Is B-Raf a Good Therapeutic Target for Melanoma and Other Malignancies?

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Abstract

The RAF family members, A-Raf, B-Raf, and C-Raf (or Raf-1), are intermediate molecules in the mitogen-activated protein (MAP) kinase [Ras/Raf/MAP kinase/extracellular signal–regulated kinase (Erk) kinase (MEK)/Erk] pathway, which relays extracellular signals from the cell membrane to the nucleus via a cascade of phosphorylation events ultimately promoting cancer development. This pathway is activated by mutation in ~7% of all human cancers. B-Raf is one of the proteins frequently mutated to an active form during tumor development. Therefore, B-Raf is an attractive cancer target but lack of clinical efficacy using agents targeting this protein has raised serious doubts about its therapeutic utility. Design of more effective B-Raf inhibitory agents, targeting other members of the signaling cascade for greater clinical efficacy or inhibiting B-Raf in combination with other targets, is being evaluated to resolve these perplexing issues. Here, we discuss recent progress, using preclinical models and clinical studies, to resolve the controversy of whether B-Raf would be a good therapeutic target for melanoma and other malignancies.

Background

B-Raf is central to mitogen-activated protein kinase signaling and frequently activated by mutation in cancer. B-Raf is one of three serine/threonine kinases in the Raf family, which includes A-Raf, B-Raf, and C-Raf (or Raf-1; refs. 1, 2). Raf family members are intermediate molecules in the mitogen-activated protein (MAP) kinase [Ras/Raf/MAP kinase/extracellular signal–regulated kinase (Erk) kinase (Mek)/Erk] pathway, a signal transduction cascade relaying extracellular signals from cell membrane to nucleus via an ordered series of consecutive phosphorylation events, which are detailed in Fig. 1A (1). Alterations activating members of the pathway are implicated in development of carcinomas of the skin (27–70%), ovary (3–30%), thyroid (36–53%), colon (5–22%), and pancreas (~33%; refs. 1, 2). B-Raf in this cascade is the most mutated gene in melanomas, with >60% of advanced tumors containing constitutively active mutant protein.

Among different B-Raf mutations, a single-base missense substitution (T to A at nucleotide 1,799) that changes valine to glutamic acid at codon 600 (V600E) in exon 15 is prevalent in ~90% of melanoma tumors with mutation of B-Raf (3). V600E-B-Raf protein leads to kinase activity 10.7 times higher than occurs in normal cells and causes hyperactivity of the MAP kinase pathway promoting tumor development (2, 3). Activation occurs as a result of a conformational change in protein structure due to glutamic acid acting as a phosphomimetic between the Thr598 and Ser601 phosphorylation sites (2, 4). Activating B-Raf mutations are acquired, somatic, post-zygotic events and are not inherited in families (5). Cell culture and/or animal based studies have shown the oncogenic potential conferred by V600E-B-Raf to developing cancer cells (1, 2).

Abnormally high activation of the MAP kinase pathway can inhibit cellular growth in a wide variety of normal and cancer cells by promoting cellular senescence (6). This senescence is suggested to be caused by induction of a variety of cyclin-dependent kinase inhibitors, such as p21Cip1, p16INK4a, and p27Kip1, which are proposed to act as a putative defense mechanism of normal cells to overcome oncogene activation (6). In contrast, moderate levels of MAP kinase pathway activation have been shown to promote transformation and immortalization of mouse melanocytes, increased in vitro colony formation, and elevated Erk1/2 activities (1, 2). Recent evidence suggests that V600E-B-Raf initially promotes nevi development but the resulting high, intense activation of the MAP kinase pathway inhibits further tumor progression. Furthermore, additional genetic changes such as loss of p16INK4a or elevation in Akt3 activity are needed for the senescent melanocytic cells to reenter the cell cycle. Recently, Akt3 activity in early melanocytic lesions has been shown to phosphorylate V600E-B-Raf to reduce its activity and the MAP kinase pathway activity to levels promoting, rather than inhibiting, proliferation to overcome the senescence block.

