Perspective

KRAS-related noncoding RNAs in pancreatic ductal adenocarcinoma

Shuang-Ni Yu a, Yi-Hui Ma a,b, Wu-Gan Zhao a,b, Xiang-Lan Jin a,c, Hai-Yan Yang a,d, Ping-Ping Liu a,e, Jie Chen a,*

a Department of Pathology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tsinghua University, Beijing 100730, China
b Department of Pathology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China
c Department of Pathology, Peking University Shenzhen Hospital, Shenzhen, Guangdong 518036, China
d Department of Pathology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei 050000, China
e Department of Pathology, China-Japan Friendship Hospital, Beijing 100029, China

Received 11 September 2016
Available online 22 December 2016

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.

© 2016 Chinese Medical Association. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 America2 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

http://dx.doi.org/10.1016/j.cdtm.2016.11.012
2095-882X/© 2016 Chinese Medical Association. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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; 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. KRAS mutations are found in 30% of human tumors 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. The KRAS gene encodes a small GTPase that mediates cellular signaling downstream of growth factor receptors. 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.

An increasing number of studies have shown that noncoding RNAs (ncRNAs) are closely associated with the carcinogenesis and prognosis of PDAC. NcRNAs include highly abundant and functionally important RNAs, such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), small nucleolar RNAs (snRNAs), 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 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. Although these molecules represent only 3% of the human genome, they regulate 20–30% of protein-coding genes.

MiRNAs were first found to control the timing of Caenorhabditis elegans larval development in 1993 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. In PDAC cases, cDNA microarrays for lncRNAs have revealed that sets of intronic lncRNAs are differentially expressed in primary and metastatic pancreatic cancer. 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.

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. 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. Furthermore, re-expression of these miRNAs suppresses KRAS activity and reduces tumorogenic
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. 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. Therefore, miR-217 could regulate KRAS and function as a tumor suppressor in PDAC.

Interestingly, miR-217 and miR-216, 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<sup>+/Cre</sup>; LSL-KRAS<sup>G12D</sup>, PDX-1-Cre; LSL-KRAS<sup>G12D</sup>, and Ela-KRAS<sup>G12D</sup> mice. Germ line deletion of this cluster was found to be embryonic lethal in mice, 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

---

**Table 1**

| KRAS-related miRNAs                | miRNAs     | Expression levels in tumors | Remarks                                                                 |
|-----------------------------------|------------|----------------------------|------------------------------------------------------------------------|
| 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-HMG2/KRAS                                                    |

PDAC: pancreatic ductal adenocarcinoma; miRNAs: microRNAs; ERK: extracellular signal-regulated kinase; Spry2: Sprouty2; MAPK: mitogen-activated protein kinase; lncRNAs: long noncoding RNAs; HMG2: high mobility group A1-hook 2.
targeting its 3′-UTR and reduced downstream signaling activity of phosphorylated Akt and extracellular signal-regulated kinase (ERK).\textsuperscript{30}

\textbf{miR-126}

In a report comparing the miRNA expression signatures of pancreatic benign cystic tumors and PDACs, Jiao et al\textsuperscript{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.

\textbf{miR-143/miR-145 (miR-143/145)}

Kent et al\textsuperscript{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\textsuperscript{35} 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.

\textbf{let-7}

The relationship between let-7 and its target RAS was first described by Johnson et al\textsuperscript{35} in lung cancer in 2005. Many studies have further verified this relationship in PDAC,\textsuperscript{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\textsuperscript{39} indicated that inhibition of KRAS caused by let-7 \textit{in vitro} failed to alter tumor progression.

Single nucleotide polymorphisms (SNPs) occur once every several hundred base pairs throughout the whole genome.\textsuperscript{39} Some studies have shown 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\textsuperscript{40} and epithelial ovarian cancer.\textsuperscript{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.\textsuperscript{42} Furthermore, some studies have shown that the KRAS variant is a prognostic marker in head and neck squamous cell carcinoma\textsuperscript{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.\textsuperscript{44} Unfortunately, no other reports have described KRAS variants in PDAC, suggesting that further SNP studies in PDAC are urgently needed.

\textbf{miR-206}

Keklikoglou et al\textsuperscript{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 \textit{in vitro} and \textit{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 \textit{KRAS} mRNA, and their suppressive efficacy in PDAC cells has been shown by gain or loss of expression tests \textit{in vitro} and \textit{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 \textit{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.

