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Review

BRAF as therapeutic target in melanoma

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ABSTRACT

BRAF is a member of the RAF kinase family, which acts in the ERK/MAP kinase pathway, a signalling cascade that regulates cellular proliferation, differentiation and survival. Single point mutations can turn BRAF into an oncogene, but there appears to be a cell type/tumour specific relevance for BRAF kinase-activating mutations, since they are found predominantly in cutaneous melanoma. With the success of targeting other oncogenic kinases such as BCR-ABL, KIT or members of the epidermal-growth factor receptor (EGFR) family in other cancers, the expectations were high when the first RAF kinase-targeting drug (sorafenib) reached clinical trials. However, disappointingly the first studies using sorafenib in melanoma patients did not show the anticipated single agent efficacy. More recently, the resolution of the BRAF crystal structure has led to the development of better, more specific BRAF inhibitors such as the Plexxikon compound, PLX4032, which induced a dramatic response rate in phase I trials, validating BRAF as a clinically relevant target. In addition, our understanding of melanoma biology and the role BRAF is playing therein has improved significantly. The complexity in the ERK/MAP kinase pathway including important feedback mechanisms has been dissected, and the relevance of cross-talks with other signalling pathways has been revealed, suggesting strategies for the design of improved, more efficient combinatorial therapies. This review highlights the relevance of BRAF and the ERK/MAP kinase pathway for melanoma cell biology and discusses some of the recent advances in both, the understanding of BRAF function in melanoma and the development of improved BRAF targeting inhibitors.

Contents

1. Introduction

The serine threonine kinase BRAF is a member of the RAF kinase family, which is part of the RAF/MEK/ERK serine threonine kinase cascade (Fig. 1). This kinase cascade, also called the ERK/MAP kinase pathway (or 'classical' MAPK pathway) regulates cell growth, survival and differentiation, and it is activated by many different membrane-bound receptors including receptor tyrosine kinases and G-protein coupled receptors [1]. Stimulation of these receptors leads to the activation of the small G-protein RAS (Fig. 1), the upstream activator of the RAF kinase family, which consists of ARAF, BRAF and CRAF. All three RAF kinases can activate MEK1/2, which in turn activate ERK1/2 [1,2]. Activated ERK1 or ERK2 then phosphorylate their target proteins either in the cytoplasm, or they translocate into the nucleus, where their main targets are transcription factors that regulate proliferation, differentiation or survival related genes (Fig. 1).

The ERK/MAPK pathway has long been associated with human cancers because *RAS* (including *HRAS*, *KRAS* and *NRAS*) is mutated in approximately 15% of cancers [3], and ERK is hyper-activated in

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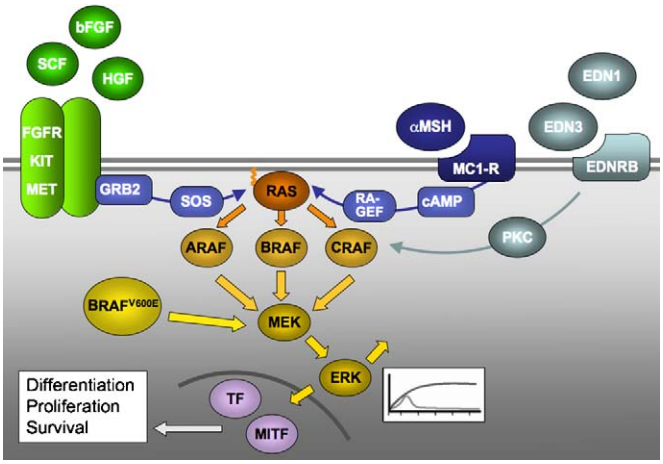

Fig. 1. The central role of ERK/MAP kinase signalling in melanocytic cells. Receptor tyrosine kinases such as FGFR, KIT and MET activate the ERK/MAP kinase pathway via GRB2 and the nucleotide exchange factor SOS. The G-protein coupled receptor MC1R activates RAS through cAMP and the exchange factor RA-GEF, whereas EDNRB appears to induce the pathway by directly activating RAF kinases. Oncogenic $BRAF^{V600E}$ constitutively activates MEK and this results in a strong and sustained activation of ERK. Downstream of all receptors, but also of $BRAF^{V600E}$ is the transcription factor MITF, which depending on the mode of activation of ERK (transient versus sustained) will regulate cell fate by activating expression from either differentiation, proliferation or survival genes.

