Review Article

BRAF in malignant melanoma progression and metastasis: potentials and challenges

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Abstract: Recent advances in gene sequencing have shown that activated BRAF mutations are present in more than 50% of malignant melanomas and contribute to constitutive signals in the MAPK pathway. Besides the importance of its mutations in cell proliferation, BRAF is associated with lymph node, brain and liver metastasis, along with the loss of PTEN expression and ATG5. Knowledge of this genetic alteration has led to the development of personalized and targeted therapy strategies which block different pathways driving melanoma pathogenesis. Several targeted therapy agents such as vemurafenib, dabrafenib and encorafenib have been approved by the FDA as BRAF inhibitors, as well as other immunotherapies such as anti-CTLA-4 (ipilimumab). However, one of the main challenges is acquired resistance via reactivation of MAPK via CRAF/COT overexpression. Resistance to current BRAF inhibitors is a clinical challenge and one of the strategies to overcome this phenomenon is combination treatment, with the most recently approved combination being BRAF/MEK inhibitors (dabrafenib and trametinib) and BRAF or MEK inhibitors with immunocheckpoint blockers. This review delineates the current role of BRAF in melanoma progression and metastasis. It discusses targeted therapies and resistance mechanisms to BRAF inhibitors, and illustrates strategies to overcome this mechanism with recently approved agents.

Keywords: BRAF, metastasis, resistance, melanoma, combination treatment, targeted therapy

What is BRAF?

Understanding genetic and epigenetic changes through gene sequencing helps to elucidate and consolidate previous knowledge of mutated BRAF in melanoma [1]. Molecular investigation of RAF gene mutations found that melanoma tumour tissues and cell lines show recurrent mutation in exon 15 T1796A of the v-RAF murine sarcoma viral oncogene homolog B (BRAF), leading to valine (V) changing into glutamic acid (E) as a result of substitution at this exon (GTG>GAG) in the second placement of codon 600 (V600E) of BRAF kinase. Although BRAFV600E is the most common mutation, around 60 variant mutations have been studied in a small cohort and these cluster mainly in the kinase domain, specifically the glycine-rich loop and the activation segment domains such as p.V600D (GTG>GAT), p.V600K (GTG>AAG), p.V600E2 (GTG>GAA) and p.V600R (GTG>AGG/CGG) [2, 3]. Activation of BRAF mutation is one of the hallmarks of melanoma and is observed to be mutated in most malignant melanoma cases [4].

BRAF in the MAPK cascade

The BRAF gene encodes RAF proteins which are of the serine/threonine kinases including ARAF, BRAF and CRAF isoforms. These proteins are part of the MAPK pathway (RAF/MEK/ERK serine threonine kinase cascade) which has been studied extensively and is known to regulate several cellular mechanisms, including proliferation, differentiation and survival. It has long been known that this cascade is activated when extracellular signals bind to cell membrane receptors such as G-protein coupled receptors and receptor tyrosine kinase (RTK). Then RAS (KRAS, NRAS and HRAS) adopts its active state, RAS-GTP, which binds in the membrane to activate its effector RAF proteins (three isoforms). Subsequently, a series of kinases are phosphorylated to activate their substrates, including MEK1 and 2, which further phosphorylate
ERK1 and 2. ERK in its activated form phosphorylates a series of substrates, consequent-ly regulating gene expression for cytoskeletal functions, metabolism, differentiation, proliferation and senescence to cellular death, thus providing essential tumour growth and maintenance functions. The emerging data indicate that collective mutations lock BRAF into its active position thus constitutively resulting in a ten-fold increase in oncogenic signalling through MEK [5].

Subsequent biochemical investigations have confirmed that BRAF is hyperactivated in the majority of melanoma cases via the mutation of BRAF, which, exclusively with other mutations such as NRAS and KRAS, render melanoma dependent on the MAPK oncogenic signal pathway. More than half of melanoma cases are characterized by BRAF mutation, V600E in most cases. NRAS is reported to be mutated in 21% of cases and KRAS mutate in 2% and 1% of cases respectively. Mutations in other RAF isoforms, ARAF and CRAF, MEK and ERK have not been reported in melanoma, indicating they are not essential for melanoma pathogenesis [6].

