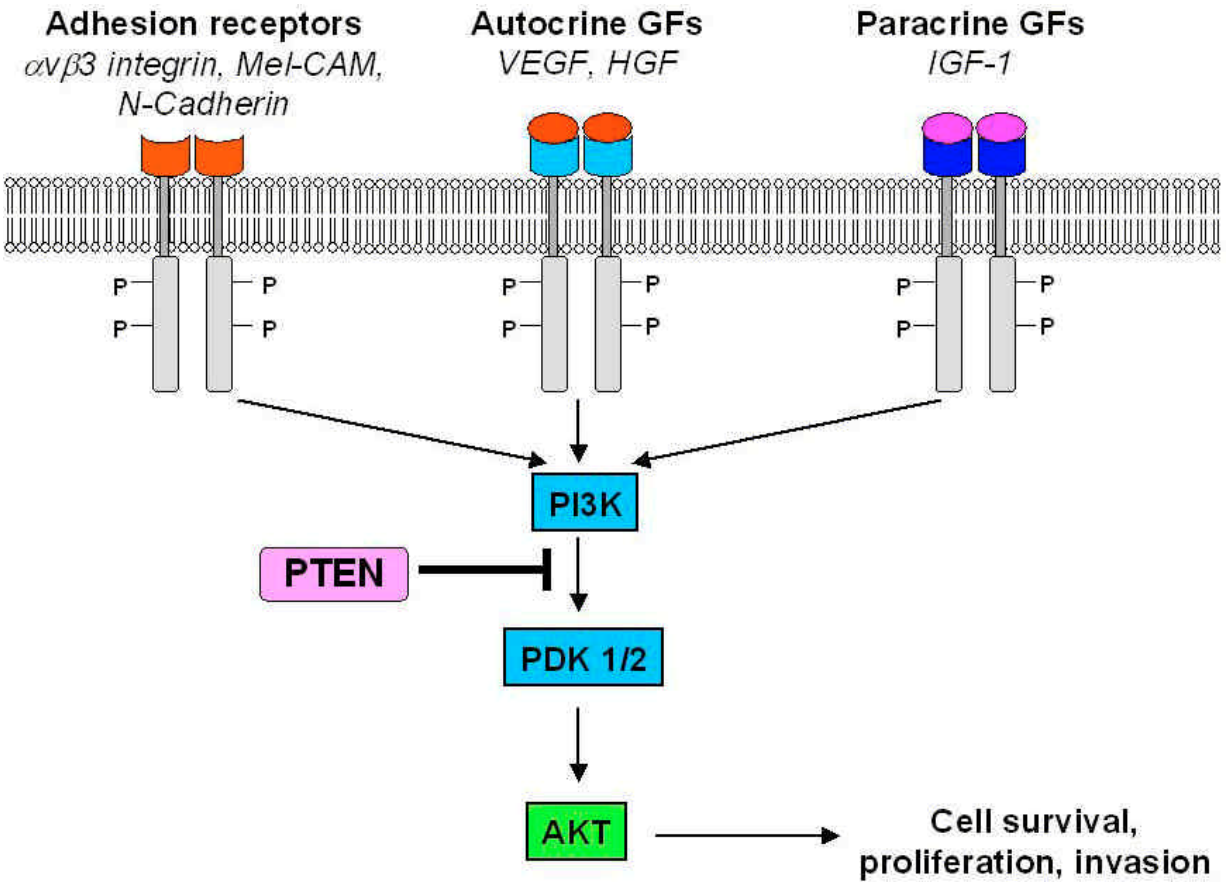

Figure 5. Activation of PI3K-AKT (AKT) signaling pathway in melanoma occurs through loss of PTEN, a negative regulator for PI3K-induced signaling, autocrine and paracrine growth factors, and adhesion receptor signaling.

translocated to the cell membrane and binds to phosphotyrosine consensus residues of RPTKs through one ore two SH2 domains in its adaptor unit. This results in allosteric activation of the catalytic subunit. Activation of the catalytic subunit leads to production of phosphatidylinositol-3,4,5-triphosphate (PIP3). PTEN (phosphatase and tensin homologue deleted from chromosome 10) is a PIP3 phosphatase and negatively regulates the PI3K-AKT pathway. PIP3 recruits signaling proteins with pleckstrin homology (PH) domains to the cell membrane. Many proteins have PH domains including AKT, also named proteinkinase B (PKB), and protein serine/threonine kinase 3'-phosphoinositide-dependent kinase 1 (PDK1). The AKT/PKB family comprises three isoforms, Akt1, Akt2 and Akt3, with up to 80% of amino acid homology though they are produced by different genes. The N-terminal region contains a PH domain with approximately 100 amino acids. The kinase domain is similar to those of proteinkinase A and proteinkinase C and holds a regulatory phosphorylation site (T308 in AKT1). The C-terminal region includes a second regulatory phosphorylation site (S473 in Akt1). T308 and S473 phosphorylation is required for maximal AKT activation. PIP3 interacts with the PH domain of AKT. The interaction likely induces conformational changes in AKT, thereby exposing the two main phosphorylation sites. PDK1 which is thought to be constitutively active phosphorylates AKT at T308. T308 phosphorylation stabilizes the activation loop in an active conformation and is required for activation of the kinase. S473 phosphorylation is required for full activation of the kinase. The responsible AKT S473 kinase/PDK2 has not yet been identified. Active AKT translocates to the nucleus where many of its substrates are localized. AKT mediates the activation and inhibition of several targets resulting in cellular survival and proliferation.

4.2. Activation of AKT signaling pathway in melanoma

AKT overexpression or activation has been documented in many types of human cancer (58). Recent evidence indicates that malignant melanoma also contains activated AKT (29, 59). Constitutive activation of AKT was observed in most melanoma cell lines and melanoma samples of different progression stages. Recently, Dai *et al.* demonstrated that increased phospho-Akt expression is significantly associated with melanoma progression and a worse patient survival (60).

PTEN (phosphatase and tensin homologue deleted from chromosome 10) dephosphorylates PIP3 at 3' inositol position and acts as a negative regulator for PI3K-induced signaling (figure 5). PTEN activity loss leads to

permanent PI3K/AKT pathway activation. PTEN is frequently mutated in advanced stages of several human cancers (61). Loss of tumor suppressor genes on chromosome 10 has been reported to contribute to the development of 30 – 60% of sporadic melanomas (62). Recent evidence suggests that PTEN is one of the genes on chromosome 10 of which the loss may play an important role in melanoma development (63). Furthermore, Stahl *et al.* reported recently that selective activation of ***Akt3*** occurs in 43 – 60% of sporadic melanomas as a result of a combination of decreased PTEN protein activity due to loss or haploinsufficiency of the PTEN gene and increased Akt3 expression accompanying copy number increases of the Akt3 gene (64). Permanent PI3K/AKT pathway activation may also occur in tumor cells expressing constitutively active ***Ras*** (65, 66).

