NOVELTIES IN DERMATOLOGY

New Therapies Targeting the Genetic Mutations Responsible for Different Types of Melanoma

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Abstract

A number of molecular alterations have been described for melanoma. Melanomas with BRAF mutations tend to be located in areas of intermittent sun exposure, whereas melanomas with KIT mutations mostly appear in acral areas, the mucosa, and areas of chronic sun exposure. Sorafenib, a BRAF inhibitor, has a cytostatic effect on most melanomas with mutations affecting the mitogen-activated protein kinase (MAPK) pathway, and is also capable of triggering apoptosis in a small subgroup of these melanomas. By inhibiting KIT, imatinib has a cytostatic and cytotoxic effect on melanomas with KIT mutations, and probably has the same effect on another subgroup of melanomas with other as yet imperfectly understood KIT mutations. For therapy to be effective, agents should be selected according to the pathways associated with the genetic mutations present in the melanoma.

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PALABRAS CLAVE

Melanoma; MAPK; BRAF; KIT; Sorafenib; Imatinib

Diferentes alteraciones genéticas causan diferentes melanomas y nuevas posibilidades terapéuticas

Resumen

Se han descrito diversas alteraciones moleculares en el melanoma. Los melanomas con mutaciones de BRAF suelen localizarse en zonas con exposición solar intermitente mientras que las alteraciones genéticas de KIT ocurren con mayor frecuencia en los melanomas acrales, mucosos y en los que se localizan en áreas con exposición solar crónica. Sorafenib, un inhibidor de BRAF, tiene un efecto citostático en la mayoría de
**Introduction**

Melanoma is a malignant tumor whose incidence has been steadily increasing in recent years. Although early diagnosis has resulted in better survival for patients, a finding of distant metastasis implies a poor prognosis, with median survival of less than 1 year.¹ No significant progress in the treatment of metastatic melanoma has been made in recent decades. Dacarbazine therefore continues to be the most widely used agent for chemotherapy, even though a sustained response to this drug is rare.

The most important prognostic factors in localized skin melanoma are Breslow thickness and ulceration.² However, melanomas with similar thickness and ulceration often develop very differently, prompting the hypothesis that significant genetic, molecular, and immunological differences exist between phenotypically similar melanomas. Our understanding of melanoma has advanced in recent years with the identification of abnormalities in various molecular pathways. Of crucial importance is the fact that molecular changes are associated with specific anatomical regions (the case of mutations affecting the c-KIT tyrosine kinase in mucosal and acral melanomas), with greater or lesser exposure to sunlight, and with histological type. Mutations in BRAF, for example, are more common in melanomas that develop in unexposed areas and tend to be associated with superficial spreading melanoma. These advances in our understanding have opened up new treatment possibilities based on modifying molecular pathways that have undergone changes, that are implicated in the development of melanoma, or that participate in the body’s immune response to this tumor.

**Selective Treatments That Target Molecular Pathways**

Tumors have traditionally been diagnosed on the basis of histological appearance and the expression of certain immunohistochemical markers, with the tumor cells reproducing the tissue characteristics and cell group where they originate. This is the case in melanomas, where the epidermal connection, basal layer origins, and cell clustering mimic melanocytes; it is also the case in the benign melanocyte proliferation observed in melanocytic nevi. Diagnosis is confirmed by the expression of melanocytic immunohistochemical markers such as the S100 protein or MelanA/MART1.

Although the incidence of melanoma has increased rapidly in recent years, the mortality rate has increased more slowly. Mean survival for patients diagnosed with melanoma has improved substantially in the last 40 years, going from 60% in 1960 to 89% in 1990.³ The higher incidence is not only due to a genuine increase in melanoma cases but also to diagnostic advances, although it is not possible to separate out the relative weights of these 2 factors. These trends reinforce the hypothesis held by some researchers regarding the existence of a group of melanomas that, despite being locally invasive, grow slowly and do not threaten the life of the patient.⁴ It is therefore a matter of some urgency to identify molecular markers that will enable a distinction to be drawn between potentially aggressive melanomas requiring extensive surgery followed by adjuvant treatment, and other melanomas with a good prognosis that merely require tumor extirpation and regular monitoring.

