Molecular Mechanisms of Melanoma

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There has been a rapid accumulation of knowledge regarding the altered molecular pathways in melanoma. With the development of targeted therapies such as vemurafenib and other therapies in clinical trials, melanomas may eventually be classified based on their altered molecular mechanisms. Knowledge of these emerging molecular melanoma subtypes is not only testable on boards but also is important for potentially understanding the future direction of melanoma therapy. What are your thoughts on targeted therapies? Despite high expectations, vemurafenib for many patients only prolongs survival by months with high rates of melanoma resistance developing thereafter. Thus far vemurafenib has not been a miracle drug but represents a new approach to cancers that may offer future cures. Herein I will review the presently known altered molecular pathways in melanoma, focusing on information that may be tested on board examinations. I do advise though that cancer biology is incredibly complex with substantial overlap and cross-talk between the numerous signaling pathways, which makes any discussion a challenge.

The deregulation of kinases is one of the principle mechanisms by which cancer develops.[1] After a cell surface receptor binds its ligand, a signal transduction pathway is activated to communicate the information to the nucleus where gene transcription is altered and an appropriate response to the signal is generated such as cell survival and growth. Signal propagation occurs by the kinase-dependent phosphorylation of proteins. Kinases perform this activity by transferring a phosphate from adenosine triphosphate to the hydroxyl group of serine, threonine, or tyrosine.[1] Once phosphorylated, targeted proteins propagate the cascade by either turning on other proteins to amplify the signal or entering the nucleus and altering gene transcription. Mutations enhancing kinase activity or disrupting their intracellular regulation allow for inappropriate cell survival and growth. Usually cancer progression requires the deregulation of multiple important proliferation and survival pathways.

Described genetic alterations include amplifications, deletions, and mutations in kinases, their regulators, and receptors. For melanoma, the important oncogenes are neuroblastoma retrovirus-associated DNA sequence (N-*ras*) viral oncogene homolog, v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), Akt protein kinase, c-kit receptor, guanine nucleotide binding protein alpha q polypeptide (Gnaq), and guanine nucleotide binding protein (G protein) alpha 11 (Gna11) (Figure). Any oncogene that undergoes a mutation causing it to gain function contributes to cancer evolution. Important tumor suppressor genes are cyclin-dependent kinase inhibitor 2A (*CDKN2A*), phosphatase and tensin homolog (*PTEN*), and a 53-kd protein. Tumor suppressor genes serve as regulators by curbing cell survival and proliferation, especially after a cellular insult resulting in DNA damage. Alterations in tumor suppressor genes are usually defined by a loss of function. Therapeutically, the inhibition of an oncogene is more feasible, such as targeting an overexpressed oncogenic protein, than replacing a deleted tumor suppressor gene.

Oncogenes

Activated N-*ras*, a membrane-bound small G protein, activates *BRAF*. N-*ras* becomes active when an appropriate receptor (usually a growth factor receptor) binds its ligand, causing it to switch guanosine diphosphate for guanosine triphosphate. *BRAF* as well as many other downstream mediators are serine/threonine protein kinases. This signaling cascade is known as the MAPK (mitogen-activated protein

