Review Article

Critical role of *KRAS* mutation in pancreatic ductal adenocarcinoma

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Abstract: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human cancers worldwide. Little progress has been made in recent years concerning its diagnosis and treatment. Genetic alterations can be found in approximately 97% of PDAC cases. Mutations in the *KRAS* gene, which encodes the KRAS protein that regulates cell proliferation, differentiation, and apoptosis via activation of downstream signal transduction pathways, occur most frequently. *KRAS* plays a pivotal role in modulating the tumor microenvironment in PDAC patients. Additionally, *KRAS* can regulate metabolic changes in PDAC cells in a variety of ways. Although previous studies have shown that *KRAS* mutation detection can be used for early diagnosis and to predict the prognosis of PDAC patients, and many paths have been proposed to suppress the effects of *KRAS*, there is still no single pathway that leads to effective treatment of *KRAS*-mutant PDAC. This review summarizes the role of *KRAS* mutation in PDAC and examines the association between *KRAS* mutation and clinical applications.

Keywords: *KRAS*; pancreatic ductal adenocarcinoma (PDAC); microenvironment; metabolic reprogramming; diagnosis; prognosis; therapeutics

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human cancers (1), with a 5-year survival rate of less than 7%, and a great proportion of patients die within 6 months after diagnosis. Previous reports have indicated that in 2017 more than 91,500 patients died of PDAC in the European Union (2). Various hereditary changes—for instance, genetic deletions, amplifications, translocations, frameshifts, inversions and substitutions—can be found in approximately 97% of PDAC cases (3). *KRAS* mutation is the most frequent mutation (more than 90%) (4) and the initiating genetic event for PDAC, and it is found in primary tumors, metastatic tumors and even in pancreatic intraepithelial neoplasia (PanIN), the earliest preneoplastic stage in PDAC progression (5).

As one of the four main driver genes (*KRAS*, *TP53*, *CDKN2A* and *SMAD4*) in PDAC, the *KRAS* gene, located at chromosome 12p12.1, is a member of the *RAS* gene family and encodes the KRAS protein (21 kDa), which has GTPase activity and thus binds GTP in the activated state and GDP in the deactivated state. Ras regulates cell

proliferation, differentiation, and apoptosis by activating several signaling pathways, including the RAF/MEK/ERK, PI3K/AKT/mTOR, PLC/PKC, and RAL pathways (6). *KRAS* mutations are found at codon12, codon13, codon60 and codon61 in PDAC, and these mutations cause KRAS protein to remain activated in the absence of signal stimulation, resulting in an uncontrollable functional status. This review summarizes the current knowledge regarding the critical applications of *KRAS* mutation in PDAC.

Microenvironment

The tumor microenvironment, which is a dynamic network primarily comprising the matrix, soluble factors and cellular components, along with large quantities of inflammatory stroma, plays a critical role in PDAC occurrence and progression. Using genetically engineered mouse (GEM) models of PDAC, researchers have investigated the initial steps of pancreatic tumorigenesis in the context of the microenvironment and the role of *KRAS* in the microenvironment (7). Pancreatic stellate cells (PaSCs) have structures and functions similar to those of hepatic stellate cells and usually exist in the resting state. In the earliest stages of PanIN, mesenchymal cells are transformed into fibroblasts and pancreatic stellate cells upon injury induction. PaSC proliferation and activation are central to the development of pancreatic fibrosis. Activated PaSCs synthesize many extracellular matrix components (8), and infiltration of these cells can be influenced by *KRAS* gene activity.

The expression of *KRAS*-dependent factors, such as interleukin-6 (IL-6) and Sonic hedgehog (Shh), in the tumor microenvironment is modulated by *KRAS* (9). The Hedgehog signaling pathway plays a significant role in stromal desmoplasia in PDAC and accelerates the progression of oncogenic disease driven by *KRAS* (10). *KRAS G12D* upregulates Hedgehog signaling to mediate paracrine interactions in the microenvironment. Additionally, overexpression of the soluble ligand Shh stimulates PDAC cells and promotes the formation of desmoplastic stroma brimming with fibroblasts (11). In the iKRAS mouse model, when *KRAS* is inactivated, Shh expression in epithelial cells is decreased to regular levels (9). IL-6, an inflammatory cytokine that has been associated with PDAC development, plays a critical role in various biologic activities (12). The serum concentration of IL-6 is increased in PDAC, and its expression is enhanced by *KRAS*.