BRAF in metastasis

The invasive behaviour of melanoma cells is a critical transition step during melanoma progression. It is well-known that melanoma can be metastasized as satellite or in-transit metastasis either through the blood or lymphatic system. The satellite metastasis nodule is embedded within two centimeters of the initial tumour bulk, whereas the in-transit is developed within the dermal and subdermal lymphatics in the drainage area prior to the involvement of the regional lymph node basin [7]. A rapid systemic dissemination is almost noticed for all organs but the most common target sites are the brain, liver and bone [8]. Clinical investigation highlights the severity of brain metastasis in melanoma patients; it is the most common cause of death in these patients, with an average survival duration of around four months [9, 10].

BRAF mutation is detected in early melanogenesis in a high percentage of melanocytic nevi, hence it cannot induce melanoma progression alone and needs additional genetic alterations at a later stage of progression, such as deletion of phosphatase with tensin homolog (PTEN), autophagy related 5 (ATG5) or cyclin-dependent kinase inhibitor 2A (CDKN2A) to give an advantage in the propagation of melanocytic cells to be transferred to melanoma cells [11]. Researchers have developed transgenic mouse models expressing BRAF, finding that it induces melanoma development, which, when combined with PTEN loss, leads to hyperactivation of the PI3K-AKT-mammalian target of the rapamycin (mTOR) pathway, which stimulates the constitutive proliferative signal, mouse-developed metastatic pattern with short survival time [12].

A recent illustration using genetically engineered mice confirmed the role of ATG5 in melanoma pathogenesis in combination with BRAF [13]. ATG5 facilitates cellular bypass to oncogene-induced senescence, thus inhibiting cellular transformation into malignant cells. Garcia et al show that the heterozygous knockdown of ATG5 accelerates melanoma metastasis while homozygous deletion has a counterpoint effect by reducing the melanoma lesion metastasis rate in different anatomical regions. Also, heterogenous deletion compromises the model response to BRAF inhibitors in clinical use (dabrafenib) [14]. Genetic alteration of CDKN2A, a tumour suppressor gene, has been involved in invasive and cell cycle progression via losing the inhibition of cyclin-dependent kinase 4 (CDK4)/cyclin D1 (CCND1) [15]. North’s group has reported recently that bi-allelic deletion of CDKN2A results in invasive melanocyte behaviour by the activation of the lineage-restricted transcription factor BRN2, a regulator of melanocyte development and differentiation [16, 17]. These deletions of the regulators along with BRAF mutation contribute to melanoma cells invading adjacent or distant organs.

There is a link between angiogenesis and metastatic potential, as melanoma cells acquire the ability for regional spread and shift from radial growth to advanced vertical growth through the angiogenesis process. Furthermore, new blood vessels are generated from the pre-existing vasculature to ensure tumour growth and survival beyond a hundred microns in diameter from the initial site [18]. Disruption of anti- and pro-angiogenic signals such as vascular endothelial growth factor (VEGF) has been reported in metastatic melanoma. BRAF not only has a role in melanogenesis but it also promotes vas-
BRAF in malignant melanoma progression and metastasis: potentials and challenges

cular development by activating the secretion of VEGF and tumour metastasis through the regulation of pro-angiogenic factors (interleukin-8) and other proteins involved in migration, integrin signalling and cell contractility [19].

Melanoma therapy

Surgery and chemotherapy are the standard therapy options for local and malignant melanoma respectively. Chemotherapy is considered the first treatment option for malignant melanoma and dacarbazine, an alkylating agent, has been the standard drug approved by FDA since 1974. Studies confirm that less than 5% of treated cases show a complete response, with a 5 year survival rate in 2-6% [20]. However, when melanoma reaches advanced stages and becomes resistant to the available treatment, new strategies are needed, such as targeted chemotherapy and immunotherapy. The latter inhibits the essential checkpoints in immune system, thereby stimulating the patient’s immune system to fight cancer cells. In tandem with the discovery of BRAF and other mutations, melanoma treatment has shifted towards targeted, personalized therapy which has become a relatively effective strategy for melanoma treatment when tailored according to the detected mutations [21].

Targeted therapy

Recent advances in the molecular approach indicate that targeted therapy is largely based on targeting melanomas that harboring mutations such as BRAF, RAS, MEK and PTEN. Moreover, targeting mutations in critical growth regulatory genes in melanogenesis and metastasis, such as BRAFV600E, leads to resetting the disruption of intracellular signal such as MAPK and consequently suppression of melanoma progression. Data validate BRAF as a therapeutic target and several FDA approved drugs (Table 1) are in clinical use as a result of drug discovery programmes [6].