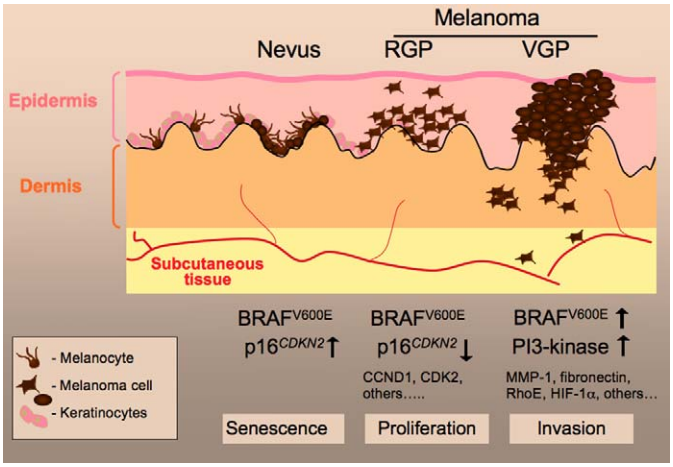

Fig. 2. Mutation of BRAF is an early event in melanoma development. BRAF mutations are found in benign nevi, but the increased expression of $p16^{CDKN2}$ contributes to oncogene induced senescence. Loss of $p16^{CDKN2}$ and increased expression of cell cycle regulators such as cyclin D1 (*CCND1*) and *CDK2* correlate with the radial growth-phase (RGP). PI3-kinase signalling cooperates with onogenic BRAF in the invasive stages of melanoma development, and $BRAF^{V600E}$ can further contribute to advanced disease by up-regulating metastasis-related factors such as fibronectin and MMP-1.

approximately 30% of cancers [4]. When BRAF was identified as an oncogene, displaying oncogenic mutations in approximately 60% of cutaneous melanoma [3], this came rather as a surprise, because until then CRAF was considered to be the RAF kinase with the highest transforming potential [1]. However, it appears that in contrast to BRAF the possibility of CRAF turning into an oncogenic protein by a single point mutation is – due to differences in their regulation – rather unlikely [5,6].

2. The relevance of the ERK/MAP kinase pathway for melanocytic cells

The striking overrepresentation of BRAF mutations in cutaneous melanoma suggests an important role of this kinase and its related signalling pathways in melanoma cells. Indeed, the ERK/MAP kinase pathway is central to the biology of melanocytes, the cells from which melanoma originates. Melanocytes are highly specialised pigment cells, which are located at the epidermal basement membrane in the skin as individual cells surrounded by adjacent keratinocytes (Fig. 2). Under physiological conditions, exposure of the skin to UV radiation induces melanocyte differentiation (tanning response), but it can also trigger proliferation and the ERK/MAP kinase pathway plays a crucial role in both processes [7].

At the molecular level UV radiation induces cAMP mediated signalling downstream of the alpha-melanocyte stimulating hormone (αMSH) receptor MC1R (melanocortin-1 receptor), which triggers a very transient (≤60 min) and weak activation of ERK and ultimately results in differentiation (see Fig. 1, [8]).

On the other hand, when the ERK/MAP kinase pathway is activated by the synergistic action of melanocyte growth factor receptors such as stem cell factor (SCF) receptor KIT or the fibroblast growth factor receptor (FGFR), or the hepatocyte growth factor (HGF) receptor MET, this results in strong activation of MEK and ultimately in the sustained phosphorylation and activation of ERK [9]; importantly strong and sustained ERK activation triggers proliferation of melanocytes (Fig. 1).