Metabolic reprogramming

The process of tumor cell metabolic reprogramming is one of the most typical mechanisms by which tumors to adapt to the microenvironment, maintain cell survival and meet the needs of macromolecule synthesis (13,14). In this sense, tumors are also considered a metabolic disease (15). Under hypoxia deficiency (16), PDAC metabolic processes exhibit significant changes (17). Moreover, the Warburg effect and altered mitochondrial metabolic activity are typical metabolic changes in pancreatic cancer (18,19). Even with sufficient oxygen, PDAC utilizes aerobic glycolysis to replace oxidative phosphorylation (OXPHOS) in normal tissue cells to provide energy, and this process is called the Warburg effect. In one aspect, *KRAS* adjusts to the metabolic changes in PDAC by increasing the expression of glycolytic enzymes, such as hexokinase 1 and 2, glucose transporters, phosphofructokinase and lactate dehydrogenase. On the other hand, the synthesis of proteins, nucleic acids and fatty acids needed for PDAC cell proliferation is supported by *KRAS* mainly through stimulation of glucose uptake and glucose mid products into the hexosamine and pentose phosphate pathways (20,21).

Another role of *KRAS* in promoting PDAC growth affects the glutamate metabolic pathway. Most cells require glutamate dehydrogenase to convert glutamine into α-ketoglutarate within the mitochondria to fuel the tricarboxylic acid cycle. However, in PDAC, glutamine-derived aspartate is transported to the cytoplasm and converted to oxaloacetate by aspartate aminotransferase, and oxaloacetate is subsequently converted to malic acid and finally becomes pyruvate, followed by an increase in the NADPH/NADP ratio to maintain the redox levels in PDAC cells. Pancreatic cancer cells are highly dependent on this metabolic pathway. *KRAS* mutations lead to increased aspartate aminotransferase expression and promote glutamine metabolism in PDAC, maintaining redox levels and promoting cancer cell growth (22).

Additionally, *KRAS* leads to metabolic changes that alter the generation of mitochondrial reactive oxygen species (ROS) (23). PDAC cells develop several mechanisms to resist the excessive ROS levels, which are detrimental to tumor cells. Therefore, the cancer cells can reduce cellular damage caused by ROS (24). Currently, *KRAS* mutation is considered to activate nuclear factor-erythroid 2-related factor 2 (Nrf2) to start the antioxidant mechanism (25),

which activates a series of antioxidant genes. More than 100 genes have been reported to be regulated by Nrf2, including NADPH regulators, drug efflux pumps and growth factors (26). The inhibitor KEAP1 strictly controls Nrf2 levels by binding to Nrf2 and mediating Nrf2 ubiquitination; thus, under normal conditions, Nrf2 levels remain low (27). One study found that mutant KRAS signals mainly through the Mek-Erk-Jun pathway to promote Nrf2 nuclear localization and antioxidative gene expression (28). In pancreatic cancer, human PanIN and PDAC, Nrf2 is more active than in normal pancreatic ductal cells, and the ROS level remains low. By contrast, because of the effect of KEAP1, Nrf2 in normal pancreatic duct cells is maintained at a very low level. Unlike many other tumors, few somatic mutations lead to inactivation of NRF2 in pancreatic cancer (29). Therefore, in *KRAS*-mutant pancreatic cancer, Nrf2-mediated antioxidant activity is regulated mainly by *KRAS* mutation. By contrast, knockdown of *KRAS* or *MAPK* signaling blocks Nrf2 expression and increases intracellular ROS levels (28).

Early diagnosis

Compared with resectable PDAC, the prognosis of patients with unresectable PDAC is worse (30); thus, an early diagnosis is essential for these patients. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) can scan the lesion within a short range and has few complications. Because pathological diagnosis is still the gold standard for PDAC diagnosis, EUS-FNA has become the preferred method to diagnose pancreatic disease and has been increasingly used clinically (31). EUS-FNA can be used to detect gene mutations in tissues or cells obtained by aspiration (32). Combination of a *KRAS*-mutation assay with cytopathology is better than cytopathology alone in increasing the sensitivity, negative predictive value and accuracy in inconclusive or doubtful diagnoses based on cytopathology and thus is central to inconclusive or doubtful diagnoses from cytopathology. This *KRAS*-mutation detection approach cannot replace histology but complements histology (33), especially for patients with an indeterminate mass in the pancreas (34). However, EUS-FNA may lead to the release of cellular material from tumors to the bloodstream. The detection of mutant *KRAS* and the concentration of cfDNA in plasma are increased after the procedure (35). Thus, further studies are needed.