Key Finding

Lessons learned from preclinical studies about the therapeutic potential of targeting B-Raf. MAP kinase pathway activation is central to cancer development by enhancing multiple oncogenic processes (4). Therefore, therapies targeting mutant V600E-B-Raf activity or other components of the MAP kinase cascade could halt progression of malignant tumors by slowing tumor growth, preventing angiogenesis, inhibiting invasion and metastasis, inducing tumor cell death, or promoting tumor differentiation (2, 7–9). Targeted therapies of this type can be effective; for example, inhibiting Abl and KIT tyrosine kinases with the small-molecule inhibitor imatinib mesylate (Gleevec, ST1571) was shown as an effective monotherapy for treating chronic myelogenous leukemia and gastrointestinal stromal tumors (10).

The potential to inhibit tumor development by targeting B-Raf signaling has been clearly shown in multiple preclinical animal models but remains controversial in the clinical setting (2, 8).

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Figure 1. A, diagrammatic representation of the MAP kinase signaling pathway. Shown are selected proteins in the signaling cascade affected by constitutively activated B-Raf mutation and effect on cell survival, invasion, growth, and angiogenesis. Pharmacologic agents inhibiting the pathway at various locations in the pathway are also shown. Typically the cascade is activated when extracellular stimuli such as mitogens, hormones, or neurotransmitters induce receptor tyrosine kinase dimerization leading to Ras-activation by increasing cellular Ras-GTP levels. Activated Ras recruits dimerized Raf kinase to the plasma membrane where it is activated by autophosphorylation or by other kinases and assembled into a MAP kinase signaling complex consisting of Mek, Erk, and scaffolding proteins. Phosphorylation and activation of Mek1 and Mek2 by activated Raf kinase initiates MAP kinase signaling, which subsequently phosphorylates (activates) downstream Erk1 and Erk2. Activated Erk1 and Erk2 dissociate from the MAP kinase complex and phosphorylate several cytoplasmic proteins, kinases, and nuclear transcription factors that ultimately lead to expression of proteins playing important roles in cell growth, differentiation, and survival. B, melanoma tumor development was inhibited with siRNA to B-RAF but not with siRNA to C-RAF or scrambled siRNA (see ref. 8). SiRNA-mediated reduction of B-Raf led to decreased tumorigenic potential of melanoma cells. SiRNAs against B-Raf, C-Raf, and scrambled siRNA were introduced into UACC 903 melanoma cells (white arrow) and, 36 h later, cells were injected into nude mice (black arrow). Size of tumors was measured at 2-d intervals; bars, SE. SiRNA-mediated down-regulation of B-Raf lasted for up to 8 d in culture and reduced the tumorigenic potential of melanoma cells. Control cells were nucleofected with buffer only, a scrambled siRNA, or siRNA against C-Raf. C, inhibition of B-Raf signaling using sorafenib retards melanoma tumor development (see ref. 8). The effects of sorafenib (BAY 43-9006) treatment are shown on melanoma tumor development. UACC 903 melanoma cells were injected into nude mice and tumor development was allowed to occur to day 6, at which point mice were injected i.p. every 2 d with sorafenib dissolved in DMSO (indicated by arrowheads). Control animals were given DMSO only. The Raf kinase inhibitor sorafenib reduced the tumorigenic potential of melanoma cells containing mutant V600E-B-Raf protein at concentrations ≥50 mg/kg by decreasing vascular development followed by a decrease in proliferation and an increase in the tumor cell apoptosis. D, inhibition of V600E-B-Raf and downstream proteins reduces formation of metastatic tumors in the lungs of mice (see ref. 9). SiRNA-mediated reduction of mutant V600E-B-Raf, Mek, Erk, and cyclin D1 proteins decreased formation of melanoma metastases in the lungs of mice. SI RNA against each respective protein or a scrambled siRNA control was introduced into green fluorescent protein–tagged 1205Lu melanoma cells; 36 h later cells were i.v. injected into nude mice, and 17 d later lung metastases were scored.
Targeting B-Raf using siRNA or pharmacologic Raf kinase inhibitor sorafenib (BAY 43-9006) reduced MAP kinase signaling, thereby decreasing tumor and/or metastasis development in animals (Fig. 1B and C; refs. 8, 9). Treating mice containing melanomas, colon, breast, or lung tumors significantly reduced cell growth and/or vascular development thereby delaying tumorigenesis (8). Specifically, siRNA- and sorafenib-mediated inhibition of B-Raf in melanoma decreased cellular proliferation and/or vascular endothelial growth factor (VEGF) secretion, which retarded vascular development by acting as an angiogenesis inhibitor (2, 8). More recently, V600E B-Raf has also been shown to regulate melanoma metastasis through overexpression of fibronectin and by promoting interaction of melanoma cells with neutrophils to facilitate extravasation across the endothelial lining as well as enhance proliferation in the lung microenvironment (7, 11). Inhibiting these processes decreased lung metastases by 4- to 5-fold of cells containing but not those lacking the V600E B-Raf mutation (9). Thus, active B-Raf can be targeted to reduce the tumorigenic and metastatic potential of cancer cells by reducing cellular proliferation or vascular development in animal models, making it a likely clinical therapeutic target.