First generation RAF inhibitors

This class was developed to inhibit MAPK pathway signalling before the discovery of BRAF mutations. They were designed to target RAS which is mutated in many cancer cells, but were unable to disrupt RAS interactions with its upstream factor, and were thus not suitable for further development. It was hoped that farnesyltransferase inhibitors might perturb the RAS signal via interference with RAS localization to the membrane [6]. Unfortunately, the results of phase II clinical studies were not promising, as these agents were not specific with off-target effects [22]. Later, the development of small-molecule acts as ATP-competitive for RAF were reported to be both CRAF and BRAF inhibitors [23]. The first promising result reported from these hard efforts was sorafenib, which was initially generated as an inhibitor of CRAF isoforms with 12 nM (IC_{50}), and later shown to be effective against BRAFV600E [24]. Several MEK inhibitors were also validated for use against malignant melanoma with the BRAFV600 mutation, such as trametinib, cobimetinib and selumetinib [25].

Second generation RAF (selective BRAF) inhibitor

The discovery of BRAF mutation in 2002 identified effective ‘druggable’ targets that could provide effective long-term treatment strategies and generated interest in developing new agents for BRAF inhibition [23]. Vemurafenib is a selective BRAF inhibitor that was approved by the FDA in 2011. It was developed with a structure-guided approach that blocks melanoma cell proliferation carrying BRAFV600 mutation at nanomolar level (Figure 1). Recent evidence has confirmed its ability to cause tumour regression in 90% of treated patients [20]. Chapman et al conducted a phase 3 randomized clinical trial to compare vemurafenib with dacarbazine in 675 metastatic melanoma patients with the BRAFV600E mutation and concluded there was an improvement in overall survival with vemurafenib (84%), as compared to dacarbazine (64%), with the ability to reduce the risk of death to 63% and disease progression to 74% [26]. This inhibitor has worked either as monotherapy or in combination with other chemotherapeutics or immunotherapy agents. Dabrafenib is another selective BRAF inhibitor, approved by the FDA in 2013 that targets BRAFV600E/K either alone or in combination with other MEK inhibitors. These two inhibitors demonstrate similar high clinical response (14-26%) in patients with second side tumours such as keratoacanthomas and squamous-cell carcinomas [26, 27]. Other BRAF inhibitors are ongoing clinical trials such as encorafenib,
**Table 1. Different malignant melanoma treatment regimens**

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<th>Targeted chemotherapy</th>
<th>Immunotherapy</th>
<th>Combinatorial therapy</th>
<th>Status</th>
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<td>1st generation inhibitors</td>
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<td>Sorafenib</td>
<td>High-dose interleukin-2 (HDIL-2)</td>
<td>BRAF and MEK inhibitors (dabrafenib and trametinib)</td>
<td>Approved by FDA</td>
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<td>Interferon-α (IFN-α)</td>
<td>BRAF and MEK inhibitors (vemurafenib and cobimetinib)</td>
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<td>2nd generation inhibitors</td>
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<tr>
<td>Vemurafenib, dabrafenib</td>
<td>Anti-PD1 (Lambrolizumab, Nivolumab and Spartalizumab) or anti-PD-L1 (Atezolizumab)</td>
<td>BRAF and MEK inhibitors (encorafenib with binimetinib)</td>
<td>Approved by FDA</td>
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<td></td>
<td>Anti-CTLA-4 (Ipilimumab)</td>
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<td>3rd generation (pan-RAF) inhibitors</td>
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<td>LY3009120, TAK-580, CCT196969, CCT241161, BGB659</td>
<td>Heat shock protein 90 inhibitors (XL888) with vemurafenib and Cobimetinib</td>
<td>Approved by FDA</td>
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<td>MEK inhibitors</td>
<td>AKT inhibitor (GSK2141795) with dabrafenib and trametinib</td>
<td>BRAF and MEK inhibitors (dabrafenib and trametinib)</td>
<td>Approved by FDA</td>
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<td>MDM2 inhibitor (AMG 232) with dabrafenib and trametinib (NCT02110355)</td>
<td>BRAF and MEK inhibitors (vemurafenib and cobimetinib)</td>
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<td>Dabrafenib and ipilimumab</td>
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<td>Dabrafenib, trametinib, and ipilimumab</td>
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<td>Dabrafenib, trametinib and spartalizumab</td>
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<td>Vemurafenib, cobimetinib, and atezolizumab</td>
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<td>Nivolumab and ipilimumab</td>
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<td>BRAF and MEK inhibitors (encorafenib with binimetinib)</td>
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**Combination**

