Oncogenic B-Raf mutations: Crystal clear at last

The Raf/MEK/ERK pathway is a conserved signaling module controlling cell growth, proliferation, apoptosis, and differentiation. Constitutive activation of this pathway is involved in malignant transformation by several oncogenes, most notably, Ras. The recent discovery by Davies et al. of somatic mutations in the *B-RAF* gene in human tumors has generated enormous interest in how Raf kinases are regulated and how mutations in *B-RAF* lead to transformation. A recent study in *Cell* by Wan et al. reports the crystal structure of the B-Raf kinase domain, providing important new insights into these questions.

In mammals, the Raf family of serine/threonine kinases comprises three members: Raf-1, A-Raf, and B-Raf. The best-studied representative, Raf-1, features a complex regulation that involves binding to activated Ras, dephosphorylation of inhibitory sites and phosphorylation of activating sites, and interactions with scaffolds, effectors, and lipid and protein regulators (Kolch, 2000). B-Raf regulation appears to be much simpler, with Ras binding and activation loop phosphorylation on T598 and S601 sufficing for full activation. Once activated, all Raf kinases can phosphorylate MEK, which in turn phosphorylates and activates ERK. Recently, B-Raf has taken center stage due to the discovery that it is mutated in prevailing human cancers, including 60% of malignant melanomas and 5%–15% of colon, ovarian, and thyroid carcinomas (Davies et al., 2002). The most common mutation is V599E, which dramatically enhances B-Raf activity, presumably because its negative charge mimics activation loop phosphorylation. However, substitution of V599 by positively charged residues was also found in tumors. Even more puzzling, a small (~10%) number of tumors contain mutations in the ATP binding site (P loop) and the conserved DFG motif at the beginning of the activation loop, which only modestly activate or even impair B-Raf activity. These findings pose the questions of how B-Raf is activated and how these mutations contribute to tumorigenesis.

A recent study sheds new light on these important questions. Wan et al. (2004) solved the structure of the isolated B-Raf and B-RafV599E kinase domain. As B-Raf was dephosphorylated and crystallized in the presence of the Raf inhibitor BAY43-9006, the structure probably captures the inactive conformation of B-Raf. It exhibits the characteristic bilobal structure of protein kinases, most closely resembling that of c-Abl and p38. B-Raf residues G595-V599 of the activation loop engage in hydrophobic interactions with residues G463-V470 of the P loop. In this conformation, the catalytic residues are not aligned for ATP and substrate binding. Oncogenic B-Raf mutations either in the P loop or activation loop destabilize their interaction and disrupt the inactive conformation (Figure 1A). However, only few mutations enable the formation of critical new interactions that fold the kinase into a catalytically competent structure. Thus, the crystal structure beautifully explains why the P loop and the activation loop are preferred mutational targets. However, it leaves as a riddle why some tumors select for mutations that impair catalytic activity.

Wan et al. (2004) classify B-Raf mutants into three groups possessing high, intermediate, or low in vitro kinase activity. The high-activity group exceeds wild-type B-Raf activity 100- to 700-fold, with the V599E mutation being 460-fold activated. The intermediate group contains B-Raf mutants with 1- to 10-fold activity, whereas the low-activity group is less active than B-Raf. Surprisingly, this large variation in mutant B-Raf kinase activities did not translate into differential ERK activity. The high and intermediate B-Raf mutants only raised ERK activity 2- to 4.6-fold. How is this wide range of B-Raf activities buffered into a narrow bandwith of ERK activities? Obviously, there must be regulatory gates that control the signal flow from Raf to MEK and ERK. Candidates are scaffolding proteins such as KSR and protein interaction disruptors such as RKIP (Kolch, 2000). A future challenge will be to identify these gatekeepers and their physiological function. Why is ERK activity so strictly controlled? Hyperactivation of the ERK pathway can induce cell cycle arrest (Ravi et al., 1998) and senescence (Zhu et al., 1998), events which a tumor obviously must avoid. A more subtle facet is that the kinetics and amplitude of ERK signaling can specify different biological programs, such as proliferation or differentiation of PC12 cells (Marshall, 1995). Conceivably, a tumor would select for ERK activities that program the cell for growth and survival.

Remarkably, three out of four low-activity B-Raf mutants also induced ERK activation. Wan et al. show that this is due to the activation of Raf-1 by the respective B-Raf mutants (Wan et al., 2004). High- and intermediate-activity B-Raf mutants also activated Raf-1 but in contrast to low-activity mutants did not

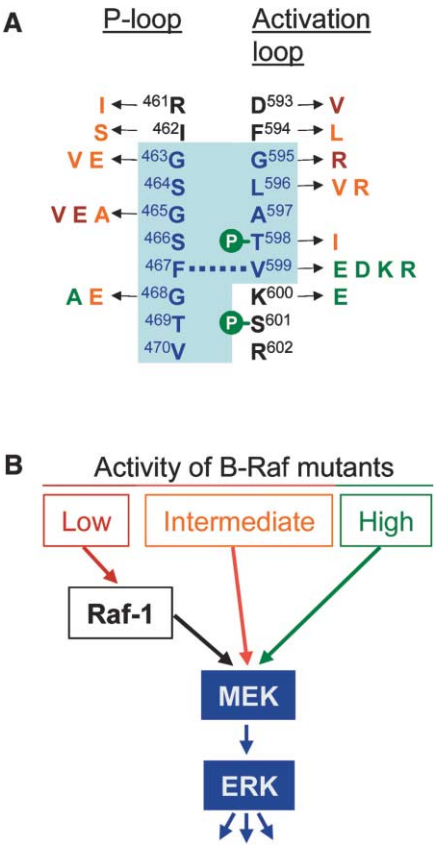

Figure 1. B-Raf activation by mutation

A: B-Raf mutations cluster in the P loop and activation loop. These loops interact via a hydrophobic interface (shaded blue) including an important contact between F467 and V599. Activating phosphorylation on T598 and S601 is indicated. Mutations analyzed by Wan et al. are color coded (red, low activity; orange, medium activity; green, high activity).

