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New Molecular Targets for the Systemic Therapy of Melanoma

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1. Introduction

Melanoma is one of the most deadly forms of skin cancer. The incidence of melanoma has been steadily increasing over the last several decades. It is estimated that in 2010 68,130 adults were diagnosed with melanoma, and 8,700 patients died of this disease (Jemal et al.). Melanoma is highly curable when it is diagnosed at early stages. However, patients with distant metastases have a median overall survival of only 6-8 months (Balch et al. 2009). Chemotherapy regimens have not improved survival in patients with metastatic melanoma, and immunotherapies have generally benefited only a small percentage of patients (Koon and Atkins 2006). Thus, there is a critical need to develop more effective therapeutic approaches for this disease. Recently, dramatic results have been reported with agents that specifically target proteins or pathways that are aberrant in this disease, such as *BRAF* and *c-KIT* (Flaherty et al. ; Hodi et al. 2008). These results support the rationale for continued investigation into the molecular events that characterize and contribute to melanoma. This review will describe existing knowledge about several of the molecules and pathways that have been implicated in melanoma, and review the results of clinical studies focused on these targets.

2. Melanoma molecular targets

Melanoma has traditionally been classified based on the clinical and pathological features of the tumor. The most commonly observed type of melanoma is cutaneous melanoma (CM), arising from skin with either intermittent or chronic sun exposure. While ultraviolet radiation likely has a significant causative role in these tumors, its role in certain other subtypes is less clear. Cutaneous melanomas can arise in areas with limited sun/UV radiation exposure such as palms, soles and the area under nails (acral lentiginous melanoma). Other melanomas arise from mucosal surfaces of the body, including the upper aerodigestive, gastrointestinal, and genitourinary tracts, and are termed mucosal melanomas. Melanomas also originate from melanocytes in the uveal tract of the eye (uveal/ocular melanoma). In addition to anatomic differences, recent research has demonstrated that the different melanoma subtypes are characterized by distinct regions of DNA copy number gain and loss (Curtin et al. 2005). This finding suggested that each of these tumor types could be characterized by distinct molecular mechanisms, a hypothesis that is also supported by the marked variance of recently described oncogenic mutations across the different melanoma subtypes.

2.1 RAS/RAF/MAPK pathway

The RAS/RAF/MAPK cascade is a critical growth and survival signaling pathway in cells. The pathway is generally triggered by activation of cell surface receptor(s) [i.e., receptor tyrosine kinases (RTK), G-protein coupled receptors (GPCR), etc] following ligand binding or cell-to-cell contact. The receptors induce activation of RAS through guanine exchange factors (GEFs), which promote the exchange of RAS-GDP to RAS-GTP. GTP-bound RAS recruits and activates the RAF (A-, B- and C-RAF) family of serine-threonine kinases, which then phosphorylate and activate Mitogen Activated Kinase Kinase [MAPKK or MAP/ERK kinase (MEK)]. Phosphorylated MEK, which is also a kinase, activates the downstream Extracellular Regulatory Kinase (ERK1/2 or P44/42 MAPK) through phosphorylation. Once activated, ERK translocates to the nucleus where it regulates the expression of several genes involved in differentiation, survival and proliferation by phosphorylating transcription factors such as ETS, MYC etc. The MAPK pathway also regulates the apoptotic machinery in cells through post-translational regulation of BAD, BIM, MCL-1 and BCL-2 proteins (George, Thomas, and Hannan).

In addition to RAF, the RAS proteins activate several other effectors that contribute to the pro-survival and proliferative phenotype, including phospholipase C (PLC), phosphatidyl inositol-3-Kinase (PI3K), Ral, Rac and Rho-GTPases. Mutations in the *RAS* family genes (*HRAS*, *NRAS* and *KRAS*) have been detected in approximately one-third of all cancers, including pancreatic, colon, leukemia and thyroid cancers (Bos et al. 1987; Bos et al. 1985; Almoguera et al. 1988; Forrester et al. 1987; Padua, Barrass, and Currie 1985). Activating mutations of *RAS* have been reported in 15-20% of melanomas, and almost exclusively involve the *NRAS* isoform (Tsao et al. 2000). *NRAS* mutations are highly conserved in melanoma, as over 90% of the detected mutations occur in codons 12, 13 and 61 (Hocker and Tsao 2007). *NRAS* mutations occur in 26% of cutaneous and 14% of mucosal melanomas, but only in 4% of acral and less than 1% of uveal melanomas (Hocker and Tsao 2007). Mutant RAS proteins have very little GTPase activity, and thus remain constitutively active. This results in aberrant regulation of its downstream signaling pathways and subsequent uncontrolled cell proliferation and survival. RAS also promotes suppression of $p16^{INK4a}$ and p53 in melanoma models, and knockdown of mutated *H-RAS* ($H\text{-}Ras^{V12G}$) using siRNAs in an doxycycline inducible melanoma mouse model resulted in tumor regression (Chin et al. 1997; Chin et al. 1999).