\textbf{MiRNAs indirectly regulate the KRAS oncogene}

\textit{miR-27a}

\textit{miR-27a} has been shown to be abnormally upregulated in other types of cancers and has been identified as an oncogenic factor in tumorigenesis.\textsuperscript{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 \textit{miR-27a} expression in PDAC. In our study, we found that inhibition of \textit{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 \textit{miR-27a} and can be upregulated by transfection with an \textit{miR-27a} inhibitor.\textsuperscript{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.\textsuperscript{50,51} Therefore, in this PDAC cohort, \textit{miR-27a} may promote \textit{KRAS} activity by relieving the inhibition of Spry2.

\textit{miR-21}

Recent studies have shown that \textit{miR-21} downregulates Spry2, thereby triggering malignancy in human gliomas\textsuperscript{52} and modulating human mesenchymal stem cell differentiation during adipogenesis and osteogenesis.\textsuperscript{53} \textit{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, \textit{miR-21} has been shown to downregulate phosphatase and tensin homolog (PTEN), thereby increasing the activity of Akt and enhancing NF-\textit{kB} activation in cancers.\textsuperscript{54} Elevated \textit{miR-21} level has been reported in PDAC precursor lesions, high-grade pancreatic intraepithelial neoplasia,\textsuperscript{55} and PDAC tissues.\textsuperscript{56} Moreover, its multiple targets, including \textit{PTEN}, \textit{PDCD4}, FoxO1, and \textit{MMP} mRNAs, have been verified in pancreatic cancer.\textsuperscript{57–59}

Surprisingly, one study by Frezzetti et al\textsuperscript{60} showed that \textit{miR-21} was upregulated both \textit{in vitro} and \textit{in vivo} by oncogenic RAS, thus linking this miRNA to the \textit{RAS} oncogene in human thyroid cancers and nonsmall cell lung cancers and suggesting an important role for \textit{miR-21} in oncogenic RAS-induced cell proliferation.

Interestingly, the aberrant upregulation of \textit{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.\textsuperscript{61} Additionally, inhibition of \textit{miR-21} by transfection with antisense oligonucleotides results in decreased migration/invasive capacity in stellate cells. These results suggest that upregulation of \textit{miR-21} expression may confer a certain degree of aggressiveness to pancreatic cancer cells.

\textbf{KRAS-related lncRNAs in PDAC}

The lncRNA \textit{MIR31HG}

\textit{MIR31HG} is a recently identified 2166-nucleotide lncRNA. Our study found that the lncRNA \textit{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 G1/S arrest. Additionally, we found an inverse correlation between \textit{MIR31HG} and \textit{miR-193b} in PDAC specimens and verified that \textit{MIR31HG} is negatively regulated by \textit{miR-193b}, which directly targets \textit{MIR31HG}.\textsuperscript{62} \textit{miR-193b} has also been shown to directly target the \textit{KRAS} gene by negatively regulating PDAC.\textsuperscript{30} Importantly, \textit{MIR31HG} could recover the \textit{miR-193b}-induced inhibition of \textit{KRAS} through acting as an endogenous ‘sponge’ by competing for the \textit{miR-193b} binding site. Therefore, these results demonstrate that \textit{MIR31HG} functions as an oncogenic lncRNA by promoting tumor progression and affects \textit{KRAS} function by competing with \textit{miR-193b} in PDAC.
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.\(^\text{29}\) 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 *in 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,\(^\text{63–66}\) 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.\(^\text{67}\) In a recent study, Ma et al\(^\text{68}\) 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 downregulation 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.

**Acknowledgements**

This study was supported by grants from the National Natural Science Foundations of China (81172334, 81472326, 81400664 and 81672648).

