Guilty as charged: B-RAF is a human oncogene

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RAF proteins are components of a conserved signaling pathway that regulates cellular responses to extracellular signals. Despite over 20 years of research into the regulation and function of the RAF proteins, it was only realized recently that the B-RAF isoform is mutated at a high frequency in human cancer. Here we review the rapid progress made in our understanding of B-RAF as an oncogene and of its role in cancer.

Introduction

There are three RAF paralogs in humans: A-RAF, B-RAF, and C-RAF (also called Raf-1) (Marais and Marshall, 1996). These serine/threonine protein kinases are components of a conserved signaling pathway downstream of the membrane-bound small G protein RAS, which is activated by growth factors, hormones, and cytokines (Robinson and Cobb, 1997). RAS stimulates RAF activation, which then activates a second protein kinase called MEK, which in turn activates a third protein kinase called ERK (Figure 1). ERK regulates gene expression, cytoskeletal rearrangements, and metabolism to coordinate responses to extracellular signals and regulate proliferation, differentiation, senescence, and apoptosis. This pathway is hyperactivated in ~30% of cancers (Hoshino et al., 1999) with activating mutations in *RAS* occurring in approximately 15%–30% of cancers (Bos, 1989), and recent data has shown that *B-RAF* is mutated in about 7% of cancers (Davies et al., 2002), identifying it as another important oncogene on this pathway.

A short course on RAF regulation

RAF proteins share three conserved regions; CR1 and CR2 in the N terminus (Figure 2) are largely regulatory, whereas CR3 at the C terminus encompasses the kinase domain. RAF regulation is complex and involves many steps (for reviews see Avruch et al., 2001; Mercer and Pritchard, 2003). In its simplest form, RAS recruits cytosolic RAF to the plasma membrane for activation (Figure 1), with one of the essential steps being the phosphorylation of two amino acids (T598 and S601 of B-RAF; see Note at the end of the main text for amino acid numbering used in this review) within the activation segment of the kinase domain (Figure 2) (Zhang and Guan, 2000). Phosphorylation of a motif called the *n*egative-charge regulatory region (N region) is also required, and critically, this region has important consequences for the differential regulation of the RAF isoforms. S338 and Y341 of C-RAF, and similarly S299 and Y302 of A-RAF, must be phosphorylated for maximal activation (Figure 2) (Fabian et al., 1993; Mason et al., 1999). In B-RAF, S445 (equivalent to S338 of C-RAF) is constitutively phosphorylated, and the equivalent of Y341 is replaced by an aspartic acid (D448; Figure 2). Thus, unlike A-RAF and C-RAF, the N region of B-RAF carries a constitutive negative charge, and so B-RAF is primed for activation, and this occurs when it is recruited to the plasma membrane for activation segment phosphorylation. By contrast, C-RAF and A-RAF activation is more complex, because, like B-RAF, they also require membrane recruitment and activation segment phosphorylation, but in addition, they require N region phosphorylation.

The A to Z of B-RAF mutations

The highest incidence of *B-RAF* mutations is in malignant melanoma (27%–70%), papillary thyroid cancer (36%–53%), colorectal cancer (5%–22%), and serous ovarian cancer (~30%), but they also occur at a low frequency (1%–3%) in a wide variety of other cancers (Table 1). Over 40 different missense mutations in *B-RAF*, involving 24 different codons, have been identified (Figure 2). Most mutations are extremely rare, accounting for 0.1%–2% of all cases. However, a thymidine to adenosine transversion at nucleotide 1796, converting valine 599 (V599) to glutamate predominates (Davies et al., 2002). It