**Status**

Approved by FDA
Ongoing phase I trial
Phase I trial
Phase II trial
Phase III trial
Ongoing phase I trial
Phase I trial
Phase II trial
Phase III trial
Ongoing phase I trial
Phase I trial
Phase II trial
Phase III trial

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either alone (NCT01436656) in phase III clinical trials, or combined with other agents (NCT-02159066/NCT01909453), or with immunotherapies (NCT02902042) [20]. Initial results indicate its ability to prolong survival time for treated patient in comparison to vemurafenib or dabrafenib, giving positive insights into its clinical profile [23].

**Third generation of BRAF inhibitors (pan-RAF inhibitors)**

RAF dimerization is a problem which many drug discovery programmes are striving to overcome by testing agents that may act as inhibitors of this process, thus preventing paradoxical ERK activation [28]. ERK activation occurs if the RAF inhibitor concentration is un-saturated, which leads to the stable binding of drug-bound RAF promoters to RAS and results in the transcription of the drug-free RAF promoters and consequent paradoxical activation of MAPK. Moreover, ATP-competitive RAF inhibitors (second generation) act in opposing ways as they either inhibit or paradoxically activate the MAPK signalling output, depending on the activation signal, either through mutated BRAF or other upstream regulators such as RAS or RTK [29]. The third generation of BRAF inhibitors is the pan-RAF which is classified as DFG-OUT/αC-IN’ binding, and addresses the problems by interfering with monomeric and dimeric RAF complexes as well as interacting with ATP binding to preclude RAF dimerization [28].

These drug discovery approaches involve several pan-RAF inhibitors in clinical trial phase, such as LY3009120, TAK-580, CCT196969, CCT241161 and BGB659. Preclinical data has shown the potential action of LY3009120 on...
melanoma driven from RAS mutation, BRAFV-600 monomers and non-mutated BRAFV600 dimers. TAK-580 similarly has a high affinity to RAF monomers/dimers and inhibits paradoxical ERK signalling. The synergistic effect was characterized when TAK-632 and MEK inhibitor (TAK-733) was administered to the developed model [30]. CCT196969 and CCT241161 have shown additional advantages by targeting Src family kinases (SFK) which are highly expressed in a vemurafenib-treated model. This is especially important because it suggests the possibility of using CCT196969 and CCT241161 as a second choice for vemurafenib resistance cases [28].

**Immunotherapy**

Researchers are continually seeking effective regimens against melanoma and attention has lately turned towards immunotherapy since melanoma is the most immunogenic cancer type. From the early 70s greater understanding of how the immune system fights tumour progression has led to advances in immunotherapy, i.e. the development of agents to boost the ability of the immune system to efficiently target cancer cells and destroy them [31]. Advanced investigations have highlighted BRAF’s ability to contribute to immune system suppression, thus treatment with BRAF inhibitors can modulate the immune system and augment the effect of immunotherapies [32].

Recent research has focused on the development of agents which target tumour-specific antigens that promote tumour progression. Two of the earliest immunotherapies were high-dose interleukin-2 (HDIL-2), a cytokine promoting T-cell proliferation, and interferon-α (IFN-α) which are approved for metastatic melanoma treatment, but which are unfortunately also associated with high toxicity [31]. Toxicity limits the effect of immunotherapy due the fact that when T-cells are activated, they attack normal cells too. Thus, different interactions should be targeted to prevent T-cell cytotoxicity and inhibit the immune checkpoints that function to prevent autoimmune diseases, and are expressed in high rate at melanoma cells. For example as shown in Figure 1, antibodies against PD1 (lambrolizumab, nivolumab, spartalizumab) or PD-L1 (atezolizumab) accelerate T-cell responses to destroy cancer cells with BRAF inhibitors, thus extending the positive clinical response and allowing greater response and survival rates [21, 33]. In clinical trials of patients with metastasis of melanoma to the brain, response in 81% of patients was found to be durable upon the administration of lambrolizumab, with 7 months as the overall progression-free survival time [34]. Moreover, ipilimumab is an IgG1 monoclonal antibody that blocks the interaction by blocking cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), a negative regulator of T cells, resulting in the augmentation of the activity and proliferation of T-cells to promote their anti-tumour activity. Clinical data show an improvement of overall survival time of up to 10 months. These approved agents are suitable for patients who have not responded to targeted therapy, either alone or in combination with BRAF/MEK inhibitors [21].

