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OUTLOOK

Anticipating resistance to KRAS inhibition: a novel role for USP21 in macropinocytosis regulation

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers. Virtually all PDAC harbors an oncogenic mutation in the KRAS gene, making it the prime target for therapy. Most previous attempts to inhibit KRAS directly have been disappointing, but recent success in targeting some KRAS mutants presages a new era in PDAC therapy. Models of PDAC have predicted that identifying KRAS inhibitor resistance mechanisms will be critical. In this issue of *Genes & Development*, Hou and colleagues (pp. 1327–1332) identify one such mechanism in which the deubiquitinase USP21 up-regulates the nutrient-scavenging process of macropinocytosis, rescuing PDAC cells from *Kras* extinction.

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and the third most deadly cancer in the United States (Siegel et al. 2021). Very little progress has been made in treating PDAC over the past several decades, due in part to most cases presenting as metastatic disease upon diagnosis and its resistance to conventional and targeted therapies. Its relatively homogenous mutational landscape, including oncogenic mutation of *KRAS*, found in virtually all cases of PDAC, together with inactivation of tumor suppressor genes such as *TRP53*, *CDKN2A*, and *SMAD4*, has facilitated the development of some of the most useful preclinical mouse models of any tumor type (Hingorani et al. 2005; Ying et al. 2012). Among other things, these mouse models have confirmed that mutant *Kras* (*Kras**) is sufficient to initiate transformation in the pancreas and that extinguishing *Kras** expression after progression to adenocarcinoma leads to widespread tumor regression (Ying et al. 2012), indicating that, even in these later stages, pancreatic tumor cells remain addicted to constitutive *Kras** signaling, reinforcing its status as the prime therapeutic target in the treatment of PDAC.

Unfortunately, KRAS* has long been considered "undruggable" for a variety of reasons. That status changed very recently with the development of small molecules that specifically target the G12C KRAS* protein, which has shown clinical efficacy (Herdeis et al. 2021). However, this specific *KRAS* mutation is found in only a small fraction (~1%) of human PDACs compared with G12D (41%), G12V (34%), or G12R (16%) (Waters and Der 2018). Fortunately, small molecules that target some of these more common *KRAS* mutants are in development (Herdeis et al. 2021), giving hope for the future of effective treatment of the vast majority of PDAC patients.

With viable KRAS inhibitors finally making headway, anticipating mechanisms of resistance to Kras inhibition is paramount. Indeed, while extinguishing *Kras** expression in PDAC mouse models causes tumor regression, resistant tumors do arise over time. In previous studies, DePinho and colleagues (Kapoor et al. 2019) have found that up-regulation of the Hippo pathway transcription factor, YAP1, is one mechanism that compensates for loss of *Kras** activity in ~30% of resistant tumors examined. In a subsequent study, the same group used a gain-of-function screen using a library encoding 284 epigenetic regulators, leading to the identification of HDAC5 expression as sufficient to compensate for the loss of *Kras** by modifying the inflammatory tumor microenvironment (Hou et al. 2020).

This prior epigenetic regulator screen revealed several additional suspects that had the potential to help PDAC overcome its *Kras** addiction. The second most prominent hit was *Usp21*, a deubiquitinase that is amplified and overexpressed in ~20% of PDAC patient samples (Hou et al. 2019). USP21 is best known for its nuclear function as a histone deubiquitinase, although nuclear USP21 promotes pancreatic tumor growth by deubiquitinating the transcription factor TCF7, amplifying canonical Wnt signaling, and enhancing tumor stem cell properties (Hou et al. 2019). In the current study, Hou

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et al. (2021) present an elegant, textbook example of scientific sleuthing to uncover a novel mechanism behind USP21's ability to overcome the loss of *Kras** activity.

Using mutant forms of USP21 that enforce localization to the nucleus or the cytoplasm, the investigators make the surprising discovery that the relevant *Kras**-bypassing activity occurs in the cytoplasm, rather than in the nucleus. USP21 did not break cells free of *Kras** dependency through a straightforward reactivation of pathways downstream from *Kras**, as MEK/ERK signaling remained low, nor was it associated with up-regulation of *Yap* activity, a previously identified mechanism of *Kras** escape. Using transcriptomic analysis, the investigators discovered that USP21 overexpression up-regulates mTOR-associated signaling, with metabolomic analysis revealing an association with increased amino acid levels, a known activator of mTOR. Sifting through the usual suspects that could be responsible for this increase in amino acids, the investigators found no associated up-regulation of de novo amino acid biosynthesis, no reduction in protein translation, and no alterations in amino acid transporter expression. They also did not find an association with the breakdown of intracellular proteins, as USP21 activity decreased autophagosome formation and autophagy. Having systematically eliminated each of these candidate activities, one remained: macropinocytosis, a mechanism of nutrient scavenging that is critical for Kras mutant PDAC cell survival. Indeed, USP21 expression restored macropinocytosis in *Kras**-extinguished cells, and macropinocytosis inhibitors thwarted USP21-induced *Kras** escape.

Finally, to identify the direct target of USP21 activity, the investigators used the protein as bait to capture a single interacting culprit: MARK3, a microtubule-binding kinase and regulator of microtubule dynamics (Sandí et al. 2017). Closing the case, the investigators confirmed that MARK3 is deubiquitinated by USP21 and is necessary and sufficient for Kras*-independent growth, tumor formation, and macropinocytosis, with the latter being necessary for *Mark3*-induced tumor growth.

With their collective studies (Hou et al. 2019), together with USP21 ablation in mice having no obvious deleterious effects (Pannu et al. 2015), the investigators have made a compelling case for USP21 as a viable therapeutic target in PDAC, either on its own or in combination with KRAS* inhibition. However, as with any compelling story, some mysteries remain. For instance, the original impetus for exploring the role of USP21 was its amplification in a significant number of pancreatic cancers (Hou et al. 2019). Would its overcoming the metabolic stress caused by KRAS* inhibition be limited to these USP21-amplified tumors, or is USP21 amplification itself indicative of an adaptive mechanism that deploys when cells are faced with such stress? It would also be fascinating to explore how the cytoplasmic and nuclear functions of USP21 may complement one another, or even synergize. Finally, the finding that MARK3 is required for macropinocytosis in this system is itself a novel discovery. If it is required for macropinocytosis in general, it also may qualify as a novel therapeutic target for treating PDAC. These and other questions will likely be answered in future studies. In the interim, while the case of effective KRAS* inhibitors is being cracked, the more mechanisms of resistance to the loss of *KRAS** activity that are identified, the better prepared we will be to defeat this devastating and deadly disease once and for all.

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