Ligand-dependent activation of protein tyrosine kinase receptors, receptors coupled with G proteins or adhesion molecules such as integrins results in PI3K activation. As surface receptors are permanently active or overexpressed in many human cancers, their downstream signaling pathways are also active. In melanoma cells, autocrine ***hepatocyte growth factor*** (HGF) causes constitutive activation of both the MAPK and PI3K-AKT signaling pathways (figure 5) and downregulation of E-cadherin, thereby freeing melanoma cells from the control by keratinocytes and facilitating melanoma dissemination (21). A study by Graells *et al.* suggests an autocrine loop between *vascular endothelial growth factor*$_{165}$ (VEGF$_{165}$) and its receptor VEGFR-2 that promotes melanoma cell survival and growth through MAPK and PI3K signaling pathways (figure 5) (67). Also, *insulin-like growth factor-1* (IGF-1) produced by fibroblasts in the tumor stroma induces survival, growth and migration of RGP melanoma cells through activation of both MAPK and AKT signaling pathways (figure 5) (26). Several *in vitro* and *in vivo* studies have documented the important role of the adhesion molecules *N-cadherin*, *MelCAM* and *alphavbeta3 integrin* in melanoma progression (68, 30, 69, 29, 27, 70, 49). Recent work provided evidence that the adhesion molecules N-cadherin and MelCAM activate the AKT signaling pathway (figure 5) (29, 30), and that the adhesion molecule alphavbeta3 integrin activates the MAPK and AKT signaling pathways (figure 5) (28).

4.3. Role of AKT signaling pathway in melanoma survival/apotosis

AKT activates a number of diverse downstream antiapoptotic pathways (71). AKT phosphorylates and activates the transcription factor cyclic AMP response element-binding protein (CREB) and the ĸB kinase that activates the transcription factor nuclear factor-κB (NF-κB). Both CREB and NF-κB regulate the expression of survival genes. AKT inactivates by phosphorylation the proapoptotic factors Bad and procaspase-9 as well as the Forkhead family of transcription factors which induce the expression of proapoptotic factors such as Fas ligand. Moreover, activated AKT appears to mediate resistance to apoptosis induced by TNF-Related Apoptosis-Inducing Ligand (TRAIL)/APO-2L (72, 73, 74). A recent study identified the downregulation of the GTPase RhoB, a suppressor of transformation, invasion and metastasis, as a mechanism by which the Ras-PI3K-AKT signaling pathway induces tumor survival (75). Adenoviral-mediated expression of a kinase-dead mutant of AKT induced apoptosis selectively in tumor cells and suppressed tumor growth in mice (76).

Several reports have evoked the role of the PI3K-AKT signaling pathway in melanoma tumorigenicity and resistance to apoptosis (77, 78, 79). Adenoviral transfer of PTEN into melanoma cells containing wild type PTEN alleles led to inhibition of AKT phosphorylation, and to tumor-specific apoptosis and growth inhibition (79). Dhawan *et al.* confirmed that AKT is constitutively activated in human melanomas and demonstrated that AKT enhances melanoma cell survival through ***NF-kB*** (p50/65) activation (59). A recent study demonstrated that the PI3K-AKT signaling pathway mediates protection against TRAIL-induced apoptosis in human melanocytes (80). TNF-related apoptosis-inducing ligand (***TRAIL***) promoted apoptosis of human melanocytes. Stem cell factor (SCF), a melanocyte growth factor that activates both the PI3K-AKT and the MAPK signaling pathways, protected melanocytes from TRAIL-induced apoptosis. Inhibition of PI3K or AKT completely blocked the antiapoptotic effect of SCF, while inhibition of ERK had a moderate effect. Furthermore, sustained activation of the PI3K-AKT signaling pathway by forced expression of an activated PI3K catalytic subunit blocked TRAIL-induced apoptosis in melanocytes, whereas sustained activation of ERK by expression of an active BRAF construct did not affect TRAIL-induced melanocyte apoptosis. These observations point out to the key role of the PI3K-AKT signaling pathway in survival of melanocytes, thereby favoring melanocyte growth and transformation.

N-cadherin dependent AKT activation has been shown to enhance melanoma cell survival (30). N-cadherin mediated cell adhesion activated AKT which subsequently inactivated the proapoptotic protein Bad and stabilized the antiapoptotic protein beta-catenin. Blocking of N-cadherin mediated intercellular interaction by N-cadherin specific antibodies increased the number of cells undergoing apoptosis. A recent study provided evidence that the adhesion molecule ***MelCAM*** and PI3K-AKT are reciprocally regulated forming a bidirectional signaling network (29). This study showed that in melanoma cells, 1) the PI3K-AKT signaling pathway is constitutively activated, 2) activation of the PI3K-AKT signaling pathway positively regulates the expression of the adhesion molecule MelCAM, and 3) the expression of the adhesion molecule MelCAM activates the PI3K-AKT signaling pathway and subsequently inactivates the proapototic protein BAD resulting in increased cell survival. Furthermore, inhibition of MelCAM in a highly metastatic melanoma cell line led to increased apoptosis in human skin reconstructs and decreased tumorigenicity in mice (69, 81). These results suggest that the adhesion molecule MelCAM is a survival factor for melanoma by activating AKT signaling pathway and inhibiting proapoptotic protein BAD.

4.4. Role of AKT signaling pathway in melanoma proliferation

AKT modulates the function of several substrates involved in the regulation of cell cycle progression and cell growth (57). A key mechanism for AKT modulation of specific substrate activity is the regulation of their cytoplasmic or nuclear localization by phosphorylation. AKT inhibits glycogen synthase kinase-3 (GSK3). Inhibition of GSK3 catalytic activity prevents phosphorylation of the cytoplasmic signaling molecule β-catenin and impedes its degradation. β-catenin translocates to the nucleus, combines with various transcription factors and induces the expression of several genes such as cyclin D1 that induces cell cycle progression. Inhibition of GSK3 catalytic activity by AKT also decreases phosphorylation of cyclin D1 and stabilizes it (82). AKT phosphorylates the cyclin-dependent kinase inhibitors p21 and p27, retains them within the cytoplasm and inhibits their antiproliferative effects (83, 84). AKT phosphorylates Mdm2 that enters the nucleus which leads to inhibition of p53 regulated processes. (85). Finally, AKT stimulates growth by phosphorylating the tumor suppressor TSC2 (tuberous sclerosis complex 2) and inhibiting formation of a TSC1:TSC2 complex. The TSC1:TSC2 complex inhibits the p70 ribosomal protein S6 kinase 1, an activator of translation, and activates the eukaryotic initiation factor 4E binding protein 1, an inhibitor of translational initiation. These functions of the TSC1:TSC2 complex are mediated by inhibition of mammalian target of rapamycin (mTOR) (86, 87, 88, 89, 90). Inhibition of *in vitro* proliferation and *in vivo* tumor growth of human tumor cells has been described following blockade of AKT signaling by adenoviral-mediated expression of a kinase-dead mutant of AKT (76).