**References**

1. Hackeng WM, Hruban RH, Offerhaus GJ, Brosens LA. Surgical and molecular pathology of pancreatic neoplasms. *Diagn Pathol*. 2016;11:47.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin*. 2016;66:7–30.
3. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2015;66:115–132.
4. Yeo CJ, Cameron JL. Prognostic factors in ductal pancreatic cancer. *Langenbecks Arch Surg*. 1998;383:129–133.
5. Benassai G, Mastorrelli M, Quarto G, Cappiello A, Giani U, Mosella G. Survival after pancreaticoduodenectomy for ductal adenocarcinoma of the head of the pancreas. *Chir Ital.* 2000;52:263–270.
6. Ryan DP, Hong TS, Bardeesy N. Pancreatic adenocarcinoma. *N Engl J Med*. 2014;371:1039–1049.
7. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science*. 2008;321:1801–1806.
8. Rishi A, Goggins M, Wood LD, Hruban RH. Pathological and molecular evaluation of pancreatic neoplasms. *Semin Oncol*. 2015;42:28–39.
9. Wilson CY, Tolias P. Recent advances in cancer drug discovery targeting RAS [published online ahead of print August 6, 2016]. *Drug Discov Today*. http://dx.doi.org/10.1016/j.drudis.2016.08.002.
10. Wood LD, Hruban RH. Pathology and molecular genetics of pancreatic neoplasms. *Cancer J.* 2012;18:492–501.

11. Almoguera C, Shibata D, Forrester K, Martin J, Arnhem N, Peruchó M. Most human carcinomas of the exocrine pancreas contain mutant ε-K-ras genes. *Cell.* 1988;53:549–554.

12. Hong SM, Vincent A, Kanda M, et al. Genome-wide somatic copy number alterations in low-grade PanINs and IPMNs from individuals with a family history of pancreatic cancer. *Clin Cancer Res.* 2012;18:4303–4312.

13. Vogelstein B, Kinzler KW. Cancer genes and the pathways they encode. *Cancer J.* 2010;16:789–799.

14. Taucher V, Mangge H, Haybaeck J. Non-coding RNAs in pancreatic neuroendocrine tumors. *Clin Oncol (Lond).* 2011;23:242.

15. Velioglu I, Sutaria DS, Jiang J, et al. miR-216 and miR-217 expression is reduced in transgenic mouse models of pancreatic adenocarcinoma, knockout of miR-216/miR-217 host gene is embryonic lethal [published online ahead of print August 19, 2016]. *Pflugers Arch.* doi:10.1007/s00424-016-0512-1.

16. Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncoproteins in pancreatic cancer: challenges and opportunities for clinical application. *Cell Oncol (Dordr).* 2016;39:295–318.

17. Iorio MV, Croce CM. MicroRNAs in cancer: small molecules with a huge impact. *J Clin Oncol.* 2009;27:5848–5856.

18. Bentwich I, Avniel A, Karov Y, et al. Identification of hundreds of conserved and nonconserved human microRNAs. *Nat Genet.* 2005;37:766–770.

19. Carthew RW. Gene regulation by microRNAs. *Curr Opin Genet Dev.* 2006;16:203–208.

20. Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell.* 1993;75:843–854.

21. Muers M. RNA: genome-wide views of long non-coding RNAs. *Nat Rev Genet.* 2011;12:742.

22. Chen X, Yan YG. Novel human lncRNA-disease association inference based on lncRNA expression profiles. *Bioinformatics.* 2013;29:2617–2624.

23. Harries LW. Long non-coding RNAs and human disease. *Biochem Soc Trans.* 2012;40:902–906.

24. Tahira AC, Kiburusy MS, Faria MF, et al. Long noncoding intronic RNAs are differentially expressed in primary and metastatic pancreatic cancer. *Mol Cancer.* 2011;10:141.

25. Kim K, Jutooru I, Chadalapaka G, et al. HOTAIR is a negative prognostic factor and exhibits pro-oncogenic activity in pancreatic cancer. *Oncogene.* 2013;32:1616–1625.

26. Bhardwaj A, Arora S, Prajapati VK, Singh S, Singh AP. Cancer “stemness”-regulating microRNAs: role, mechanisms and therapeutic potential. *Curr Drug Targets.* 2013;14:1175–1184.

27. Yu S, Lu Z, Liu C, et al. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. *Cancer Res.* 2010;70:6015–6025.

28. Jiao LR, Frampton AE, Jacob J, et al. MicroRNAs targeting oncogenes are down-regulated in pancreatic malignant transformation from benign tumors. *PLoS One.* 2012;7:e32068.

29. Zhao WG, Yu SN, Lu ZH, Ma YH, Gu YM, Chen J. The miR-217 microRNA functions as a potential tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS. *Carcinogenesis.* 2010;31:1726–1733.