Although immunotherapies modulate the immunomicroenvironment, immune response is limited due to the increase in the rate of PD1 positive melanoma cells that may ensure tumour relapse. Blocking immunocheckpoints is an effective approach for maximizing the therapeutic response of BRAF inhibitors. Recent work suggests that macrophage infiltration contributes towards the resistance of melanoma to different treatment options, since when the MAPK pathway is activated paradoxically in macrophages after the administration of BRAF inhibitors, VEGF is produced by macrophage. This consequently activates MAPK in melanoma cells and promotes melanoma progression. One study has shown that BRAF inhibitors trigger the transformation of macrophages from being passengers to drivers of melanoma growth. This gives insight that targeting macrophage infiltration may overcome melanoma resistance and improve the therapeutic effect of BRAF inhibitors [32].

**Combination therapy**

It is clear that melanoma is driven by the disruption of different signalling pathways through mutations of oncogenes or tumour suppressors. Recent data has confirmed that it is necessary to target multiple dysregulated points in a single pathway by two or more monotherapies to achieve effective outcomes in treated patients, illustrating the importance of the concept of combination therapy. Thus, understanding the molecular biology of melanoma pathogenesis in depth in an individual’s tumour helps
in rationally designing the correct drug combination [6]. In addition, combination therapy is essential to overcoming tumour recurrence and delaying acquired resistance, resulting in longer duration of response to the drug programme; this can be combined chemotherapeutics or biochemotherapy (chemo-immunotherapy) [35].

Combined chemotherapy

Clinical data has proved the efficacy of several combination treatments and one of the first was BRAF with MEK inhibitors (dabrafenib and trametinib) for metastatic BRAF mutated melanoma. This combination achieved slightly better progression-free survival than dabrafenib in phase III clinical trials, with 37% reduction in the risk of death [36]. Similar results in terms of progression-free survival were observed in vemurafenib and cobimetinib (phase III study) to delay the onset of resistance at the cost of high toxicity [37]. A phase Ib/II clinical study confirmed that a different combination of encorafenib with binimetinib was well tolerated in BRAF mutated melanoma with 11.3 months of progression-free survival, and an ongoing phase III clinical trial is under way to test these as monotherapies as well as in combination in compared to vemurafenib. Other ongoing clinical studies combining BRAF and other inhibitors involve heat shock protein 90 inhibitors (XL888) with vemurafenib and cobimetinib (NCT02721459), AKT inhibitor (GSK2141795) with dabrafenib and trametinib (NCT01902173) and MDM2 inhibitor (AMG 232) with dabrafenib and trametinib (NCT02110355) [35].

Combined chemo-immunotherapy

This approach is applicable in melanoma treatment either with conventional drugs such as dacarbazine, cisplatin and vinblastine combined with HDIL-2 or IFN-α or targeted chemotherapeutics; BRAF or MEK inhibitors with immunocheckpoint blockers. The latter has attracted a lot of attention recently as targeted therapy response rates have reached up to 70% with the essential roles of immunocheckpoint blockers in tumour microenvironments. However, the early phases of several clinical trials have reported significant toxicity for different combinations; dabrafenib and ipilimumab or dabrafenib, trametinib, and ipilimumab in metastatic melanoma patients. More recent phase III trials investigating dabrafenib, trametinib and spartalizumab in patients with advanced BRAF melanoma have shown fewer side effects. The initial results showed a manageable safety profile with promising efficacy. Other clinical trials are ongoing for triplet combinational therapies of vemurafenib, cobimetinib, and atezolizumab [38]. Also, immunotherapy combinations have shown therapeutic response in metastatic melanoma. The median progression-free survival in metastatic melanoma was significantly extended with the combination of nivolumab and ipilimumab or nivolumab alone in phase III clinical trials rather than ipilimumab alone, whereas with PD-L1-negative tumour patients, the combination was more effective than either monotherapy [39, 40]. Another phase II study demonstrated considerable efficacy for such combination in melanoma patients with untreated brain metastasis [41].