In melanoma, adenoviral transfer of PTEN (Ad-PTEN) into melanoma cells containing wild type PTEN alleles led to tumor specific growth inhibition with concomitant inhibition of AKT phosphorylation (79). Radial growth phase WM35 melanoma cells and metastatic MeWo melanoma cells were treated with Ad-PTEN. Three days after infection melanoma cells were harvested and subjected to cell cycle analysis. In both, radial growth phase and metastatic melanoma cells, Ad-PTEN caused G2/M block. This is in line with a study by Bedogni *et al.* (55). Immunodeficient mice that received s.c. injection with 1984-1 melanoma cells, derived from a TPras mouse melanoma, were topically treated with the PI3K inhibitor Ly294002. When comparing tumors from the control and Ly294002-treated mice, tumors treated with the PI3K inhibitor Ly294002 exhibited a significant decrease in cell proliferation.

4.5. Role of AKT signaling pathway in melanoma invasion

AKT contributes to invasiveness of tumor cells by inhibiting anoikis, a form of apoptosis induced by loss of adhesion or adhesion to inappropriate ECM, and stimulating matrix metalloproteinase secretion (57). Treatment of 1984-1 melanoma cells with the PI3K inhibitor Ly294002 blocked their invasive behavior *in vitro* and *in vivo* which correlated with reduced expression and activity of ***MMP-2*** (55). Recently, downregulation of the GTPase ***RhoB***, a tumor suppressor, has been recognized as a mechanism by which the Ras-PI3K-AKT signaling pathway induces tumor cell survival, invasion and metastasis (75). Jiang *et al.* demonstrated that Ras downregulates RhoB expression through PI3K-AKT but not MAPK signaling. Blockade of PI3K/AKT led to upregulation of RhoB expression. Ectopic RhoB expression inhibited Ras, PI3K and AKT induced tumor cell migration and invasion, induced apoptosis and anoikis, and inhibited melanoma metastasis to the lung in mice.

PI3K-AKT blockade by adenoviral transfer of PTEN into metastatic melanoma cells suppressed melanoma cell migration accompanied by increased levels of cell surface ***E-cadherin*** (79). These findings suggest that the PI3K-AKT signaling pathway is involved in the regulation of cadherin-mediated cell-cell adhesion and migration processes. Consistent with these findings, Li *et al.* reported that in melanoma cells, autocrine hepatocyte growth factor (HGF) causes constitutive activation of both the PI3K-AKT and MAPK signaling pathways and subsequently downregulates E-cadherin (21). The loss of E-cadherin expression during melanoma development is accompanied by the gain of ***N-cadherin*** expression. A study by Li *et al.* (30) demonstrated that the adhesion molecule N-cadherin mediates homotypic aggregation among melanoma cells as well as heterotypic adhesion of melanoma cells to dermal fibroblasts and endothelial cells, which may improve their ability to migrate through stroma and enter the vasculature. Furthermore, N-cadherin mediated intercellular interactions activated the PI3K-AKT signaling pathway, and promoted survival of melanoma cells as well as migration of melanoma cells over dermal fibroblasts. Together, these results indicate that the cadherin subtype switching from E- to N-cadherin during melanoma development not only frees melanocytic cells from the control by keratinocytes but also provides growth and possibly metastatic advantages to melanoma cells. The adhesion molecule ***MelCAM*** and PI3K-AKT are reciprocally regulated (29). Mel-CAM is usually not expressed in benign melanocytic lesions (91). In contrast, it is strongly expressed in melanomas, the degree of expression being correlated to tumor thickness and metastatic potential (92). MelCAM-transfected melanoma cells exhibit an enhanced MMP-2 activity (93). MelCAM-negative melanoma cells with low tumorigenic potential became highly tumorigenic and metastatic upon transfection with MelCAM (92, 69, 81). Inhibition of MelCAM in a highly metastatic melanoma cell line resulted in reduced invasion in human skin reconstructs and decreased tumorigenicity in mice (69, 81). Overall, the results of the research conducted on this issue to date indicate that the adhesion molecule MelCAM plays a multifunctional role in melanoma invasion and metastasis.

5. CONCLUSION AND FUTURE PERSPECTIVES

The Ras-Raf-MEK-ERK (MAPK) and the PI3K-AKT (AKT) signaling pathways modulate the function of numerous substrates involved in the regulation of cell survival, proliferation and invasion. In melanoma, both the