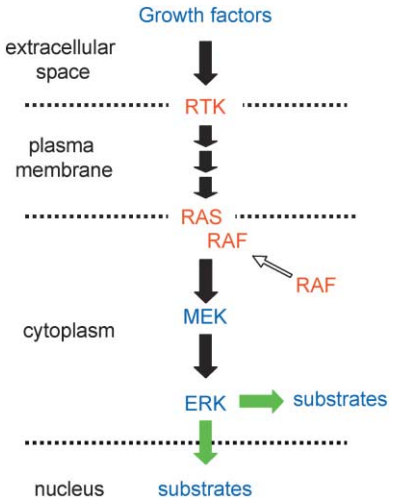

Figure 1. The RAS-ERK signaling pathway

The classical RAS-ERK signaling pathway is depicted. Growth factors bind to and activate receptor tyrosine kinases (RTKs), which, through a series of adaptor proteins and exchange factors, stimulate RAS activation. RAS proteins are attached to the inner surface of the plasma membrane. In their active form, they bind to and recruit RAF proteins from the cytosol to the plasma membrane, which is where RAF is activated. RAF then phosphorylates and activates MEK, which in turn phosphorylates and activates ERK. ERK phosphorylates proteins in the cytosol, and it also translocates to the nucleus, where it phosphorylates proteins such as transcription factors. The RTKs, RAS, and RAF are highlighted in red to reflect the fact that they are all mutated in cancer.

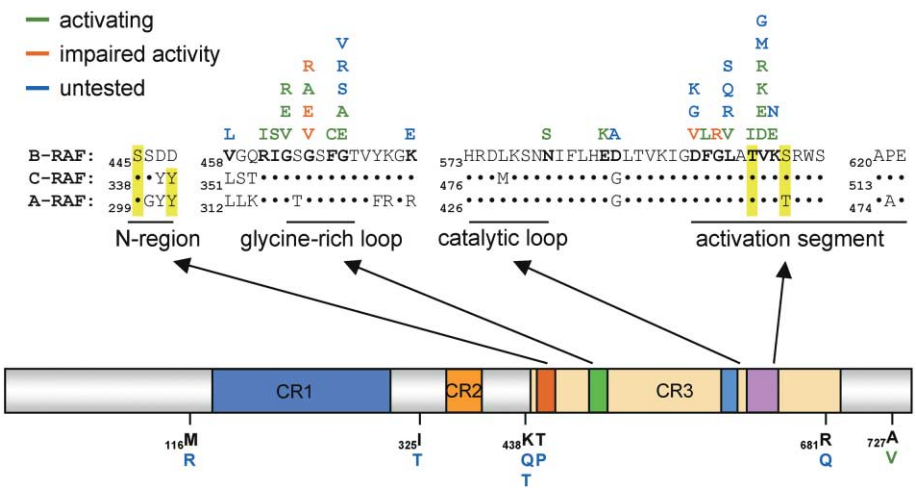

Figure 2. Cancer-associated B-RAF mutations

The structure of B-RAF is shown schematically. The three regions that are conserved in all RAF proteins, CR1 (blue), CR2 (orange), are CR3 (straw) are shown. The kinase domain is encompassed within CR3. The amino acid sequence of the N region, the glycine-rich loop, the catalytic loop, and the activation segment (single amino acid code) for B-RAF, C-RAF, and A-RAF are aligned above the schematic. The yellow bars highlight residues whose phosphorylation is required for kinase activity. The residues that are mutated in B-RAF in cancer are shown in bold, with the amino acid substitutions above the sequence. In addition, mutated residues outside these core regions are shown below the schematic, with the amino acids that they are converted to below them. The mutated residues are color-coded for their effect on B-RAF kinase activity. Activating substitutions are shown in green, those that impair B-RAF kinase activity are in red, and untested mutants are in blue. Amino acids in A-RAF and C-RAF that are conserved in B-RAF are indicated by a dot.

accounts for most (over 90%) of the mutations in melanoma and thyroid cancer and for a high proportion of those in colorectal cancer, but is comparatively rare in non-small cell lung cancer (Fukushima et al., 2003; Kimura et al., 2003; Kumar et al., 2003; Nikiforova et al., 2003; Brose et al., 2002; Davies et al., 2002; Rajagopalan et al., 2002; Yuen et al., 2002). It is worth noting that in the absence of the V599E substitution, B-RAF mutations in cancer would be rather rare. V599 can also be mutated to other residues (Table 1), although at a much lower frequency (0.1% to 2%). Presumably this is because, although their activities are similar to that of ^{V599E}B-RAF (Wan et al., 2004), they arise through tandem nucleotide changes that are very rare. This is supported by the observation that a tandem mutation that generates ^{V599E}B-RAF is also very rare (<0.1%) (Houben et al., 2004).