**The MAPK Pathway**

Melanomas develop from a melanocyte in a process marked by successive genetic changes. Although the development sequence is not always the same, the process typically commences with the development of a melanocytic nevus; a dysplastic nevus follows, and finally, in situ melanoma appears. A radial growth phase then commences, followed by a vertical growth phase, after which some melanomas metastasize. The most typical molecular change in melanomas involves the intracellular mitogen-activated protein kinase (MAPK) signaling pathway, also known as the extracellular-related kinase (ERK) pathway (Figure 1). Four kinases—RAS, RAF, MEK and ERK—participate in this pathway. Mutations in the neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS) have been detected in 15% of melanomas and BRAF mutations in half of melanomas (27% to 70%, depending on the series).⁵ ⁶ These 2 mutations are mutually exclusive. The frequency of BRAF mutations is similar in melanocytic nevi and melanomas.⁷ The most frequent BRAF mutation occurs at position 600, where thymine-to-adenine transversion results in valine-to-glutamic acid substitution. This change alters the kinase domain that leads to permanent BRAF activation, and by extension, MAPK pathway activation.⁸
The mutation of any of these kinases permanently activates the MAPK pathway and should theoretically lead to ongoing cell proliferation. The proliferation that follows a mutation in any single kinase, however, will be checked by a cell-death mechanism triggered by the activation of an oncogenic pathway. More specifically, in human melanocytes, BRAF-triggered MAPK pathway activation is kept under control by increased expression of a cell cycle inhibitor known as the cyclin-dependent kinase inhibitor 4A (INK4A),\textsuperscript{10} which arrests cell growth in melanocytic nevi. Progression towards melanoma requires an additional mutation in the tumor suppressor genes that are normally responsible for inhibiting the process initiated by a mutation in the MAPK pathway. A mutation in the \textit{CDKN2A} gene has been found in around 25% to 40% of familial melanomas.\textsuperscript{11} \textit{CDKN2A} encodes 2 proteins, p16\textsubscript{INK4A} and p14\textsubscript{ARF}, whose function is to arrest uncontrolled cell proliferation. In contrast, in 25% to 50% of nonfamilial melanomas the mutation occurs in another tumor suppressor gene, the phosphatase and tensin homolog (\textit{PTEN}).\textsuperscript{12,13} MAPK pathway mutations, therefore, appear to play a role in initiating changes in melanocytes; however, in most cases, cell-death mechanisms are launched that abort neoplastic progression to a malignant phenotype, leading instead to the appearance of melanocytic nevi.

The \textit{BRAF} gene is not associated with a hereditary predisposition to cancer. The incidence of cancer is not high among individuals with germ cell mutations in this gene; rather, these individuals develop the cardiofaciocutaneous syndrome.\textsuperscript{14} Particularly interesting is the relationship between \textit{BRAF} mutations and exposure to UV light, and, by extension, between \textit{BRAF} mutations and different kinds of melanoma. The characteristic \textit{BRAF\textsuperscript{V600E}} mutation in melanoma does not generally occur in response to exposure to UV light, or, at least, not to chronic exposure. \textit{BRAF} mutations are more common in melanomas located in parts of the body exposed intermittently to sunlight, such as the torso and arms (59%); they are less common in acral (23%) and mucosal melanomas (11%), and do not occur at all in uveal melanomas.\textsuperscript{15} These mutations occur in only 11% of melanomas that develop in chronically sun-exposed areas. Melanoma phenotypes associated and unassociated with \textit{BRAF} mutations have been identified. With \textit{BRAF} mutation there is cell migration to the upper epidermis and coalescence (pagetoid growth), thickening of the epidermis, and clear differentiation from the surrounding skin. These melanoma cells are also larger, rounder, and more highly pigmented.\textsuperscript{16}

Although chemically inhibiting the MAPK pathway originally seemed to offer promise, the usefulness of this approach is now questioned after scrutiny of experimental evidence. The MAPK pathway dependence observed in vitro in melanoma cell lines and mouse xenotransplant models\textsuperscript{17,18} was mitigated in vivo by alternative autocrine and paracrine ERK pathway activation.\textsuperscript{19} Furthermore, although \textit{BRAF} mutations play a crucial role in initiating the development of a melanoma tumor, they do not fully account for definitive melanocyte transformation and maintenance.\textsuperscript{20}

In view of the importance of the MAPK pathway in the development of melanoma, several molecules have been developed to selectively inhibit some of the kinases.