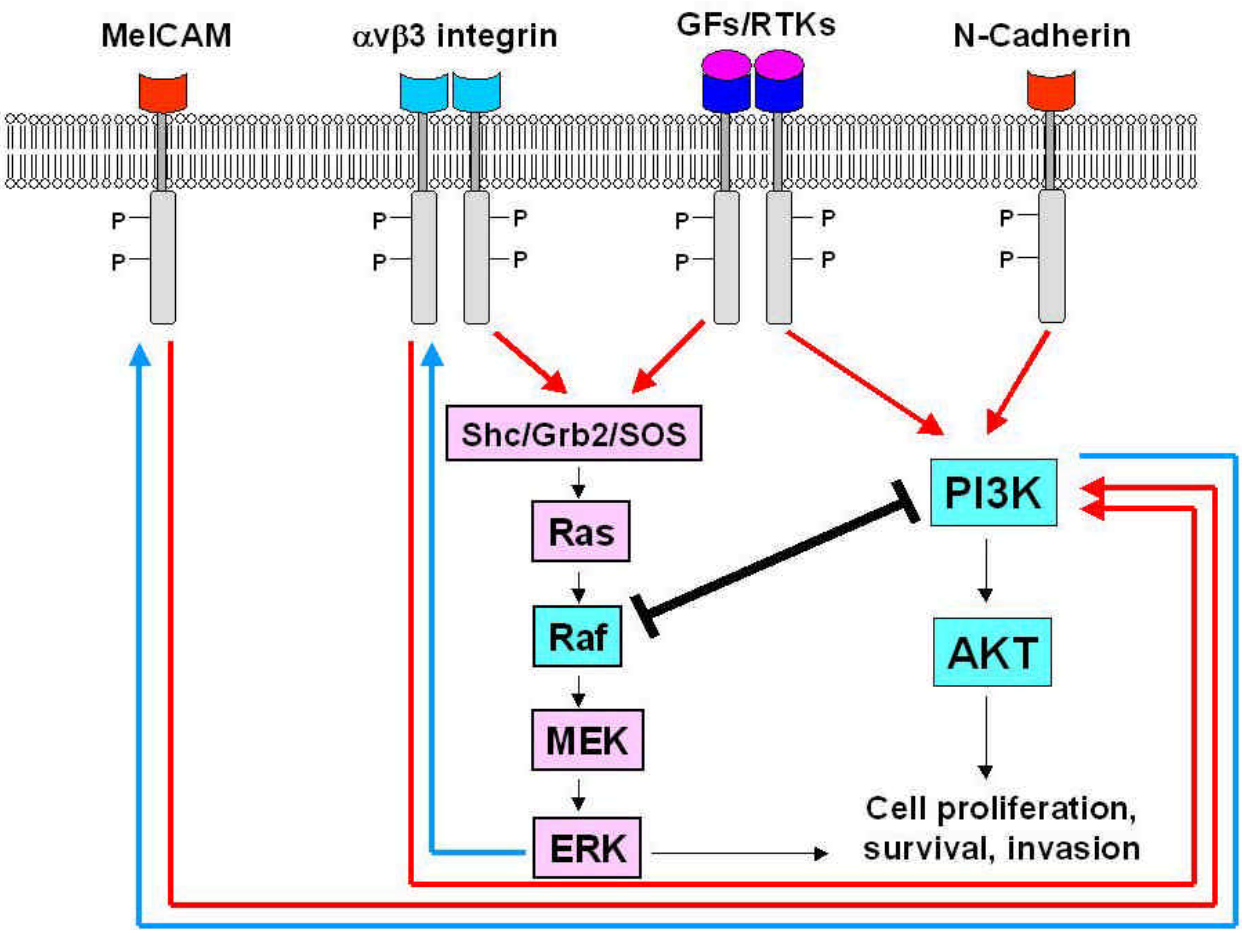

Figure 6. The Ras-Raf-MEK-ERK (MAPK)and PI3K-AKT (AKT) signaling pathways present promising molecular targets for the effective treatment of malignant melanoma. The MAPK and AKT signaling pathways are constitutively activated in melanoma and assume key functions in melanoma development and progression. Various molecules known to play key roles in melanoma development and progression such as the adhesion molecules MelCAM, alphavbeta3 integrin and N-cadherin as well as several growth factors are regulated by these pathways and/or activate the same. In melanoma, therefore, effective therapeutical targeting may involve both MAPK and AKT signaling pathways. ECM = extracellular matrix; GFs = growth factors; RTKs = receptor tyrosine kinases; Shc = adapter protein linking RTK and Grb2; Grb2 = growth-factor-receptor-bound protein 2 adapter protein; SOS = GDP/GTP exchange factor son of sevenless.

MAPK and AKT signaling pathways are constitutively activated through multiple mechanisms, and thus exert several key functions in melanoma development and progression. Intriguingly, several molecules known to play key roles in melanoma development and progression such as the adhesion molecules E-/N-cadherin, MelCAM and alphavbeta3 integrin are regulated by these pathways and/or activate the same (figure 6). Overall, the results of the research conducted on this issue to date strongly indicate that in melanoma effective therapeutical targeting may involve both MAPK and AKT signaling pathways (figure 6). This hypothesis is supported by the recent observation that BAY 43-9006, a potent RAF kinase inhibitor, is not potent enough to elicit a sufficient response as a monotherapy. A study by Karasarides *et al.* (37) showed that BAY43-9006 targets B-Raf signaling *in vitro* and *in vivo*, and induces a substantial growth delay in human melanoma xenografts in mice. However, the RAF kinase inhibitor BAY43-9006 was unable to completely cure the mice. Moreover, in a phase II randomized discontinuation trial the RAF kinase inhibitor BAY 43-9006 had very modest activity as a single agent in patients with advanced melanoma (94). Interestingly, a phase I/II trial of BAY43-9006 administered in combination with carboplatin and paclitaxel demonstrated preliminary antitumor activity in metastatic melanoma with a favorable safety profile (95).

The biological properties of tumor cells are thought to be the sum of activation of many genes. However, there may be key molecules which represent unique points of vulnerability for tumor cells and thus present targets for curative therapies. Indeed, despite the complexity of genetic and epigenetic alterations in malignant tumors, recent evidence strengthens the hope that the specific targeting of one or perhaps a few critical

molecules may be sufficient to elicit a significant clinical response. As example, the treatment of patients with chronic myelogenous leukemia (CML) or gastrointestinal stromal tumor (GIST) with imatinib mesylate, a small molecule inhibitor of the ABL and KIT tyrosine kinases, induces dramatic remissions with minimal toxicity (96, 97). The results in CML and GIST are highly encouraging and suggest that essential pathways may exist in other malignant tumors. The results of the research to date indicate that in melanoma both the MAPK and the AKT signaling pathways may represent therapeutic targets analogous to ABL and KIT**.**

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7. REFERENCES

1. Hall HI, D.R.Miller, J.D.Rogers &B.Bewerse: Update on the incidence and mortality from melanoma in the United States. *J Am Acad Dermatol* 40, 35-42 (1999)

2. Perlis C &M.Herlyn: Recent advances in melanoma biology. *The Oncologist* 9, 182-187 (2004)

3. Balch CM, A.C.Buzaid, S.J.Soong, M.B.Atkins, N.Cascinelli, D.G.Coit, I.D.Fleming, J.E.Gershenwald, A.J.Houghton, J.M.Kirkwood, K.M.McMasters, M.F.Mihm, D.L.Morton, D.S.Reintgen, M.I.Ross, A.Sober, J.A.Thompson &J.F.Thompson: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 19, 3635-3648 (2001)

4. Hilger RA, M.E.Scheulen &D.Strumberg: The Ras-Raf-MEK-ERK pathway in the treatment of cancer. *Onkologie* 25, 511-518 (2002)