Furthermore, the endothelin receptor B (EDNRB), which regulates melanocyte differentiation, but also the proliferation and survival of melanoblasts is an activator of the ERK/MAP kinase pathway [10,11]. The dual and strikingly opposing functions of the EDNRB could be explained by the fact that although ERK activation is transient similar to MC1R induced activation, it is much stronger compared to what is achieved by the MC1R.

In addition, EDNRB activated ERK/MAP kinase signalling has been linked to the dual regulation of one protein that is central to the execution of both, differentiation and proliferation of melanocytic cells [12]. This protein is microphthalmia associated transcription factor (MITF), a tissue specific transcription factor expressed in melanocytes and melanoma [13,14]. MITF regulates fate decision by inducing the expression from a repertoire of genes that are regulating either differentiation (*e.g. TYROSINASE*, *MART1* [15,16]), proliferation (*e.g.* $p16^{INK4a}$, *CDK2*, *CDK4* [17–19]) or survival (*e.g. BCL2* [20]). Most importantly MITF is differentially modulated downstream of the ERK/MAP kinase pathway in the context of activation of each individual receptor described above (see Fig. 1). Thereby, phosphorylation of MITF by ERK results in ubiquitin-mediated degradation and thus decreased protein levels [21–23]. On the other hand ERK can stimulate the activation of transcriptions factors such as CREB or BRN2 and thereby increase transcription from the *MITF* gene [9,12,19]. Importantly, the impact of ERK on the two different ways of MITF regulation also depends on its mode of activation (transient versus sustained; strong versus weak). Thus, the ERK/MAP kinase pathway has stringent control over MITF expression levels, and this has been shown to be crucial for its fate decision function downstream of BRAF [19,21].

In summary, the fact that the mode of action of the ERK/MAP kinase pathway impacts on melanocyte fate by modulating the melanocyte specific transcription factor and fate decision regulator MITF might explain why this pathway is so particularly critical in the biology of a melanocytic cell and hence in melanoma. It also suggests that targeting this pathway at other levels than BRAF (especially when the pathway is activated by mutations in RAS or KIT, which also occur in melanoma) represents a reasonable therapeutic strategy.

3. The role of BRAF in melanoma

The first study that identified BRAF as an oncogene in cutaneous melanoma reported mutations in up to 70% of analysed tumour

samples [3]. Numerous subsequent large-scale sequencing studies confirmed the presence of BRAF mutations in melanoma with a frequency ranging from 40–70% [24]. During the last 8 years over 35 amino acids within the BRAF protein have been identified as targets for mutation in melanoma [24]. However, the most predominantly targeted amino acid, representing over 95% of all BRAF mutations in melanoma, is the valine at position 600, and mutations at this residue result in hyper-activation of the kinase (see below) [1,24,25].

Most strikingly apart from mutations in primary tumours and metastases, V600 mutant BRAF is also found in benign nevi (Fig. 2, [26,27]). This suggests that although mutated BRAF is the most prominent oncogene in melanoma, and acquiring an activating mutation in BRAF clearly seems to create an advantage in the propagation of melanocytic cells, signalling from activated BRAF is not sufficient to fully transform normal human melanocytes.

Nevertheless, it is thought that mutated BRAF will initially stimulate sustained and strong activation of ERK and thus trigger melanocyte proliferation and clonal expansion. This situation is comparable with physiological conditions, in which UV radiation can induce the production of growth factors that trigger the benign proliferation of melanocytes. However in both cases it appears that this clone of melanocytes will eventually senesce (that is undergo irreversible growth arrest), which is in line with the finding that nevi mainly consist of senescent cells with increased expression of $p16^{CDKN2A}$ [28]. Importantly, mutated hyper-activated BRAF can induce senescence and expression of the cell cycle inhibitor $p16^{CDKN2A}$ in melanocytes [28,29]. Thus, the presence of BRAF mutations in benign nevi appears to elicit the phenomenon of oncogene induced senescence. It should be mentioned however that although $p16^{CDKN2A}$ is a target of mutant BRAF and is strongly expressed in nevi, BRAF induced senescence in melanocytes can occur independently of $p16^{CDKN2A}$ [29] and appears to involve additional factors. Cytokines such as IL-6 are suggested to be involved in oncogenic BRAF induced senescence in melanocytes [30]. Another secreted factor inducing $BRAF^{V600E}$ induced senescence might be IGFBP7 [31], but in contrast to $p16^{CDKN2A}$, a correlation between BRAF mutation status and IGFBP7 expression is still debated [32].