The pattern of *B-RAF* mutations raises two important questions. First, why are *B-RAF* mutations common in some cancers but not in others? In part, the answer comes from the observation that *B-RAF* mutations occur in many of the same cancers in which *RAS* is mutated, but concomitant mutations are extremely rare (Davies et al., 2002; Rajagopalan et al., 2002; Singer et al., 2003). This is elegantly illustrated in papillary thyroid cancer, where 66% of tumors have activating mutations in *RET* (a receptor tyrosine kinase that signals through RAS), *RAS*, or *B-RAF*, but strikingly, there is no coincidence between them (Kimura et al., 2003). Thus, *B-RAF* mutations appear to occur in cancers where there is strong selection for aberrant ERK signaling, and the mutations causing this can occur at different levels within the pathway. The biology of cancer precursor cells may also provide a partial answer to the question. Melanocytes and thyrocytes are neuronal in origin and stimulation with appropriate hormones elevates their intracellular cAMP levels, activating B-RAF and inducing proliferation (Busca et al., 2000; Iacovelli et al., 2001). The interplay between B-RAF, cAMP, and proliferation in these cells may provide strong selection for B-RAF mutations, particularly ^{V599E}B-RAF.

The second question is: why do V599 mutations predominate? The selection does not appear to be based on catalytic activity, because mutants such as ^{G468A}B-RAF have similar activ-

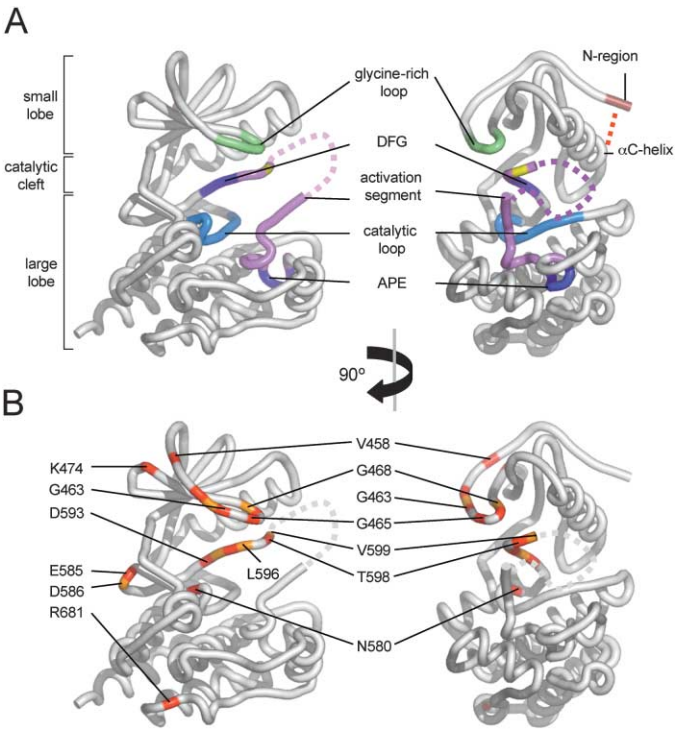

Figure 3. B-RAF kinase domain structure

The structure of the B-RAF kinase domain is shown (residues 447–725). For clarity, BAY43-9006, the inhibitor that B-RAF was crystallized with, has been omitted.

A: Important catalytic and structural features. The small lobe, large lobe, and catalytic cleft are indicated. The N region is colored rust, with the interaction that occurs between D447 of this region and R505 of the αC-helix being depicted by the red dashed line. The glycine-rich loop is green, the catalytic loop is blue, the activation segment is magenta, and the DFG and APE motifs are purple. A portion of the activation segment is disordered and is indicated by the dashed magenta line. T598, the activation segment phosphorylation site, is colored yellow.

B: Mutations in B-RAF in cancer. The same views shown in **A** are presented, but with the mutated residues colored red or orange (alternate colors are used for clarity, but have no additional significance).