Activating mutations in the serine/threonine kinase *BRAF* were first reported by Davies and colleagues in 2002, who demonstrated in a small cohort of tumors and cancer cell lines that 66% of melanomas harbored somatic mutations in *BRAF*, which were also detected in smaller fractions of gliomas, colon and ovarian cancer samples. A recent meta-analysis reported *BRAF* mutations in 43% of melanoma clinical specimens and 65% of human melanoma cell lines (Hocker and Tsao 2007). *BRAF* mutations were detected most frequently in cutaneous melanomas (42.5%), but were markedly less common in acral (18.1%), mucosal (5.6%), and uveal (<1%) melanomas. Some studies have also reported significantly lower rates of *BRAF* mutations in cutaneous melanomas with chronic sun damage (Curtin et al. 2005), but this has not been recapitulated in other studies (Handolias, Salemi et al. 2010). Approximately 40 different *BRAF* mutations have been identified in melanoma. The most frequent mutation (approximately 90% of mutations in clinical samples) arises due to a T→A transversion in position 1799 of the *BRAF* gene (T1799A) resulting in the substitution of glutamic acid for valine at position 600 ($BRAF^{V600E}$) of the BRAF protein acid, which has markedly increased catalytic activity compared to the wild-type BRAF protein (Davies et al. 2002; Wan et al. 2004; Hocker and Tsao 2007). Interestingly, some of the *BRAF* mutations that have been detected in cancer do not increase the catalytic activity of the BRAF protein,

but still result in hyperactivation of MEK and ERK through efficient dimerization with other RAF isoforms (Garnett et al. 2005; Heidorn et al. 2010).

In melanoma, activating *BRAF* and *NRAS* mutations are almost always mutually exclusive, but overlap can occur with non-activating *BRAF* mutations (Heidorn et al. 2010; Tsao et al. 2004). While *BRAF* mutations are extremely common in melanoma, there is significant evidence that they must be complemented by additional genetic events in melanomagenesis. Pollock *et al* (2002) reported that the $BRAF^{V600E}$ mutation is detectable in 82% of benign nevi, which have virtually no malignant potential. In addition, expression of the $BRAF^{V600E}$ mutation alone in melanocytes failed to induce transformation in several preclinical models, including zebrafish and mice (Patton et al. 2005). Invasive lesions were only seen when other molecules were inactivated concurrently, such as p16/Ink4a, p53, and *PTEN* or *p53* (Dankort et al. 2009; Chudnovsky et al. 2005).

The dual specificity kinases MEK1/2 that lie downstream of BRAF are activated in majority of the cancers with deregulated RAS/RAF/MAPK signaling. The MEK kinases phosphorylate ERK1/2 downstream and mediate cell survival signaling through MAPK signaling cascade. Emery *et al*. (2009), using random mutagenesis and massive parallel sequencing approaches identified mutations in the drug binding and regulatory domains of MEK1 kinase that led to increased phosphorylation of ERK and a MEK inhibitor-resistance phenotype. Subsequently, *MEK1* point mutations P124L and C121S have been detected in melanoma patients who progressed after initial clinical responses to MEK or BRAF inhibitors (Wagle et al. ; Emery et al. 2009). To date no *MEK1* or *MEK2* mutations have been reported *de novo* in melanoma.