5. Cobb MH: MAP kinase pathways. *Prog Biophys Mol Biol* 71, 479-500 (1999)

6. Smalley KS: A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? *Int J Cancer* 104, 527-532 (2003)

7. van Elsas A, S.Zerp, S.van der Flier, M.Kruse-Wolters, A.Vacca, D.J.Ruiter &P.Schrier: Analysis of N-ras mutations in human cutaneous melanoma: tumor heterogeneity detected by polymerase chain reaction/single-stranded conformation polymorphism analysis. *Recent Results Cancer Res* 139, 57-67 (1995)

8. Carr J, R.M.MacKie: Point mutations in the N-ras oncogene in malignant melanoma and congenital naevi. *Br J Dermatol* 131, 72-77 (1994)

9. Davies H, G.R.Bignell, C.Cox, P.Stephens, S.Edkins, S.Clegg, J.Teague, H.Woffendin, M.J.Garnett, W.Bottomley, N.Davis, E.Dicks, R.Ewing, Y.Floyd, K.Gray, S.Hall, R.Hawes, J.Hughes, V.Kosmidou, A.Menzies, C.Mould, A.Parker, C.Stevens, S.Watt, S.Hooper, R.Wilson, H.Jayatilake, B.A.Gusterson, C.Cooper, J.Shipley, D.Hargrave, K.Pritchard-Jones, N.Maitland, G.Chenevix-Trench, G.J.Riggins, D.D.Bigner, G.Palmieri, A.Cossu, A.Flanagan, A.Nicholson, J.W.Ho, S.Y.Leung, S.T.Yuen, B.L.Weber, H.F.Seigler, T.L.Darrow, H.Paterson, R.Marais, C.J.Marshall, R.Wooster, M.R.Stratton &P.A.Futreal: Mutations of the BRAF gene in human cancer. *Nature* 417, 949-954 (2002)

10. Wan PT, M.J.Garnett, S.M.Roe, S.Lee, D.Niculescu-Duvaz, V.M.Good, C.M.Jones, C.J.Marshall, C.J.Springer, D.Barford &R.Marais: Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 116, 855-867 (2004)

11. Maldonado JL, J.Fridlyand, H.Patel, A.N.Jain, K.Busam, T.Kageshita, T.Ono, D.G.Albertson, D.Pinkel &B.C.Bastian: Determinants of BRAF mutations in primary melanomas. *J Natl Cancer Inst* 95, 1878-1890 (2003)

12. Edwards RH, M.R.Ward, H.Wu, C.A.Medina, M.S.Brose, P.Volpe, S.Nussen-Lee, H.M.Haupt, A.M.Martin, M.Herlyn, S.R.Lessin &B.L.Weber: Absence of BRAF mutations in UV-protected mucosal melanomas. *J Med Genet* 41, 270-272 (2004)

13. Pollock PM, U.L.Harper, K.S.Hansen, L.M.Yudt, M.Stark, C.M.Robbins, T.Y.Moses, G.Hostetter, U.Wagner, J.Kakareka, G.Salem, T.Pohida, P.Heenan, P.Duray, O.Kallioniemi, N.K.Hayward, J.M.Trent &P.S.Meltzer: High frequency of BRAF mutations in nevi. *Nat Genet* 33, 19-20 (2003)

14. Cohen C, A.Zavala-Pompa, J.H.Sequeira, M.Shoji, D.G.Sexton, G.Cotsonis, F.Cerimele, B.Govindarajan, N.Macaron &J.L.Arbiser: Mitogen-actived protein kinase activation is an early event in melanoma progression. *Clin Cancer Res* 8, 3728-3733 (2002)

15. Schuierer MM, F.Bataille, S.Hagan, W.Kolch &A.K.Bosserhoff: Reduction in Raf kinase inhibitor protein expression is associated with increased Ras-extracellular signal-regulated kinase signaling in melanoma cell lines. *Cancer Res* 64, 5186-5192 (2004)

16. Maldonado JL, L.Timmerman, J.Fridlyand &B.C.Bastian: Mechanisms of cell-cycle arrest in Spitz nevi with constitutive activation of the MAP-kinase pathway. *Am J Pathol* 164, 1783-1787 (2004)

17. Arbiser JL: Activation of B-raf in non-malignant nevi predicts a novel tumor suppressor gene in melanoma (MAP kinase phosphatase). *J Invest Dermatol* 121, xiv (2003)

18. Tanami H, I.Imoto, A.Hirasawa, Y.Yuki, I.Sonoda, J.Inoue, K.Yasui, A.Misawa-Furihata, Y.Kawakami &J.Inazawa: Involvement of overexpressed wild-type BRAF in the growth of malignant melanoma cell lines. *Oncogene* 23, 8796-8804 (2004)

19. Rodeck U, D.Becker &M.Herlyn: Basic fibroblast growth factor in human melanoma. *Cancer Cells* 3, 308-311 (1991)

20. Kinkl N, J.Sahel &D.Hicks: Alternate FGF2-ERK1/2 signaling pathways in retinal photoreceptor and glial cells in vitro. *J Biol Chem* 276, 43871-43878 (2001)

21. Li G, H.Schaider, K.Satyamoorthy, Y.Hanakawa, K.Hashimoto &M.Herlyn: Downregulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development. *Oncogene* 20, 8125-8135 (2001)

22. Satyamoorthy K, G.Li, M.R.Gerrero, M.S.Brose, P.Volpe, B.L.Weber, P.Van Belle, D.E.Elder &M.Herlyn: Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. *Cancer Res* 63, 756-759 (2003)

23. Herlyn M, I.M.Shih: Interactions of melanocytes and melanoma cells with the microenvironment. *Pigment Cell Res* 7, 81-88 (1994)

24. Herlyn M, J.Thurin, G.Balaban, J.L.Bennicelli, D.Herlyn, D.E.Elder, E.Bondi, D.Guerry, P.Nowell &W.H.Clark: Characteristics of cultured human melanocytes isolated from different stages of tumor progression. *Cancer Res* 45, 5670-5676 (1985)

25. Kanter-Lewensohn L, A.Dricu, M.Wang, J.Wejde, R.Kiessling &O.Larsson: Expression of the insulin-like growth factor-1 receptor and its anti-apoptotic effect in malignant melanoma: a potential therapeutic target. *Melanoma Res* 8, 389-397 (1998)

26. Satyamoorthy K, G.Li, B.Vaidya, D.Patel &M.Herlyn: Insulin-like growth factor-1 induces survival and growth of biologically early melanoma cells through both the mitogen-activated protein kinase and beta-catenin pathways. *Cancer Res* 61, 7318-7324 (2001)

