Impact of BRAF mutation status in the prognosis of cutaneous melanoma: an area of ongoing research

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Abstract: This review is intended to provide an updated role of molecular genetics and various targeted therapies that have been developed to treat advanced stages of melanoma. Because of the declining success in melanoma therapy, the curative treatment for advanced stage melanoma has been a challenge for clinicians. Several mutations such as *N-RAS*, *p53*, *BRAF* including mutant-*BRAF* that lead to activation of kinase pathway, are implicated in the development of malignant melanoma. However, the current literature depicts that the prognostic role of *BRAF* mutation in disease progression is still controversial. While its higher level in advanced stage disease is associated with decreased overall survival (OS), some studies show that it failed to confer as an independent prognostic predictor of the disease. This has also led researchers to accomplish newer therapeutic strategies that lead to improved disease-response and grant survival benefits. Vemurafenib, a *BRAF* inhibitor agent, is one of the few available targeted therapies that is FDA approved and provides promising results in metastatic disease. However, its resistance at an early stage is of great concern. Recent implementation of combinational therapies including "targeted therapy", immunotherapy, and biological agents has appealed many researchers to define the adjunctive role of available therapies and their limitations in advanced stage and metastatic melanoma. This commends the need for future multi-institutional studies to confirm the clinical validity of different therapeutic strategies on a large scale population.

Keywords: Malignant melanoma; disease progression; mutations; vemurafenib; immunotherapy

Introduction

A wide variety of *BRAF* mutations have been observed in human cancers. Over 30 somatic mutations in the *BRAF* gene have been identified in cancers such as melanoma, colorectal cancers, papillary thyroid cancer, breast and lung cancers (1,2). Inherited *BRAF* gene mutations cause birth defects of the heart and face and can affect cognitive development. In 2002, Davies *et al*. reported somatic *BRAF* as a mutated target that activates kinases. It was detected in 66% of malignant melanomas and other human cancers such as colorectal cancers, lung cancers and ovarian cancers (2). This finding prompted novel insights toward effective therapies against these cancers, including metastatic melanoma. Malignant melanoma, the most aggressive skin cancer, majorly affects young adults. Primary cutaneous melanoma can be cured by effective surgical resection. However, one of the major concerns occurs when the tumor displays visceral spread. Metastatic disease proves to be fatal, with long-term survival rates of less than a year (3,4). *BRAF*-mutated melanoma has recently cumulated a great interest in the field of oncology. One of the earliest trials with dacarbazine, an alkylating agent, showed no survival benefits in patients with advanced metastatic disease. The development of *BRAF* inhibitors may lead to a potential therapy to overcome resistance in advanced stages of melanoma.

