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Perspective

KRAS-related noncoding RNAs in pancreatic ductal adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with a poor overall prognosis. However, curative resection during the early stages of the disease can greatly improve survival rates, highlighting the importance of early screening and detection. Studies of noncoding RNAs, primarily microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), provide important insights into strategies for the early detection of *KRAS*-driven PDAC. Here, we summarize our studies and review current reports on research investigating *KRAS*-related miRNAs and lncRNAs, emphasizing their aberrant expression, mechanisms, carcinogenic effects, and prognostic and predictive capacities in PDAC.
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Keywords: Pancreatic ductal adenocarcinoma; *KRAS*; Noncoding RNAs; microRNAs; Long noncoding RNAs

Introduction

Pancreatic ductal adenocarcinoma (PDAC), which accounts for approximately 90% of all pancreatic tumors,[1] is the fourth leading cause of cancer-related deaths in the United States of America[2] and the ninth leading cause of cancer-related deaths in China, having the second highest ratio of mortality to incidence (approximately 88%) among all types of cancers.[3] Surgery remains the only effective curative treatment for localized disease, and chemotherapy offers some palliation in advanced disease. Only 15–20% of patients with PDAC are surgical candidates with resectable masses at the time of diagnosis.[4] However, for patients with early-stage disease who undergo pancreaticoduodenectomy, the 5-year survival rate is greatly improved to 52.9% in patients who have negative resection margins, negative lymph nodes, and a tumor size of less than 3 cm.[5] Therefore, it is

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essential to detect and screen out these patients with localized PDAC during the early stages of the disease.

Unfortunately, no effective screening modalities for localized PDAC have been developed[6]; although the widespread use of high-resolution computed tomography (CT) and serum tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9), has provided insights into markers of disease progression, these modalities are neither sensitive nor specific for early detection and diagnosis. Accordingly, many genomic alterations associated with pancreatic tumorigenesis and progression have been explored, and several major genetic variations have been identified in this lethal cancer, including the *KRAS* oncogene and related tumor-suppressor genes, such as *CDKN2A*, *SMAD4*, and *TP53*.[7,8] *KRAS* mutations are found in 30% of human tumors[9] and in more than 90% of PDAC cases. Moreover, *KRAS* plays an important role in carcinogenesis; mutations in *KRAS* occur throughout premalignancy and then in early and advanced stages of PDAC carcinogenesis.[6,10–12] The *KRAS* gene encodes a small GTPase that mediates cellular signaling downstream of growth factor receptors.[13] Constitutive KRAS activation leads to stimulation of a number of complex downstream pathways, such as the mitogen-activated protein kinase (MAPK), phosphoinositol-3 kinase (PI3K), and transforming growth factor-β (TGF-β) signaling pathways, which govern proliferation, cell survival, differentiation, and gene expression.[8]

An increasing number of studies have shown that noncoding RNAs (ncRNAs) are closely associated with the carcinogenesis and prognosis of PDAC.[14] NcRNAs include highly abundant and functionally important RNAs, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), small nuclear RNAs (snRNAs), exosomal RNAs (exRNAs), piwi-interacting RNAs (piRNAs), small Cajal body-specific RNAs (scaRNAs), and long noncoding RNAs (lncRNAs). Of these, miRNAs and lncRNAs are the most frequently studied ncRNAs in cancer biology, diagnosis, and therapy.

MiRNAs are small RNA molecules of approximately 22 nucleotides; these molecules interact with messenger RNAs (mRNAs) and serve as negative regulators of gene expression[15,16] by completely or incompletely binding to complementary regions in the 3′-untranslated region (UTR; some rare miRNAs may bind in the 5′-UTR) of target mRNAs to facilitate mRNA cleavage or translational repression. MiRNAs comprise one class of abundant gene regulatory molecules in multicellular organisms and influence the output of many protein-coding genes. Moreover, miRNAs have been shown to influence cell differentiation, proliferation, morphogenesis, development, apoptosis, and stress responses.[17] Although these molecules represent only 3% of the human genome, they regulate 20–30% of protein-coding genes.[18,19] MiRNAs were first found to control the timing of *Caenorhabditis elegans* larval development in 1993[20] and have now been profiled in many different malignancies. Current data have suggested that miRNAs may be promising biomarkers for the early detection of cancer.