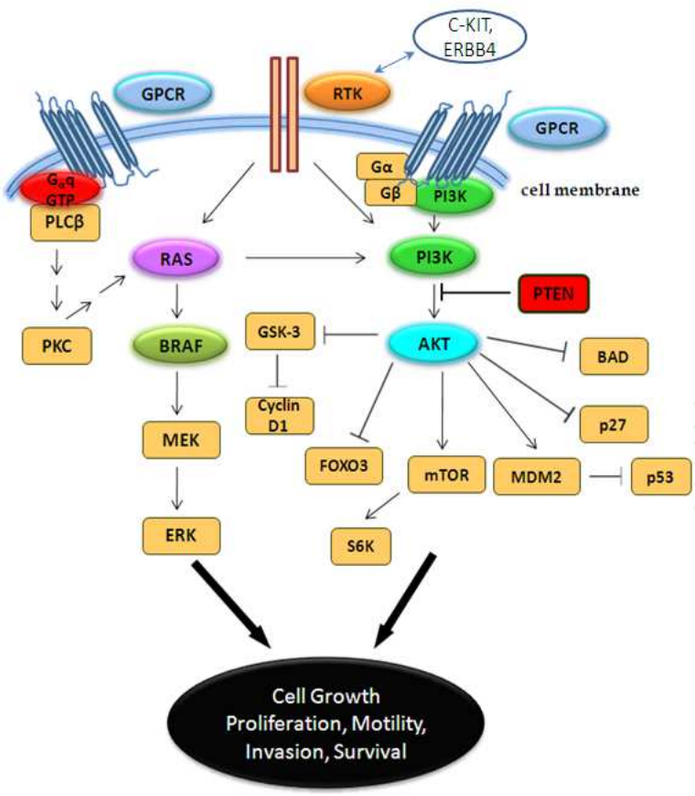


Fig. 1. Molecular targets in melanoma. The diagram illustrates pathways that are affected by prevalent genetic alterations in melanoma.

2.2 PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR pathway is one of the most important intracellular signaling pathways. The pathway regulates many important cellular processes, including proliferation, differentiation, motility, metabolism, survival, invasion and intracellular transport (Engelman, Luo, and Cantley 2006). The Phosphatidyl Inositol-3 Kinases (PI3K) are a family of lipid kinases that are composed of an adaptor/regulatory subunit (i.e. p85) and a catalytic unit (i.e. p110). Similar to RAS/RAF/ERK, the PI3K/AKT/mTOR pathway is activated by a variety of signals, including receptor tyrosine kinases and RAS proteins. Activation of PI3K results in phosphorylation of phosphatidylinositols in the cell membrane at the 3'-hydroxyl group. This reaction generates the lipid species PI $(3,4)P_2$ and PI$(3,4,5)P_3$. PI $(3,4)P_2$ and PI$(3,4,5)P_3$ act as second messengers, recruiting proteins that contain a pleckstrin homology (PH) domain to the cell membrane, such as the serine/threonine kinases AKT and PDK1. Upon recruitment to the cell membrane, AKT is phosphorylated at two critical residues, serine 473 and threonine 308. Once phosphorylated, the activated AKT translocates to the cytosol where it promotes cell proliferation and survival by phosphorylating numerous substrate proteins including mTOR, GSK3, FOXO, and BAD, among others.

The activity of the PI3K/AKT/mTOR pathway is normally controlled by the lipid phosphatase PTEN (Phosphatase and Tensin Homolog), which dephosphorylates phosphatidyl inositols (PI) at the 3' position, thereby inhibiting PI3K-mediated signaling (Maehama and Dixon 1998). PTEN, which is a tumor suppressor, is inactivated in a variety of tumor types, through both genetic and epigenetic mechanisms (Li et al. 1997; Myers et al. 1998; Mirmohammadsadegh et al. 2006). Tumors with loss of PTEN are characterized by markedly increased basal activation of AKT (Davies et al. 1999; Davies et al. 1998; Davies et al. 2009).

In melanoma, *PTEN* loss is observed in up to 20% of tumors and 30% of melanoma cell lines (Tsao et al. 1998; Tsao, Mihm, and Sheehan 2003; Tsao et al. 2000). This prevalence is primarily defined for cutaneous melanomas; the prevalence in other subtypes is poorly described. Similar to BRAF, loss of PTEN appears to be mutually exclusive with the presence of an *NRAS* mutation in melanoma tumors and cell lines. While this pattern suggests functional redundancy, quantitative analysis of AKT activation demonstrated that PTEN loss correlated with significantly higher levels of phosphorylated AKT than *NRAS* mutations in both clinical specimens and cell lines (Davies et al. 2009). In contrast to NRAS, PTEN loss frequently occurs in melanomas with a concurrent activating *BRAF* mutation (Tsao et al. 2000; Goel et al. 2006; Tsao et al. 2004). The functional nature of this pattern is supported by mouse studies, which demonstrated that crossing mice with the $BRAF^{V600E}$ mutation in melanocytes with mice that harbour PTEN loss resulted in frankly invasive and metastatic melanomas, which did not occur with either lesion alone (Dankort et al. 2009).