Despite the proven involvement of *BRAF* in melanoma confirmed by various studies, its role as a prognostic marker remains unclear (5-7). Various clinicopathological parameters such as thickness, mitotic rate and ulceration are helpful in determining the risk of recurrence of the
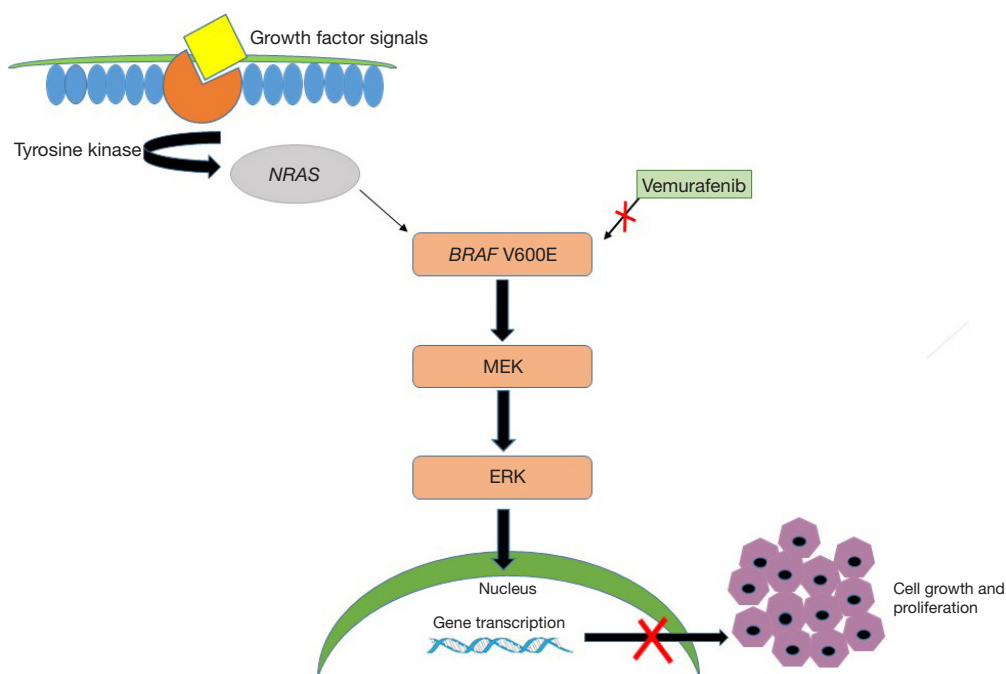
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Figure 1 Image displaying signaling pathways triggered by binding of growth factors to tyrosine kinase receptor that triggers RAS, RAF, MAPK and ERK pathways leading to cell growth and proliferation. Mutations in BRAF (V600E) which can lead to accelerated cell growth and cancer formation of melanoma cells. Inhibition of mutant BRAF by Vemurafenib (red cross) in the melanoma cells shuts down the signaling pathway causing tumor regression following cell apoptosis, tumor antigen expression and decreased release of cytokines and VEGF. MAPK, mitogen-activated protein kinase; ERK, extracellular-signal regulated kinase; EGF, vascular endothelial growth factor.

disease (8). With the aim to determine the overall survival (OS) and disease-free survival (DFS) in patients with advanced stage melanoma harboring *BRAF* mutations, a large number of clinical trials were conducted. Recently published series have compared *BRAF* V600E mutated melanoma with wild-type *BRAF*. They concluded that OS and DFS were lower for *BRAF* V600E than the wild-type *BRAF* (9). We conducted a review of literature to determine the prognostic role of *BRAF* mutations in advanced stage melanoma.

BRAF at the molecular level

Multiple factors combine to make the management of melanoma a great challenge to clinicians. Clinical factors, genetic aberrations and response to standard chemotherapeutic agents have not been able to definitively predict tumor recurrence or survival benefit (9). A wide variety of genomic aberrations are seen frequently in melanoma, such as N-RAS, p53 and p16^{INK4a}, of which

BRAF contributes to a majority of the mutations in the disease. It is estimated that *BRAF* mutation is present in approximately 50-60% of cutaneous melanomas. *BRAF*, a proto-oncogene, belongs to the family of growth signal transduction RAF kinases (6,7). It is responsible for regulation of the mitogen-activated protein (MAP) kinases pathway that mediates cell division, differentiation and secretion. Most *BRAF* mutations result from single point mutation with valine (V) being substituted for by glutamic acid (E) at codon 600 (*BRAF* V600E) (5,6,10). This substitution leads to elevated levels of *BRAF* that further stimulate extracellular regulated kinase (ERK) activity. The altered growth programming of cells results from accumulated genetic mutations which ultimately induce cancer formation (*Figure 1*). Mutant *BRAF* also produces a number of immunosuppressive factors that further favor tumor growth (11). The oncogenic role of the mutant form of *BRAF* in melanoma cell lines has been further confirmed by various authors (2,6,7). Moreover, circulating methylated DNA that carries *BRAF* mutations

has been hypothesized to predict disease recurrence and response to chemotherapy (12) in melanoma patients.

