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RAF-isotype switching: from B to C through PDE

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Abstract

A new study reveals that rewiring of MAPK signaling in cells expressing mutant RAS includes ERK-mediated BRAF inactivation and the concomitant activation of CRAF, partly through amplification by the phosphodiesterase 4 isoform.

BRAF-to-CRAF signal switching in melanoma is due to the presence of mutant RAS¹, although precisely how this switch occurs has remained unclear until now. The current study by Marquette *et al.*² provides new insights into BRAF-to-CRAF isoform switching, showing how mutant RAS contributes to BRAF inactivation and how specific phosphodiesterase (PDE) isoforms amplify CRAF signaling.

Interplay between the RAF isotypes BRAF and CRAF, important upstream components in the mitogen-activated protein kinase (MAPK) signaling pathway, is essential to the rewiring of MAPK- and cAMP-related signal transduction pathways in melanoma. Assessments of clinical outcomes following the use of BRAF inhibitors to treat melanoma have revealed increasing levels of cross-talk and compensatory signaling between these two factors^{3–5}. Treatment with BRAF inhibitors during monotherapy results in acquired resistance due to altered activity of receptor tyrosine kinases such as platelet-derived growth factor receptor (PDGFR), acquired mutations in RAS (N-RAS), or upregulation of extracellular signal-related kinase (ERK) and CRAF^{6–8}. The complexity of BRAF-CRAF cross-talk was previously highlighted by Heidorn *et al.*, who showed that although they effectively block BRAF-mediated ERK signaling, BRAF inhibitors can induce ERK signaling through activation of CRAF in mutant RAS-bearing melanoma⁹. These observations indicate that a better understanding of rewired RAF-MAPK signaling cascades is required for a more effective design of multipronged strategies to combat melanoma.

Marquette *et al.* showed that mutant G^{12V}RAS drives ERK-mediated phosphorylation of BRAF on Ser151, suppressing BRAF activity by blocking its ability to interact with RAS (see Fig. 1). Therefore, although BRAF cannot interact with G^{12V}RAS unless ERK is synthetically inhibited or BRAF residue Ser151 is mutated, CRAF can. Consequently, in mutant RAS cells, signaling to MEK is consolidated through CRAF. However, because CRAF is subject to negative regulation by protein kinase A (PKA), the authors evaluated the

possibility that cAMP-mediated PKA inhibition of CRAF could be perturbed in mutant RAS cells. In investigating how regulation of CRAF by cAMP signaling might be altered, they uncovered the striking role of PDEs.

Phosphodiesterases function primarily to downregulate cAMP and cGMP levels by specifically catalyzing cAMP and cGMP hydrolysis, thereby modulating G protein–coupled receptor (GPCR) signaling cascades and allowing receptor resensitization to enable subsequent ligand stimulation. PDEs comprise a diverse group of 11 subfamilies, containing up to 21 possible splice variants¹⁰. Despite their diversity of structure and substrate specificity, all PDEs contain a conserved catalytic metal-dependent phosphohydrolase domain (HD motif).

Previous studies have reported the expression of numerous PDEs in melanoma, although their contribution to tumor pathology has until lately remained obscure^{11–13}. Recent work by Khaled *et al.* has highlighted the importance of PDE4D3 in the formation of signaling circuits that homeostatically attenuate ligand-stimulated signaling through the melanocortin 1 receptor (MC1R)¹⁴, a GPCR expressed in melanocytes that is both important for normal melanocyte biology and implicated in melanoma risk and development¹⁵. Downstream of MC1R, cAMP was found to trigger transient activation of microphthalmia-associated transcription factor (MITF), driving transcription of PDE4D3, which, in turn, negatively regulates the pathway. Accordingly, perturbation of these negative feedback circuits should potentiate cAMP-driven cascades. Indeed, repression of *PDE5A* gene transcription by the POU domain, class-3 transcription factor 2 (BRN2), whose expression is driven by mutant BRAF, elevated cGMP and Ca²⁺ levels sufficiently to promote cellular contractility and increased invasiveness and metastatic behavior of melanoma cells¹⁶.