BRAF mutations have also been identified in intra-epidermal lesions called radial growth-phase (RGP) melanoma or melanoma *in situ*, a stage at which a reduction in the cell cycle inhibitor $p16^{CDKN2A}$ expression is observed (Fig. 2). Reduced expression or loss of $p16^{CDKN2A}$ is a hallmark of melanoma and at this stage it is thought to contribute to deregulated cell cycle progression [33]. Because oncogenic BRAF stimulates the expression of cell cycle progression genes such as *CCND1*, *CDK4* and *CDK2* [19,34], the absence of $p16^{CDKN2A}$ allows BRAF to contribute to the hyper-proliferative phenotype of RGP melanoma. In line with such a hyper-proliferative phenotype the majority of additional genetic changes that have been identified in RGP melanoma compared to nevi, appear to be in genes involved in cell cycle regulation and proliferation [35].

RGP cells can progress to the vertical growth-phase (VGP), a stage where the cells have metastatic potential with nodules or nests of cells invading the dermis (Fig. 2). At this stage an increase in the presence of BRAF mutations can be observed [36], which suggests a role for BRAF in melanoma progression. Accordingly, numerous tumour progression-related genes have been identified downstream of oncogenic BRAF, such as the extracellular matrix protein fibronectin, the matrix metalloproteinase MMP-1, the hypoxia inducible factor HIF-1alpha, the Rho GTPase Rnd3/RhoE and inducible nitric oxide synthase, iNOS [37–41]. BRAF also contributes to neo-angiogenesis by inducing autocrine VEGF secretion [42].

Despite this plethora of BRAF downstream events, it becomes increasingly apparent that one particular pathway is required to cooperate with oncogenic BRAF in tumour progression, and this is PI3-kinase induced signalling. First indications for the relevance of this cooperation came from genetic data demonstrating that while *NRAS* and *BRAF* mutations are mutually exclusive – presumably due to the fact that NRAS acts upstream of BRAF – BRAF mutations and loss of PTEN, a PI3-kinase antagonist, are found to coincide with high frequency [43], although in general loss of PTEN occurs less frequently (approximately 30%) [44]. The requirement of the cooperativity between ERK/MAPK and PI3-kinase signalling for melanoma progression *in vivo* has been demonstrated by two independent studies using either mouse or zebrafish as model organisms [45,46].

Importantly, PI3-kinase induced signalling is hyper-activated in melanoma [25]. Besides the loss of function of the phosphatase PTEN, this can be due to mutationally activated *NRAS*, which also acts upstream of PI3-kinase [46–48]. Furthermore, over-expression of the PI3-kinase effector protein kinase B (PKB/AKT) is proposed to contribute to PI3-kinase activation in melanomas [49]. In line with a role in tumour progression, PI3-kinase signalling regulates cell survival, proliferation, growth (increase in cell mass) and motility [50]. Thus, targeting the PI3-kinase pathway (*e.g.* PI3-kinase, PKB/AKT, mTOR) in addition to BRAF appeals as a promising approach, and has already been taken into consideration in the design of current clinical trials.

Overall oncogenic BRAF activates constitutive ERK signalling, and stimulates proliferation and survival. Moreover, the wealth of studies of recent years demonstrates that BRAF regulates not only melanoma initiation and progression, but also provides essential tumour maintenance functions [51], which validates this kinase as an important therapeutic target for the treatment of melanoma.