30. Jin X, Sun Y, Yang H, et al. Deregulation of the miR-193b-KRAS axis contributes to impaired cell growth in pancreatic cancer. *PLoS One.* 2015;10:e0125515.

31. Ali S, Ahmad A, Aboukameel A, et al. Increased Ras GTPase activity is regulated by miRNAs that can be attenuated by CDF treatment in pancreatic cancer cells. *Cancer Lett.* 2012;319:173–181.

32. Keklikoglou I, Hosaka K, Bender C, et al. MicroRNA-206 functions as a pleiotropic modulator of cell proliferation, invasion and lymphangiogenesis in pancreatic adenocarcinoma by targeting ANXA2 and KRAS genes. *Oncogene.* 2015;34:4867–4878.

33. Azavedo-Pouly AC, Sutaria DS, Jiang J, et al. miR-216 and miR-217 expression is reduced in transgenic mouse models of pancreatic adenocarcinoma, knockout of miR-216/miR-217 host gene is embryonic lethal [published online ahead of print August 19, 2016]. *Pmcnt Int Genomics.* doi:10.1007/s10459-016-9417-2.

34. Kent OA, Chivukula RR, Mullendore M, et al. Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. *Genes Dev.* 2010;24:2754–2759.

35. Johnson SM, Grosshans H, Shingara J, et al. RAS is regulated by the let-7 microRNA family. *Cell.* 2005;120:635–647.

36. Torrisani J, Bournet B, du Rieu MC, et al. miR-7 MicroRNA transfer in pancreatic cancer-derived cells inhibits in vitro cell proliferation but fails to alter tumor progression. *Hum Gene Ther.* 2009;20:831–844.

37. Appari M, Babu KR, Kaczorowski A, Gross W, Herr I. Sulforaphane, quercetin and catechins complement each other in elimination of advanced pancreatic cancer by miR-let-7 induction and K-ras inhibition. *Int J Oncol.* 2014;45:1391–1400.

38. Sharma SB, Ruppert JM. MicroRNA-based therapeutic strategies for targeting mutant and wild type RAS in cancer. *Drug Dev Res.* 2015;76:328–342.

39. Levy S, Sutton G, Ng PC, et al. The diploid genome sequence of an individual human. *PLoS Biol.* 2005;3:e254.

40. Chin L, Ratner E, Leng S, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. *Cancer Res.* 2008;68:8535–8540.

41. Bundu ST, Nallur S, Paranjape T, Boeke M, Weidhaus JB, Slack FI. KRAS alleles: the LCS6 3'UTR is associated with HER2-overexpressed and poorly-differentiated breast cancer in hormone replacement therapy users: a case control study. *BMC Cancer.* 2012;12:105.

42. Christensen BC, Moyer BJ, Avissar M, et al. A let-7 microRNA-binding site polymorphism in the KRAS 3' untranslated region increases non-small cell lung cancer risk. *Carcinogenesis.* 2009;30:1003–1007.

43. Zhang W, Winder T, Ning Y, et al. A let-7 microRNA-binding site polymorphism in 3'-untranslated region of KRAS gene predicts response in wild-type KRAS patients with metastatic colorectal cancer treated with cetuximab monotherapy. *Ann Oncol.* 2011;22:104–109.

44. Mertens-Talcott SU, Chintharlapalli S, Li X, Safe S. The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. *Cancer Res.* 2007;67:11001–11011.

45. Guttilka IK, White BA. Coordinate regulation of FOXY1 by miR-217a, miR-96, and miR-182 in breast cancer cells. *J Biol Chem.* 2009;284:23204–23216.

46. Liu T, Tang H, Lang Y, Liu M, Li X. MicroRNA-27a functions as an oncogene in gastric adenocarcinoma by targeting prohibitin. *Cancer Lett.* 2009;273:233–242.

47. Chintharlapalli S, Papineni S, Abdelrahim M, et al. Oncogenic microRNA-27a is a target for anticancer agent methyl 2-cyano-3,11-dioxo-18beta-olean-1,12-dien-30-oate in colon cancer cells. *Int J Cancer.* 2009;125:1965–1974.
49. Ma Y, Yu S, Zhao W, Lu Z, Chen J. miR-27a regulates the growth, colony formation and migration of pancreatic cancer cells by targeting Sprouty2. *Cancer Lett.* 2010;298:150–158.

