

Melanoma Genomics and Immunotherapy

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ABSTRACT

Over the last decade the molecular characterization of melanoma has progressed. Since a majority of melanoma cases arise from repeated intermittent ultra violet radiation (UVR) exposure, the role of UVR has been evaluated extensively. Recent work has identified two mechanisms in which the carcinogenesis of melanoma may result; Ultra violet radiation has been demonstrated to lead to down regulation in immune responses and result in pyrimidine dimerization. Given these links and more significant immunogenic antigen profile of melanoma, as compared to other malignancies, there has been significant therapeutics breakthroughs based on these molecular pathways.

KEYWORDS: Tumor profiling, Melanoma, Immunotherapy

MELANOMA RATES ARE RISING RAPIDLY

Over the past 30 years, the incidence has doubled among women and tripled among men. More than 75,000 new cases of invasive melanoma will be diagnosed in the US in 2015. Melanoma claims 8,776 lives annually. The cost of treatment for melanoma is 3.3 billion per year and continues to rise.

In the majority of cases, melanoma arises from repeated intermittent sun exposure especially in individuals with a history of multiple severe sunburns in childhood and adolescence. Melanoma is associated with a more immunogenic antigen profile compared to other malignancies. Ultra Violet radiation exposure leads to down regulation in immune responses. The absorption of UV radiation leads to a release of mediators that can affect antigen-presenting cells locally and systemically. This generates antigen specific T-cells capable of regulating immunity. In addition, UV exposure can lead to pyrimidine dimerization on DNA. If these products are not removed by cellular repair activities, after DNA replication, they may cause mutations.

Regardless of the cause of the rise there has been an increase in survival due to the development of new treatments. The new treatments are targeted therapies which have emerged from advances in genetic profiling of molecular targets. Three key molecular pathways have been identified as highly deregulated in melanoma and include: mitogen-activated protein kinase (MAPK), PI3K/AKT and CDKN2A [p16] pathway.

I. MAPK Pathway

Advances in melanoma have emerged from an increased understanding of melanoma biology and signaling pathways. The mitogen activated protein kinase (MAPK) pathway is a signaling cascade that has been studied extensively in melanoma. Deregulation occurs as a result of acquired mutations along this pathway.

This cascade includes several upstream signals that are funneled through RAF, MEK and ERK. Downstream molecules such as phosphatases, communicate with higher levels in the pathway to appropriately reduce signaling in normal cells. In melanoma, cells with an activating BRAF mutation, feedback is inhibited, which keeps the pathway switched on. The most prevalent are the BRAF mutations seen in at least 45% of cases. RAS mutations have a prevalence of 15% and the surface receptor tyrosine kinase, c-Kit is rare with 2-3% prevalence. Downstream mutations include amplification of CDK4 [30%], CCND [10%] and the survival oncogene microphthalmia associated transcription factor [MITF] [10%].

BRAF is a serine/threonine kinase, a component of the MAPK pathway downstream of RAS. BRAF activation is a very strong signal, when it is activated it triggers the phosphorylation of MEK. BRAF mutations occur early in melanogenesis. About 50% of all melanomas have BRAF mutations. V600E mutations represent 80-90% followed by V600K, V600D and V600R that account for 5-15% of all BRAF mutations. These are more common in intermittently sun exposed skin and superficial spreading melanomas.

BRAF inhibition is associated with a robust clinical
response. Vemurafenib and Debrafenib inhibit cells with a BRAF mutation. Immunohistochemistry of tumor biopsies showed a positive association between tumor response and the percentage decrease in cytoplasmic phosphorylated ERK supporting the proposed mechanism of action. A 3-year overall survival rate of 30% was observed in long-term follow-up with a significant improvement over standard therapy, Dacarbazine (DTIC). BRAF inhibition response is heterogeneous and rapid but resistance to therapy is seen within a year.

Within the MAPK pathway 37% of patients have a secondary RAS mutation that co-exists with the BRAF mutation. Alternative splicing, which allows BRAF to dimerize and increase signaling occurs in 20% of patients. BRAF amplification, which may produce a 50-fold increase in BRAF copies, occurs in 30% of patients. Downstream mutations in MEK 1/2 (2%) and cdk4 (11%) are less common. The pattern of acquired BRAF inhibitory resistance follows a branched rather than linear path. This heterogeneity supports the rationale for early use of combined BRAF and MEK inhibitors in pursuit of a durable response. When resistance-related disease progression occurs while taking a BRAF inhibitor a secondary response may occur when a MEK inhibitor is added. Debrafenib, a BRAF inhibitor, used in combination with Trametinib, a MEK inhibitor, is associated with an improved 12-month OS of 72% vs 63% in the monotherapy group and a MPFS (Median Progression Free survival) of 11 months vs 7.3 months. The combination therapy delays resistance and is associated with lower treatment-related toxicity.