4. The regulation of BRAF kinase activity and mutant BRAF signalling

In recent years our understanding of BRAF function and its regulation has increased tremendously. With the relevance of RAS for cellular transformation, the mode of activation of RAF kinases by RAS had already been studied in detail before the discovery of BRAF mutations in cancer. This regulation includes the recruitment of RAF kinases to the membrane, phosphorylation and dephosphorylation events, conformational changes and interaction with scaffolding proteins [1]. However, the identification of the individual mutations in BRAF allowed new insight into its regulation. In addition the crystal structure of the BRAF kinase domain was solved, which helped to understand the mode of activation of the kinase [5]. This knowledge is of great importance for the development of BRAF specific inhibitors, but it is also crucial in order to predict and confront the emergence of resistance as seen for example with imatinib (Gleevec®, STI571).

The crystal structure of the BRAF kinase domain revealed the complex conformational changes involved in the activation of the catalytic domain [5]. Unlike other kinases the BRAF kinase domain is folded into a conformation in which an atypical intra-molecular protein–protein interaction between the glycine-rich loop and the activation segment induces an inactive conformation. It is thought that mutations, which disrupt the interaction between the glycine-rich loop and the activation segment (*e.g.* V600E, K601E) mimic phosphorylation, which under physiological conditions would take place at residues adjacent to V600 and allow the kinase to fold into the active conformation. These mutants will then activate MEK and ultimately lead to constitutive activation of ERK. Thus, patients carrying an activating BRAF mutation would qualify not only for treatment with a BRAF specific inhibitor, but also for instance for a MEK inhibitor. In fact it has been shown that cell lines carrying the

activating BRAF mutation V600E show increased sensitivity to MEK inhibition [52].

The model of BRAF activation described above can explain the majority of mutations found in the activation segment and the glycine-rich loop. However, a series of mutations do not significantly increase BRAF activity, with some of them displaying even decreased activity (*e.g.* G469E, G466A). Analysis of these so-called 'intermediate' and 'impaired' mutants revealed a novel mechanism in which CRAF acts as a BRAF effector [5,53]. In this situation the BRAF mutants activate CRAF and this results in efficient stimulation of MEK and ERK in a BRAF but also CRAF dependent manner.

CRAF is also the RAF kinase that is activated in melanoma cells expressing oncogenic RAS [54], and it appears that when cells signal through CRAF, their survival signalling is dependent on this RAF isoform in a MEK independent manner [55]. This is in line with the fact that CRAF is known to be able to activate survival pathways independently of MEK [56,57], and might also explain why melanoma cells expressing 'intermediate' or 'impaired' BRAF mutants are more sensitive to inhibition by sorafenib [58].

Using the information acquired from the BRAF structure and the mode of action of the various BRAF mutants, much effort is going into developing inhibitors that will specifically inhibit mutant activated BRAF. However, the necessity for also targeting CRAF is becoming increasingly evident, since for instance BRAF mutant cell lines can acquire resistance towards BRAF specific inhibitors through increased CRAF expression [59], and CRAF expression appears to be increased in melanomas when compared to benign nevi [60]. Moreover, several recent studies show that the complexity of RAF isoform specific signalling and cross-activation could provide melanoma cells with an escape mechanism from BRAF inhibition, and even result in tumour promoting effects in the presence of a BRAF specific inhibitor [61–64]. The BRAF specific inhibitors GDC-0879 and PLX-4720 very efficiently and specifically block $BRAF^{V600E}$ induced ERK activation and xenograft growth [64,65]. However, strikingly these drugs activate ERK and increase proliferation in oncogenic RAS expressing cells, in which all RAF isoforms are activated downstream of RAS [61,62,64]. A similar observation was made with PLX-4032 (a structural analog of PLX-4720) [63]. The mechanism appears to be based on the ability of drug-bound BRAF to translocate to the membrane where it can activate CRAF [61,62]. Thus, care has to be taken as to who is treated with a BRAF specific inhibitor and under certain conditions the strategy of using pan-RAF kinase inhibitors may even be the better option.