LncRNAs are a new class of regulatory RNAs that also do not have protein-coding capacity. Many studies have demonstrated that lncRNAs play essential roles in a wide variety of biological processes and are involved in the progression of many human diseases, including cancer.[21–23] In PDAC cases, cDNA microarrays for lncRNAs have revealed that sets of intronic lncRNAs are differentially expressed in primary and metastatic pancreatic cancer.[24] Additionally, in another study, the lncRNA hox transcript antisense intergenic RNA (HOTAIR) was shown to have pro-oncogenic activity and was a negative prognostic factor in pancreatic cancer.[25]

In this review, we describe the role of *KRAS*-related noncoding RNAs, primarily miRNAs and lncRNAs, and evaluate their aberrant expression patterns, mechanisms, carcinogenic effects, and prognostic and predictive capacities in PDAC (Table 1). To identify papers, we searched for articles on the combined terms of *KRAS*, miRNA/lncRNAs, and PDAC. The inclusive relationship used in our study meant there was a direct or indirect correlation found within each cited paper. All literature presented in this study was published in English.

KRAS-related miRNAs in PDAC

MiRNAs directly targeting the KRAS oncogene

Multiple mutations in the evolution of PDAC are influenced by miRNAs, which serve as tumor promoters or suppressors by targeting functional or regulated genes and further silencing or promoting downstream pathways.[26] Many miRNAs, including miR-96, miR-217, miR-216, miR-193b, miR-126, miR-143, miR-145, miR-206, and let-7, directly target *KRAS* and have been shown to be downregulated in PDACs compared with those in control tissues.[27–32] Furthermore, re-expression of these miRNAs suppresses *KRAS* activity and reduces tumorigenic

Table 1
KRAS-related noncoding RNAs in PDAC.

Groups	miRNAs	Expression levels in tumors	Remarks
KRAS-related miRNAs			
Directly targeting *KRAS*	miR-96	Downregulated	Potent regulator, potential therapeutic target
	miR-217	Downregulated	Regulates *KRAS* and functions as a tumor suppressor
	miR-216	Downregulated	Verified in *KRAS* mutant mouse models
	miR-193b	Downregulated	Inhibits *KRAS*, Akt, and ERK
	miR-126	Downregulated	Interacts with a "seedless" motif
	miR-143/145	Downregulated	miRNA-*RAS*-associated feed-forward mechanism; miRNA-mediated therapy
	let-7	Downregulated	Inhibits *KRAS*, but fails to alter tumor progression
	miR-206	Downregulated	Pleiotropic modulator, tumor microenvironment
Indirectly regulating *KRAS*	miR-27a	Upregulated	miR-27a-Spry2-*RAS*/MAPK
	miR-21	Upregulated	Multiple targets, multifunctional roles
KRAS-related lncRNAs	MIR31HG	Upregulated	MIR31HG-miR-193b-*KRAS*
	MALAT1	Upregulated	miR-217-MALAT1/*KRAS*
	H19	Upregulated	H19-let-7-HMGA2/*KRAS*, metastatic tendency

PDAC: pancreatic ductal adenocarcinoma; miRNAs: microRNAs; ERK: extracellular signal-regulated kinase; Spry2: Sprouty2; MAPK: mitogen-activated protein kinase; lncRNAs: long noncoding RNAs; HMGA2: high mobility group AT-hook 2.

properties, suggesting that these miRNAs function as tumor suppressors. Additionally, recent studies have shown that some of these miRNAs can influence downstream *KRAS*-associated signaling pathways involved in cell survival and proliferation.

miR-96

We have identified several miRNAs that directly target the *KRAS* oncogene and function as tumor-suppressive miRNAs in PDAC. MiR-96 was one of the first miRNAs identified. In 10 pairs of fresh clinical specimens from humans, miR-96 was found to be downregulated or deleted and was associated with KRAS upregulation, consistent with results in PDAC cell lines. Ectopic expression of miR-96 inhibited *KRAS*, blocked Akt signaling, and triggered apoptosis in PDAC cells. *In vitro* and *in vivo* assays established that miR-96 decreased cancer cell invasion and migration and slowed tumor growth in association with *KRAS* downregulation.[27] These findings identified miR-96 as a potent regulator of *KRAS* and may provide a novel therapeutic strategy for the treatment of pancreatic cancer and other *KRAS*-driven cancers.