B: Low-activity B-Raf mutants stimulate ERK through Raf-1.

rely on Raf-1 for ERK stimulation (Figure 1B). The mechanism of transactivation remains unsolved. It is not due to autocrine stimulation but rather seems related to the fact that Raf-1 forms heterodimers with B-Raf and equally efficiently with B-Raf mutants. However, while most B-Raf mutants activated Raf-1, wild-type B-Raf and two B-Raf mutants devoid of kinase activity failed. The latter include the tumor-derived D593V mutant in the DFG motif and an engineered mutation, K482M, which is commonly used to generate kinase-negative mutants by changing the critical catalytic lysine. These observations suggest that B-Raf must be both in an activated conformation and retain at least some kinase activity to activate Raf-1. Interestingly, B-Raf has been identified biochemically as component of a 400 kDa Ras-induced Raf-1 activator complex (Mizutani et al., 2001). Hence, transactivation of Raf-1 may involve activating transphosphorylation, recruitment of other Raf-1 activators, or inactivation of Raf-1 inhibitors by B-Raf. It will be interesting to elucidate what the physiological role of the Raf-1/B-Raf complex is and how exactly B-Raf mutations subvert its regulation.

Another intriguing finding of the Wan et al. study (Wan et al., 2004) is that the low-activity B-Raf mutants did not transform NIH 3T3 fibroblasts although they could stimulate ERK. This could represent a simple threshold phenomenon, as transformation may require a certain level of ERK activity. However, it also could reflect a more subtle function of this class of B-Raf mutants, as hinted to by the phenotype of a Raf-1 mutant where the inhibitory S259 was changed. This Raf-1 mutant activates ERK to levels that are indistinguishable from oncogenic v-Raf and stimulates the proliferation of NIH 3T3 cells yet fails to transform (Dhillon et al., 2003). Thus, similar ERK activities may produce distinct biological responses depending on the upstream mode of activation. This reemphasizes the question of what role the different B-Raf mutants play in tumorigenesis. Ras and strongly activating B-Raf mutations generally are not found in the same tumor, arguing that B-Raf can fully substitute for Ras and is its main effector in oncogenesis (Davies et al., 2002). However, where Ras and B-Raf mutations coincide, such as in some colon carcinomas, B-Raf mutants fall in the intermediate- and low-activity groups (Yuen et al., 2002), suggesting that Ras and B-Raf mutations are coselected because they supply complementary functions to the tumor. It will be instructive to reveal what these functions are.

A related question is why there are so many different B-Raf mutations. The frequency of B-Raf mutations but not of K-Ras mutations is elevated in mismatch repair-deficient colon tumors, suggesting that B-Raf mutations are favored by intrinsic faults in DNA repair, while Ras is mainly targeted by environmental carcinogens. B-Raf mutations are typically somatically acquired and occur early, often in premalignant lesions. Most strikingly, ~80% of nevi harbour B-RafV599E mutations without featuring ERK hyperactivation and progression to malignancy. On the other hand, mutated B-Raf seems to be required at later stages of melanoma progression (Mercer and Pritchard, 2003). This apparent paradox could point to a dual function of B-Raf or the existence of B-Raf inhibitors that are lost during progression. Wan et al. (2004) have set the stage to unravel the molecular function of B-Raf in tumorigenesis. The major challenge ahead is now to define its pathophysiological role.

Amardeep S. Dhillon[1] and Walter Kolch[1,2,*]

[1]The Beatson Institute for Cancer Research
Garscube Estate
Switchback Road
Bearsden
Glasgow G61 1BD
United Kingdom
[2]Institute of Biomedical and Life Sciences
Sir Henry Wellcome Functional Genomics Facility
University of Glasgow
Glasgow G12 8QQ
United Kingdom
*E-mail: wkolch@beatson.gla.ac.uk

Selected reading

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., et al. (2002). Nature *417*, 949–954.

Dhillon, A.S., Meikle, S., Peyssonnaux, C., Grindlay, J., Kaiser, C., Steen, H., Shaw, P.E., Mischak, H., Eychene, A., and Kolch, W. (2003). Mol. Cell. Biol. *23*, 1983–1993.

Kolch, W. (2000). Biochem. J. *351*, 289–305.

Marshall, C.J. (1995). Cell *80*, 179–185.

Mercer, K.E., and Pritchard, C.A. (2003). Biochim. Biophys. Acta *1653*, 25–40.

Mizutani, S., Inouye, K., Koide, H., and Kaziro, Y. (2001). FEBS Lett. *507*, 295–298.

Ravi, R.K., Weber, E., McMahon, M., Williams, J.R., Baylin, S., Mal, A., Harter, M.L., Dillehay, L.E., Claudio, P.P., Giordano, A., et al. (1998). J. Clin. Invest. *101*, 153–159.

Wan, P.T., Garnett, M.J., Roe, S.M., Lee, S., Niculescu-Duvaz, D., Good, V.M., Cancer Genome Project, Jones, C.M., Marshall, C.J., Springer, C.J., Barford, D., Marais, R. (2004). Cell *116*, 855–867.

Yuen, S.T., Davies, H., Chan, T.L., Ho, J.W., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Tsui, W.W., Chan, A.S., et al. (2002). Cancer Res. *62*, 6451–6455.

Zhu, J., Woods, D., McMahon, M., and Bishop, J.M. (1998). Genes Dev. *12*, 2997–3007.