5. Sorafenib/BAY 43-9006 (Nexavar®) and second generation BRAF inhibitors

Coinciding with the discovery of BRAF mutations in melanoma was the development of the targeted inhibitor sorafenib/BAY 43-9006 originally designed to inhibit BRAF's 'brother' CRAF, which was until then the RAF kinase considered to possess the highest oncogenic potential. Sorafenib is a biaryl modified urea molecule that competes with ATP for binding to RAF kinases [66]. The crystal structure of sorafenib in complex with the kinase domain of BRAF revealed that the distal pyridyl ring of sorafenib interacts directly with three amino acids in the ATP adenine binding pocket [5]. These amino acids are present also in CRAF, and sorafenib inhibits both RAF isoforms with similar potency by binding to and stabilizing the inactive state of the protein. Sorafenib also inhibits the V600E mutant form of BRAF (less potently than the wild-type form), as well as a number of other protein kinases including VEGFR2 and -3, PDGF, p38 MAPK, FLT3 cKIT, FMS and RET [67]. Thus sorafenib is in reality a multi-kinase inhibitor. While interfering with multiple targets increases the chance of toxicity, it can prove useful in multifactorial disease like melanoma where multiple kinases are hyperactive and stimulate numerous processes such as proliferation, invasion and angiogenesis. In truth, sorafenib is well tolerated, with a maximum tolerated dose (MTD) of 400–600 mg bid [68,69]. The most common toxicities associated with sorafenib are hand-foot skin reaction (HFS), rash and diarrhoea. However, these adverse events are predominantly mild to moderate in severity and easily manageable.

Preclinical studies demonstrated that sorafenib could retard the growth of human melanoma cells engrafted in mice and induced near complete inhibition of MEK phosphorylation [55], and near complete suppression of ERK phosphorylation these xenografts [67]. Sorafenib was however ineffectual in preventing lung metastases in an experimental mouse model [70]. Pharmacodynamic studies in man revealed almost complete suppression of ERK phosphorylation in peripheral lymphocytes at 400 mg bid [68], but only partial inhibition in melanoma tumours [69,71]. Perhaps for this reason, sorafenib had only modest activity as a single agent in treating advanced melanoma in a phase I trial. Only one objective response among 34 assessable patients was observed although a small cohort of patients with previously progressive disease maintained stable disease for more than 6 months [71]. This disappointing outcome, notwithstanding, trials were then performed comparing the efficacy of conventional chemotherapy to the same therapy combined with sorafenib. A phase I trial combining sorafenib with paclitaxel and carboplatin suggested improvement in objective response rate and progression free survival [72], which however was not reproduced in a subsequent phase III trial [73]. Combining sorafenib with dacarbazine or its congener temozolomide has also shown benefits to progression free survival compared to chemotherapy alone [74,75]. However, in the above cited trials, clinical outcome did not correlate with $BRAF^{V600E}$ status. Coupled with the clinical efficacy of sorafenib in renal cell and hepatocellular carcinoma, where oncogenic RAF is not implicated, it is likely that the clinical target of sorafenib is another kinase, perhaps VEGFR2 or PDGFR, and not BRAF.