miR-217/miR-216

Our group also investigated the expression and possible role of miR-217 in PDAC. From a set of 21 PDAC specimens, data obtained using locked nucleic acid *in situ* hybridization (LNA-ISH) and real-time quantitative polymerase chain reaction showed that miR-217 was downregulated in 76.2% of PDAC tissues; similar results were observed in all tested PDAC cell lines when compared with the corresponding normal pancreatic tissue. Data from dual-luciferase reporter gene assays showed that *KRAS* was a direct target of miR-217. Upregulation of miR-217 inhibited tumor cell growth *in vitro* and *in vivo*, decreased KRAS protein level, and reduced the constitutive phosphorylation of downstream Akt. In contrast, downregulation of miR-217 expression in PDAC cells increased cell anchorage-independent colony formation and KRAS protein level. Furthermore, miR-217 expression was found to be negatively correlated with KRAS protein expression in PDAC cell lines.[29] Therefore, miR-217 could regulate *KRAS* and function as a tumor suppressor in PDAC.

Interestingly, miR-216 and miR-217, which are located in the same cluster within a ~30-kb region on 11qA3.3, were found to be downregulated in the pancreas of *KRAS*-mutant PDAC model mice, e.g., P48$^{+/Cre}$; LSL-KRAS^{G12D}, PDX-1-Cre; LSL-KRAS^{G12D}, and Ela-KRAS^{G12D} mice. Germ line deletion of this cluster was found to be embryonic lethal in mice,[33] indicating the importance of these two miRNAs in murine embryonic development.

miR-193b

Our team found that miR-193b levels were lower in 11 surgically resected PDAC specimens than in matched adjacent benign tissues and further confirmed these results using LNA-ISH in 22 PDAC specimens. Re-expression of miR-193b inhibited pancreatic cancer cell growth and proliferation by functioning as a cell-cycle brake in PDAC cells and was associated with suppression of apoptosis. We also verified that miR-193b regulated the expression of KRAS by directly

targeting its 3′-UTR and reduced downstream signaling activity of phosphorylated Akt and extracellular signal-regulated kinase (ERK).[30]

miR-126

In a report comparing the miRNA expression signatures of pancreatic benign cystic tumors and PDACs, Jiao et al[28] showed that some miRNAs, including miR-16, miR-126, and let-7d, were downregulated, the latter two of which target *KRAS*. Moreover, they found that miR-126 regulated KRAS protein translation by interacting with a "seedless" motif in its 3′-UTR, not the canonical seed regions of most miRNAs.

miR-143/miR-145 (miR-143/145)

Kent et al[34] evaluated miRNAs associated with the feed-forward mechanism that potentiates RAS signaling. They demonstrated that miR-143/145 targets *KRAS* and RAS-responsive element-binding protein (RREB1) and that KRAS activation leads to repression of the miR-143/145 cluster in some diverse model systems, including PDAC cell lines. Interestingly, miR-143/145 downregulation requires RREB1, which represses the miR-143/145 promoter. Additionally, loss of miR-143/145 expression was observed frequently in *KRAS* mutant pancreatic cancers, and restoration of these miRNAs abrogated tumorigenesis. In another study, Ali et al[31] showed that increased RAS GTPase activity was regulated by loss of miR-143 and let-7, which can be attenuated by diflourinated-curcumin (CDF), a novel synthetic analog of curcumin, treatment in pancreatic cancer cells, thus providing an miRNA-mediated therapeutic strategy for *KRAS*-associated PDAC.

let-7

The relationship between let-7 and its target *RAS* was first described by Johnson et al[35] in lung cancer in 2005. Many studies have further verified this relationship in PDAC,[36–38] and let-7 expression has been shown to be strongly reduced in PDAC samples and in poorly differentiated cancer samples compared with that in adjacent tissues. Restoring let-7 levels strongly inhibits cell proliferation, KRAS expression, and MAPK activation. However, one study by Torrisani et al[36] indicated that inhibition of *KRAS* caused by let-7 *in vitro* failed to alter tumor progression.

Single nucleotide polymorphisms (SNPs) occur once every several hundred base pairs throughout the whole genome.[39] Some studies have showed that the SNP within the let-7 binding site of *KRAS* (rs61764370), referred to as the *KRAS* variant, is strongly associated with increased risk of non-small cell lung cancer[40] and epithelial ovarian cancer.[41] Additionally, investigators found that the *KRAS* variant is also associated with human epidermal growth factor receptor 2 (HER2)-positive tumors and tumors of higher histopathologic grade in breast cancer.[42] Furthermore, some studies have shown that the *KRAS* variant is a prognostic marker in head and neck squamous cell carcinoma[43] and is associated with significantly reduced survival. In a study of metastatic colorectal cancer, researchers demonstrated that patients with the *KRAS* variant had poorer overall and progression-free survival, regardless of whether they also harbored *KRAS* protein-coding gene mutants. Researchers also found that those with the *KRAS* variant but without *KRAS* protein-coding mutations had better responses to cetuximab monotherapy.[44] Unfortunately, no other reports have described *KRAS* variants in PDAC, suggesting that further SNP studies in PDAC are urgently needed.