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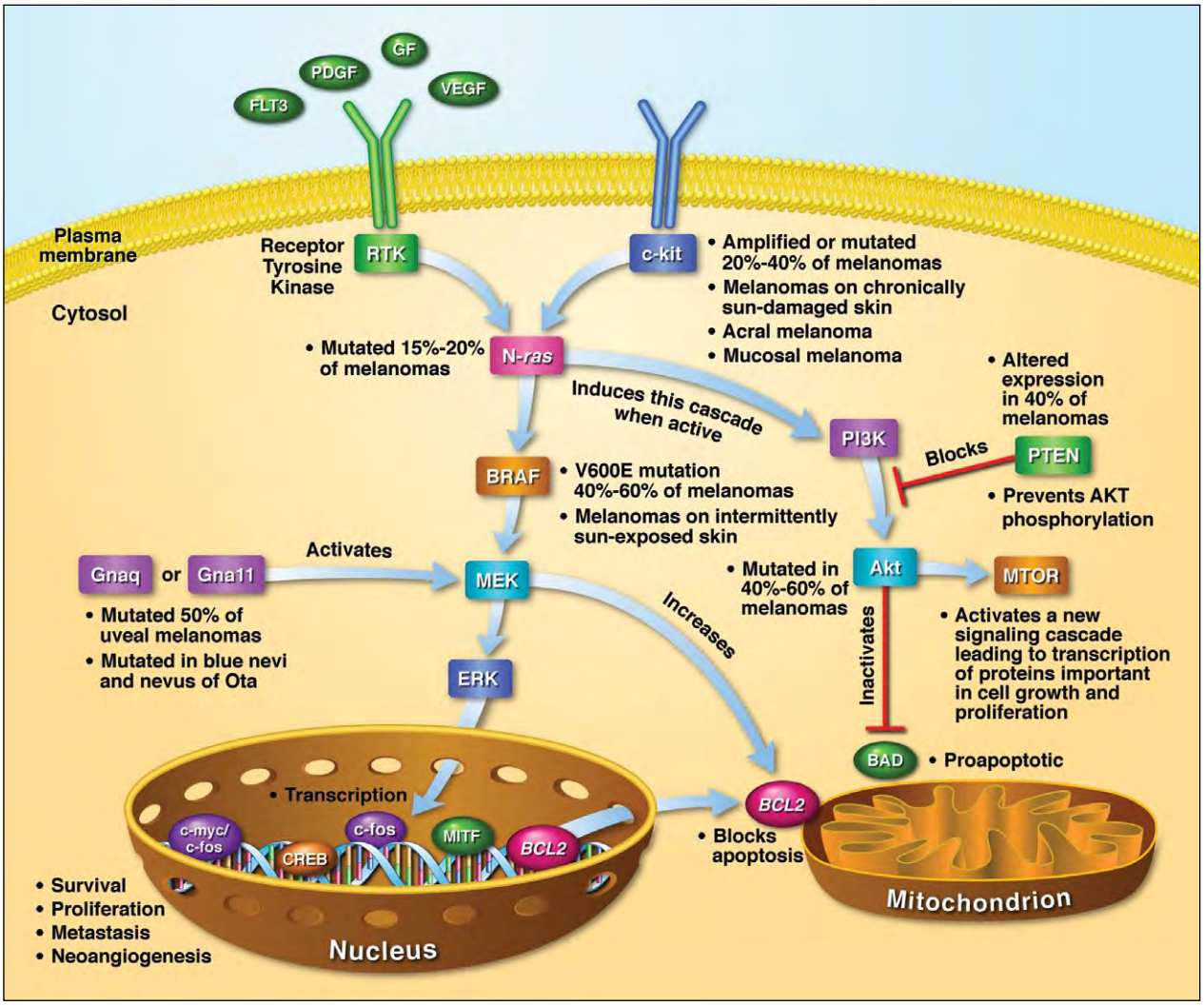

Melanomagenesis. FLT3 indicates FMS-like tyrosine kinase 3; PDGF, platelet-derived growth factor; GF, growth factor; VEGF, vascular endothelial growth factor; N-*ras*, neuroblastoma retrovirus-associated DNA sequence; PI3K, phosphatidylinositol 3-kinases; PTEN, phosphatase and tensin homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; MTOR, mechanistic target of rapamycin; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinases; Gnaq, guanine nucleotide binding protein alpha q polypeptide; Gna11, guanine nucleotide binding protein (G protein) alpha 11; *BCL2*, b-cell lymphoma 2; BAD, *BCL2*-associated agonist of cell death; CREB, cAMP response element binding; MITF, microphthalmia-associated transcription factor.

kinases)/ERK (extracellular signal-regulated kinases) pathway. The end result of the cascade is altered DNA transcription leading to cell cycle progression and survival. In 15% to 20% of melanomas, N-*ras* is mutated. N-*ras*, when turned on, also activates the phosphatidylinositol 3-kinases (PI3K)/AKT pathway.[2] Harvey rat sarcoma (H-*ras*), another member of the *ras* family, mutations are found in spitz nevi. Up to 60% of melanomas on nonchronically sun-damaged skin (intermittent sun exposure) contain acquired mutations in *BRAF*.[3] Inherited mutations in *BRAF* cause cardiofaciocutaneous syndrome, a disease characterized by heart defects, mental retardation, cutaneous abnormalities (ie, xerosis, ichthyosis, keratosis pilaris), and a distinctive facial appearance.[4] Other cancers associated with *BRAF* mutations include non-Hodgkin lymphoma, colorectal cancer, papillary thyroid carcinoma, and non-small cell lung carcinoma. Benign nevi are well-known to have *BRAF* mutations, and the presence of a *BRAF* mutation alone is usually insufficient for malignant transformation. *BRAF* mutations usually work in concert with others, which explains why *BRAF*-positive melanomas may overcome the *BRAF* inhibitor vemurafenib. More

than 95% of the mutations affecting *BRAF* switch a valine residue at the 600 amino acid for a glutamic acid, resulting in constitutive activation of the kinase.[5]