In addition to loss of PTEN, the PI3K/AKT/mTOR pathway may also be activated by gene amplifications and gain of function mutations in other pathway components. Rare activating mutations in *PI3KCA* have been detected in 2-3% of melanomas (Omholt et al. 2006). Studies by Stahl *et al*, identified activation of AKT3 in 43 to 60% of sporadic melanomas, which was associated with an increase in copy number of the *AKT3* gene along with a simultaneous decreased activity of PTEN, either due to loss or haploinsufficiency of the *PTEN* gene. Knockdown of *AKT3* by siRNA induced apoptosis and reduced melanoma tumor development (Stahl et al. 2004). Davies *et al* recently also reported rare gain of function point mutations in the regulatory pleckstrin homology domains of *AKT1* and *AKT3* (*AKT1 E17K*,

AKT3 E17K) in ~2% melanoma cell lines and tumor specimens (Davies et al. 2008). Every melanoma with an *AKT1* or *AKT3* mutation also had a concurrent *BRAF* mutation.

2.3 Receptor tyrosine kinases

Activating mutations or amplifications of receptor tyrosine kinases are implicated in multiple tumor types, including gastrointestinal stromal tumors (GIST) (*c-KIT*), breast (*HER2/neu*), and lung (*EGFR*) cancers. However, until recently there has been little evidence of significant aberrations in melanoma. The relatively low rate of *BRAF* and *NRAS* mutations in non-cutaneous melanomas led to focused searches for other oncogenic drivers in these tumor types. Comparative genome hybridization (CGH) analysis identified selective amplification of the 4q12 chromosomal region in acral and mucosal melanomas (Curtin et al. 2005). Detailed analysis of the genes in this region identified focal amplifications of the *c-KIT* gene (Curtin et al. 2006). C-KIT is a receptor tyrosine kinase which is affected by activating mutations in ~80% of GISTs (Hirota et al. 1998). Subsequent to the discovery of gene amplifications, sequencing demonstrated that the *c-KIT* gene is also frequently mutated in the same melanoma subtypes in which amplifications had been detected(Curtin et al. 2006). Overall, *c-KIT* gene amplification or mutation was identified in 39% of mucosal and 36% of acral melanomas. Among cutaneous melanomas, *c-KIT* gene alterations were also detected in 28% of cutaneous melanomas with chronic sun damage (CSD), but no *c-KIT* gene aberrations were reported in cutaneous melanomas without CSD (Curtin et al. 2006). However, other studies have reported lower rates of *c-KIT* alterations in cutaneous melanomas with CSD (Handolias, Salemi et al. 2010). The mutations in *c-KIT* gene generally affect the same exons that are mutated in GIST, although the distribution in melanoma shows a higher prevalence of mutations in exons associated with resistance to many c-KIT inhibitors. Interestingly, while most *c-KIT* mutations in GIST are short insertions or deletions, the overwhelming majority of changes in melanoma are point mutations, with the most common event being the L576P substitution at exon 11 of the juxtamembrane region (Beadling et al. 2008). The finding of activating *c-KIT* mutations was surprising, as previous reports had demonstrated that although c-KIT is essential for the development of normal melanocytes, c-KIT activation suppressed the growth of melanoma cells, and melanoma progression was associated with loss of c-KIT expression (Huang et al. 1996; Lassam and Bickford 1992). However, the lack of mutations in *c-KIT* in cutaneous melanomas suggests that other lineage-specific genetic or environmental factors in the non-cutaneous melanocytes may critically interact with the *c-KIT* mutations.

More recently, high-throughput sequencing analysis of all protein kinases identified novel somatic mutations in 19 different genes (Prickett et al. 2009). The most frequently mutated gene was *ERBB4* (24 missense mutations in 15 patients; 19% prevalence in the cohort), which encodes a receptor tyrosine kinase that is a member of the epidermal growth factor receptor family (EGFR, HER2, HER3). *ERBB4* mutations have previously been reported in lung, colon, stomach and breast cancers (Soung et al. 2006; Ding et al. 2008). Interestingly, the mutations in the melanomas were distributed throughout the entire *ERBB4* gene. Despite this unusual pattern for an activating event, Pickett et al., found that every tumor-derived mutation *ERBB4* tested had higher levels of receptor tyrosine kinase activity, promoted anchorage independent growth, and induced cellular transformation (Prickett et al. 2009). In contrast to the distinct patterns seen with other mutations, *ERBB4* mutations were not mutually exclusive with *BRAF* or *NRAS* mutations. Further investigation is needed to gain a

better understanding of the role of ERBB4 in melanoma, and to understand the therapeutic potential of inhibitors against this target.

