THERAPEUTICS

Iterations of BRAF

GETTY

Activation of the RAS–RAF–MEK–ERK pathway is associated with various types of cancer. BRAF frequently undergoes activating mutation in cancer, which has provided a rationale for targeting this kinase. However, in cells lacking BRAF mutations, MEK–ERK signalling can be activated in the presence of BRAF inhibitors, which is thought to result from RAS-driven BRAF–RAF1 heterodimerization. It has therefore been suggested that targeting the conserved dimer interface (DIF) that mediates RAF dimerization might overcome MEK–ERK signalling. So, Röring and colleagues sought to further understand RAF dimerization and the implications for RAF inhibitors.

The authors found that MEK phosphorylation was substantially reduced when BRAF DIF mutants were expressed in Plat-E cells. Similarly, the BRAF-E586K tumour-associated mutant, which increases homodimerization, was sensitive to the introduction of DIF mutations. However, the cancer-associated mutants BRAF-V600E, BRAF-G469A and BRAF-insT retained substantial activity when the DIF was mutated despite the fact that DIF mutations reduced homodimerization and formation of large complexes by BRAF-V600E.

Next, the authors investigated whether mutating the BRAF DIF affects transformation. Consistent with the downstream activation of MEK–ERK, mutating the DIF did not abrogate the ability of BRAF-V600E to transform mouse embryonic fibroblasts (MEFs) and Caco-2 human intestine epithelial cells, *in vitro* and in three-dimensional culture.

Does mutating the DIF affect heterodimerization? Using co-immunoprecipitation of exogenously expressed tagged proteins, the authors found that basal and drug-induced heterodimerization between BRAF and RAF1, ARAF or KSR1 is differentially affected by DIF mutations in an inhibitor-specific manner.

The authors also used a kinase-dead mutant of BRAF, BRAF-D594A, to investigate downstream MEK–ERK signalling activation when BRAF kinase activity is inhibited (to mimic the effects of BRAF inhibitors). Mutation of the DIF in the BRAF-D594A mutant reduced MEK and ERK phosphorylation despite still being heterodimerized with RAF1. Furthermore, in the presence of oncogenic RAS, mutating the DIF of BRAF or RAF1 substantially reduced MEK phosphorylation. Importantly, the treatment of MEFs with BRAF inhibitors, PLX4720 and L779450, abrogated the phosphorylation of MEK and ERK when the BRAF DIF was mutated.

> “BRAF inhibitors … abrogated the phosphorylation of MEK and ERK when the BRAF DIF was mutated.”

This paper highlights the complexity of RAF signalling and dimerization, which requires further investigation to understand whether targeting the DIF might overcome resistance to BRAF inhibitors.

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