27. Hsu MY, D.T.Shih, F.E.Meier, P.Van Belle, J.Y.Hsu, D.E.Elder, C.A.Buck &M.Herlyn: Adenoviral gene transfer of beta3 integrin subunit induces conversion from radial to vertical growth phase in primary human melanoma [see comments]. *Am J Pathol* 153, 1435-1442 (1998)

28. Stupack DG, D.A.Cheresh: Get a ligand, get a life: integrins, signaling and cell survival. *J Cell Sci* 115, 3729-3738 (2002)

29. Li G, J.Kalabis, X.Xu, F.Meier, M.Oka, T.Bogenrieder &M.Herlyn: Reciprocal regulation of MelCAM and AKT in human melanoma. *Oncogene* 22, 6891-6899 (2003)

30. Li G, K.Satyamoorthy &M.Herlyn: N-cadherin-mediated intercellular interactions promote survival and migration of melanoma cells. *Cancer Res* 61, 3819-3825 (2001)

31. Bao W, S.Stromblad: Integrin alphav-mediated inactivation of p53 controls a MEK1-dependent melanoma cell survival pathway in three-dimensional collagen. *J Cell Biol* 167, 745-756 (2004)

32. Welsh CF, K.Roovers, J.Villanueva, Y.Liu, M.A.Schwartz &R.K.Assoian: Timing of cyclin D1 expression within G1 phase is controlled by Rho. *Nat Cell Biol* 3, 950-957 (2001)

33. Pages G, P.Lenormand, G.L'Allemain, J.C.Chambard, S.Meloche &J.Pouyssegur: Mitogen-activated protein kinases p42mapk and p44mapk are required for fibroblast proliferation. *Proc Natl Acad Sci U S A* 90, 8319-8323 (1993)

34. Smalley K, T.Eisen: The involvement of p38 mitogen-activated protein kinase in the alpha-melanocyte stimulating hormone (alpha-MSH)-induced melanogenic and anti-proliferative effects in B16 murine melanoma cells. *FEBS Lett* 476, 198-202 (2000)

35. Smalley KS, T.G.Eisen: Differentiation of human melanoma cells through p38 MAP kinase is associated with decreased retinoblastoma protein phosphorylation and cell cycle arrest. *Melanoma Res* 12, 187-192 (2002)

36. Kortylewski M, P.C.Heinrich, M.E.Kauffmann, M.Bohm, A.MacKiewicz &I.Behrmann: Mitogen-activated protein kinases control p27/Kip1 expression and growth of human melanoma cells. *Biochem J* 357, 297-303 (2001)

37. Karasarides M, A.Chiloeches, R.Hayward, D.Niculescu-Duvaz, I.Scanlon, F.Friedlos, L.Ogilvie, D.Hedley, J.Martin, C.J.Marshall, C.J.Springer &R.Marais: B-RAF is a therapeutic target in melanoma. *Oncogene* 23, 6292-6298 (2004)

38. Sumimoto H, M.Miyagishi, H.Miyoshi, S.Yamagata, A.Shimizu, K.Taira &Y.Kawakami: Inhibition of growth and invasive ability of melanoma by inactivation of mutated BRAF with lentivirus-mediated RNA interference. *Oncogene* 23, 6031-6039 (2004)

39. Hingorani SR, M.A.Jacobetz, G.P.Robertson, M.Herlyn &D.A.Tuveson: Suppression of BRAF(V599E) in human melanoma abrogates transformation. *Cancer Res* 63, 5198-5202 (2003)

40. Huntington JT, J.M.Shields, C.J.Der, C.A.Wyatt, U.Benbow, C.L.J.Slingluff &C.E.Brinckerhoff: Overexpression of collagenase 1 (MMP-1) is mediated by the ERK pathway in invasive melanoma cells: role of BRAF mutation and fibroblast growth factor signaling. *J Biol Chem* 279, 33168-33176 (2004)

41. Goodall J, C.Wellbrock, T.J.Dexter, K.Roberts, R.Marais &C.R.Goding: The Brn-2 transcription factor links activated BRAF to melanoma proliferation. *Mol Cell Biol* 24, 2923-2931 (2004)

42. King R, K.N.Weilbaecher, G.McGill, E.Cooley, M.Mihm &D.E.Fisher: Microphthalmia transcription factor. A sensitive and specific melanocyte marker for MelanomaDiagnosis. *Am J Pathol* 155, 731-738 (1999)

43. McGill GG, M.Horstmann, H.R.Widlund, J.Du, G.Motyckova, E.K.Nishimura, Y.L.Lin, S.Ramaswamy, W.Avery, H.F.Ding, S.A.Jordan, I.J.Jackson, S.J.Korsmeyer, T.R.Golub &D.E.Fisher: Bcl2 regulation by the melanocyte master regulator Mitf modulates lineage survival and melanoma cell viability. *Cell*109, 707-718 (2002)

44. Eisenmann KM, M.W.VanBrocklin, N.A.Staffend, S.M.Kitchen &H.M.Koo: Mitogen-activated protein kinase pathway-dependent tumor-specific survival signaling in melanoma cells through inactivation of the proapoptotic protein bad. *Cancer Res* 63, 8330-8337 (2003)

45. Zhang XD, J.M.Borrow, X.Y.Zhang, T.Nguyen &P.Hersey: Activation of ERK1/2 protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. *Oncogene* 22, 2869-2881 (2003)

46. Woods D, H.Cherwinski, E.Venetsanakos, A.Bhat, S.Gysin, M.Humbert, P.F.Bray, V.L.Saylor &M.McMahon: Induction of beta3-integrin gene expression by sustained activation of the Ras-regulated Raf-MEK-extracellular signal-regulated kinase signaling pathway. *Mol Cell Biol 7* 21, 3192-3205 (2001)

47. Petitclerc E, S.Stromblad, T.L.von Schalscha, F.Mitjans, J.Piulats, A.M.Montgomery, D.A.Cheresh &P.C.Brooks: Integrin alpha(v)beta3 promotes M21 melanoma growth in human skin by regulating tumor cell survival. *Cancer Res* 59, 2724-2730 (1999)

48. Hsu MY, M.J.Wheelock, K.R.Johnson &M.Herlyn: Shifts in cadherin profiles between human normal melanocytes and melanomas. *J Investig Dermatol Symp Proc* 1, 188-194 (1996)

49. Hsu MY, F.Meier &M.Herlyn: Melanoma development and progression: a conspiracy between tumor and host. *Differentiation* 70, 522-536 (2002)