In addition to EUS-FNA, a new emerging detection method, cancer-specific DNA detection, was recently identified. This method, called a liquid biopsy, detects cancer-specific DNA in peripheral blood and noninvasively examines tumor characteristics. *KRAS* mutations can be detected both in plasma and serum DNA (36,37). Carbohydrate antigen 19-9 (CA19-9) is currently the most meaningful and widely used biomarker in PDAC, and has a sensitivity and specificity of 79–81% and 82–90% respectively. However, approximately 5% to 10% of the PDAC patients are Lewis negative individuals which are documented to have scarce or no CA19-9 secretion (38). Therefore, measurement of ctDNA for detection and quantitative monitoring of *KRAS* mutations may offer an additional diagnostic biomarker to CA19-9 (39). Baseline ctDNA *KRAS* detection rate was 93.7% (86.4% in patients with non-elevated CA19-9) with the application of an ultrasensitive ctDNA *KRAS* assay (40). However, the *KRAS*-mutation subtype in peripheral blood may be heterogeneous compared with that in the primary tumor (41). Additionally, *KRAS* mutations can be detected through genomic DNA in exosomes derived from the serum of PDAC patients. Exosomes are small vesicles (50–150 nm) of endocytic origin that are shed from viable cells into the circulation, and serum exosomes from patients with PDAC contain genomic DNA spanning all chromosomes (42). Exosomal DNA (exoDNA) can be detected even in the early stage of PDAC. Additionally, *KRAS* mutations in exoDNA were found in 7.4%, 66.7%, 80%, and 85% of age-matched controls and localized, locally advanced, and metastatic PDAC patients, respectively, which is higher than the detection levels in cfDNA (14.8%, 45.5%, 30.8%, and 57.9%, respectively) (43). However, mutant *KRAS* in the circulation has also been detected in healthy samples, possibly limiting its usefulness as a marker for early diagnosis.

Prognosis

There is still no consensus on whether *KRAS* mutations affect the prognosis of PDAC patients. Many studies have found that the presence of a *KRAS* mutation and a *KRAS* mutational subtype were both associated with a poor prognosis in PDAC patients (44,45). A single-nucleotide mutation induces a replacement of the GGT sequence (encoding glycine) by the GAT (aspartic acid-*G12D-c35* G>A), GTT (valine-*G12V-c35* G>T), CGT (arginine-*G12R-C34* G>C), or GCT (alanine-*G12A-c35* G>C) sequence. The *KRAS G12* mutation accounts for 99% of

all mutations. A point mutation can also happen on codon 13 (*G13D*) or 61 (*Q61L* or *Q61H*) but is less frequent (46,47). Whole-exome sequencing of PDAC revealed that codon Q61 alleles of *KRAS* are specifically associated with a better prognosis (48). Using next-generation sequencing, Qian *et al.* (49) analyzed DNA alterations in four main driver genes in 356 patients with resected PDAC. They found that patients who had *KRAS*-mutant tumors had a worse disease-free survival (DFS) and overall survival (OS) than those with *KRAS* wild-type tumors. Additionally, patients with *KRAS G12D*-mutant tumors had particularly different outcomes and the worst DFS. Ako *et al.* (36) proposed the contrasting idea that the prognosis of *KRAS G12V*-mutant tumors was poorer than other subtypes. In metastatic pancreatic cancer patients, *KRAS G12V* mutation was also correlated with poor OS based on subgroup analysis (50). Hamidi *et al.* (51) used a growth inhibition assay to determine the sensitivity to MEK inhibition in different *KRAS* mutational subtypes and copy number variations. They found that cell lines with *KRAS G12V* mutation and *KRAS* gain or loss were ~10 times more resistant than the other subtypes. Copy number variation may be an important biomarker for PDAC. Thus, multicenter investigations in a larger homogeneous cohort of PDAC patients are certainly needed in the future to reach a definitive conclusion.