2.4 G proteins

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are a diverse family of proteins that regulate and propagate signals from G-Protein Coupled Receptors (GPCRs) that are expressed at the cell membrane. The complex of G-proteins and GPCRs activate several key signaling pathways involved in cell survival, proliferation, and transformation. There is growing evidence that this family of genes may play a significant role in certain subtypes of melanoma.

A role for G proteins in melanoma was first suggested by a preclinical study that was designed to identify genes that promote melanin synthesis and pigmentation in mice. Two different G protein alpha subunits, *GNAQ* and *GNA11,* were identified in this screen (Van Raamsdonk et al. 2004). In order to determine the clinical relevance of these genes in patients, panels of melanomas and nevi were then screened for alterations in these genes. Remarkably, point mutations in *GNAQ* were identified in ~50% of primary uveal melanomas (Onken et al. 2008; Van Raamsdonk, Bezrookove, Green, Bauer, Gaugler, O/'Brien et al. 2009). *GNAQ* mutations were also identified in 50-80% of blue nevi, and in 6% of rare lesions called nevi of Ota, which are associated with an increased risk of uveal melanoma (Van Raamsdonk, Bezrookove, Green, Bauer, Gaugler, O/'Brien et al. 2009). In contrast, no *GNAQ* mutations were identified in cutaneous melanomas without chronic sun damage, acral melanomas, or mucosal melanomas; 1 of 27 cutaneous melanomas with chronic sun damage tested had a mutation. Further sequencing identified point mutations of *GNA11* in 32% of primary uveal melanomas, all of which were mutually exclusive with *GNAQ* mutations (Van Raamsdonk et al. 2010). Interestingly, analysis of a cohort of uveal melanoma metastases identified a higher prevalence of *GNA11* (56%) than *GNAQ* (22%) mutations. Overall, somatic mutations in *GNAQ* and *GNA11* were detected in over 80% of all uveal melanomas analyzed (Van Raamsdonk, Bezrookove, Green, Bauer, Gaugler, O'Brien et al. 2009).

Over 90% of the reported mutations in *GNAQ* and *GNA11* affect the Q209 residue. This occurs in a RAS-like domain of these proteins, and is specifically analogous to the Q61 residue that is the most common site of point mutations in the *RAS* gene. Functional studies of the Q209L mutation in both *GNAQ* and *GNA11* demonstrated that this mutation promotes anchorage independent growth, tumorigenicity, and activation of the RAS/RAF/MAPK pathway (Van Raamsdonk, Bezrookove, Green, Bauer, Gaugler, O/'Brien et al. 2009; Van Raamsdonk et al. 2010). These findings suggest that these mutations may obviate the requirement for *BRAF* or *NRAS* mutations, which are virtually non-existant in uveal melanomas (Cohen et al. 2003; Rimoldi et al. 2003). Studies to determine the various functions of *GNAQ* and *GNA11* mutations in uveal melanoma, and determine the therapeutic potential of inhibiting these genes or their effectors, are currently ongoing.

In addition to these mutations in uveal melanoma, high-throughput sequencing for mutations in G protein family members in cutaneous melanomas identified 18 non-synonymous somatic mutations in G protein subunits spanning seven genes (Cardenas-Navia et al.). Mutations were identified in *GNA12, GNG10, GNAZ, GNG14, GNA15, GNA11, and GNB3* (Cardenas-Navia et al.). Further work needs to be done to understand the pathways and processes affected by these mutations.

2.5 Other affected genes

Alterations in several regulators of cell cycle progression have also been implicated in melanoma. Allelic alterations in *CDK4* and *CCND1* have been reported in melanoma (Curtin et al. 2005; Smalley et al. 2008). Inactivation of the tumor suppressor *p53,* which is associated with DNA damage and metabolic stress, has also been reported. (Yang, Rajadurai, and Tsao 2005; Jonsson et al. 2007). P53 may also be functionally inactivated by loss of function of *p16^{INK4a}/p14^{ARF}* genes (Pomerantz et al. 1998; Stott et al. 1998; Zhang, Xiong, and Yarbrough 1998). Loss of function of p16^{INK4a}/p14^{ARF} is present in most familial melanomas (Cannon-Albright et al. 1992; Goldstein et al. 2007). Loss of both *p16* and *p14* is seen in both melanoma cell lines and primary tumors and is a selection factor for the survival of melanoma cells *in vitro* (Daniotti et al. 2004; Rakosy et al. 2008). However, currently there are no therapies in place to restore the expression of these tumor suppressors.