50. Van Belle PA, R.Elenitsas, K.Satyamoorthy, J.T.Wolfe, D.Guerry, L.Schuchter, T.J.Van Belle, S.Albelda, P.Tahin, M.Herlyn &D.E.Elder: Progression-related expression of beta3 integrin in melanomas and nevi. *Hum Pathol* 30, 562-567 (1999)

51. Hofmann UB, J.R.Westphal, A.A.Van Kraats, D.J.Ruiter &G.N.Van Muijen: Expression of integrin alpha(v)beta(3) correlates with activation of membrane-type matrix metalloproteinase-1 (MT1-MMP) and matrix metalloproteinase-2 (MMP-2) in human melanoma cells in vitro and in vivo. *Int J Cancer* 87, 12-19 (2000)

52. Benbow U, G.B.Tower, C.A.Wyatt, G.Buttice &C.E.Brinckerhoff: High levels of MMP-1 expression in the absence of the 2G single nucleotide polymorphism is mediated by p38 and ERK1/2 mitogen-activated protein kinases in VMM5 melanoma cells. *J Cell Biochem* 86, 307-319 (2002)

53. Ge X, Y.M.Fu &G.G.Meadows: U0126, a mitogen-activated protein kinase kinase inhibitor, inhibits the invasion of human A375 melanoma cells. *Cancer Lett* 179, 133-140 (2002)

54. Tower GB, C.C.Coon, U.Benbow, M.P.Vincenti &C.E.Brinckerhoff: Erk 1/2 differentially regulates the expression from the 1G/2G single nucleotide polymorphism in the MMP-1 promoter in melanoma cells. *Biochim Biophys Acta* 1586, 265-274 (2002)

55. Bedogni B, M.S.O'Neill, S.M.Welford, D.M.Bouley, A.J.Giaccia, N.C.Denko &M.B.Powell: Topical treatment with inhibitors of the phosphatidylinositol 3'-kinase/Akt and Raf/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathways reduces melanoma development in severe combined immunodeficient mice. *Cancer Res* 64, 2552-2560 (2004)

56. Denkert C, A.Siegert, A.Leclere, A.Turzynski &S.Hauptmann: An inhibitor of stress-activated MAP-kinases reduces invasion and MMP-2 expression of malignant melanoma cells. *Clin Exp Metastasis* 19, 79-85 (2002)

57. Fresno VJ, E.Casado, J.de Castro, P.Cejas, C.Belda-Iniesta &M.Gonzalez-Baron: PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 30, 193-204 (2004)

58. Nicholson KM, N.G.Anderson: The protein kinase B/Akt signalling pathway in human malignancy. *Cell Signal* 14, 381-395 (2002)

59. Dhawan P, A.B.Singh, D.L.Ellis &A.Richmond: Constitutive activation of Akt/protein kinase B in melanoma leads to up-regulation of nuclear factor-kappaB and tumor progression. *Cancer Res* 62, 7335-7342 (2002)

60. Dai DL, M.Martinka &G.Li: Prognostic significance of activated Akt expression in melanoma: a clinicopathologic study of 292 cases. *J Clin Oncol* 23, 1473-1482 (2005)

61. Simpson L, R.Parsons: PTEN: life as a tumor suppressor. *Exp Cell Res* 264, 29-41 (2001)

62. Bastian BC, P.E.LeBoit, H.Hamm, E.B.Brocker &D.Pinkel: Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res* 58, 2170-2175 (1998)

63. Stahl JM, M.Cheung, A.Sharma, N.R.Trivedi, S.Shanmugam &G.P.Robertson: Loss of PTEN promotes

tumor development in malignant melanoma. *Cancer Res* 63, 2881-2890 (2003)

64. Stahl JM, A.Sharma, M.Cheung, M.Zimmerman, J.Q.Cheng, M.W.Bosenberg, M.Kester, L.Sandirasegarane &G.P.Robertson: Deregulated Akt3 activity promotes development of malignant melanoma. *Cancer Res* 64, 7002-7010 (2004)

65. Rodriguez-Viciana P, P.H.Warne, A.Khwaja, B.M.Marte, D.Pappin, P.Das, M.D.Waterfield, A.Ridley &J.Downward: Role of phosphoinositide 3-OH kinase in cell transformation and control of the actin cytoskeleton by Ras. *Cell* 89, 457-467 (1997)

66. Kauffmann-Zeh A, P.Rodriguez-Viciana, E.Ulrich, C.Gilbert, P.Coffer, J.Downward &G.Evan: Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB. *Nature* 385, 544-548 (1997)

67. Graells J, A.Vinyals, A.Figueras, A.Llorens, A.Moreno, J.Marcoval, F.J.Gonzalez &A.Fabra: Overproduction of VEGF concomitantly expressed with its receptors promotes growth and survival of melanoma cells through MAPK and PI3K signaling. *J Invest Dermatol* 123, 1151-1161 (2004)

68. Hsu MY, F.E.Meier, M.Nesbit, J.Y.Hsu, P.Van Belle, D.E.Elder &M.Herlyn: E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. *Am J Pathol* 156, 1515-1525 (2000)

69. Satyamoorthy K, Muyrers J, Meier F, Patel D, & erlyn M: Mel-CAM-specific genetic suppresor elements inhibit melanoma growth and invasion through loss of gap junctional communication. 20, 4676-4684 (2001)

70. Sturm RA, K.Satyamoorthy, F.Meier, B.B.Gardiner, D.J.Smit, B.Vaidya &M.Herlyn: Osteonectin/SPARC induction by ectopic beta(3) integrin in human radial growth phase primary melanoma cells. *Cancer Res* 62, 226-232 (2002)

71. Testa JR, A.Bellacosa: AKT plays a central role in tumorigenesis. *Proc Natl Acad Sci U S A* 98, 10983-10985 (2001)

72. Kandasamy K, R.K.Srivastava: Role of the phosphatidylinositol 3'-kinase/PTEN/Akt kinase pathway in tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in non-small cell lung cancer cells. *Cancer Res* 62, 4929-4937 (2002)

73. Wang Q, X.Wang, A.Hernandez, M.R.Hellmich, Z.Gatalica &B.M.Evers: Regulation of TRAIL expression by the phosphatidylinositol 3-kinase/Akt/GSK-3 pathway in human colon cancer cells. *J Biol Chem* 277, 36602-36610 (2002)

74. Yuan XJ, Y.E.Whang: PTEN sensitizes prostate cancer cells to death receptor-mediated and drug-induced apoptosis through a FADD-dependent pathway. *Oncogene* 21, 319-327 (2002)