Table 1. Cancer-associated mutations of B-RAF

Amino acid[a]	Coding variant[b]	Frequency[c]	Cancer type[d] (incidence)
M116	R	0.1	Mel (1)
I325	T	0.1	Breast (1)
K438	Q, T	0.2	Lung (2)
T439	P	0.1	Lung (1)
V458	L	0.1	Lung (1)
R461	I	0.1	Colo (1)
I462	S	0.1	Colo (1)
G463	E, V, R	0.5	Colo (3), liver (1), ova (1)
G465	A, E, R, V	0.9	Mel (7), lung (2)
F467	C	0.1	Colo (1)
G468	A, E, R, S, V	1.9	Mel (4), leukemia (4), colo (3), nhl (3), liver (2), lung (2), bar (1)
K474	E	0.1	Mel (1)
N580	S	0.1	Colo (1)
E585	K	0.1	Ova (1)
D586	A	0.1	Colo (1)
D593	G, K, V	1.0	Colo (6), stom (2), mel (1), nhl (1)
F594	L	0.3	Colo (2), liver (1)
G595	R	0.2	Colo (2)
L596	Q, R, S, V	0.8	Mel (3), lung (3), liver (1), ova (1)
T598	I	0.1	Colo (1)
T598-insertion	Two amino acid insertion (T-T)	0.1	Mel (1)
V599	D, E, G, K, M, R	91[e]	Mel (442), thy (187), colo (179), ova (24), liver (11), sarcoma (7), stomach (5), glioma (4), Bar (2), breast (1), epen (1), lung (1)
V599-insertion/ deletion	Six amino acids (V599-K604) replaced by an aspartic acid	0.1	Mel (1)
K600	E, N	1.0	Thy (4), mel (3), colo (3)
R681	Q	0.1	Endo (1)
A727	V	0.1	ALL (1)

[a]The mutations described in *B-RAF* in cancer are listed in column 1.
[b]The residues they are mutated to (coding variant) are shown in column 2.
[c]In column 3, the frequency with which each residue is mutated with respect to reported *B-RAF* mutations is provided. These data are compiled from 37 publications with over 900 B-RAF mutation-positive tumor samples and cell lines. Due to editorial limitations we are unable to cite all the references used to compile this table and we apologize to the authors of those papers not cited. For a detailed description of the B-RAF mutations in cancer, please see the COSMIC website (www.sanger.ac.uk/genetics/CGP/cosmic/).
[d]Finally, in column 4, the occurrence of each mutation in specific cancers is shown: mel, melanoma; colo, colorectal; thy, thyroid; ova, ovarian; endo, endometrial; nhl, non-Hodgkin's lymphoma; bar, Barret's adenocarcinoma; epen, ependymoma; ALL, acute lymphoblastic leukemia. Incidence refers to the total number of times the mutations at the indicated position have been identified in that cancer type.
[e]Altogether, the V599 substitutions account for 91% of the mutations in cancer. However, the V599E mutation accounts for approximately 95% of these, and therefore approximately 86% of all mutations in cancer.

ity to ^{V599E}B-RAF and are also generated through a single nucleotide substitution, but account for less than 1% of mutations (Davies et al., 2002). The high incidence of V599E mutations in melanoma, a disease whose only risk factor is exposure to sunlight (De Vita et al., 2001), suggests that this mutation is induced by ultraviolet (UV) light. Indeed, V599E mutations are absent in mucosal melanoma (Edwards et al., 2004) and are also rare in melanomas from regions of sun-protected skin (Maldonado et al., 2003). However, the T>A transversion associated with V599E mutations is distinct from the C>T or CC>>TT pyrimidine dimer mutations common to UV-induced DNA damage (Daya-Grosjean et al., 1995). Moreover, B-RAF mutations are not found in other forms of skin cancer such as basal or squamous cell carcinomas, and UV exposure is clearly not responsible for the V599E mutations in colorectal, thyroid, and ovarian cancers. Thus, although the V599E mutations may be an indirect consequence of UV exposure in melanoma, in other cancers, it is presumably the context of the DNA surrounding this codon combined with the biology and/or the environment of the cells that accounts for the high frequency of this mutation.