BRAF resistance mechanisms

Although understanding the mechanisms of action of the various BRAF mutations led to the initial success of targeted therapy with BRAF inhibitors, the development of resistance mechanisms in many patients is a significant challenge as shown by disease recurrence or lack of response to treatment in 15% of cases. Research into the molecular basis of resistance mechanisms against BRAF focuses on both primary and secondary resistance, with the secondary being the most frequently occurring and also called acquired [36]. Earlier data from tumour biopsies from patients and the reported high recurrence rate in treated malignant melanoma indicates the initiation and development of new mutations, promoting disease progression [3]. Primary resistance is developed based on pre-treatment factors such as cell cycle regulator and mutation status, including PTEN loss and hepatocyte growth factor (HGF) [42]. Mechanisms of secondary resistance include the reactivation of the MAPK pathway via upstream mediators such as NRAS mutation, COT overexpression or elevated levels of BRAF and CRAF [36].

Intrinsic resistance

Melanoma cells were able to confer resistance through the dysregulation of key mediators of the sensitivity of BRAF inhibitors such as cell cycle regulators (cyclin D1) and the amplifica-
tion of this emerging predictive biomarker was detected in a panel of BRAF-mutant cell lines. It was found that BRAF-mutant cell lines with high levels of cyclin D1 are more resistant to BRAF inhibitors. Another predictor of melanoma resistance is PTEN loss, as initial data show the association of PTEN loss in BRAF-mutated cells with recurrence of melanoma after BRAF inhibitor treatment [42]. In addition, the importance of cellular growth factor (HGF) and its interaction with receptor CMET renders the ability of melanoma cells to develop intrinsic BRAF resistance. Proteomic analysis reveals the HGF/CMET interaction is essential for resistance and an addition of antibodies against HGF or CMET reinstates the BRAF inhibitor effect [43].

Acquired resistance

Given the importance of elucidating resistance mechanisms, it would be of particular interest to study secondary resistance mechanisms against BRAF inhibitors. Clinical response to targeted therapy is largely confounded by acquired resistance. De novo resistance could be either through ERK phosphorylation (ERK dependent) or without (ERK independent).

ERK dependent pathways

Upregulation of the MAPK pathway signalling can use the effects of a BRAF inhibitor to establish a growth advantage via different mechanisms; RTK upregulation, NRAS mutation, CRAF dysregulation, splice versions of BRAF or amplification of alternative BRAF, although BRAF signalling is suppressed by BRAF-targeting agents [42].

CRAF

The necessity to target CRAF is becoming increasingly evident since BRAF mutant cells acquire resistance to BRAF inhibitors through the overexpression of CRAF, which is highly expressed in melanoma cells when compared with benign nodes. Since melanoma cells escape BRAF inhibitors through the complexity of RAF isoforms and their cross activation, much effort is being put into developing agents that specifically inhibit the reactivated MAPK through the CRAF isoform [44]. The development of CRAF-mediated models confirms the critical role of prohibitins, which control cell cycle, senescence and tumour suppression, and which bind to CRAF, allowing melanoma cells to become resistant to BRAF inhibitors. Analysis shows that disrupting this interaction reduces MEK/ERK activation and thus cellular proliferation with a high rate of apoptotic death [45]. Another model has utilized wild-type BRAF and the localization of wild-type BRAF in the presence of BRAF inhibitors has been observed. Furthermore, recruitment of CRAF to the plasma inner membrane has been documented upon the activation of RAS signalling to transfer signals through the MAPK pathway. So, the wild-type BRAF configuration remains inactive due to the effect of class I RAF inhibitors and serves as a scaffold to enhance CRAF heterodimerity and thus the pathway output has been turned on constitutively (Figure 2). This is unlikely to occur if pan-RAF inhibitors have been administered as they demonstrate similar activity against BRAF and CRAF isoforms, inhibiting the MAPK pathway signalling output. This knowledge is of great importance in that ERK signalling is largely under RAF control and RAF inhibitors are significant in melanoma treatment [3, 46].