A comparative genetic analysis of melanomas with other tumor types identified selective amplifications of the gene encoding the microphthalmia-associated transcription factor (*MITF*) in melanoma (Garraway et al. 2005*). MITF* is a transcription factor that regulates many genes associated with melanin production and melanocyte development (Levy, Khaled, and Fisher 2006). Initial studies demonstrated the MITF could function as an oncogene, and was able to cooperate with the mutant *BRAF* gene to induce transformation of normal melanocytes (Garraway et al. 2005). Amplification of the MITF locus occurs in 10-20% melanomas, and subsequent studies have also detected rare somatic mutations in the gene in melanomas (Cronin et al. 2009).

3. Clinical targeting of activated pathways in melanoma

The treatment of many cancers has changed dramatically due to an improved understanding of the genes and pathways that contribute to the aggressive nature of many of these diseases. The discovery of activating events in kinase signaling pathways in melanoma rapidly led to clinical testing of a number of targeted therapies for this disease. The early results illustrate both the promise and challenge of this strategy.

3.1 The RAS/RAF/MAPK pathway

The high prevalence of mutations in components of the RAS/RAF/MAPK pathway in melanoma, particularly in the most common subtype (cutaneous), strongly supports the rationale to test the clinical efficacy of drugs against it. After the discovery of activating *BRAF* mutations (Davies et al. 2002), the first drug against the pathway to be tested clinically was sorafenib. Sorafenib is a small molecule that inhibits a number of kinases, including BRAF, CRAF, vascular endothelial growth factor receptor (VEGFR), platelet derived growth factor receptor (PDGFR), and c-Kit (Strumberg 2005). Preclinical studies demonstrated that sorafenib slowed the growth of melanoma xenografts with activating *BRAF* mutations, but did not result in tumor eradication (Karasarides et al. 2004). Subsequently, in a single-agent phase II trial, treatment with sorafenib resulted in only 1 clinical response among 34 evaluable patients (Eisen et al. 2006). More promising results were observed in a phase I trial that tested the safety of combined treatment with sorafenib, carboplatin, and paclitaxel. Ten clinical responses were observed, all of which occurred in patients with metastatic melanoma [n=24; 40% overall response rate (ORR)] (Flaherty et al. 2008). Of note, the clinical benefit among the melanoma patients did not correlate with the presence of activating *BRAF* mutations. Despite these promising results, a subsequent randomized phase III trial of treatment with carboplatin and

paclitaxel with or without sorafenib definitively showed that sorafenib did not improve the ORR or progression-free survival (PFS) that was achieved with the chemotherapy agents alone (Hauschild et al. 2009). Combined with preclinical studies that showed the high prevalence of *BRAF* mutations in benign nevi, and induction of cellular senescence only following expression of mutant *BRAF* in normal melanocytes, these results raised doubts about the value of *BRAF* as a therapeutic target.

The identification of mutant *BRAF* as a therapeutic target in melanoma has now been confirmed by clinical trials with potent, selective second-generation BRAF inhibitors. PLX4032 (vemurafenib) is a small molecule that has an *in vitro* IC50 for the $BRAF^{V600E}$ protein of ~ 10 nM. This is one log lower than the IC50 for the wild-type BRAF protein, and 2-3 logs lower than the IC50 for other related kinases (Tsai et al. 2008). Experiments in xenografts models demonstrated that PLX4720, a closely related compound used for preclinical studies, eradicated melanomas with a $BRAF^{V600E}$ mutation (Yang et al. 2010). More importantly, the phase I trial of PLX4032 in patients with advanced melanoma reported an unconfirmed ORR of 81% among patients with the $BRAF^{V600E}$ mutation (Flaherty et al. 2010). The selectivity of the agent *in vivo* is supported by the relatively mild toxicity of the drug, which was well-tolerated by patients. In addition, no clinical responses were observed in the 5 patients included in the trial who had a wild-type *BRAF* gene. In fact, 4 of those patients demonstrated clinical progression of disease at their initial restaging. This clinical finding is consistent with work in preclinical models that demonstrated that treatment of human melanoma cell lines with a wild-type *BRAF* gene with PLX4720 and other selective *BRAF* inhibitors resulted in hyperactivation of MEK and MAPK, and increased growth of cancer cells *in vitro* and *in vivo* (Halaban et al. 2010; Hatzivassiliou et al. 2010; Heidorn et al. 2010; Poulikakos et al. 2010). A second selective inhibitor of the $BRAF^{V600E}$ protein, GSK2118436, has demonstrated similar activity, with a 62% ORR in phase I testing in advanced melanoma patients with a *BRAF* mutation (Kefford et al. 2010).

