Viewpoint

Increased mutant *KRAS* gene dosage drives pancreatic cancer progression: evidence for wild-type KRAS as a tumor suppressor?

Oliver A. Kent

Princess Margaret Cancer Centre, University Health Network, Toronto Medical Discovery Tower, Toronto, Canada

Correspondence to: Oliver A. Kent. Princess Margaret Cancer Centre, University Health Network, Toronto Medical Discovery Tower, 101 College Street TMDT 12-701, Toronto ON M5G 1L7, Canada. Email: kent.uhn@gmail.com.

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RAS genes are most commonly associated with gain-of function mutations that promote oncogenic behavior. Activating mutations in *KRAS* occur in 90–95% cases of pancreatic ductal adenocarcinoma (PDAC) a deadly and highly metastatic disease. Currently the fourth leading cause of cancer death in the United States, PDAC presents with a dismal 5-year survival rate of less than 5% (1). Acquisition of *KRA*S mutation is regarded as an initiating event in the development of PDAC, but what is the role of the wild-type *KRAS* allele in disease initiation and progression?

The human genome encodes three distinct RAS genes: *KRAS*, *NRAS*, and *HRAS*. The *KRAS* gene has two splice variants of the 4th exon that give rise to *KRAS4A* and *KRAS4B* (2). The majority of *KRAS* mutations occur at either codons G12, G13 or Q61. When *KRAS* is mutated, both *KRAS4A* and *KRAS4B* expressed from the mutant allele will be mutated (3). Alteration of codon 12 changes glycine to aspartic acid (G12D) and locks KRAS in the constitutively active and therefore oncogenic configuration (2). Oncogenic KRAS engages downstream effectors including the RAF-mitogen activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways, which promotes enhanced cellular proliferation, survival, and motility all of which are commonly perturbed in cancer (2).

The recent paper by Mueller and colleagues elegantly demonstrated that gene dosage gain of oncogenic KRAS ($KRAS^{MUT}$) was associated with loss of wild-type KRAS ($KRAS^{WT}$) in PDAC. Collectively, Mueller *et al.* show that gain of oncogenic *KRAS* underlies aggressive phenotypes driving PDAC and affects downstream biology including further oncogenic gains and tumor suppressor alterations leading to tumorigenesis and early dissemination (4). These results coupled with other lines of evidence outlined below, suggest $KRAS^{WT}$ must be lost for tumor initiation and progression and therefore may function as a tumor suppressor. The evidence is particularly strong for mouse models of *Kras* mutant leukemia which often display suppression or loss of $Kras^{WT}$ (3).

In an attempt to correlate mutational landscapes with tumor initiation and metastatic progression of PDAC, Mueller *et al.* characterized somatic mutations, gene expression, and copy-number changes in primary PDAC cultures derived from 38 mice expressing a conditional pancreas specific $Kras^{G12D}$ allele (mPDAC). The authors cross-referenced the mPDAC data to micro-dissected human PDAC to establish cross species comparison associated with molecular features of PDAC evolution. The most common amplification affected the *Kras* locus; in total four different $Kras^{G12D}$ gene dosage states were identified. The authors found that two-thirds of the cancers analyzed had allelic imbalances that caused increased $Kras^{G12D}$ gene dosage ($Kras^{G12D\text{-}iGD}$). In addition, two tumors displayed loss of $Kras^{WT}$ mRNA coincident with high $Kras^{MUT}$ expression which revealed additional mechanisms for oncogenic *Kras* gain (4). These observations demonstrated a correlation between allelic gain of $Kras^{G12D}$ with associated loss of the $KRAS^{WT}$ allele.

The progression of PDAC has been well documented by histologically distinct precursor lesions called pancreatic intraepithelial neoplasia (PanIN) which harbor many of the same genetic aberrations found in the cancer (5). Activating *KRAS* mutations occur in early low grade PanIN-1, whereas inactivating mutation and/or loss of tumor suppressor genes *CDKN2A* and *TP53* encoding the cyclin-dependent kinase p16 and the transcription factor p53 respectively, occur in intermediate to late lesions (5). Mueller *et al.* found hPanIN-1 and hPanIN-2 had a high frequency of increased $KRAS^{MUT}$ allele dosages. This result suggested $Kras^{G12D\text{-}iGD}$ acquisition is conserved between human and mouse and has a critical role in early PDAC progression and metastasis. Indeed, $Kras^{G12D\text{-}iGD}$ cancers had increased metastatic potential whereas $Kras^{G12D\text{-}HET}$ mPDAC were predominantly non-metastatic which explained early dissemination observed in human and mouse pancreatic cancer (4).

