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Roles and Mechanisms of MicroRNAs in Pancreatic Cancer

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Abstract

Pancreatic cancer (PC) is an aggressive malignancy with poor survival. The discovery of microRNAs (miRNAs) has provided a new opportunity to study the disease. Thus far, altered expression of miRNAs has been reported in a wide range of malignancies, including PC, and some miRNAs are associated with PC cell proliferation, invasion, chemoresistance, and patient survival. This review summarizes recent advances with respect to the roles and mechanisms of miRNAs in PC and discusses potential clinical applications.

Introduction

Pancreatic cancer (PC) is the fourth leading cause of cancer-related death in the United States. It is characterized by late clinical presentation, early and aggressive local and systemic invasion, and poor prognosis; the five-year survival rate is less than 5%, regardless of treatment [1, 2]. Therefore, there is an urgent need to understand the molecular mechanisms of tumorigenesis and the progression of PC, and to develop new diagnostic and therapeutic strategies to improve the outcome for patients suffering from this malignant disease.

The recent discovery of microRNAs (miRNAs) has revealed new mechanisms for the regulation of gene expression and provided new directions for cancer research. MicroRNAs are a class of small noncoding RNAs that target multiple messenger RNAs (mRNAs), triggering translational repression and/or mRNA degradation [3, 4]. Extensive studies have indicated highly diverse roles of miRNAs in cancer development, invasion, diagnosis, prognosis, and treatment [5–7].

MicroRNAs are predicted to regulate the activity or gene expression of approximately 30% of all the protein-coding genes in mammals. So far, more than 700 human miRNAs have been identified [5, 6].

During their biogenesis, miRNAs initially are transcribed from genomic DNA into large primary miRNAs (pri-miRNAs), which are up to several thousand nucleotides in length. Primary miRNAs are then cleaved by Drosha RNase III endonuclease in the nucleus into a hairpin loop structure of up to 70 nucleotides in length that is known as precursor miRNA (pre-miRNA). Pre-miRNAs are subsequently transported into the cytoplasm by a nuclear membrane transporter Exportin 5, where they are further processed by another RNase III Dicer into mature miRNAs measuring 21–25 nucleotides in length. Single-stranded mature miRNAs are incorporated into the RNA-induced silencing complex (RISC); they then guide

the RISC to find specific target mRNAs by complementary base pairing interactions with the 3' untranslated regions (3'UTRs) of specific target mRNAs. The RISC eventually causes translational downregulation and/or degradation of target mRNAs, thus inhibiting gene expression [5, 6, 8].

Aberrant expression of miRNAs has been found in many human diseases, including cancer [5, 6]. MicroRNAs also exhibit tissue-specific and disease-specific expression, and these unique miRNA expression profiling signatures can classify different types of cancer and distinguish cancer from benign tissues; this may provide the basis for developing new diagnostic and therapeutic strategies [5, 6]. In this review, we focus on the recent advances of miRNA research in PC. Specific roles and molecular mechanisms of miRNAs in tumorigenesis and tumor progression in PC are discussed, as are potential clinical applications.

Aberrant miRNA expression patterns in PC

A variety of patterns of dysregulation of miRNA expression has been observed in PC specimens with respect to normal tissue. An early comprehensive study of miRNA precursor expression profiling in PC was performed by a real-time PCR technique by Lee et al. in 2006 [9]. In their study, 100 miRNAs of 201 miRNA precursors examined were found to be dysregulated in PC. Most of the top aberrantly expressed miRNA precursors also were demonstrated to correlate with the expression of their active mature miRNAs. The most highly upregulated miRNAs in PC were miR-221, miR-424, miR-301, miR-100, miR-376a, miR-125b-1, miR-21, miR-16-1, miR-181a,c, miR-92-1, miR-15b, miR-155, let-7f-1, miR-212, miR-107, miR-24-1,2, and let-7d, and the most downregulated miRNAs were miR-345, miR-142-P, and miR-139. In another investigation [10], Szafranska et al. analyzed the expression of 377 miRNA using miRNA array technology in tissue samples from normal pancreas, chronic pancreatitis, and pancreatic ductal adenocarcinoma (PDAC). One important finding in that study was that the expression of some miRNAs, such as miR-29c, miR-96, miR-143, miR-148b, and miR-150, was dysregulated in both the chronic pancreatitis and the PDAC samples, whereas miR-196a, miR-196b, miR-203, miR-210, miR-222, miR-216, miR-217, and miR-375 were aberrantly expressed only in the PDAC samples. The authors concluded that the aberrant expression of only two miRNAs, miR-217 and miR-196a, could distinguish PDAC from normal pancreas and pancreatitis. In a similar study [11], Bloomston et al. demonstrated that upregulation of miRNAs—including miR-155, miR-181a,b,c,d, miR-21, and miR-221—and downregulation of miRNAs—including miR-148a,b and miR-375—differentiated PC from normal pancreas and pancreatitis tissue samples. Using real-time polymerase chain reaction (PCR) technology, our group has examined the expression profiling of 95 cancer-related miRNAs in PC. We found that eight miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b, and miR-95) were significantly upregulated in most PC tissues (70%) and pancreatic cell lines (100%) [12]. These data provide important information for additional study of the functional roles of these miRNAs in PC.