\textbf{Figure 1}  Representation of the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) pathways, and of the activation points of the cyclin-dependent kinase inhibitor 2A (CDKN2A) and the phosphatase and tensin homolog (PTEN) tumor suppressor proteins.
implicated. One of the first to be developed was sorafenib (BAY 43-9006), a bi-aryl urea that inhibits both normal BRAF and BRAF \textsuperscript{V600E} kinases. Sorafenib also acts against other kinases, such as CRAF, vascular endothelial growth factors (VEGFR-3 and VEGFR-2), platelet-derived growth factor receptor, c-KIT, and FMS-like tyrosine kinase-3. Despite the promising in vitro results for inhibition of the MAPK pathway, sorafenib’s pharmacological properties mean that adequate effect requires very high concentrations, as demonstrated in melanoma cell lines and mouse xenotransplant models.\textsuperscript{9}

Since sorafenib appears to have a cytostatic antitumor mechanism of action, ongoing administration is required to inhibit tumor growth. Furthermore, the largest dose possible is administered to inhibit the pathway as fully as possible, the only constraint being the tolerated level of toxicity. The main side effects are asthenia, anorexia, diarrhea, exanthema with desquamation, and hand-foot syndrome. Phase 1 studies have established the most suitable dose as 400 mg twice daily.\textsuperscript{21}

The US Food and Drug Administration has approved sorafenib to treat metastatic kidney cancer. Angiogenesis is clearly implicated in the pathogenesis of this tumor, whereas BRAF mutations play no role. For this reason, it is thought that the antitumor action of sorafenib is more related to the antiangiogenic activity resulting from VEGFR inhibition than to BRAF suppression.

The results of clinical trials of sorafenib monotherapy in patients with metastatic melanomas have been disappointing. In a phase 1/2 study performed in 2005, only a partial response was obtained for 1 of the 22 enrolled patients with melanoma.\textsuperscript{22} In another study of 37 patients with metastatic melanoma, Eisen and coworkers\textsuperscript{23} confirmed that sorafenib antitumor activity in monotherapy was minimal: 1 patient had a partial response, and disease was stabilized in 16% of the cases. This study also controlled for whether or not tumors had the BRAF kinase mutation—given that this seems to be a necessary condition if sorafenib’s antimalanoma effect is indeed mediated by the inhibition of this molecule; it was found that the effects of sorafenib occurred independently of the tumor’s BRAF kinase status. The best results for sorafenib to date have been observed when it has been combined with dacarbazine. A 2008 randomized double-blind trial compared the dacarbazine and sorafenib combination with a dacarbazine and placebo combination in patients who had not previously received chemotherapy, and with either stage-3 irresectable melanoma or distant metastasis.\textsuperscript{24} Although the dacarbazine and sorafenib combination did not improve overall survival, it did show a statistically significant survival advantage in the absence of disease progression; furthermore, the associated toxicity was tolerable. Nonetheless, since it was not established prior to inclusion whether or not the patients in the study had BRAF mutations, it is quite possible that the effectiveness of sorafenib has been underestimated.

In the absence of drugs that would achieve more selective and more complete BRAF inhibition, a number of molecules have been developed that block the MAPK pathway. MEK is a kinase located downstream from BRAF in the same intracellular signaling pathway. Although MEK mutations have not been detected, drugs inhibiting this kinase have been shown to be useful in blocking the pathway activation triggered by BRAF mutations. Of the drugs developed for this purpose, PD0325901 and ARRY-142886 have been demonstrated in preclinical studies to have properties that make them eligible for trial; these properties are an ability to inhibit MAPK activity in tumor cell lines by reducing phosphorylation of ERK (the last kinase in this pathway), antitumor activity against a panel of human tumor xenografts, and suitable pharmacokinetic and pharmacodynamic properties.\textsuperscript{9}

The latest discoveries for sorafenib paint a slightly different picture to the one described here. As already mentioned, a very high proportion of melanomas have an activated MAPK pathway, and this may result from NRAS or BRAF mutations. In certain melanomas, signaling is due to activation of the CRAF kinase, even though there is no evidence of mutation in this kinase itself. CRAF and BRAF are at the same location and, under normal conditions, CRAF remains inactivated and so does not participate in MAPK pathway activation. BRAF only activates the MAPK pathway, whereas CRAF also activates other cell proliferation pathways (mammalian STE20-like kinase 2, apoptosis signal-regulating kinase 1, and nuclear factor-kb); furthermore, the mitochondria-associated CRAF regulates the BCL2 and BCL2-associated death promoter proteins and so directly controls apoptosis.\textsuperscript{25}

The most frequent BRAF mutation is V600E; however, at least 70 more such mutations are known to lead to less intense activation, a fact that explains why they are referred to as low-activity mutations.\textsuperscript{26}