A number of other small-molecule inhibitors, including Raf265 (Novartis), XL281 (Exelixis/Bristol Myers Squibb), AZ628 (AstraZeneca), SB-590885 (GlaxoSmithkline) and PLX-4720 (Plexxikon/Roche) have since been developed that are more selective than sorafenib for RAF kinases, are much more potent MAPK signalling inhibitors *in vivo*, and retain excellent antitumour activity in xenograft models [65,75–79]. With these inhibitors it is hoped that the validity of oncogenic BRAF as a therapeutic target can be properly evaluated in the clinic. Of these second generation inhibitors, Raf265, XL281 and PLX-4032 (a structural analog of PLX-4720) are currently under clinical investigation as single agents in advanced melanoma (www.cinicaltrials.gov). Results disclosed at the ASCO 2009 Annual Meeting for PLX-4032 in particular have ignited optimism among oncologists. PLX-4720 was designed by Plexxikon according to the atomic structure of $BRAF^{V600E}$, and as such is highly selective for BRAF and demonstrates 10-fold greater potency for $BRAF^{V600E}$ versus wild-type in kinase assays and over 100-fold selectivity in cell proliferation assays [65]. Further, PLX-4032 possesses good oral bioavailability and because of its selectivity for the oncogenic form of the kinase displays little toxicity; 960 mg BID is currently under evaluation as the MTD. In a phase I trial, 13 of 16 metastatic melanoma patients who were positive for $BRAF^{V600E}$ and received $\geq$240 mg BID showed tumour regression (an astounding 80% response rate), including those with liver, lung and bone metastases. Some responding patients remained progression free at the end of the trial, up to 14 months, with treatment continuing. Interim median progression free survival was at least 6 months. As expected, patients negative for $BRAF^{V600E}$ failed to respond to the drug. Although numbers in this trial were small, and relapse

(resistance) was encountered, and a survival benefit has yet to be proven, this study strongly indicates that selective targeting of early driver mutations, and in particular oncogenic BRAF in the case of melanoma, can induce tumour regression in man.

6. Combined targeted therapies

While the experience with PLX-4032 indicates that monotherapy can stabilize disease or even cause regression of malignant lesions, complete and durable responses (might one even risk using the word cure) are likely to require combining potent RAF inhibitors either with conventional chemotherapy (as outlined above for sorafenib) or with other targeted therapies. As mentioned above (Section 3), there is compelling evidence to indicate that simultaneous suppression of ERK/MAPK signalling and PI3-kinase signalling are more effective at killing melanoma cells than blockade of either pathway alone. Thus simultaneous pharmacological antagonism of MEK and PI3-kinase was more effective than blockade of either alone in preventing melanoma progression in TPRas transgenic mice [80]. Similarly, both ERK and PI3-kinase signaling modules had to be suppressed to induce apoptosis in organotypic cultures of human melanoma cells [81,82]. *In vivo*, siRNA-mediated knockdown of AKT3 and $BRAF^{V600E}$ augmented the suppression of melanoma xenograft growth [83,84]. Combined administration of rapamycin (a selective mTOR inhibitor) and PD325901 (a highly selective MEK inhibitor) resulted in melanoma regression in mutant mice expressing $BRAF^{V600E}$ and lacking PTEN; monotherapies were only effective at preventing disease onset or stabilizing disease [45].

In terms of pharmacological inhibitors of PI3-kinase signaling, rapamycin (sirolimus) analogs are the most clinically developed. These bind to FK506-binding protein-12 (FKBP12) to form a complex that interacts with and antagonizes mTOR. A phase II clinical trial failed to detect activity for CCI-779 (temsirolimus) as a single agent in advanced melanoma [85]. However, preclinical studies demonstrated a co-operative effect between sorefenib and rapamycin in inhibiting melanoma cell proliferation and invasion [86], and new clinical trials have been devised to test the efficacy of combined sorafenib and CCI-779 in advanced melanoma patients. Regardless of the outcome, it would be prudent to evaluate the combination of these and other mTOR and PI3-kinase inhibitors with second generation RAF inhibitors.

7. Conclusions

The discovery of BRAF mutations in melanoma in 2002 hugely encouraged the development of targeted drugs for this cancer and stimulated a fundamental change in the approach to melanoma therapies. Further studies aimed to genetically subgroup melanoma led to the identification of other targets such as KIT, and contributed to a much improved understanding of the genetic basis of this cancer. In addition, a plethora of cell biological studies has helped to shed some light on the cellular signalling that is relevant for melanoma initiation as well as progression to metastatic disease. With the current exciting situation of novel BRAF specific inhibitors showing promising results, and the possibility to identify appropriate melanoma subgroups, the concept of combinatorial therapies with either traditional cytotoxic drugs or specific drugs targeting further critical molecules (probably in a different signalling pathway) appears to be key to better improved therapies in the future.

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