While the high response rate with minimal toxicity with PLX4032 and GSK2118436 is unprecedented, it is now becoming clear that resistance will be a major problem with these agents. In the phase I trial of PLX4032, virtually all patients who responded clinically went on to develop disease progression, with a median duration of response of approximately 7 months (Flaherty et al. 2010). While the experience with resistance to targeted therapies in other diseases made it reasonable to hypothesize that secondary *BRAF* mutations could cause this, to date the analysis of tumors and cell lines with secondary resistance to selective BRAF inhibitors have failed to identify any such mutations (Nazarian et al. 2010; Villanueva et al. 2010). Instead, cells lines and tumors have developed changes that either maintain activation of the RAS/RAF/MAPK pathway in the presence of the BRAF inhibitors, or changes that allow cells to survive even when that pathway is inhibited. Mechanisms that result in continued activation of the RAS/RAF/MAPK pathway include (1) concurrent *NRAS* or *MEK1* mutation (Nazarian et al. 2010; Wagle et al.), (2) induction of the serine-threonine kinase COT1 (Johannessen et al. 2010), or (2) utilization of all 3 RAF isoforms to activate MEK (Villanueva et al. 2010). Mechanisms that result in resistance to cell killing despite continued inhibition of MEK and MAPK generally implicate activation of the PI3K-AKT pathway, either through the increased expression of receptor tyrosine kinases or through the loss of PTEN (Nazarian et al. 2010; Villanueva et al. 2010). Activation of the PI3K-AKT pathway by loss of PTEN also results in *de novo* resistance to cell killing by BRAF inhibitors, but the relationship between PTEN loss and clinical responsiveness in patients has yet to be determined (Paraiso et al. 2011).

In addition to BRAF inhibitors, MEK inhibitors have shown promise in the treatment of metastatic melanoma. The initial presentation of the preliminary results of the phase I trial of GSK1120212, an orally available MEK inhibitor with a very long half life, reported a 40% ORR response among patients with metastatic melanoma (Infante et al. 2010). This response rate is higher than previous reports with other MEK inhibitors, such as AZD6244 (Dummer et al. 2008). Preclinical studies demonstrated that, similar to the results with BRAF inhibitors, loss of PTEN correlates with increased resistance to cell killing by MEK inhibitors (Gopal et al. 2010). Interestingly, several cells with normal PTEN expression but similar resistance developed activation of the PI3K-AKT pathway following treatment with MEK inhibitors. This compensatory mechanism, which was mediated by the insulin-like growth factor-1 receptor, gives further support to the rationale for testing the effects of targeted therapy combinations to improve clinical results with both BRAF and MEK inhibitors.

3.2 c-KIT

Imatinib, a small molecule inhibitor of a number of kinases, is approved by the FDA for the first-line treatment of metastatic GISTs, which are characterized by a high (~80%) prevalence of activating mutations in the *c-KIT* gene. This clinical experience gave cause for optimism for the use of imatinib in melanoma, a mesenchymal tumor like GIST with very poor responsiveness to cytotoxic chemotherapies. Prior to the identification of *c-KIT* mutations in acral and mucosal melanomas, three different phase II trials with imatinib were conducted in advanced melanoma patients (Kim et al. 2008; Ugurel et al. 2005; Wyman et al. 2006). The cumulative ORR was only 1.5% for these trials. However, the patients overwhelmingly consisted of patients with cutaneous primary melanomas, and thus were unlikely to harbor activating *c-Kit* mutations.

There are now several case reports describing impressive clinical responses to c-KIT inhibitors in melanoma patients with mutations in the *c-KIT* gene (Handolias, Hamilton et al. 2010; Hodi et al. 2008). In addition, relatively large clinical trials are ongoing testing the efficacy of c-KIT inhibitors in this patient population. An initial report from one of the imatinib trials reported an ORR of approximately 50% among patients with *c-KIT* mutations, but 0 of 10 patients with only gene amplification of the wild-type gene responded (Fisher et al. 2010). In addition, while c-KIT inhibitors have induced some durable responses, other dramatic responses have lasted for only a few months (Woodman et al. 2009). Thus, the further development of therapies for melanoma patients with *c-KIT* mutations will likely require an improved understanding of mechanisms of resistance to these agents, and combinatorial approaches.

