The KRAS oncogene is frequently mutated in cancers that comprise the top three causes of cancer-related death in the United States: lung, colorectal, and pancreatic ductal adenocarcinoma (PDAC) cancers (1, 2). Despite the occurrence of over 150 distinct missense mutations in KRAS (COSMIC), the RAS isoform most frequently mutated in cancer, the different mutant KRAS proteins have largely been grouped together as one homogeneous group, with presumed equivalent mechanistic roles as cancer drivers. Thus, a majority of anti-KRAS development has focused on pan-mutant RAS therapies for the treatment of all RAS-mutant cancers (3). With the recent entry into clinical evaluation of the first direct RAS inhibitors, selective for only one KRAS mutant, G12C (1), a more concerted research effort has emerged to address an issue that has perplexed the research community—the striking tissue-specific differences in the KRAS mutational spectrums seen in different cancer types. Do they simply reflect tissue-selective DNA mutational frequencies or is biology to blame?

Cancer-associated KRAS missense mutations occur primarily at three hotspot sites, positions G12, G13, and Q61 (1, 2). An evaluation of colorectal cancer and PDAC reveals striking tissue-specific differences in the frequencies of specific KRAS mutations (Fig. 1A). At one extreme, 92% of KRAS mutations in PDAC are found at residue G12. Contrasting with this, G12 mutations comprise but 64% of mutations in colorectal cancer, followed by G13, A146, Q61, and K117 mutations. Although A146 is the fourth most common site of KRAS mutation (COSMIC), mutations at this site are largely limited to colorectal cancer and hematopoietic cancers. In the study by Poulin and colleagues in this issue (4), the authors determined the biochemical and biological defects caused by the A146T mutation and addressed why this KRAS mutant is found in such a limited tissue spectrum. They applied an impressive array of experimental approaches to profile the A146T mutation, using structural biology, mass spectrometry profiling of the cellular proteome, and mouse models to compare the driver functions of KRAS\textsubscript{A146T} and KRAS\textsubscript{G12D} when expressed in different tissue types.

Poulin and colleagues first compared the consequences of the A146T mutation with the most prevalent KRAS mutation, G12D, on regulation of the GDP–GTP cycle. Although KRAS\textsubscript{G12D} retains wild-type (WT) intrinsic and SOS1-stimulated nucleotide exchange activities, this mutation impairs intrinsic and GAP-mediated GTP hydrolysis (5). Other G12 substitutions, as well as those at Q61, also impair GAP-mediated hydrolysis.

In contrast, KRAS\textsubscript{A146T} retained GTP hydrolysis activities similar to those of WT and instead exhibited increased intrinsic and GEF-mediated nucleotide exchange (Fig. 1B). The G13D mutation causes increased nucleotide exchange as well as decreased GAP-mediated hydrolysis (5). Thus, the A146T mutation contrasts with the predominant G12, G13, and Q61 mutations in that it favors formation of KRAS-GTP by increasing exchange and not by disrupting GAP responsiveness. Interestingly, the rare cancer-associated K117 mutations that are restricted to colorectal cancer also activate RAS by increased exchange rather than by decreased GAP function (6).

Although a previous study found that HRAS\textsubscript{A146V} exhibited enhanced nucleotide exchange and a weakly transforming phenotype in mouse fibroblasts (7), a surprising observation in this study was that this mutation also caused a 100-fold increased binding affinity to the guanine nucleotide exchange factor (GEF) SOS1, which further accelerates the intrinsic rate of exchange. Surprisingly, the increased SOS1 activity was indirectly excluded, by SHP2 inhibition, as being important for full KRAS\textsubscript{A146T} activation. As RAS GEFs that are not dependent on SHP2 for activation may still be involved, how KRAS\textsubscript{A146T} achieves its full activated state remains to be fully elucidated. Thus, the conclusion from this study is that enhanced intrinsic exchange is the basis for the activated state of KRAS\textsubscript{A146T}, with its reduced oncogenic potency due, simply, to the relative steady-state level of KRAS-GTP formed. However, that KRAS\textsubscript{G12D} is frozen in the GTP-bound state, whereas KRAS\textsubscript{A146T} exhibits a “fast cycling” phenotype, may also distinguish these two mutants. That colorectal cancer is relatively unique in the high occurrence of such “fast cycling” mutants (G13D, K117, and A146) suggests this possibility.

A surprise from the X-ray crystal structural analyses was that, although A146 lies in a tightly packed hydrophobic
region of the protein, the A146T mutation disrupts this packing (Fig. 1C). The end result of this disruption is the movement of switch I (SI), a region critical for effector binding, away from the nucleotide-binding pocket, where it forms an extended loop structure as well as a unique beta sheet interaction. This disrupts magnesium binding and destabilizes SII, decreasing the affinity of the guanine nucleotide and increasing intrinsic exchange. Surprisingly, despite the key importance of SI in binding to the key KRAS effector RAF, no apparent impairment in RAF binding was seen. However, whether other effectors that contribute to KRAS-dependent cancer growth (e.g., PI3K) have disrupted binding was not determined.