A molecular understanding of B-RAF mutations

Our understanding of B-RAF regulation was greatly increased recently when the crystal structure of the B-RAF kinase domain bound to the small molecule inhibitor BAY43-9006 was solved (Wan et al., 2004). The kinase adopts the overall fold typical of active kinases, with a small and large lobe separated by a catalytic cleft (Figure 3A). The N region aspartic acid at position 447 helps to stabilize the small lobe. The glycine-rich loop, identified by the GXGXXG motif (G = glycine, X = variable) which clamps ATP into the catalytic cleft (Johnson et al., 1998) is well defined (Figures 2 and 3A). A portion of the activation segment, a flexible region between the conserved DFG and APE motifs

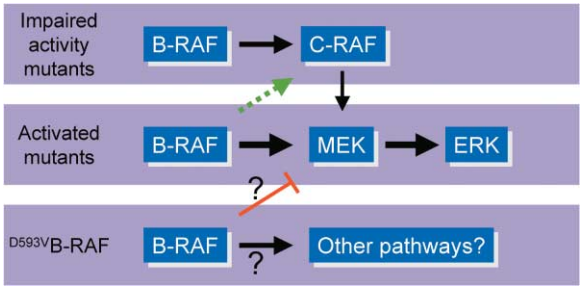

Figure 4. B-RAF signaling in cancer

The signaling pathways stimulated by B-RAF mutants in cancer are shown. The impaired activity mutants cannot signal to MEK directly, but do so by activating C-RAF. The activated mutants signal to MEK directly, and although they can also activate C-RAF, this does not appear to be necessary for signaling to MEK. ^{D593V}B-RAF does not signal to either MEK or C-RAF and may signal to other unknown pathways. Alternatively, it could act as a dominant-negative to suppress MEK activation in some cellular contexts.

(Figures 2 and 3A) that is likely to facilitate substrate recognition and to position key catalytic residues (Johnson et al., 1998), is also well defined.

Intriguingly, there is a distinctive hydrophobic interaction between the glycine-rich loop and activation segment of B-RAF, and this displaces the DFG motif to a position that is incompatible with catalysis (Figure 3A). As mentioned above, phosphorylation of T598 within the activation segment is essential for B-RAF activation, and this residue is at the interface of the glycine-rich loop/activation segment interaction domain. Its phosphorylation is predicted to disrupt the interaction, displacing the activation segment and thereby permitting the DFG motif to adopt the active conformation (Wan et al., 2004), explaining how activation segment phosphorylation stimulates B-RAF kinase activity. Since the kinases that phosphorylate T598 seem to be at the membrane, the structure also explains why B-RAF recruitment to the plasma membrane is necessary. Furthermore, it partly explains why N region charge is necessary for B-RAF activity and provides a rational explanation for why N region phosphorylation of A-RAF and C-RAF is required—presumably it also stabilizes the small lobe. It probably also explains why A-RAF and C-RAF are not mutated in cancer (Davies et al., 2002; Fransen et al., 2004), despite the fact that many of the residues mutated in B-RAF are conserved (Figure 2). In the absence of N region-mediated small lobe stabilization, substitutions that reorganize the activation segments of A-RAF and C-RAF will be insufficient to induce the active conformation, and so they will not be selected in cancer.

The majority of the mutations that occur in cancer activate B-RAF, as has been shown by direct measurements of their abilities to phosphorylate MEK in vitro and stimulate ERK signaling in vivo (Figure 2) (Davies et al., 2002; Ikenoue et al., 2003, 2004; Houben et al., 2004; Wan et al., 2004). However, the activities of the mutants range from 1.3- to 700-fold relative to ^{WT}B-RAF, and again, the structure explains the underlying mechanism. The mutations cluster to the glycine-rich loop and activation segment and involve many of the residues that stabilize the interaction between these regions (Figures 2 and 3) (Wan et al., 2004). Importantly, many of these residues, such as the glycines (G463, G465, and G468) of the glycine-rich loop, the Asn (N580) of the catalytic loop, and the phenylalanine (F594) of the DFG motif are highly conserved within the kinase superfamily (Hanks and Hunter, 1995; Johnson et al., 1998). This is because they serve key catalytic functions, and yet, despite this, when mutated in B-RAF in cancer, they can be activating (Figure 2), although their levels of activity tend to be lower than the mutants involving the nonconserved residues (Wan et al., 2004). A unifying feature of the mutations is that they disrupt the gycine-rich loop/activation segment interaction, destabilizing the inactive conformation and stimulating B-RAF activity up to 700-fold. However, since some of the mutations involve important catalytic residues, the full activation potential is not reached, and therefore some mutants have only modestly increased kinase activity.