As commented earlier, melanomas with the BRAF\textsuperscript{V600E} mutation require an additional mutation in other cell pathways (eg, the phosphoinositide 3-kinase [PI3K] or Janus kinase/signal transducers and activators of transcription pathways, which are implicated in both cell proliferation and apoptosis). Therefore, sorafenib, by inhibiting BRAF\textsuperscript{V600E} and blocking only the MAPK pathway, has a reversible cytostatic effect. Nonetheless, sorafenib suppresses CRAF more selectively than BRAF, and, for this reason, may be very effective for 2 particular kinds of melanomas in which MAPK pathway activation occurs through the CRAF rather than BRAF kinase. These melanomas, which represent but a small proportion of melanomas, have either low-activity BRAF mutations or NRAS mutations.\textsuperscript{26,27} Given the involvement of CRAF in a number of cell proliferation and apoptosis pathways, sorafenib would have a cytostatic and cytotoxic effect (Figure 2), which is crucial for treatment to be effective. It has been demonstrated in melanoma cell lines with CRAF mutations that the cytotoxic effect is mediated by apoptosis induced by reduced BCL2 expression.\textsuperscript{25}

In terms of molecular changes, the most common BRAF mutation is V600E. The group of melanomas with this mutation is also the most heterogeneous from a genetic perspective; in addition to the BRAF\textsuperscript{V600E} mutation, they probably also have mutations in one of the following: PTEN, cyclin D1, the cyclin-dependent kinase 2 and 4 genes (CDK2 and CDK4), the microphthalmia-associated transcription factor gene, and the v-akt murine thymoma viral oncogene homolog 3 gene.\textsuperscript{28} This would explain the incomplete tumor regression observed for drugs that target the
inhibition of BRAF or MEK, as these tumors use alternative pathways that enable them to bypass a blocked pathway. Therefore, effective treatment of this group of melanomas would appear to call for drugs that simultaneously target the mutated molecular pathways these tumors rely on. Preclinical studies have demonstrated that melanomas with BRAF<sub>V600E</sub> mutations that are resistant to BRAF/MEK inhibition respond to dual inhibition of the MAPK and PI3K pathways or of the MAPK and mammalian target of rapamycin pathways. If these results are confirmed, successful treatment of this group of melanomas is likely to depend on the development of drugs that are highly efficacious in blocking each of these pathways. Naturally, the success of these treatments requires that genetic changes in the tumors to be studied prospectively in patients due to receive each treatment.

**The KIT Pathway**

KIT was initially described as an oncoprotein encoded by a feline sarcoma retrovirus. The KIT protooncogene codes a tyrosine-kinase receptor whose ligand is the stem cell factor, a growth factor that plays a key role in hematopoiesis and the formation of other kinds of cells such as melanocytes and intestinal motility cells. Mutations in the KIT receptor produce permanent activation without any binding to the ligand. KIT mutations have been identified in gastrointestinal stromal tumors, in certain leukemias, and in mastocytoses and seminomas. In gastrointestinal stromal tumors in humans, KIT mutations tend to affect the juxtamembrane portion of the receptor. In this KIT receptor and in other similar KIT receptors, the juxtamembrane domain has an inhibiting function that is canceled when the receptor is affected by mutations. It is important to identify tumors with KIT mutations, given the availability of imatinib, a drug which is capable of inactivating this and other KIT receptors.

KIT involvement in melanoma is still poorly understood and has been the subject of debate. Two important studies published in 2005 and 2006 found that imatinib was ineffective in treating melanoma, independently of whether or not the KIT receptor was expressed on the surface of the tumor. Nonetheless, the role of this receptor in different types of melanoma has subsequently been analyzed more precisely. Curtin and coworkers found mutations or an increased number of KIT gene copies in mucosal and acral melanomas, and, to a lesser degree, in melanomas in chronically sun-exposed skin. Melanomas located in intermittently exposed parts of the body, on the other hand, had very few KIT mutations. These results would indicate that melanomas with KIT mutations are characterized by a lentiginous growth pattern: the melanoma cells, before invading the dermis, are isolated in the basal layer, in a pattern seen in mucosal and acral melanomas and in tumors in chronically sun-exposed skin. In contrast, BRAF mutations are more frequent in melanomas with a pagetoid growth pattern, which is marked by upward cell migration in the epidermis and coalescence in clusters. This pattern is characteristic of the superficial spreading melanomas typically found in intermittently sun-exposed skin.

A 2008 report of 2 cases of metastatic mucosal melanoma with KIT mutations which responded very well to treatment with imatinib support the use of this treatment; a temporary reduction in the imatinib dose that was necessary for 1 of the patients led to reappearance of the metastasis, but once the imatinib dose was increased again, the positive response returned.