Targeted chemotherapy in melanoma

After initially frustrating results to target *BRAF* in melanoma, extensive clinical trials led to the advent of *BRAF* inhibitors. The discovery of targeted chemotherapy for melanoma has emerged as a milestone development in oncological research. One of the first drugs that was developed in RAF-targeted agents in melanoma was sorafenib (13). However, the responses were suboptimal due to its principal inhibitory effect on tyrosine kinase and limited ability to target RAF-1. This led to the innovation of newer drug therapies that could selectively inhibit mutant forms of *BRAF* (14-17). Vemurafenib, a drug that selectively acts on mutant-*BRAF*, inhibits ERK phosphorylation, leading to programmed cell death in melanoma cell lines. In 2011, because of the potential efficacy of Vemurafenib (earlier known as PX4032) towards melanoma, it received US Food and Drug Administration (FDA) approval (18). Other selective inhibitors such as dabrafenib and trametinib also selectively target *BRAF*. This novel discovery acted as an impetus to understand the detailed underlying molecular and genetic alterations that further impart resistance to anti-cancer therapy. A phase III clinical trial was conducted to compare vemurafenib with dacarbazine in metastatic melanoma patients with the *BRAF* V600E mutation. In addition to overall and progression-free survival, response rate, response duration and safety of the drug were also evaluated. They concluded that vemurafenib produced a significantly increased OS rate of 84%, with a 63% relative reduction in the risk of death from disease, while dacarbazine was associated with an OS of 64% (14). A newer concept has been established recently where liver-X nuclear hormone receptor (LXR) acts as a therapeutic target in malignant melanoma. LXRβ works by enhancing transcription of tumor and stromal Apo-lipoprotein E (apoE) which further suppresses the progression and metastatic activity of the disease (17). Pencheva *et al*. investigated the therapeutic role of LXR agonists in a genetically driven mouse model. Administration of oral LXR agonist agents was shown to decrease lymph node metastases and significantly increase OS in melanoma cell lines (17).

The ability of melanoma to effectively respond to distinct modes of immunotherapy has attracted a broad audience for combinational therapy. In general, immunotherapy alone with high-dose interleukin-2, adoptive cell transfer therapy (ACT) and anti-CTLA4 agents have been proven beneficial in advanced stages of melanoma (19-21). However, their association with an increased number of adverse events precludes their use in all but a small subset of patients. The rapidly metastasizing nature of malignant melanoma can be disastrous. Therefore, combination therapy of *BRAF* inhibitors and immunotherapy can be of paramount importance in achieving durable destruction of tumor cells and prolonging survival in patients. There has been an increasing trend to perform trials of this combination therapy in metastatic disease (11,22-25). The addition of MEK inhibition to *BRAF* prevents MAPK reactivation, up regulates melanocyte differentiation antigen expression, and increases recruitment of tumor-infiltrating lymphocytes (TILs). Furthermore, this inhibitory mechanism makes the tumor niche favorable to T-lymphocytes by decreasing the immunosuppressive factors IL-6 and -10, and vascular endothelial growth factor. It also leads to a reduction in the number of drug toxicities and decreased development of secondary cutaneous malignancies linked to use of these agents. An added advantage of this strategy is the reduction in paradoxical activation of *BRAF*-wild type. However, side effects such as hepatotoxicity and severe skin rash have been observed with this combination (26). Flaherty and colleagues conducted phase 1 trials using combination therapy with dabrafenib (*BRAF* inhibitor) and trametinib (MEK inhibitor) for metastatic melanoma. They compared this combination to monotherapy with dabrafenib. Progression-free survival with combination and monotherapy were 9.4 and 5.8 months, respectively. Moreover, it was also observed that the response rate with combination treatment (76%) was significantly higher than monotherapy (54%) (26).

Prognostic implications of BRAF

The bleak prognosis of advanced stage melanoma has led various researchers to determine the factors that result in failure of targeted therapy, decreased response rate, and recurrence of disease. In addition to various other prognostic factors such as age, gender, ECOG status, metastatic sites, and LDH levels, the detection of *BRAF* status post-chemotherapy plays a critical role in determining prognosis. Though this topic is disputable, authors are still in search of finding profound clinical utility with respect to disease progression. Shinozaki and colleagues studied the prognostic effect of mutant-*BRAF*V600E in patients