50. Shaw AT, Meissner A, Dowdle JA, et al. Sprouty-2 regulates oncogenic K-ras in lung development and tumorigenesis. *Genes Dev.* 2007;21:694–707.

51. Tsavachidou D, Coleman ML, Athanasiadis G, et al. SPRY2 is an inhibitor of the ras/extracellular signal-regulated kinase pathway in melanocytes and melanoma cells with wild-type BRAF but not with the V599E mutant. *Cancer Res.* 2004;64:5556–5559.

52. Kwak HJ, Kim YJ, Chun KR, et al. Downregulation of Spry2 by miR-21 triggers malignancy in human gliomas. *Oncogene.* 2011;30:2433–2442.

53. Mei Y, Bian C, Li J, et al. miR-21 modulates the ERK-MAPK signaling pathway by regulating SPRY2 expression during human mesenchymal stem cell differentiation. *J Cell Biochem.* 2013;114:1374–1384.

54. Choudhury SN, Li Y. miR-21 and let-7 in the Ras and NF-κB pathways. *Microrna.* 2012;1:65–69.

55. du Rieu MC, Torrisani J, Selves J, et al. MicroRNA-21 is induced early in pancreatic ductal adenocarcinoma precursor lesions. *Clin Chem.* 2010;56:603–612.

56. Moriyama T, Ohuchida K, Mizumoto K, et al. MicroRNA-21 modulates biological functions of pancreatic cancer cells including their proliferation, invasion, and chemoresistance. *Mol Cancer Ther.* 2009;8:1067–1074.

57. Wei X, Wang W, Wang L, et al. MicroRNA-21 induces 5-fluorouracil resistance in human pancreatic cancer cells by regulating PTEN and PDCD4. *Cancer Med.* 2016;5:693–702.

58. Song W, Li Q, Wang L, Wang L. Modulation of FoxO1 expression by miR-21 to promote growth of pancreatic ductal adenocarcinoma. *Cell Physiol Biochem.* 2015;35:184–190.

59. Funel N. The role of miR-21 and miR-211 on MMP9 regulation in pancreatic ductal adenocarcinoma: cooperation in invasiveness behaviors? *Epigenomics.* 2015;7:333–335.

60. Frezzetti D, De Menna M, Zoppoli P, et al. Upregulation of miR-21 by Ras in vivo and its role in tumor growth. *Oncogene.* 2011;30:275–286.

61. Ali S, Suresh R, Banerjee S, et al. Contribution of microRNAs in understanding the pancreatic tumor microenvironment involving cancer associated stellate and fibroblast cells. *Am J Cancer Res.* 2015;5:1251–1264.

62. Yang H, Liu P, Zhang J, et al. Long noncoding RNA MIR31HG exhibits oncogenic property in pancreatic ductal adenocarcinoma and is negatively regulated by miR-193b. *Oncogene.* 2016;35:3647–3657.

63. Jiao F, Hu H, Han T, et al. Long noncoding RNA MALAT-1 enhances stem cell-like phenotypes in pancreatic cancer cells. *Int J Mol Sci.* 2015;16:6677–6693.

64. Zhu L, Liu J, Ma S, Zhang S. Long noncoding RNA MALAT-1 can predict metastasis and a poor prognosis: a meta-analysis. *Pathol Oncol Res.* 2015;21:1259–1264.

65. Li L, Chen H, Gao Y, et al. Long noncoding RNA MALAT1 promotes aggressive pancreatic cancer proliferation and metastasis via the stimulation of autophagy. *Mol Cancer Ther.* 2016;15:2232–2243.

66. Wang Y, Xue D, Li Y, et al. The long noncoding RNA MALAT-1 is a novel biomarker in various cancers: a meta-analysis based on the GEO database and literature. *J Cancer.* 2016;7:991–1001.

67. Doucrasy S, Coll J, Barrois M, et al. Expression of the human fetal bac h19 gene in invasive cancers. *Int J Oncol.* 1993;2:753–758.

68. Ma C, Nong K, Zhu H, et al. H19 promotes pancreatic cancer metastasis by derepressing let-7s suppression on its target HMGA2-mediated EMT. *Tumour Biol.* 2014;35:9163–9169.

Edited by Pei-Fang Wei