Any interpretation of RAF regulation based on the structure must be considered in light of the fact that BAY43-9006 may induce an inactive conformation in the protein that does not reflect the "native" inactive conformation of B-RAF. However, the model does provide a satisfactory explanation of why activation segment phosphorylation is necessary for activity and of how the mutants activate B-RAF. It also explains why the mutants are selected for in cancer and why they cluster to the glycine-rich loop and activation segment. Thus, it seems that BAY43-9006 induces a conformation in B-RAF that does reflect the normal inactive state, and hence the structure provides a great deal of insight into how B-RAF is regulated.

It should also be noted that the structure does not reveal how the kinase domain interacts with the rest of the molecule, and intriguingly, some of the mutations do not appear to involve amino acids involved in stabilizing the glycine-rich loop/activation segment interaction. For example, E585, D586, and R681 are all at the "back" of the kinase domain (Figure 3B). They appear to define a large surface that is remarkably similar to one found in the tyrosine kinase Abl and which is responsible for its negative regulation through an intramolecular interaction with the noncatalytic N-terminal domains (Nagar et al., 2003). This provides an exciting possibility that B-RAF is regulated by its N terminus through a similar mechanism, and these mutations may disrupt this interaction. Another example is A727, which is within a 14-3-3 binding site C-terminal to the kinase domain (Figure 2); it is not clear how its mutation to valine activates B-RAF. Finally, K438 and T439 are outside the kinase domain in a region of B-RAF that is implicated in negative regulation by protein kinase B (Figure 2) (Guan et al., 2000). Presumably, some of the mutants destabilize the inactive conformation through alternative mechanisms, possibly by disrupting intramolecular interactions, or by affecting negative regulation by other pathways, demonstrating that B-RAF can be activated by multiple mechanisms, some of which are not fully understood.

The biology of B-RAF in cancer

^{V599E}B-RAF is one of the most active mutants, its in vitro kinase activity being ~500 fold greater than that of ^{WT}B-RAF (Wan et al., 2004). In NIH3T3 cells and murine melanocytes, ^{V599E}B-RAF stimulates constitutive ERK signaling, induces proliferation and transformation, and allows these cells to grow as tumors in nude mice (Davies et al., 2002; Ikenoue et al., 2003, 2004; Houben et al., 2004; Wan et al., 2004; Wellbrock et al., 2004). These data show that B-RAF is an oncogene. However, ^{V599E}B-RAF is detected in a high proportion of naevi (Pollock et al., 2003; Yazdi et al., 2003), benign melanocytic skin lesions in

which the cells are thought to be senescent, and it is also found in premalignant colon polyps and the early Duke's stages (A/B) of colorectal cancer (Rajagopalan et al., 2002; Yuen et al., 2002). Thus, B-RAF activation occurs early in tumorigenesis and may be a founder event, but by itself it is not sufficient to induce cancer. Finally, ^{V599E}B-RAF also activates NF-κB (Ikenoue et al., 2003, 2004) and protects cells from apoptosis (Hingorani et al., 2003; Karasarides et al., 2004), both of which may be important for transformation.