receiving chemotherapy for melanoma (12). Their study concluded that circulating mutant-*BRAF* was significantly associated with decreased OS of 13 months compared to 30.6 months in those who did not possess mutant-*BRAF*. They also showed that 70% of patients in the non-responder group retained *BRAF* mutations compared to 10% in the responder group. Another study by Ardekani and colleagues revealed similar results (27). It was observed that higher *BRAF* expression was associated with significantly poor OS in primary melanoma patients. Additionally, high *BRAF* expression showed a significant correlation with thickness and ulceration of the tumor and higher AJCC stages. In contrast, some authors concluded that although *BRAF* was observed in a higher proportion of tumors, it failed to influence OS in melanoma (8,28). Results of Ugurel and colleagues were in concordance where mutant-*BRAF* was associated with decreased OS; however, the results were insignificant. Thus, it was concluded that *BRAF* did not confer an independent prognostic factor of OS (29).

Although *BRAF* does not show significant correlation with other prognostic markers of disease progression, its role in the determination of survival benefits still warrants continued interest. It is important to clarify the distinct genetic mechanisms that are associated with disease progression rendering the potential therapy ineffective in controlling metastatic disease. To validate the efficacy of *BRAF* mutation in the determination of poor survival outcomes, it becomes imperative to identify other biochemical markers in order to confirm the prognostic role of *BRAF*.

Current trends and limitations of targeted therapy

Currently, adjuvant biological agents such as interferon-α (IFN-α) have outsourced remote therapies such as radiotherapy and immune-modulator agents in the treatment of advanced stage melanoma (25). This transitioned use of adjuvant therapy has provided significant survival benefits to melanoma patients. However, the related toxicities and cost of the therapy impede their use in many countries. The use of chemotherapeutic agents has yielded optimal benefits in metastatic melanoma. Targeted inhibition of the MAP/ERK cascade has gained great popularity in the field. Despite the beneficial performance of chemotherapeutic agents against melanoma, their limitations have impacted a large group of the population. The use of *BRAF* inhibitors is associated with a diverse side effect profile, most commonly nausea, fatigue, rash, arthralgia, and alopecia. It

has also been reported to cause a photosensitivity reaction that can be prevented by following sun-exposure protective measures (26). Another major toxicity that is of critical concern with administration of drugs like vemurafenib is the development of keratoacanthomas and/or squamous cell carcinoma. Authors have reported an 18-20% incidence of cutaneous malignancies that were treated with simple surgical resection (14,22). Another obstacle with the use of Vemurafenib is its acquired secondary resistance, which has been well established in various clinical trials (30). There have been several proposed mechanisms of resistance to *BRAF* inhibitors. The general concept of a "gatekeeper" mutation that prevents binding of the drug to the targeted oncogene does not confer resistance to *BRAF* targeted agents. Though this question remains unanswered, various studies have revealed distinct mechanisms that play critical roles in tumor progression (*Figure 2*).

- ❖ Multiple studies have suggested that the primary mechanism of resistance is due to MAPK pathway reactivation. It has also been observed to be due to sustained mutant-*BRAF* presence during tumor progression (30);
- ❖ *De novo* activation of the MAPK pathway via oncogenic mutated NRAS has been observed in some trials (31,32). The continued activation of CRAS causes NRAS to evade *BRAF* inhibition, resulting in enhanced activation of MEK and ERK;
- ❖ A variant of *BRAF*[V600E], splice *BRAF*[V600E], also results in acquired resistance to selective *BRAF* inhibitors through RAF dimerization (15,16).