The Mueller *et al.* study then connected $KRAS^{MUT}$ acquisition in early tumorigenesis to the complete or partial loss of tumor suppressor genes *CDKN2A* and/or *TP53*. Through examination of mPDAC copy-number changes, Mueller *et al.* found the most frequent deletion in mPDAC affected *Cdkn2a* and they were able to delineate the sequence of events leading to $Kras^{G12D}$ allelic imbalance. Specifically, the majority of cancers with homozygous loss of *Cdkn2a* exhibited $Kras^{G12D\text{-}iGD}$ and high $Kras^{G12D}$ expression. In contrast, those tumors with heterozygous loss of *Cdkn2a* or wild-type *Cdkn2a* were predominantly $Kras^{G12D\text{-}HET}$ with low $Kras^{G12D}$ expression. Where a reconstructable sequence of events permitted, the results argued that *Cdkn2a* deletion preceded $Kras^{G12D\text{-}iGD}$ acquisition and was contingent on *Cdkn2a* homozygous inactivation. Similarly, homozygous loss of *Trp53* also predisposed tumors to $Kras^{G12D\text{-}iGD}$ acquisition. An *in vivo* model using mice with pancreas specific $Kras^{G12D}$ and *Cdkn2a* deletion demonstrated complete penetrance of $Kras^{G12D\text{-}iGD}$ acquisition confirming this was the preferred evolutionary mechanism upon homozygous *Cdkn2a* loss (4).

The consequences of *Cdkn2a* loss in pancreatic tumorigenesis have been described previously in the seminal paper by Qiu and colleagues (6). The inactivation of *Cdkn2a* alone in a mouse model was not sufficient to initiate pancreatic tumorigenesis but required simultaneous $Kras^{G12D}$ activation (6). All of the compound mice with pancreas specific *Cdkn2a* inactivation and $Kras^{G12D}$ activation developed the full spectrum of mPanIN lesions and mPDAC with metastatic burden consistent with the human disease (6). Similar to the work described by Mueller *et al.* above, the $Kras^{WT}$ allele was lost during the progression from primary tumors to metastases in the pancreas from $Cdkn2a^{Null}$-$Kras^{G12D}$ mice. Considering both *in vivo* and *in vitro* data, these results showed that loss of heterozygosity (LOH) at the *Kras* locus engendered aggressive phenotypes in pancreatic tumor cells that favored growth and promoted metastasis. Thus, $Kras^{WT}$ had a bona fide suppressive effect on $KRAS^{MUT}$ through an as of then, unknown mechanism. Interestingly, the aggressive phenotypes were not a consequence of increased MAPK signaling as no discernible differences in phosphorylated ERK1/2 were observed in cancer cells with or without LOH at *Kras*.

In work published earlier this year, Ambrogio *et al.* discerned that the $KRAS^{WT}$ allele imparts a growth inhibitory effect to oncogenic *KRAS* via dimerization of RAS molecules (7). Previously, KRAS was found to form stable homodimers creating two major dimer interfaces which are required to bring together and activate two molecules of RAF (8). Upon examination of RAS-dimer crystal structures, Ambrogio *et al.* identified a critical residue within the dimer interface that mediated RAS dimerization. Homodimerization was required to sustain the oncogenic function of mutant KRAS and activate downstream signaling through the RAF-MAPK cascade. The inhibitory effect of wild-type KRAS was found to be caused by dimerization with mutant KRAS. A dimerization-deficient wild-type KRAS was unable to impart a growth-inhibitory effect on mutant KRAS (7).

In summary, the Mueller *et al.* paper proposes a "comprehensive conceptual framework" for the molecular mechanisms involved in the initiation and development of PDAC. Since gain-of-function mutation in RAS genes are among the most common events in human tumorigenesis (9), the importance of the Mueller *et al.* study extends beyond pancreatic cancer. The work emphasizes defining principles of RAS-driven oncogenesis and corroborates observations seen in mutant RAS-driven mouse models of tumorigenesis and patient tumors that $KRAS^{WT}$ likely serves as a tumor suppressor. However, the function of wild-type RAS is complicated by the expression of multiple RAS isoforms and likely is inhibitory only to the oncogenic RAS of the same isoform (2). Future therapeutics aimed at targeting KRAS may need to consider targeting oncogenic KRAS specifically without inhibiting wild-type KRAS function or gene dosage.

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Footnote

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