Affected Gene	Subtypes Affected	Classes of Inhibitors
BRAF	Cutaneous >> Acral > Mucosal	Selective BRAF inhibitors Non-Selective BRAF Inhibitors MEK inhibitors
NRAS	Cutaneous > Acral > Mucosal	Farnesyl transferase inhibitors
PI3K/PTEN/AKT	(Undefined)	AKT inhibitors PI3K inhibitors Dual PI3K/mTOR inhibitors mTORC1/2 inhibitors
c-KIT	Mucosal > Acral >> Cutaneous	c-KIT inhibitors
ERBB4	(Undefined)	HER-family inhibitors
GNAQ, GNA11	Uveal	MEK inhibitors

Table 1. Molecular Targets in Melanoma

4. Summary

The testing and treatment of melanoma patients is evolving rapidly due to an improved understanding of the genes and pathways that are genetically altered in this disease. The dramatic responses of melanoma patients with *BRAF* and *c-KIT* mutations to inhibitors against these targets demonstrate the power and benefit of research to uncover the underpinnings of cancer. However, the early experience with these targets has also illuminated the challenges of this approach.

There is a clear need to improve our understanding of the factors that are present at baseline that allow resistance to occur to BRAF and c-KIT inhibitors, as well as changes that evolve over time to manifest the resistance. An improved understanding of pre-treatment factors that facilitate the eventual emergence of resistance may suggest rational combinatorial approaches that can prevent resistance from developing. Such factors may also serve as markers that clinically distinguish patients who need combinatorial treatments, which are likely to incur additional toxicities, from those who may achieve significant benefit from single-agent therapy. Similarly, determining the changes that evolve over time and correlate with functional resistance will also suggest rational combinatorial approaches that can be used after single-agent therapies fail. While it is reasonable to hypothesize that many of the critical mechanisms that underlie resistance will involve changes in signaling pathways in the tumors, the possibility of other factors should not be dismissed. For example, recent research has demonstrated that targeted therapies against the RAS/RAF/MAPK pathway can influence both the ability of immune cells to recognize melanomas, and their proliferation and survival (Boni et al. 2010). As immunotherapies have been associated with relatively low response rates but durable benefit when they occur, it is possible that strategies that combine such approaches with targeted therapies may have synergistic clinical benefit.

While there are now clearly defined challenges for patients with *BRAF* and *c-KIT* mutations, the picture remains much less clear for other patients. To date, effective treatment strategies for tumors with mutations in *RAS* family members have not been validated clinically. Research clearly needs to be undertaken to develop such approaches for patients with *NRAS* mutations, and perhaps the analogous mutations in *GNAQ* and *GNA11* in uveal melanomas. Furthermore, a significant number of patients (i.e. ~30% of cutaneous melanomas) do not have a detectable mutation in *BRAF, NRAS,* or *c-KIT*. As has been described here, the number of other mutations that have been identified in melanoma is now rapidly increasing, but the functions and therapeutic implications of many of these events remain poorly characterized. It is highly likely that many more events will be identified in the future, as the first whole-genome sequencing effort revealed almost 200 non-synonymous coding region substitutions in a single patient-derived melanoma (Pleasance et al. 2010). Unraveling the functional interactions and significance of the multiple mutations that are present in each tumor will require extensive testing and innovation. In addition, additional pathways, such as metabolism, oxidative stress, and angiogenesis likely play key functional roles, and may be important therapeutic targets without being directly involved by genetic alterations.

Overall, recent discoveries have provided new hope and therapeutic options for patients with melanoma. These advances highlight the potential of translational research, and provide the impetus for continued research of this highly aggressive disease.

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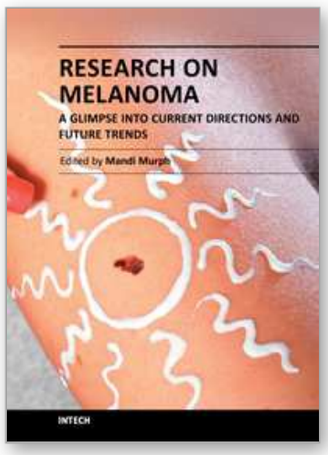
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Research on Melanoma - A Glimpse into Current Directions and Future Trends

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The book Research on Melanoma: A Glimpse into Current Directions and Future Trends, is divided into sections to represent the most cutting-edge topics in melanoma from around the world. The emerging epigenetics of disease, novel therapeutics under development and the molecular signaling aberrations are explained in detail. Since there are a number of areas in which unknowns exist surrounding the complex development of melanoma and its response to therapy, this book illuminates and comprehensively discusses such aspects. It is relevant for teaching the novice researcher who wants to initiate projects in melanoma and the more senior researcher seeking to polish their existing knowledge in this area. Many chapters include visuals and illustrations designed to easily guide the reader through the ideas presented.

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