II. PI3K/AKT: RAS and PTEN

The RAS proteins belong to a family of p21 proteins. These are all part of a complex network of pathways resulting in the release of nuclear transcrsion factors leading to expression of genes involved in mitogenesis and apoptosis. Three closely related proto-oncogenes encoding the HRAS, KRAS and NRAS are found in mutated forms in human tumors. NRAS mutations are common in myeloid leukemia and melanomas. About 20% of melanomas have NRAS mutations. NRAS and BRAF mutations are mutually exclusive. There are no successful therapeutic targets for mutant NRAS mutant melanomas.

The pathways that could be targeted in NRAS mutant melanoma include MEK, P13K/m-TOR and cell related targets. Monotherapy with MEK inhibitors was associated with partial responses of 20% in this group of patients.

The PTEN gene is located on chromosome 10. Mutations in PTEN are found in 10%-20% of melanomas. PTEN has lipid phosphatase activity, which prevents formation of intracellular signaling molecules required for conformational change, which results in activation of the AKT protein kinase family. Activation of AKT pathway suppresses apoptosis through phosphorylation and inactivation of pro-apoptotic proteins. DNA copy gain of the AKT3 locus is found in 40%-60% of melanomas and results in activation of the AKT protein kinase. AKT3 expression correlates with melanoma progression. Thus, inactivation of PTEN allows signaling through the AKT pathway, which contributes to cell growth and anti-apoptosis. Evidence suggests that there is cooperation between loss of PTEN and BRAF mutations.

Adaptive responses by the tumor are reflected in pathway alterations contributing to acquired resistance to therapy. In more than half of the cases, the MAPK pathway, previously blocked by the BRAF inhibitors is reactivated, and p13k-PTEN-AKT alteration is involved in 4% of resistance development.

III. AKT: CDKN2A/p16

The CDKN2a gene products, p16 [tumor suppressor molecule] and p14 are cell cycle regulators that are frequently nonfunctional, especially in tumors arising from chronically sun-damaged skin. CDK4 and CDKN2A mutations are more common in acral and mucosal melanomas. The p16 protein binds to CDK4/6 kinase, blocking phosphorylation of the retinoblastoma protein and therefore leading to cell cycle arrest and inhibition of melanogenesis. Dysfunction in the proteins involved in this pathway promotes cell growth. P14 protein inhibits oncogenic activity of Bax/bcl-2 proteins that are responsible for effective apoptosis and are associated with resistance to anti cancer therapy.

OTHER MOLECULAR PATHWAYS

C-Kit is a receptor tyrosine kinase [RTK] activated by binding of a stem cell factor. C-Kit plays an important role in proliferation, development, and survival of melanocytes, hematopoietic cells and germ cells. C-Kit mutations or amplifications activate a signal transduction pathway that ultimately leads to melanogenesis. Mutations in C-Kit are found in mucosal, acral and permanently exposed skin melanomas. C-Kit mutations or copy number gains are found in 39% of mucosal melanomas and 36% of acral melanomas. C-Kit mutations have also been shown to occur in up to 88% of oral mucosal melanomas and 15% of anal melanomas. Acral melanomas and mucosal surfaces appear to be the most aggressive subtypes. C-Kit mutations are rare in melanoma but inhibitors such as Imatinib and Nilotinib have shown promising activity in patients with exon 11 and 13 mutations. Phase II data showed overall disease control rate of 50%. The presence of NRAS mutations is associated with resistance to Imatinib in the C-Kit mutant melanomas. BRAF mutations are less frequent in these melanomas.