A recent mouse model–based analysis addressed whether oncogenic potency may influence tissue-specific KRAS mutation profiles, but only focused on mutations at G12 and G13 (8). To specifically address a basis for A146 mutations in different cancer types, Poulin and colleagues generated new mouse strains harboring the A146T mutation in the endogenous Kras locus, identical otherwise to previously well-characterized KrasG12D-driven mouse models of lung and pancreatic cancers (9, 10). In the colon, A146T caused an intermediate phenotype relative to WT (weakest) and G12D (strongest). However, in hematopoietic stem cells, induction of A146T led to similar qualitative levels of myelodysplastic syndrome/myeloproliferative neoplasm, but with a significantly delayed onset. And finally, when KrasA146T was expressed in the pancreas, a cancer that rarely has KrasA146T mutations, histologic evidence of pancreatic intraepithelial neoplasia (PanIN) formation, an early preneoplastic state of PDAC, was not observed at any time point evaluated, including up to 300 days, whereas G12D-expressing mice showed PanIN formation by 8 weeks of age. These findings support the likelihood that the tissue-specific KRAS mutation patterns seen in cancers are driven by the cancer driver potency of the mutant.

Taking these studies one step further, Poulin and colleagues looked into the contribution of co-occurring mutations in tumor formation. In the Fabp1-Cre mouse containing a conditional allele of the Apc gene, the initiating step in colorectal cancer development, mice expressing KRASA146T showed increased survival and decreased tumor burden compared with KRASG12D mice, similar to what has been observed in human patients. However, when coupled with mutation of the p53 tumor suppressor, 9 of 12 mice expressing KRASA146T developed invasive pancreatic tumors, compared with 100% of the KRASG12D-expressing mice. Survival favored the KRASA146T mice, as median survival was approximately 120 days and was markedly increased compared with KRASG12D, indicative of the relative strength of this oncogenic mutant. What is interesting is that p53 mutations are quite common in PDAC, and thus one has to wonder why KRASA146T is not more commonly observed in PDAC. This apparent paradox may be explained by the fact that genetically engineered mouse...
models of cancer do not accurately model the temporal onset of rare mutations that drive human cancer development.

Thus, these data suggest that oncogenic potency is a major determinant for the observed RAS mutation frequencies in human cancers. To begin to assess whether KRAS\(^{A146T}\) differs from KRAS\(^{G12D}\) in qualitative terms, Poulin and colleagues used global proteomic analyses to profile KRAS\(^{A146T}\) and KRAS\(^{G12D}\) expressed in the colon, pancreas, and spleen. Perhaps not surprisingly, they found that samples from the same tissue clustered together; for example, colorectal cancer is more similar to itself regardless of which KRAS mutation is expressed. In addition, KRAS\(^{A146T}\) clustered more closely with KRAS\(^{WT}\) tissue than with KRAS\(^{G12D}\) tumors, further highlighting the relatively weak role of this mutation in driving tumor formation. Interestingly, although both mutants tended to discordantly regulate a number of pathways, many of the altered pathways were different depending on the mutation and tissue of origin. What was perhaps most fascinating is that even in pancreas, where the KRAS\(^{A146T}\) mutation was unable to drive PanIN formation alone, the proteome was significantly altered even though no histologic phenotype was observed. Thus, KRAS is likely able to promote tumor formation through a variety of different signaling mechanisms.

Finally, although the data showed that there are distinct signaling differences, the authors next validated how KRAS signaling regulates cellular behavior in the context of intact tissue. Using Paneth cells as a readout, in which Paneth cell differentiation is sensitive to high levels of ERK signaling, only KRAS\(^{G12D}\)-mutant colons lacked Paneth cells. This phenotype was reversed on MEK inhibition using trametinib. Thus, the authors concluded that different mutant forms of KRAS can produce quantitative and qualitative phenotypes, in that low levels of ERK activation lead to increased proliferation, whereas high levels of ERK activation lead to differentiation. They extended this result into PDAC, where ERK activation driven by KRAS\(^{A146T}\) may be too low to drive cell differentiation and failed to cross the threshold necessary to disrupt pancreatic homeostasis.

In summary, Poulin and colleagues provide compelling evidence that KRAS\(^{A146T}\) preferentially drives colorectal but not pancreatic cancer development, due in part to reduced ERK signaling as well as tissue-selective signaling activities. An important lesson learned from this study and those of KRAS\(^{G12C}\) is that KRAS mutations are definitely not created equal, opening the door for the development of KRAS mutation-selective as well as cancer type-specific therapies.

**Disclosure of Potential Conflicts of Interest**

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