The presence of these factors can lead to tumor progression and decrease the efficacy of the drug. It has been observed that approximately 10% of patients will have tumor progression post-therapy, and a majority of those will lead to tumor prolapse within a year. Interestingly, recent results have emerged that also contribute to offering resistance to Vemurafenib in both ERK dependent and independent pathways. A recent study by Boussemart and group confirmed the role of the eIF4F complex in mutant-*BRAF* melanoma resistance and metastases (33). The eIF4F complex is a eukaryotic translational initiation complex, persistent formation of which is associated with resistance to treatment. Their findings also confirmed that the formation of this complex is increased in cases where metastasis has occurred, and decreased in tumors that respond well to Vemurafenib therapy. Therefore, it is suggested that these therapeutic targets can convene a rationale for treatment of metastatic as well as drug-
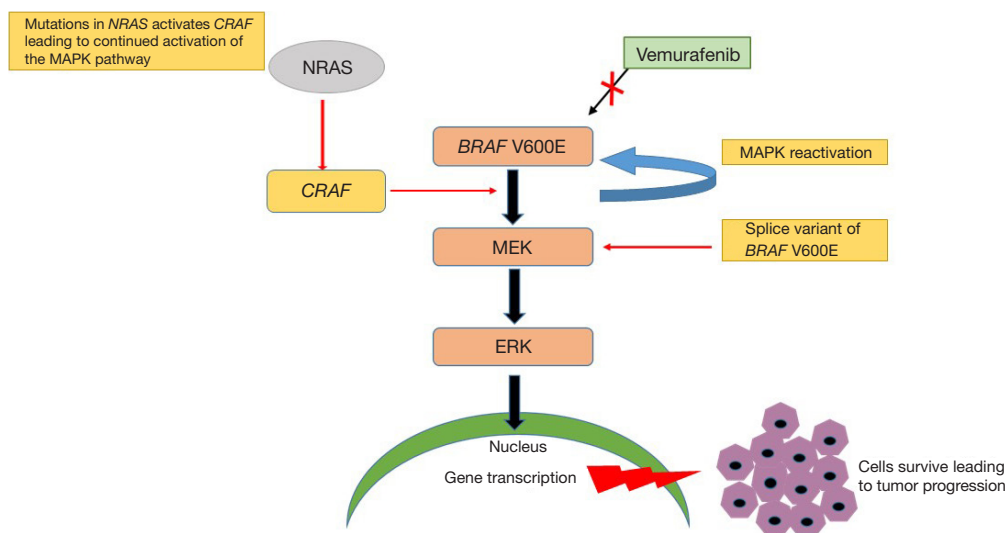
Figure 2 Image displaying multiple hypotheses for acquired BRAF inhibition resistance to chemotherapeutic agents (BRAF inhibitors) and mechanisms causing MAPK pathway reactivation. This process further leads to tumor relapse thus suggesting trials of combinational therapy (BRAF inhibitors, MEK inhibitors and/or Immunotherapy) at different levels of the pathway to prevent tumor progression. MAPK, mitogen-activated protein kinase.

resistant mutant-*BRAF* melanoma. Recently, Sun and colleagues have examined the idea of reversing acquired BRAF-resistance in melanoma (34). This is a strikingly new finding where authors have discussed the upregulated expression of epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor β (PDGFRB) only in the presence of anti-*BRAF* and anti-*MEK* drug treatment resistance. The acquired expression of EFGR is due to activation of TGF-β (due to suppressed levels of *SOX10*) after resistance to *BRAF/MEK* inhibitors in melanoma, which was observed in approximately 37% of EGFR-positive melanoma samples. However, it was noted that the higher expression of EGFR (low *SOX10*) was reversed on discontinuation of the drug. Thus, interruption in treatment causes an upsurge in SOX10 expression and, subsequently, increases drug sensitivity. This evidence indicates that *BRAF*-inhibitor resistant melanoma patients with EGFR expression can be conveniently re-treated with the same drugs after a "drug holiday" period.

Conclusions

After years of continued research, no single therapy has been found to improve the survival rate of metastatic melanoma. Although adjuvant therapy with IFN-α has provided survival benefit in high-risk cases, its adverse side effect profile is of great concern. Targeted *BRAF* chemotherapeutic agents have upstaged the management of cutaneous melanomas; however, these offer a palliative benefit to patients in advanced disease. Resistance to these agents disrupts management strategies. Because the role of *BRAF* has not been definitively correlated with the progression of the disease, it has become essential to clarify the mechanisms that are responsible for progression, relapse and recurrence. The drought of substantial evidence for the prognostic role of *BRAF* in metastatic melanoma opens areas of clinical trials to investigate newer prognostic markers. Moreover, it has been suggested that trials using combinational therapies such as *BRAF* inhibitors combined with biological agents such as IL-2, anti-angiogenic agents such as bevacizumab, and immunotherapy, could prove beneficial to halt the progression of metastatic melanoma.

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