In addition to exocrine PC such as PDAC, pancreatic endocrine tumors are an important area of investigation with respect to the role of miRNA. In a comparison to normal pancreatic tissues, insulinomas and nonfunctioning endocrine tumors have been shown to have higher expression of miR-103 and miR-107 and lower expression of miR-155. Interestingly, the same study demonstrated that pancreatic acinar cell carcinoma, a rare exocrine PC, showed a similar miRNA downregulation [13]. Because miR-155 has been reported to be upregulated in a variety of malignancies, including PDAC, it is important that these results be confirmed [9]. Table 1 summarizes several important miRNAs that are aberrantly expressed in PC and their validated target genes [9–12, 14–42].

Metastasis-related miRNAs

MicroRNAs have been implicated not only in tumorigenesis but also in the development of metastatic potential. MicroRNAs appear to be involved at several points along the tumor's pathway to acquisition of migratory and invasive properties.

Several miRNAs appear to promote metastasis. For example, EP300 is a histone acetyltransferase that regulates transcription through chromatin remodeling. It plays an essential role in modulating cell growth and division, prompting cells to mature and differentiate and preventing the growth of cancerous tumors. Mees et al. [43] found that expression of a group of miRNAs targeting EP300, including miR-194, miR-200b, miR-200c, and miR-429, were significantly increased in the highly metastatic PDAC cell lines when compared to low or nonmetastatic PDAC cell lines; decreased expression of EP300 mRNA also was observed in the highly metastatic cells. EP300 is recognized widely as a metastasis suppressor, so downregulation of EP300 and upregulation of its targeting molecules may play a key role in the metastasis of PC.

miR-224 and miR-486 also appear to promote PC metastasis; a recent investigation revealed that several miRNAs targeting CD40, including miR-224 and miR-486, showed significantly higher expression in highly invasive PDAC than in lowly invasive PDAC. Meanwhile, CD40 is a member of the tumor necrosis factor receptor family that is important in anti-tumor immune responses. [44]. Thus, the upregulation of these miRNAs targeting CD40 may represent an important factor in PC metastasis. miR-27a is abnormally upregulated in several malignancies, including PC[45–48]. Functional inhibition of miR-27a has been shown to suppress growth, colony formation, and migration of PC cell lines Panc-1 and Mia PaCa-2. One validated target gene of miR-27a is Spry2, which is a member of the Sprouty family. Functionally, overexpression of Spry2 inhibits tumor growth and metastases through Ras/MAPK pathway inactivation [30].

Meanwhile, several miRNAs have been found to suppress metastasis. miR-146a has been reported to be lost in metastatic prostate cancer and breast cancer[49–51]. It also has been shown that ectopic expression of miR-146a reduced the metastatic potential of breast cancer [49, 50]. With respect to cancer of the pancreas, expression levels of miR-146a were decreased in PC cell lines Colo357 and Panc-1, as compared to a normal pancreatic duct epithelial cell line (HPDE). Meanwhile, ectopic expression of miR-146a inhibited the invasive capacity of these PC cells, with downregulation of epidermal growth factor receptor (EGFR), NF- κ B regulatory kinase, and interleukin 1 receptor-associated kinase 1 (IRAK-1) [21].

miR-96 is considered to be a potential tumor suppressor because it directly targets and downregulates the KRAS oncogene. In PC, miR-96 is downregulated significantly when compared with normal pancreatic tissues [10]. Ectopic expression of miR-96 has been seen to induce apoptosis and inhibit cell proliferation, migration, and invasion in PC cell lines Panc-1 and Mia PaCa-2; this is mediated by the inhibition of KRAS and Akt signaling. In human clinical specimens, an inverse correlation was observed between miR-96 and KRAS expression. Thus, miR-96 may have potential therapeutic use in KRAS-driven PC [34].

