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Methylation Matters In KRAS Oncogenesis

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Mutationally activated KRAS is a key oncoprotein driver of many human cancers. Moreover, mice with lung- or pancreas-specific expression of oncogenic *KRAS*, especially when combined with a dominant-negative form of the tumor suppressor TP53, develop cancers that recapitulate key features of the human disease. Since oncogenic KRAS has largely proven an intractable pharmacologic target, attention has turned to KRAS effector pathways to identify actionable targets for therapeutic intervention. Moreover, although RAF and PI3'-kinase are the best-validated effectors of oncogenic KRAS, alternative targets for pharmacologic intervention are under active investigation.

With that in mind, Mazur *et al.* demonstrate the importance of SMYD3, a lysine methyl transferase (KMT) consistently expressed in KRAS-driven human cancers, in the malignant progression of KRAS-induced cancers in mice. To do so, the authors employed innovative techniques spanning the gamut from genetically engineered mouse (GEM) models to proteomic screens for SMYD3 substrates. By these means, the authors conclude that SMYD3 acts in the cytoplasm, downstream of oncogenic KRAS, to mono- di- or trimethylate a single lysine residue (K260) in MAP3K2 (a.k.a. MEKK2 or MEK Kinase 2), a protein kinase previously associated with activation of the stress-induced JNK and ERK5 mitogen-activated protein kinase (MAPK) pathways and NF- κ B (Figure) [1]. Interestingly, Mazur *et al.* show that MAP3K2 is required for efficient activation of the MEK1/2→ERK1/2 MAPK pathway. To address biochemical mechanism, the authors demonstrated that MAP3K2 K260 methylation had no direct effect on the protein's intrinsic kinase activity. However, K260 methylation promotes dissociation of MAP3K2 from its negative regulator, the PP2A protein phosphatase, thereby enhancing MEK1/2→ERK1/2 signaling in tumor cells. Consistent with a role in tumorigenesis, SMYD3 silencing elicited a modest, but significant, extension in survival of mice genetically engineered with lung- or pancreas-specific expression of oncogenic KRAS. Interestingly, SMYD3 silencing in mice appeared to attenuate late-stage cancer progression without influencing early-stage tumor initiation. Strikingly, the diminished KRAS-induced pancreatic tumorigenesis observed in SMYD3 deficient mice could be rescued by systemic administration of cantharidin, a PP2A inhibitor that promotes MAP3K2 activity. However, the effects of SMYD3 silencing were not tested in mice with more aggressive cancers elicited by combined oncogenic *KRAS* plus *TP53* mutation. In sum, the authors propose that elevated SMYD3 expression promotes

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K260 methylation of MAP3K2, thereby freeing MAP3K2 from the inhibitory constraints of PP2A that, in turn, promotes more efficient activation of signaling events downstream of oncogenic KRAS (Figure).

The principle of MAPK regulation by protein methylation was first established by Andreu-Pérez *et al.*, who demonstrated that arginine methylation of either BRAF or CRAF by protein arginine methyl transferase 5 (PRMT5) promoted their degradation, thereby inhibiting flux through the MEK1/2→ERK1/2 MAPK pathway (Figure) [2]. While Andreu-Pérez *et al.* found that RAF protein methylation was inhibitory to MAPK activation, the fact that protein methylation has emerged as both a positive and a negative regulator of MAPK pathway activation suggests that this might be a ubiquitous strategy for intracellular signal regulation.