Unfortunately, clinical trials using sorafenib to target B-Raf signaling have not been effective except for kidney cancer, which has raised serious doubt about the utility of therapeutically targeting B-Raf signaling (12). Sorafenib has now been approved for use in humans with renal cell carcinoma with relatively few minor side effects (rashes, hand-foot syndrome, diarrhea, hypertension, and stomatitis) and extending average life span by almost 12 weeks (2, 12). Discrepancies between preclinical and clinical trial results in terms of effectiveness of shrinking tumors following B-Raf inhibition are serious and controversial issues that need resolution.

Searching for the solution to whether B-Raf is a suitable therapeutic target. Controversy remains about an explanation for the lack of therapeutic success when targeting mutant V600EB-Raf. One possibility is that it is not the most efficient strategy to inhibit the MAP kinase signaling cascade or whether it is due to the mechanism of action of sorafenib (2, 9). In response to this controversy, preclinical studies have evaluated whether targeting V600E B-Raf or another member of the MAP kinase pathway would be the most effective approach to inhibit this pathway. SiRNA-mediated inhibition of V600E B-Raf and proteins downstream in the MAP kinase signaling cascade (Mek1/2, Erk1/2, and cyclin D1) all significantly decreased lung metastases compared with controls (9). However, targeting Mek1/2 was the most effective strategy to reduce lung metastases, which is illustrated in Fig. 1D (9). Furthermore, melanomas containing mutated B-Raf are more responsive to agents targeting Mek in the MAP kinase pathway than tumors with wild-type B-Raf or harboring a Ras mutation (13). These were not the first studies to identify Mek as a potentially important therapeutic target in the pathway. Pharmacologic inhibition of Mek using CI-1040 had previously been shown to be effective for reducing melanoma metastasis (14). A separate study has also shown that inhibition of V600E B-Raf and Mek using sorafenib and U0126 was more effective in retarding tumor development compared with sorafenib monotherapy or siRNA-mediated down-regulation of B-Raf expression (2).

Additional controversy exists about whether the presence of B-Raf mutation affects therapeutic agent efficacy. Preclinical models suggest that it may alter responsiveness to therapeutic agents (13). However, studies assessing the clinical significance of B-Raf mutations in metastatic melanoma show varied results with respect to the clinical characteristics or outcomes between melanomas with or without B-Raf mutation (9). This has raised significant concern about B-Raf as a therapeutic target (2). Especially, if preclinical animal studies are correct, therapeutically targeting the MAP kinase signaling cascade may only be effective for the ~60% of melanoma patients whose tumors contain mutant V600E B-Raf protein and maximal inhibition may require inhibition of Mek1/2 and not B-Raf (9, 13). Thus, it may be critical to ascertain the mutational status of B-Raf in patients and use Mek1/2 inhibitors for greatest therapeutic efficacy. Discrepancies between preclinical animal models and clinical studies still need to be reconciled.