Patients with *KRAS* wild-type tumors seem to benefit from chemotherapy. A multicenter, randomized phase IIb study found that patients with *KRAS*-mutant tumors experienced a significantly poorer prognosis than those with *KRAS* wild-type tumors when administered gemcitabine combined with the monoclonal antibody nimotuzumab (52). Another phase III trial that compared gemcitabine/erlotinib followed by capecitabine with capecitabine/erlotinib followed by gemcitabine in advanced PC also showed that *KRAS* wild-type patients have an improved OS (53). Early changes in the plasma DNA concentration of mutant *KRAS* is a sensitive index of the chemotherapy effect of the start stage in PDAC (54). An increase in plasma DNA in the sample collected from patients after chemotherapy was correlated with poorer PFS and OS than observed in patients with stable/reduced plasma DNA (55). The dynamic changes in ctDNA *KRAS* mutation load from serial measurements may be used as an assessment of therapeutic response, which was independent and complementary to the commonly used biomarker CA19-9, especially in Lewis negative individuals (40). And ctDNA level over time is a better predictor of survival than the dynamics of CA19-9 (56).

Therapeutics

Because the *KRAS* gene plays a vital role in PDAC development, it is an attractive therapeutic target. However, there is still no lone path to an effective treatment for *KRAS*-mutant PDAC (57). Nonetheless, researchers have attempted to find possible paths that can suppress the effects of *KRAS* in PDAC (58).

Many studies have tried to inhibit RAS directly. Burns *et al.* (59) reported the identification of a small molecule that can link to a unique pocket on the Ras:Son of Sevenless (SOS): Ras complex and increase the rate of SOS-catalyzed nucleotide exchange in vitro. This finding provides a new target for the discovery of potent Ras signaling inhibitors, but this complex has yet to be targeted by existing compounds (*Figure 1*).

Compared with the protein inhibition approach, mRNA targeting via RNA interference (RNAi) has already been shown to be an effective alternative (60). Zorde Khvalevsky *et al.* (61) developed a local prolonged siRNA delivery system (Local Drug EluteR, LODER) that sheds siRNA against mutant *KRAS* (si*G12D* LODER) (*Figure 1*). They found that the *in vitro* growth of pancreatic cancer cells can be substantially inhibited by LODER-derived si*G12D* and that *in vivo* tumor growth can also be suppressed. Another phase 1/2a study showed that the combination of LODER-derived si*G12D* and chemotherapy demonstrates a potential treatment effect in patients with locally advanced pancreatic cancer (60). Additionally, many miRNAs have been shown to target *KRAS* in PDAC, including let-7a, miR-96, miR-126, miR-143 and miR-217 (62,63). For example, miR-126 can directly target *KRAS* at a 'seedless' binding site within its 3′ UTR. Replacing these miRNAs in *KRAS*-mutant PDAC patients may represent a new approach to preventing tumor progression and metastasis (62).

RAS must be positioned at the cell membrane to maintain its biological activity, and this association is induced by farnesyl transferase (FTase) (64). FTase attaches a 15-carbon farnesyl isoprenoid to the cysteine in the CAAX-motif, which is the first step in the CAXX modifications. Some farnesyltransferase inhibitors (FTIs), such as tipifarnib, have been tested clinically but do not show clinical benefit (65,66), likely because the *RAS* gene encodes four different proteins—HRAS, NRAS, KRAS4a, and KRAS4b—and because HRAS and NRAS do not rely on farnesylation. Another approach to interfere with RAS