A key question emerging from the work of Mazur *et al.* is how does oncogenic KRAS connect biochemically to SMYD3→MAP3K2 activation to regulate the magnitude of MEK1/2→ERK1/2 signaling in cancer cells? First, it is unclear why SMYD3 levels are elevated in RAS mutated cancers but it remains possible that RAS regulates SMYD3 expression either through effects on gene transcription or protein stability. Despite early claims that the related enzyme MAP3K1 (MEKK1) can bind directly to GTP-bound RAS proteins, it seems unlikely that MAP3K2 is a direct effector of oncogenic KRAS [3]. It has previously been demonstrated that CRAF is a relevant and direct MAP3K effector of oncogenic KRAS in lung tumorigenesis [4,5]. Furthermore, although both BRAF and MAP3K2 are required for tumor growth and metastasis in *KRAS* mutated MDA-MB-231 breast cancer cells, in that context BRAF contributed to MEK1/2→ERK1/2 activation whereas MAP3K2 contributed to MEK5→ERK5 activation [6]. One possibility that may reconcile some of these disparate observations may lie in the selective effects of SMYD3→MAP3K2 on late-stage KRAS-induced lung or pancreatic cancer. Oncogenic KRAS is known to promote the synthesis and secretion of numerous growth factors including EGF family members and inflammatory cytokines such as interleukins-6 and -8 [7–10]. Perhaps the activation of MAP3K2 is tied to an autocrine or paracrine signaling circuit that, while not essential for early-stage activation of MEK1/2→ERK1/2 signaling, is required for the elevated activation of this pathway detected in more advanced tumors [11] (Figure). To that end, EGF-mediated activation of MAP3K2 acts through SRC-dependent formation of a MAP3K2-LAD complex that allows for activation of MAP3K2 activity [12]. This would be consistent with the inhibitory effects of SYMD3 silencing on EGF-mediated activation of MEK1/2→ERK1/2 signaling observed by Mazur *et al.* Given the reported ability of MAP3K2 to activate IκB kinase (IKK) and the perceived importance of NF-κB signaling in carcinogenesis, MAP3K2 may promote activation of numerous signaling pathways important in the progression of KRAS-induced tumors (Figure) [13]. However, at least one complication with this model is the observation that MKK7, a direct downstream effector of MAP3K2, is reported to be a suppressor of KRAS-initiated lung tumorigenesis (Figure) [14].

A key question is whether patients with late-stage *KRAS* mutated lung or pancreatic cancer might derive clinical benefit from targeting the SMYD3→MAP3K2 signaling axis. To address this, Mazur *et al.* demonstrate that inhibition of SMYD3 expression inhibited the

growth of human *KRAS* mutated cancer cells and also enhanced the anti-tumor effects of trametinib, a MEK1/2 inhibitor, both in cultured cells and in GEM models (Figure). Importantly, mice lacking SMYD3 or MAP3K2 appear normal, suggesting that the therapeutic window for SMYD3→MAP3K2 inhibitors, at least in mice, may be broad. Efforts to develop pharmacologic inhibitors of SMYD family KMTs or MAP3K2 are already underway [15,16]. However, single-agent MAP3K2 inhibition may lead to dynamic rewiring of intracellular signaling such that other MAP3K enzymes assume the function of MAP3K2, hence vertical inhibition of SMYD3 and MAP3K2 may represent another promising avenue. Despite the specificity of SYMD3 for MAP3K2 claimed by Mazur *et al.*, previous work has suggested that SMYD3 acts as a nuclear histone KMT to regulate RNA polymerase II [17]. Thus inhibition of SMYD3 may carry attendant MAP3K2-independent toxicities. Regardless, the work of Mazur *et al.* has identified a fascinating mechanism that expands the regulatory importance of protein methylation in intracellular signaling. The field awaits with anticipation the therapeutic applications of this mechanism for patients with *KRAS*-mutated cancers.

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Figure. KRAS engages SMYD3 for downstream signal pathway activation

Previously, it was reported that PRMT5-mediated arginine methylation promoted proteolysis of RAF kinases thereby inhibiting MAPK pathway activation. In this work, Mazur *et al.* claim that, in cells expressing mutationally activated KRAS, the magnitude of ERK1/2, JNK and ERK5 MAPK activation is dependent on SMYD3-mediated methylation of lysine 260 of MAP3K2. However, the mechanism(s) by which mutated KRAS promote activity of the SMYD3→MAP3K2 signaling axis is not clear. In principle, there may be a direct biochemical link between KRAS and SMYD3→MAP3K2 signaling or the activity of this pathway may be sustained by an autocrine circuit due to KRAS-mediated induction of members of the EGF family of ligands (e.g. HB-EGF) or through secretion of inflammatory cytokines (e.g. IL-6).