miR-206

Keklikoglou et al[32] found that miR-206 functions to modulate intercellular communication within the tumor microenvironment. They found that miR-206 expression was abrogated in 25 PDAC specimens and 6 PDAC cell lines. Additionally, they showed that miR-206 exerted tumor-suppressive functions by directly targeting the oncogenes *KRAS* and annexin A2 in PDAC cells. Importantly, they identified miR-206 as a negative regulator of oncogenic KRAS-induced nuclear factor-κB (NF-κB) transcriptional activity, resulting in concomitant reduction of pro-angiogenic and pro-inflammatory factors. Furthermore, using *in vitro* and *in vivo* approaches, they revealed that re-expression of miR-206 in PDAC cells was sufficient to inhibit tumor blood and lymphatic vessel formation, thus leading to a significant delay in tumor growth and progression. Taken together, these findings clarified the mechanistic role of miR-206 as a pleiotropic modulator of different hallmarks of cancer through the targets of *KRAS* and annexin A2 in the context of the tumor microenvironment in PDAC.

This type of negative regulation facilitates the changes in expression of *KRAS*-targeted miRNAs in PDAC. The above-mentioned miRNAs all directly target the UTR of *KRAS* mRNA and inhibit KRAS activity in PDAC, thus acting as tumor suppressors and influencing downstream signaling pathways of KRAS and its network. Theoretically, the alterations caused by these miRNAs are realized through the inhibition or obstruction of functional genes and target, such as *KRAS*. When these miRNAs are present at normal

levels, they function in an inhibitory manner to maintain the balanced homeostasis of the organism. Whereas, in PDAC, different levels of downregulation or abrogation of these miRNAs are observed. Dual-luciferase reporter gene assays have verified the exact binding sites for these miRNAs in *KRAS* mRNA, and their suppressive efficacy in PDAC cells has been shown by gain or loss of expression tests *in vitro* and *in vivo*. However, it is difficult to evaluate whether such nonphysiological approaches are reliable in organisms.

Several miRNAs, such as miR-18a and miR-622, have been shown to directly target *KRAS* in cancer and can be searched using online databases of validated miRNA-target interactions, such as miRWalk 2.0. However, these miRNAs were identified in other types of cancer, not in PDAC. Thus, further studies are needed to determine the roles of these miRNAs in PDAC.

MiRNAs indirectly regulate the KRAS oncogene

miR-27a

miR-27a has been shown to be abnormally upregulated in other types of cancers and has been identified as an oncogenic factor in tumorigenesis.[45–48] Similarly, our miRNA microarray assays indicated that this miRNA was upregulated in PDAC, and also provided insights into the roles and mechanisms of miR-27a expression in PDAC. In our study, we found that inhibition of miR-27a suppressed the growth, colony formation, and migration of PDAC cells. Using a reporter-screening verification assay and loss of function tests, we discovered that Sprouty2 (Spry2) protein, which showed reduced expression in PDAC specimens, was targeted by miR-27a and can be upregulated by transfection with an miR-27a inhibitor.[49] Spry2 is an inhibitor of the RAS/MAPK signaling pathway and is an important modulator of vital pathways central to the development or progression of cancers.[50,51] Therefore, in this PDAC cohort, miR-27a may promote KRAS activity by relieving the inhibition of Spry2.

miR-21

Recent studies have shown that miR-21 downregulates Spry2, thereby triggering malignancy in human gliomas[52] and modulating human mesenchymal stem cell differentiation during adipogenesis and osteogenesis.[53] miR-21 is one of the mostly extensively studied miRNAs in solid cancers and has been shown to suppress multiple targets, enhancing the oncogenic action of RAS. Additionally, miR-21 has been shown to downregulate phosphatase and tensin homolog (PTEN), thereby increasing the activity of Akt and enhancing NF-κB activation in cancers.[54] Elevated miR-21 level has been reported in PDAC precursor lesions, high-grade pancreatic intraepithelial neoplasia,[55] and PDAC tissues.[56] Moreover, its multiple targets, including *PTEN*, *PDCD4*, *FoxO1*, and *MMP* mRNAs, have been verified in pancreatic cancer.[57–59]

Surprisingly, one study by Frezzetti et al[60] showed that miR-21 was upregulated both *in vitro* and *in vivo* by oncogenic RAS, thus linking this miRNA to the *RAS* oncogene in human thyroid cancers and non-small cell lung cancers and suggesting an important role for miR-21 in oncogenic RAS-induced cell proliferation.