75. Jiang K, J.Sun, J.Cheng, J.Y.Djeu, S.Wei &S.Sebti: Akt mediates Ras downregulation of RhoB, a suppressor of transformation, invasion, and metastasis. *Mol Cell Biol* 24, 5565-5576 (2004)

76. Jetzt A, J.A.Howe, M.T.Horn, E.Maxwell, Z.Yin, D.Johnson &C.C.Kumar: Adenoviral-mediated expression of a kinase-dead mutant of Akt induces apoptosis selectively in tumor cells and suppresses tumor growth in mice. *Cancer Res* 63, 6697-6706 (2003)

77. Krasilnikov M, V.N.Ivanov, J.Dong &Z.Ronai: ERK and PI3K negatively regulate STAT-transcriptional activities in human melanoma cells: implications towards sensitization to apoptosis. *Oncogene* 22, 4092-4101 (2003)

78. Murakami T, A.R.Cardones, S.E.Finkelstein, N.P.Restifo, B.A.Klaunberg, F.O.Nestle, S.S.Castillo, P.A.Dennis &S.T.Hwang: Immune evasion by murine melanoma mediated through CC chemokine receptor-10. *J Exp Med* 198, 1337-1347 (2003)

79. Stewart AL, A.M.Mhashilkar, X.H.Yang, S.Ekmekcioglu, Y.Saito, K.Sieger, R.Schrock, E.Onishi, X.Swanson, J.B.Mumm, L.Zumstein, G.J.Watson, D.Snary, J.A.Roth, E.A.Grimm, R.Ramesh &S.Chada: PI3 kinase blockade by Ad-PTEN inhibits invasion and induces apoptosis in RGP and metastatic melanoma cells. *Mol Med* 8, 451-461 (2002)

80. Larribere L, M.Khaled, S.Tartare-Deckert, R.Busca, F.Luciano, K.Bille, G.Valony, A.Eychene, P.Auberger, J.P.Ortonne, R.Ballotti &C.Bertolotto: PI3K mediates protection against TRAIL-induced apoptosis in primary human melanocytes. *Cell Death Differ* 11, 1084-1091 (2004)

81. Mills L, C.Tellez, L.Baker, J.Green, J.Gudas, X.Feng, & M.Bar-Eli: Fully human antibodies to MUC18 inhibits tumor growth and metastasis of human melanoma. *Cancer Res* 62, 5106-5114 (2002)

82. Diehl JA, M.Cheng, M.F.Roussel &C.J.Sherr: Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* 12, 3499-3511 (1998)

83. Zhou BP, Y.Liao, W.Xia, Y.Zou, B.Spohn &M.C.Hung: HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nat Cell Biol* 3, 973-982 (2001)

84. Liang J, J.Zubovitz, T.Petrocelli, R.Kotchetkov, M.K.Connor, K.Han, J.H.Lee, S.Ciarallo, C.Catzavelos, R.Beniston, E.Franssen &J.M.Slingerland: PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat Med* 8, 1153-1160 (2002)

85. Sherr CJ, J.D.Weber: The ARF/p53 pathway. *Curr Opin Genet Dev* 10, 94-99 (2000)

86. Tee AR, D.C.Fingar, B.D.Manning, D.J.Kwiatkowski, L.C.Cantley &J.Blenis: Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. *Proc Natl Acad Sci U S A* 99, 13571-13576 (2002)

87. Potter CJ, L.G.Pedraza &T.Xu: Akt regulates growth by directly phosphorylating Tsc2. *Nat Cell Biol* 4, 658-665 (2002)

88. Manning BD, A.R.Tee, M.N.Logsdon, J.Blenis &L.C.Cantley: Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. *Mol Cell* 10, 151-162 (2002)

89. Inoki K, Y.Li, T.Zhu, J.Wu &K.L.Guan: TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nat Cell Biol* 4, 648-657 (2002)

90. Dan HC, M.Sun, L.Yang, R.I.Feldman, X.M.Sui, C.C.Ou, M.Nellist, R.S.Yeung, D.J.Halley, S.V.Nicosia, W.J.Pledger &J.Q.Cheng: Phosphatidylinositol 3-kinase/Akt pathway regulates tuberous sclerosis tumor suppressor complex by phosphorylation of tuberin. *J Biol Chem* 277, 35364-35370 (2002)

91. Holzmann B, E.B.Brocker, J.M.Lehmann, D.J.Ruiter, C.Sorg, G.Riethmuller &J.P.Johnson: Tumor progression in human malignant melanoma: five stages defined by their antigenic phenotypes. *Int J Cancer* 39, 466-471 (1987)

92. Xie S, M.Luca, S.Huang, M.Gutman, R.Reich, J.P.Johnson &M.Bar-Eli: Expression of MCAM/MUC18 by human melanoma cells leads to increased tumor growth and metastasis. *Cancer Res* 57, 2295-2303 (1997)

93. Shih IM, D.Speicher, M.Y.Hsu, E.Levine &M.Herlyn: Melanoma cell-cell interactions are mediated through heterophilic Mel-CAM/ligand adhesion. *Cancer Res* 57, 3835-3840 (1997)

94. Ahmad T, R.Marais, L.Pyle, M.James, B.Schwartz, M.Gore, & T.Eisen: BAY 43-9006 in patients with advanced melanoma: The Royal Marsden experience. *Journal of Clinical Oncology*, ASCO Annual Meeting Proceedings 22, 7506 (2004)

95. Flaherty K T, M.Brose, L.Schuchter, D.Tuveson, R.Lee, B.Schwartz, C.Lathia, B.Weber, & P.O'Dwyer: Phase I/II trial of BAY 43-9006, carboplatin (C) and paclitaxel (P) demonstrates preliminary antitumor activity in the expansion cohort of patients with metastatic melanoma. *Journal of Clinical Oncology*, ASCO Annual Meeting Proceedings 22, 7507 (2004)

96. Druker BJ, C.L.Sawyers, H.Kantarjian, D.J.Resta, S.F.Reese, J.M.Ford, R.Capdeville &M.Talpaz: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344, 1038-1042 (2001)

97. Demetri GD, M.von Mehren, C.D.Blanke, A.D.Van den Abbeele, B.Eisenberg, P.J.Roberts, M.C.Heinrich, D.A.Tuveson, S.Singer, M.Janicek, J.A.Fletcher, S.G.Silverman, S.L.Silberman, R.Capdeville, B.Kiese, B.Peng, S.Dimitrijevic, B.J.Druker, C.Corless, C.D.Fletcher &H.Joensuu: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 347, 472-480 (2002)

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