It seems clear that the mere presence of the receptor on the tumor surface does not predict that there will be a response to treatment with imatinib. The ideal candidates...
for this treatment are melanomas in which phosphorylated KIT activates pathways essential for cell survival and growth.\textsuperscript{38} It has been demonstrated, for imatinib treatment of melanoma cell lines with KIT-activator mutations, that there is simultaneous inhibition of the MAPK, PI3K/AKT and JAK/STAT pathways.\textsuperscript{37} The combined suppression of all 3 of these pathways arrests the cell cycle and induces apoptosis due to reduced expression of BCL2 and survivin. Maximum clinical benefit can be expected in melanomas in which the apoptosis triggered by KIT inhibition results in cytotoxic effects (Figure 2).

At least 3 molecular changes that would explain an exacerbated KIT pathway function are known. The existence of activator mutations and an increase in the number of KIT gene copies have been discussed above. The third change is constitutional activation of the CDK4 pathway. It has been demonstrated that certain melanomas without mutations in the MAPK pathway (normal \textit{BRAF} and \textit{NRAS}) overexpress both KIT and CDK4.\textsuperscript{39} Although these melanomas do not have \textit{KIT} mutations, expression of the phosphorylated KIT receptor is high, indicating an activated receptor. In vitro experiments and in vivo melanoma models have shown that tumors with overexpression of these 2 pathways do not respond to treatment with \textit{BRAF} inhibitors, although tumor growth is inhibited by imatinib.

Regarding the relationship between KIT expression and the presence of mutations, a study has recently been published that demonstrates immunohistochemically elevated expression (over 50% of the cells) of the KIT receptor in melanomas with \textit{KIT} mutations.\textsuperscript{39} In other words, only a certain proportion of melanomas with immunohistochemical elevation of KIT expression will have mutations, but we need not expect to see mutations in melanomas with low KIT expression (fewer than 10% of cells).

Wu and coworkers\textsuperscript{40} recently reported a statistically significant immunohistochemical association between hyperpigmentation and KIT expression in melanomas. These authors studied 70 melanomas with epithelioid and fusiform histologies that were located in different parts of the body. Curiously, only 2 of the 70 melanomas had a KIT mutation and so it was not possible to demonstrate a relationship. The correlation between hyperpigmentation and increased KIT expression is logical, given the latter’s involvement in melanogenesis. Caution is required, however, given the discovery of a mutation in the KIT ligand resulting in enhanced KIT functioning in familial progressive hyperpigmentation,\textsuperscript{41} an autosomal dominant syndrome characterized by the presence in infancy of hyperpigmented patches of skin that increase in size and number with age.

Disseminated melanomas have been reported to respond to imatinib treatment in some patients without relevant \textit{KIT} mutations. Specifically, Kim and coworkers\textsuperscript{42} published a study of 21 patients with metastatic melanoma treated with imatinib, reporting that tumor cells with immunohistochemically high KIT expression achieved a full response. The melanomas studied did not have mutations in exons 13, 15, or 17, or in exons 9 or 11, which most commonly mutate in imatinib-responsive gastrointestinal stromal tumors. Responders displayed alternative splicing in exon 15 of the melanoma; 4 nonresponders, however, also had this alternative splice.

Many questions remain regarding KIT pathway aberrations in melanoma and responses to imatinib. Do melanomas with mutations respond differently from melanomas with an increased number of gene copies, or melanomas with overexpressed KIT and CDK4 pathways? Is there a group of melanomas with demonstrable immunohistochemical KIT overexpression, in which—in the absence of the 3 molecular changes described above—KIT is phosphorylated and its pathway activated? We could, perhaps, extrapolate findings for uveal melanomas to skin melanomas with increased expression of KIT and phosphorylated KIT whose growth is blocked by imatinib. However, the hyperfunctioning of this pathway is not due to mutations but to the autocrine and paracrine release of the KIT ligand (the stem cell factor) by the tumor.\textsuperscript{43}

The success of treatment with imatinib underlines, once again, the need to adequately select candidates: patients whose melanoma tumors have \textit{KIT} gene mutations. Otherwise, we run the risk of rejecting treatments whose usefulness may never result in statistical significance when assessed for a nonelected group of melanomas.\textsuperscript{44}

Conclusions

Melanoma differentiation has been made possible by molecular biology and has led to a regrouping of the different clinicopathological melanoma subtypes in terms of molecular aberrations. One of the most surprising conclusions to be drawn from the studies cited above is the correlation between the pagetoid growth pattern and \textit{BRAF} mutation on the one hand, and a lentiginous growth pattern and \textit{KIT} mutation on the other. Despite the fact that sorafenib and imatinib are proposed as treatments for particular melanoma subgroups, the future seems to be evolving towards combined treatments based on different drugs that target the specific molecular pathways that control the growth of the melanoma.

References