Interestingly, the aberrant upregulation of miR-21 has also been found in the tumor microenvironment of PDAC, such as in cancer-associated fibroblasts (CAFs) and stellate cells, as compared with normal pancreas.[61] Additionally, inhibition of miR-21 by transfection with antisense oligonucleotides results in decreased migration/invasive capacity in stellate cells. These results suggest that upregulation of miR-21 expression may confer a certain degree of aggressiveness to pancreatic cancer cells.

***KRAS*-related lncRNAs in PDAC**

The lncRNA MIR31HG

MIR31HG is a recently identified 2166-nucleotide lncRNA. Our study found that the lncRNA MIR31HG is markedly upregulated in 13 paired PDAC tissues. Functional analyses have shown that this lncRNA significantly promotes PDAC cell growth and invasion and inhibits apoptosis and G_1/S arrest. Additionally, we found an inverse correlation between MIR31HG and miR-193b in PDAC specimens and verified that MIR31HG is negatively regulated by miR-193b, which directly targets MIR31HG.[62] miR-193b has also been shown to directly target the *KRAS* gene by negatively regulating PDAC.[30] Importantly, MIR31HG could recover the miR-193b-induced inhibition of *KRAS* through acting as an endogenous ‘sponge’ by competing for the miR-193b binding site. Therefore, these results demonstrate that MIR31HG functions as an oncogenic lncRNA by promoting tumor progression and affects KRAS function by competing with miR-193b in PDAC.

The lncRNA MALAT1

Another *KRAS*-related lncRNA in our study is metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which was first identified in lung cancer and was shown to play an important role in tumorigenesis. We found that MALAT1 was upregulated in pancreatic tumors compared with that in nontumor tissues and was negatively regulated by miR-217, which was shown to function as an inhibitor of *KRAS* in our previous study.[29] Knockdown of MALAT1 in PDAC cells attenuates KRAS protein expression, and the suppression of KRAS can be rescued by inhibiting miR-217 expression. *In vitro* or *vivo* knockdown of MALAT1 reduces tumor cell growth and proliferation, impairs other tumorigenic features, and decreases the phosphorylation of MAPK kinase (MEK) and ERK1/2. Additionally, we found that miR-217 exhibits altered cellular localization consistent with changes in MALAT1 levels, suggesting that MALAT1 could bind with and sequester miR-217 to protect *KRAS* mRNA from repression. Many other studies have also demonstrated the roles of MALAT1 in promoting the proliferation, metastasis, and stem cell-like phenotypes of PDAC,[63−66] highlighting the importance of MALAT1 in the molecular regulation of PDAC.

The lncRNA H19

H19, the first lncRNA identified in human cancer in 1993, is a maternally imprinted gene on chromosome 11p15.5 that is not associated with a protein product.[67] In a recent study, Ma et al[68] found that H19 was overexpressed in PDAC than in adjacent normal tissues and was upregulated markedly in tumors with metastatic tendency. Subsequently, they found that down-regulation of H19 impaired PDAC cell invasion and migration. Furthermore, they showed that H19 promoted tumorigenesis in PDAC cells partially by increasing the high mobility group AT-hook 2 (HMGA2)-mediated epithelial-mesenchymal transition (EMT) through antagonizing let-7. Moreover, let-7 has been shown to target *KRAS*; thus, H19 may influence KRAS in PDAC. However, further studies are needed to validate these findings.

To date, many studies have shown the differential expression of lncRNAs in PDAC and their associations with tumor invasion, migration, and prognosis. Despite these studies, more work is needed to determine the mechanisms through which lncRNAs contribute to the tumorigenesis of PDAC.

Conclusion

KRAS is the most frequently activated oncogene in human PDAC and is relevant during the entire process of carcinogenesis, development, and progression. Inhibition of this crucial oncogene may have therapeutic effects in the management of this lethal disease. Efforts to target KRAS activation through ncRNAs have shown promising results owing to their effectiveness and specificity, and the number of reports addressing the role of ncRNAs in PDAC has increased over time. However, there are still many steps to be taken before the application of ncRNA-based detection and therapies will become a reality in clinical practice for patients with *KRAS*-driven PDAC.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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