Uveal melanoma arises from melanocytes of the choroid, ciliary body, and iris. Unlike cutaneous melanomas, which more frequently metastasize to lymph nodes, lung and brain, uveal melanomas often spreads to the liver. Metastatic disease is aggressive and with no effective treatment options for this group of patients. GNAQ and GNA11 pathway dysregulation appears to be responsible for the development of uveal...
melanomas. GNAQ and GNA11 are genes that up-regulate the MAPK pathway when constitutively active. Mutations in GNAQ and GNA11 are mutually exclusive and found in more than 80% of uveal melanomas. These mutations are potential targets of therapy through blockade of the mutated proteins or other signaling molecules downstream in the same signaling pathways.\(^7\)\(^16\)

**IMMUNOTHERAPY**

Over the past decade, new developments in T cell immunology have changed treatment algorithms and led to significant improvements in survival in patients with metastatic melanoma. T cell activation and proliferation are upregulated by checkpoint proteins that originate during the distinct phase of the T cell response. Several immune checkpoint molecules have been identified many of which are co-expressed on cancer specific T cells. T cell activation occurs away from the target tumor Cytotoxic T lymphocyte associated molecule-4 (CTLA-4). CTLA-4 is a negative regulatory molecule that is translocated to the surface of the T cells after activation. This molecule ligation receptor B7 on antigen presenting cells (APC) and down-regulates T cell responses within 72 hours after activation. Blocking the ligation of CTLA-4 to the APC permits proliferation of activated cells.\(^17\)

**Ipilimumab**

Ipilimumab is a monoclonal antibody to CTLA-4 approved for treatment of metastatic or unresectable melanoma. This therapy is associated with a disease control rate of about 30% and a 2-year survival of 29%, with a long-term durable response achieved in 20% of patients. There are no clear markers to predict response to therapy.\(^18\)

**Anti PD1/L1 immune blockade**

Cytokines activate T cells that subsequently proliferate and migrate to the tumor microenvironment, where terminal inhibition of activated T cells occurs. PD1 and its ligand PD L1 which is upregulated on the tumor cells upon T cell recognition, are important immune checkpoint proteins. Whereas anti CTLA-4 inhibition occurs at the lymph node checkpoint, PD 1 blockade occurs in the tumor microenvironment.

Nivolumab is an anti PD1 humanized monoclonal antibody approved for treatment of metastatic melanoma in patients who failed prior therapy with Ipilimumab or other targeted therapies. Nivolumab achieved response rates of 32% with durability ranging from 2.6-10+ months. Pembrolizumab is another IgG4 human programmed death receptor-1 engineered blocking antibody approved for patients with unresectable or metastatic melanoma and disease progression following Ipilimumab or BRAF targeted therapy. Overall response rate was 24% with ongoing responses 6 months or longer.\(^19\)

PD-L1 is an inducible ligand with several immune system functions. When expressed on tumor cells this protein down regulates the immune response. Histologic studies have shown that PD-L1 expression on the leading edge of the growing tumor further supporting the role that PD-L1 plays in the suppression of the immune system.\(^19\) Treating patients with compounds known to induce tumor PD-L1 has been suggested and this strategy is currently being investigated in clinical trials.

PD-L2 is a homologue ligand of PD-L1 both of which have normal physiologic functions in humans. Dendritic cells and macrophages express high levels of PD-L2 after immune challenge. Therefore specificity of PD antibodies is important. Results from a phase 1 study on anti PD-L1 antibodies suggested similar responses to that achieved with anti PD-1 antibodies; an overall response rate of 32% was observed and several patients achieved durable responses. Patients with >5% cell expression of the PD-L1 had better disease control rates [80%], compared to those with <5% PD-L1 staining.\(^20\)

**OTHER IMMUNE MODULATORY RECEPTOR TARGETS**

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<tr>
<th>Receptor Target</th>
<th>Function</th>
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<tr>
<td>GITR (glucocorticoid induced TNF receptor)</td>
<td>Increases expansion of the T cell population</td>
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<tr>
<td>OX40</td>
<td>Plays a role in T cell regulation</td>
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<td>CD137</td>
<td>Produces CD8 T cell activation, decrease in B natural killer cells and CD4 T cells</td>
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<tr>
<td>LAG-3</td>
<td>Inhibitory signaling molecules expressed in conjunction with PD-1 on CD8 T cells</td>
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<tr>
<td>TIM-3</td>
<td>Inhibitory signaling molecules expressed in conjunction with PD-1</td>
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**CONCLUSIONS**

Molecular differences among melanoma are extremely valuable for best therapeutic options and targets. Despite the recent advances in this field most patients ultimately relapse because of resistance. Many studies are underway investigating mechanisms and pathways to prolong treatment response and combat resistance.

**References**

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