COT

Pre-clinical studies have demonstrated that the MEK-ERK-dependent mechanism is activated via COT, which acts as MAPK pathway agonist and does not require RAF signalling (Figure 1). It is clear from the available data from tissue samples from patients experiencing recurrence following treatment with MEK or RAF inhibitors that COX overexpression is correlated with acquired resistance to BRAF inhibitors. Johannesen et al confirmed the role of COT in reducing the sensitivity of melanoma cells to vemurafenib by increasing constitutive phosphorylation of ERK without effect with kinase-dead derivatives. Furthermore, vemurafenib potentiates the outgrowth of COT-expressing cells during treatment by using quantitative real-time PCR and the COT mRNA level was significantly high in relapsing melanoma tissues compared to pre-treated or ongoing treated tissue [47]. Thus, COT is considered a potential target for recurrent melanoma.

ERK independent pathways

The possibility of reducing the sensitivity of melanoma tissues to BRAF inhibitors is achieved by RTK upregulation. Several RTK kinases
such as platelet derived growth factor receptor beta (PDGFRβ), insulin-like growth factor 1 receptor (IGF-1R), epidermal growth factor receptor (EGFR) and c-Met activate PI3K-AKT-mTOR pathway, trigger an alternative survival pathway by decreasing apoptosis in melanoma cells. IGF-1R inhibition in combination with MEK inhibitors accelerates apoptotic death dramatically, which was detected to be upregulated in BRAF-mutated cells. Thus deactivation of PI3K-AKT-mTOR pathway through inhibition of RTKs is an effective strategy to suppress melanoma resistance to BRAF inhibitors [48].

**Potentials and challenges**

Oncogenic activation of BRAF fuels melanoma growth by constitutively promoting MAPK activation as well as melanoma metastasis. Molecular understanding of BRAF mutation has informed drug discovery programmes and the development of BRAF inhibitors has progressed at a fast pace. Although BRAF inhibitors are preferred for upfront systemic therapy in advanced melanoma, melanoma recurrence and drug resistance are still major obstacles to successful treatment. ERK paradoxical activation presented to be a major challenge even for selective BRAF inhibitors through BRAF dimerization, although several potential pan-RAF blockers are either already available or are in the pipeline. Combinatory treatment has now become the standard for patients with relapse melanoma, and mostly combines chemotherapy with immunotherapy. Several regimens are now approved and others are the subject of ongoing clinical research.

The study of melanoma through innovative bench and translational approaches has highlighted a number of challenges which are being addressed using many emerging technologies. This includes (i) the high toxicity of combination therapies and (ii) the dilemma of whether to begin treatment with immunotherapy or chemotherapy as anti-PD1 will be approved soon as the frontline option for melanoma, thus clinical trials are ongoing to answer this question. A further challenge (iii) is understanding the dimerization mechanism of action, as the structure of BRAF-CRAF heterodimer is still not delineated. Such information will enable further improvement of the current inhibitors in designing tailored drugs for this mutation. (iv) Individual
BRAF in malignant melanoma progression and metastasis: potentials and challenges

response to treatment leads to the concept of personalized medicine as patients metabolize drugs at different rate, respond differently and carry other mutations affecting the therapeutic response, a major challenge for the future. (v) Pan-RAF inhibitors target mutant as well as normal RAF signalling, which may be associated with a reduction in therapeutic window. They may serve as good candidates for combination approaches targeting other signalling pathways to provide synergistic effects and reduce possible side effects. Deeper investigation into these issues will lead to the development of new strategies in order to achieve the therapeutic goals.

Disclosure of conflict of interest

None.

Abbreviations

ATG5, Autophagy related 5; CDKN2A, Cyclin-dependent kinase inhibitor 2A; CCND1, Cyclin D1; CTLA-4, Cytotoxic T-lymphocyte–associated antigen 4; EGFR, Epidermal growth factor receptor; PDGFβR, Growth factor receptor beta; HGF, Hepatocyte growth factor; HDIL-2, High-dose interleukin-2; IGF-1R, Insulin-like growth factor 1 receptor; IFN-α, Interferon-α; mTOR, Mammalian target of rapamycin; PTEN, Phosphatase with tensin homolog; SFK, Src family kinases; VEGF, Vascular endothelial growth factor.

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References


BRAF in malignant melanoma progression and metastasis: potentials and challenges


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