Pharmacologic agent selection could be the key to effectively target B-Raf pathway signaling. Whereas failure of sorafenib in clinical trials could be due to its inability to reach a concentration sufficient to inhibit B-Raf or to decreased potency against highly active mutant B-Raf compared with wild-type B-Raf, it is most likely due to off-target secondary effects. Pharmacologic agents are generally not specific but rather inhibit activity of multiple proteins. Sorafenib is one such example where, in addition to inhibiting Raf, it also decreases activity of VEGF receptor (VEGFR)-1, VEGFR2, VEGFR3, platelet-derived growth factor receptor-β, Flt-3, p38, c-Kit, and fibroblast growth factor receptor 1 (15). Therefore, inhibitory effects may be due to sorafenib or metabolized products targeting any single or combination of proteins. To address this issue, preclinical studies are evaluating the efficacy of more specific B-Raf inhibitors such as SB-590885. SB-590885 is a potent B-Raf kinase inhibitor that is ~100-fold more specific than sorafenib and acts by stabilizing the active conformation. However, use in preclinical models led to only a modest decrease in melanoma tumor volume, suggesting B-Raf might not be an ideal target (16).

Perhaps drugs need to be selected that target particular processes mediated by B-Raf signaling to be effective. Preclinical studies in animals have evaluated this possibility by identifying agents that inhibit the same tumorigenic phenotype as occurs following siRNA-mediated inhibition of B-Raf. In these studies, siRNA-mediated inhibition of V600E B-Raf showed that it was necessary for the pathway inhibitor to decrease the proliferative capacity of tumor cells for efficacy (8, 9). However, comparison of siRNA-mediated versus sorafenib-mediated inhibition showed that sorafenib significantly retarded vascular development, mediated through reduced VEGF secretion or VEGFR inhibition, whereas siRNA decreased the proliferative capacity of tumor cells (8). Studies examining the mechanism reducing metastasis development following B-Raf inhibition came to a similar conclusion showing that decreased proliferative capacity was necessary for metastasis inhibition (9). Because lung metastases in this model did not require angiogenesis, sorafenib and its antiangiogenic properties had no effect. In contrast, Mek inhibition using U0126 decreased tumor cell proliferation thereby significantly reducing lung metastases (8, 9, 17). Other reports also found sorafenib ineffective at blocking lung adenoma formation compared with the Mek inhibitor CI-1040 (14). It is confounding that sorafenib and U0126 inhibit V600E B-Raf signaling in cultured melanoma cells to similar levels but are not equally effective at inhibiting metastasis development in animals. Efficacy of an agent such as sorafenib in cultured cells does not necessarily translate into tumor inhibitory effectiveness. Thus, targeting Mek using pharmacologic agents and not mutant B-Raf might have greatest clinical efficacy.
Targeting B-Raf signaling in combination with other pathway inhibitors. Lack of clinical efficacy using sorafenib as a monotherapy and no mechanistic-based explanation for failure have led to exploration of other therapeutic regimens in which sorafenib is combined with other agents to identify combinations resulting in more effective, synergistically acting tumor inhibition. Preliminary results combining sorafenib with carboplatin and paclitaxel in 2004 looked promising with responses in melanoma patients, possibly as high as 50%, when compared with sorafenib alone (18). However, recent results of phase III trials in patients with advanced melanoma announced in 2006 that this combination failed to improve progression-free survival of patients (19). Thus, this combination might not be effective as first thought, driving the search for alternative strategies to improve the efficacy of sorafenib (18).

Recent preclinical studies combining sorafenib with other agents to increase its efficacy are providing a mechanistic basis for selection of novel agent combinations that might be clinically effective. For example, sorafenib has been combined with Rottlerin, a protein kinase C inhibitor, leading to increased synergistic inhibition of cell proliferation and apoptosis in glioma cells (20). In a similar manner, systemic liposomal ceramide has been shown to enhance efficacy of sorafenib. This combination led to inhibition acting in a cooperative synergetic manner, which has therapeutic potential for more effectively treating melanoma and breast cancer through enhanced cellular apoptosis. Thus, targeting B-Raf signaling using sorafenib may be more effective in combination with other therapeutic agents than alone. It remains to be determined whether these combinations would be more effective in the clinic. Thus, uncertainty remains whether sorafenib could be combined with another agent to improve its clinical efficacy; however, before clinical evaluation, the agent combination would need to have a solid and novel mechanistic underpinning before proceeding to the clinic.

Conclusion. B-Raf is central to MAP kinase signaling and is frequently activated in cancer. It remains uncertain whether targeting B-Raf could be an effective therapeutic approach for patients whose tumors have an active form of this protein. Currently, targeting Mek, rather than B-Raf, in the MAP kinase pathway seems to be the most promising therapeutic strategy.

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References


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