In addressing the role of altered cAMP activity in the transformation process, Marquette *et al.* observed that in G^{12V}RAS-expressing melanocytes, the MC1R ligand (α-melanocortin-stimulating hormone (α-MSH) could no longer elicit activation of downstream cAMP signaling, consistent with previous observations that cAMP signaling is impaired or uncoupled in the presence of mutant RAS¹. Suppression of cAMP signaling was similarly observed in melanoma cell lines expressing mutant RAS. Examining members of the PDE superfamily by using synthetic PDE inhibitors and siRNA silencing in melanoma cells harboring mutant RAS led the authors to identify PDE4B and PDE4D as predominant suppressors of cAMP signaling. Inhibition of either of these factors during α-MSH stimulation resulted in reactivation of the cAMP-response element-binding (CREB) protein, indicating that activated cAMP and PKA signaling in response to α-MSH was restored. However, PDE inhibition was insufficient to reactivate BRAF, indicating that RAS-mediated inactivation of BRAF and PDE upregulation are not coupled. The authors show that small hairpin RNA-mediated inhibition of PDE4B is sufficient to abrogate G^{12V}RAS transformation of normal melanocytes, and that inhibition of either PDE isoform can induce cell death in melanoma cells, but not in melanocytes.

The discovery of mutant RAS-driven negative regulation of BRAF with concomitant increased PDE4 activity that attenuates cAMP signaling and derepresses CRAF (see Fig. 1) has important implications for our understanding of melanoma biology, treatment and

therapy. Furthermore, these findings provide insight into the segregation of melanomas harboring mutant RAS rather than mutant BRAF^{17,18}. Attenuated BRAF activity due to its inactivation by mutant RAS-CRAF-MEK- ERK signaling is consistent with the finding that overexpression of CRAF antagonizes mutant BRAF signaling¹⁹, and this explains why mutant RAS and mutant BRAF are mutually exclusive. This is further substantiated by the fact that overexpression of kinase-inactive BRAF promotes aneuploidy and immortalization of murine cells by inducing CRAF²⁰. Whether BRAF inhibitors and altered PDE levels elicit the same effects through BRAF inhibition and/or upregulation of CRAF remains to be determined. An unanticipated clinical side effect of BRAF inhibitors has been the increased incidence of keratoacanthomas and squamous cell carcinomas²¹, which may be attributable to reactivation of CRAF and PDEs in keratinocytes harboring pre-existing RAS mutations.

It is increasingly apparent that PDEs, particularly members of the PDE4 and PDE5 subfamilies, orchestrate the delicate balance of feedback loops in cyclic nucleotide signaling cascades. Accordingly, as an antitumor approach, the targeted inhibition of PDEs is a topic of active exploration²². Consistent with the important role of cAMP levels in control of MTF—the central regulator of melanocyte biogenesis, proliferation and differentiation²³—deregulation of receptors and signaling components upstream of cAMP (such as MC1R and G(s)) may underlie numerous skin disorders and susceptibility to melanoma development. Individuals with the most common mutations in MC1R have fair skin and red hair, sensitivity to ultraviolet exposure and predisposition to melanoma development^{24,25}. Similarly, mutations affecting G(s), such as those observed in McCune-Albright syndrome, result in hyper-pigmented skin lesions. The extent to which changes in PDE activity contribute to the onset or development of these conditions or to melanocyte transformation deserves further study. For these reasons, the nonspecific effects of commercial PDE inhibitors used for other conditions, such as erectile dysfunction or pulmonary hypertension^{26–28}, should be monitored. Although questions remain regarding exactly how PDE expression and activities are altered during disease progression, particularly in the context of mutant RAS or BRAF inhibitor treatment, targeted inhibition of specific isoforms may eventually serve as a complementary therapeutic process in the treatment of melanoma.

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Figure 1.

From BRAF to CRAF through PDE4. (a) Normal melanocytes: RAS–BRAF drives MEK and ERK signaling, as cAMP can activate PKA to suppress CRAF. (b) Mutant RAS: upregulated PDE4 members hydrolyze cAMP and relieve PKA-mediated suppression of CRAF, which, together with mutant RAS, signal through MEK and ERK in the melanoma harboring mutant RAS. ERK phosphorylation of BRAF on Ser151 abrogates the ability of BRAF to associate with mutant RAS. (c) Mutant RAF: mutant BRAF drives elevated MEK–ERK signaling, but CRAF remains suppressed in mutant BRAF–bearing melanoma.