Many of the other mutants also transform cells and so appear, like ^{V599E}B-RAF, to be classically activated oncogenes (Davies et al., 2002; Ikenoue et al., 2003, 2004; Houben et al., 2004; Wan et al., 2004). However, there is a group of mutants that have impaired kinase activity relative to ^{WT}B-RAF (Figure 2) (Ikenoue et al., 2003, 2004; Houben et al., 2004; Wan et al., 2004). While their lack of activity is not surprising, because they involve the important catalytic residues G465 (the second Gly of the glycine-rich loop) and G595 (the Gly of the DFG motif), what is surprising is that they can still stimulate ERK signaling in cells because they activate wild-type C-RAF, which then activates MEK (Figure 4) (Wan et al., 2004). Indeed, depletion of C-RAF in cells that express these mutants blocks ERK activity (Wan et al., 2004), demonstrating the dependence of the mutants on C-RAF for signaling down this pathway. These mutants are also predicted to adopt the active conformation, but presumably cannot phosphorylate MEK due to additional disruptions induced by their specific substitutions. However, they can associate with C-RAF and presumably transmit a signal to it because they are in the active conformation. Thus, it seems that irrespective of their kinase activity, most of the B-RAF mutants stimulate ERK signaling in cells.

To stimulate proliferation, the level of ERK signaling must be carefully modulated, because if it is too strong, cells will stop cycling and differentiate or senesce (Marshall, 1995; Kerkhoff and Rapp, 1996; Sewing et al., 1997; Woods et al., 1997). This may explain why different levels of activation are seen in the B-RAF mutants. Some of the rare mutants may occur in contexts that select for weaker B-RAF signaling as a secondary event to an existing mutation already in the pathway. This model is supported by the observation that some rare B-RAF mutants are coincident with RAS mutations, suggesting that they cooperate to carefully modulate ERK signaling (Davies et al., 2002; Yuen et al., 2002). Indeed, some cancer cell lines expressing the rare B-RAF mutants still require RAS for proliferation, whereas cells expressing ^{V599E}B-RAF do not (Davies et al., 2002). Presumably, ^{V599E}B-RAF does not occur with RAS mutations, because their combined signaling is incompatible with proliferation.

Finally, the most enigmatic mutations are those involving D593, the aspartate of the DFG motif (Figure 2). ^{D593V}B-RAF cannot phosphorylate MEK in vitro and does not activate C-RAF, ERK, or NF-κB in cells (Ikenoue et al., 2003; Wan et al., 2004). It is not surprising that ^{D593V}B-RAF is a loss-of-function mutant, because D593 is a key catalytic residue (Hanks and Hunter, 1995; Johnson et al., 1998), and the other substitutions at this position are also likely to be inactive. However, together, they account for ~1% of all B-RAF mutants, too common for a random event, particularly as similar mutations are not found in C-RAF or A-RAF. Their role in cancer is unclear, but they could act as dominant-negative regulators of RAS to suppress rampant ERK signaling (Figure 4), and it is worth noting that three out of the ten D593 mutants occurred coincident with mutant RAS (Yuen et al., 2002; Houben et al., 2004), significantly more frequently than other mutants. However, it is also possible that these mutants actually function in completely different pathways in cancer, but currently, there are no candidate B-RAF effectors of these putative pathways, and presumably this signaling would be independent of B-RAF kinase activity.

Therapeutic implications

Studies with RNA interference have demonstrated that depleting oncogenic B-RAF in cancer cells reduces ERK activity, inhibits proliferation, and induces apoptosis (Hingorani et al., 2003; Karasarides et al., 2004). The C-RAF inhibitor BAY43-9006 also inhibits B-RAF in vitro and in vivo, and provides some therapeutic benefit in tumor xenografts of melanoma cells expressing oncogenic B-RAF (Karasarides et al., 2004). However, in phase II clinical trials, when administered to patients as a monotherapy for melanoma, BAY43-9006 has limited antitumor activity, and it is currently being tested in combination with other agents (T. Eisen, personal communication). Until the data analysis is complete, it is premature to try to explain why the monotherapy trials in melanoma have been disappointing, and although it sounds a note of caution, the preclinical work has shown that B-RAF is an important therapeutic target, and there is considerable interest in developing potent B-RAF inhibitors.