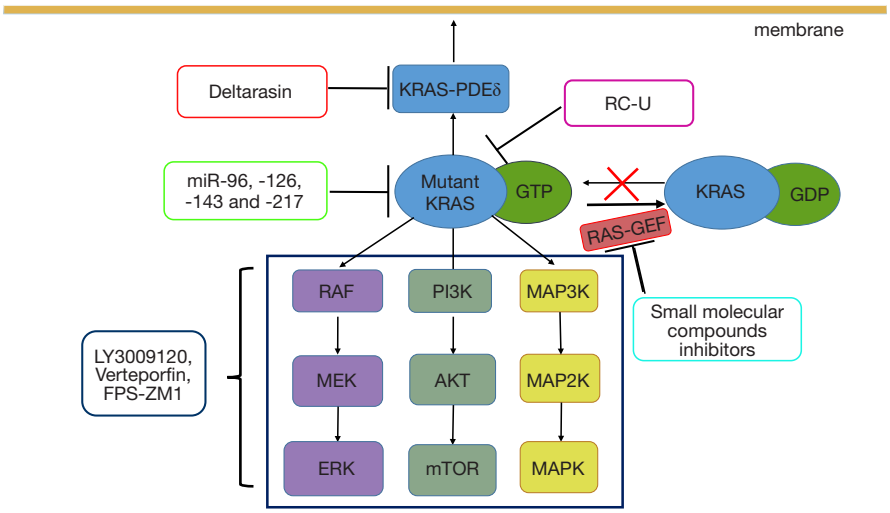

Figure 1 Model of anti-*KRAS* therapy. Deltarasin inhibits the KRAS-PDEδ interaction, which inhibits oncogenic RAS signaling and suppresses PDAC. RC-U, which targets the KRAS oncoprotein for ubiquitination and degradation, results in the reduction of PDAC cell proliferation. siRNA and miRNA target *KRAS* to inhibit the growth of PDAC cells. Additionally, numerous inhibitors, such as LY3009120, Verteporfin, and FPS-ZM1, target RAS downstream effector signaling. Furthermore, some small-molecule compound inhibitors bind directly to RAS protein and inhibit GDP-GTP regulation. PDAC, pancreatic ductal adenocarcinoma.

membrane association is blocking the RAS pathway to the plasma membrane transporters. Phosphodiesterase 6 delta (PDE δ) has a pocket that can combine with RAS protein that has been modified by FTase. PDE δ can promote RAS protein distribution in the correct position and is involved in signal transduction (67). Deltarasin is a small-molecule inhibitor that inhibits the KRAS-PDEδ interaction. Through this process, oncogenic RAS signaling and the oncogenic KRAS-dependent proliferation of human PDAC cells are suppressed (*Figure 1*) (68). This brings a new approach to suppressing oncogenic RAS signaling.

Numerous inhibitors that target RAS downstream effector signaling (*Figure 1*), such as RAF/MEK/ERK and PI3K/AKT/mTOR signaling pathways, are already being tested in clinical trials. Many inhibitor combinations for PDAC treatment have demonstrated promising results (69). LY3009120 is a pan-RAF inhibitor with activity against three RAF isoforms and avoids the induction of paradoxical downstream signaling activation (70). Verteporfin, a YAP inhibitor (71), can block the activation of a parallel AKT signaling pathway after LY3009120 treatment, significantly enhancing the antitumor efficacy of LY3009120 (72). Many inhibitor-based combinations must be informed by the activation state of each putative driver in a given treatment context (73) and will be needed for efficacy across different *KRAS*-mutant PDAC populations (74,75). Some inhibitors can block the ERK signaling pathway indirectly. For example, inhibition of receptor for advanced glycation end-products (RAGE) using the pharmacological antagonist FPS-ZM1 restrained ERK activity downstream of KRAS in PDAC cell lines (76).

Chimeric antigen receptor T cells (CAR-T) have shown huge success against CD19-ex-pressing B cell leukaemia (77). As CAR T cell therapy spread, so does the search for new biomarker targets for PDAC (78). However, there are reports on the CAR-T cells treatment which target an upstream component of the RAS signaling pathway (i.e., EGFR/HER2) in pancreatic cancer. Raj *et al.* (79) used switchable CAR-T cells to target the antigen HER2. They found that a switchable CAR-T system is effective against aggressive and disseminated tumours derived from patients with advanced PDAC while affording the potential safety of a control switch.

In addition, many alternative strategies exist for targeted *KRAS* therapy in PDAC. Ma *et al.* (80) generated an engineered E3 ubiquitin ligase (RC-U) to target the KRAS oncoprotein for ubiquitination and degradation, resulting in a reduction in PDAC cell proliferation both *in vitro* and *in vivo* (*Figure 1*). Exosomes that carry short interfering RNA or short hairpin RNA specific to *KRAS G12D*, generated by Kamerkar, have been confirmed to significantly suppress cancer development in multiple

mouse models (81).

Summary

KRAS can be used to predict the prognosis and assist in the early diagnosis of PDAC. Unfortunately, despite the many paths available to suppress the effects of *KRAS*, none of the treatments targeting these paths have been significantly successful in the clinic. More studies are required to further elucidate the effects of *KRAS* and identify therapeutic targets in PDAC.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.10.19). The authors have no conflicts of interest to declare.

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