The PI3K/AKT signal transduction cascade, similar to most pathways, can be activated by more than one mechanism including N-*ras*.[5] The crucial downstream mediator of this pathway is the Akt kinase, which has many functions but most notably stimulates the transcription of proteins preventing apoptosis. It also inhibits *BCL2*-associated agonist of cell death (BAD), a proapoptotic protein, and activates mechanistic target of rapamycin (MTOR), a serine/threonine protein kinase that has its own signaling cascade promoting cell growth and proliferation. The downstream phosphorylation of Akt kinase is tightly regulated by *PTEN*. *PTEN* is a phosphatase that switches off PI3K/AKT transmission by removing a phosphate from a key protein, thus reverting the signal transduction pathway to the off position after activation. Akt kinase deregulation occurs in more than 60% of melanomas, often in conjunction with *BRAF* mutations. V-akt murine thymoma viral oncogene homolog 1 (*AKT1*) mutations have been found to cause Proteus syndrome, not *PTEN* mutations as previously thought.[5]

c-kit Receptor mutations are found in 20% to 40% of melanomas on acral, mucosal, and chronically sun-damaged skin.[6] c-kit Is a receptor tyrosine kinase that regulates the intracellular processes of growth, division, and migration in response to the binding of its ligand stem cell factor, also known as steel factor.[5] c-kit Signal transduction is at least partly mediated through the MAPK/ERK pathway.[7] Therefore, mutations in the c-kit receptor constitutively activate several proliferative signal transduction pathways. c-kit Receptor mutations also are linked to gastrointestinal stromal tumors, chronic myelogenous leukemia, and mastocytosis.

Mutations in the G proteins Gnaq and Gna11 are responsible for blue nevi, nevus of Ota, and 50% of uveal melanomas.[5] Most mutations lock these proteins in the on position by preventing guanosine triphosphate hydrolysis. Similar to N-*ras*, intracellular signaling includes activation of the MAPK/ERK pathway. However, signaling is strongest through phospholipase C and protein kinase C transduction pathway, which also is, of course, a promoter of cell growth and survival.[5]

Tumor Suppressor Genes

The *CDKN2A* gene codes for the multiple tumor suppressor-1 protein.[5] Its 2 gene products are p14 and p16; p14 stabilizes p53 and inhibits the binding of MDM2. MDM2 is an ubiquinating protein that causes degradation of p53. (p53 Will be discussed below.) Similarly, p16 binds to cyclin-dependent kinases 4 and 6 (CDK4/CDK6), preventing their interaction with cyclin D. Cyclin D when bound to CDK4/CDK6 advances both the G1 and S phase of cell cycle. The S phase of cell cycle is advanced by the cyclin D CDK4/CDK6 complex, triggering the phosphorylation of retinoblastoma protein, leading to its degradation. Retinoblastoma protein, when present, inhibits the S phase of cell cycle by binding unphosphorylated E2F in the nucleus. E2F, when unbound, initiates the S phase of cell cycle. The *CDKN2A* gene is a key susceptibility locus for familial melanoma and may also be an acquired mutation present in nonfamilial melanomas.[5]

The name p53 comes from its molecular mass; its presence can arrest both the G1 and S phase of cell cycle. p53 Is crucial in halting cellular division after acquirement of DNA damage. If the damage is irreparable, p53 initiates apoptosis by directing the transcription and activation of proapoptotic genes. p53 Is mutated in more than half of all human cancers and in approximately 10% of melanomas.[5,8] Usually patients with p53 mutations are less responsive to chemotherapy because most chemotherapy drugs depend on apoptosis initiation pathways to have their effect.[5] Li-Fraumeni syndrome is a rare, autosomal, dominantly inherited mutation in the p53 gene. Affected individuals have dramatically increased susceptibility to cancers including the breast, brain, leukemia, and sarcomas.

PTEN protein is a phosphatase that negatively regulates the PI3K/AKT signal transduction pathway. It dephosphorylates the signaling molecule phosphatidylinositol 3,4,5-triphosphate, converting it to phosphatidylinositol 4,5-bisphosphate. Phosphatidylinositol 3,4,5-triphosphate levels correlate with PI3K activity and high levels drive the phosphorylation of Ark kinase, turning it on. Many melanomas will have loss of *PTEN* with an activating *BRAF* mutation. Loss of *PTEN* protein occurs in 20% to 40% of all melanomas.[9]

Conclusion

I am optimistic that this review was helpful and not too confusing. Cancer biology is complex and difficult to discuss in such a limited manner. My goal was to touch on the currently known genetic alterations occurring in melanoma. Hopefully the review familiarized you with some of the mutations and how they are disturbed, such as *BRAF* mutations occurring usually on intermittently sun-exposed skin. Now if you see a board question, the correct answer will be more obvious.

Melanoma resistance to vemurafenib occurs because of compensation by other mutated pathways. Understanding the collection of mutations that cause

melanoma and finding the right targets without causing substantial side effects is key to successful therapy. What do you think the most important next molecular target should be? I think the ability to replace the lost tumor suppressor genes is very important, such as *PTEN*, and could be a crucial part of cancer therapy that is still missing.

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