Initial efforts are likely to focus on developing agents that inhibit ^{V599E}B-RAF, because it is the most common mutant, but as many of the mutations activate B-RAF through a common mechanism, it is hoped that these agents will inhibit most or all of the mutants. However, previous experience warns that this may not be the case. The tyrosine kinase Abl plays a major role in the etiology of chronic myeloid leukaemia (CML). Imatinib is a small molecule inhibitor of Abl that has been used to treat CML with spectacular results, but initial excitement is tempered by clinical resistance to treatment driven by the occurrence of point mutations in Abl that block imatinib binding (Gorre et al., 2001; Shawver et al., 2002). Since B-RAF can tolerate many different point mutations within its kinase domain, it is possible that single agents will fail to inhibit all mutants, and this may also provide a mechanism for clinical resistance to develop. To preempt this likely occurrence, it will be desirable to develop an arsenal of anti-B-RAF drugs with different modes of action. Again, Abl provides the paradigm, because the compound BMS-354825, which binds to Abl through an alternative mechanism, can inhibit 14 out of 15 imatinib-resistant Abl mutants (Shah et al., 2004). Another complication of B-RAF is the finding that the impaired activity mutants can activate ERK via C-RAF. This suggests that agents that inhibit B-RAF, but allow the active conformation to persist, could stimulate ERK signaling via C-RAF. In practice, it is likely that most B-RAF inhibitors will also inhibit C-RAF, thereby blocking signaling through this pathway whether it arises directly from B-RAF, from cryptic C-RAF activation, or by other upstream events.

Conclusions

Rapid progress has been made in our understanding of the B-RAF mutations in cancer, both at the molecular and biological levels. We understand how phosphorylation regulates B-RAF and also how many of the mutations stimulate its activity. However, further work is required to understand some of the rare mutations that appear to activate B-RAF through distinct mechanisms; no doubt this understanding will reveal more about how ^{WT}B-RAF is regulated. The majority of the mutants

signal to ERK either directly through MEK or indirectly through C-RAF (Figure 4). For ^{V599E}B-RAF, this signaling stimulates proliferation and protects cells from apoptosis. Presumably, ERK signaling will also be important for the biological functions of the other mutants, but it is possible that the individual mutants will have different biological outputs. As noted, some of the mutants require RAS to stimulate cell growth, whereas others do not, either reflecting differences in the mutants themselves or differences in the cellular context in which they occur. Thus, although we may expect all B-RAF mutants to have more-or-less similar outputs, a closer examination of a range of mutants may reveal important differences in the biological functions between them. Furthermore, understanding how this differs from ^{WT}B-RAF signaling will provide further insight into the biological functions of B-RAF signaling in normal cells.

Another area that needs greater understanding is the role that C-RAF plays in mediating ERK signaling in cells with B-RAF mutations. It will be important to determine how mutant B-RAF activates C-RAF and what the biological outputs from this signaling are, to define the role that C-RAF plays in the oncogenic activity of the mutants, and to determine whether ^{WT}B-RAF also activates C-RAF in normal cells. It will also be important to investigate how B-RAF regulates apoptosis. B-RAF has been validated as an important therapeutic target because its inhibition blocks proliferation and induces apoptosis in cancer cells, and yet, we understand very little of the regulation of apoptosis by this isoform. Ultimately, it will be desirable to develop an arsenal of potent B-RAF inhibitors to allow us to test the importance of this oncogene in the clinical setting and to offer novel strategies for treating cancers that harbor mutations in this gene.

Note

The original B-RAF sequence deposited on the NCBI web site was missing a codon (three nucleotides) in the region encoding for the amino acids 31 and 32 (see http://www.ncbi.nlm.nih.gov/entrez/sutils/girevhist.cgi/girevhist.cgi?val=NM_004333). The corrected sequence is therefore one amino acid longer than originally thought and all amino acids after the alanine at 33 are displaced by one position. For example, T598 and S601 are actually T599 and S602, and the common valine mutation is actually V600 and not V599. However, in order to avoid confusion with original published data, here we use the original, if incorrect, numbering.

Acknowledgments

We thank Dr. Lori Passmore for preparing Figure 3. We also thank members of the Signal Transduction Team for helpful discussions. This work is funded by The Institute of Cancer Research and Cancer Research UK (Grant ref: C107/A3096).

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