Although there is no clear evidence showing miR-520 h deregulation in PC, ectopic expression of miR-520 h has been observed to display an inhibitory effect on PC cell migration and invasion. miR-520 h-mediated, anti-metastatic effects may be mediated through direct target inhibition of ATP binding cassette subfamily G member 2 (ABCG2) gene expression, which also is known as breast cancer resistance protein [52]. Meanwhile, miR-20a, a member of the miR-17-92 family, also appears to have metastasis-suppressing effects. miR-20a also is known to negatively regulate Stat3 protein expression and inhibit

proliferation and invasion of PC cells in vivo and in vitro, and a recent study showed that miR-20a expression is reduced in PC tissues and cell lines. As such, engineering expression of miR-20a may potentially be useful in the treatment of human PC [32].

The miR-200 family genes are potential tumor suppressor miRNAs, and their role in metastasis is still under investigation. miR-200 family miRNAs target and reduce expression of two E-box-binding transcription factors, ZEB1 and ZEB2. ZEB1/2 are negative regulators of E-cadherin, and their downregulation is responsible for epithelial-mesenchymal transition (EMT). During EMT, cells lose their capacity for adhesion to other cells or interstitial matrix and become more motile, thus increasing their metastatic capacity [25, 39, 53]. Low expression of miR-200 family genes and high expression of their targets ZEB1 and ZEB2 are common in several malignancies, including breast cancer and ovary cancer [54, 55]. However, a recent investigation has shown that most PC cells studied harbor hypomethylation and overexpression of miR-200a and miR-200b. Moreover, the same study also demonstrated that for most PCs miR-200 expression is not required for suppression of ZEB2 expression, as ZEB2 expression is silenced by the hypermethylation inactivation mechanism [56]; this indicates that the miR-200-ZEB2 pathway is not involved in metastases in most PCs. Nevertheless, further investigation is needed in order to confirm this observation as a general rule for PC.

Chemosensitivity and radiosensitivity

MicroRNAs have been shown to induce changes in the chemosensitivity or radiosensitivity of PC cells in a variety of settings. miR-21, for one, appears to convey chemoresistance to PC cells. miR-21 previously has been shown to be upregulated significantly in PC, and strong expression of miR-21 is associated with poor survival of this cancer [15], indicating its oncogenic properties. Forced overexpression of miR-21 with transfection of the miR-21 precursor increases proliferation and invasion of PC cell lines, and this appears to occur through target inhibition of the phosphatase and tensin homolog (PTEN), programmed cell death 4 (PDCD4), presence of tropomyosin 1 (TPM1), and tissue inhibition of metalloproteinases 3 (TIMP3), thereby indirectly inducing expression of matrix metalloproteinase-2, 9, and vascular endothelial growth factor (VEGF).

Meanwhile, miR-21 appears also to induce chemoresistance to gemcitabine in PC cell lines. For example, when PC cell lines Panc-1, LpC111, and LpC006 were transfected with the miR-21 precursor, those cells were resistant to gemcitabine treatment, showing reduced apoptosis or increased proliferation [31, 57]. In contrast, the inhibition of miR-21 function induced more apoptosis and decreased proliferation in PC cell line SUIT-2, which expresses relatively high levels of miR-21 [31]. In another study, Hwang et al. [20] demonstrated that miR-21 might be a useful biomarker for chemoresistance prediction, as their research data showed that the PC cells with lower miR-21 expression had higher chemosensitivity to 5-fluorouracil (5-FU).

As discussed above, miR-200 is a potential tumor suppressor that plays important roles in cancer metastases [58, 59]. Like miR-21, miR-200 may be involved in chemoresistance, or in this case, chemosensitivity. It has been reported that decreased expression of miR-200 and increased expression of miR-21 are associated with gemcitabine resistance in PC cells. Interestingly, treatment with curcumin, a major chemical component in turmeric, a spice commonly used in Indian cooking, resulted in upregulation of miR-200 expression and downregulation of miR-21 expression [60].

miR-34 also appears to sensitize PC cells to chemotherapy and radiotherapy. It has been reported that miR-34 expression is significantly lower in PC cell lines than in normal pancreatic ductal epithelial cell lines [61]. miR-34 demonstrates tumor suppressive qualities.

Its expression normally is regulated by the tumor suppressor gene p53 [62], but it also can be inactivated by aberrant CpG methylation in cancer [63]. Evidence has been found that restoration of miR-34 expression induces a G1 cell cycle arrest and apoptosis in some malignancies, including PC. Researchers also tested the inhibitory effects of miR-34 on human PC tumor-initiating cells. To do this, CD44 +/CD133 + Mia PaCa-2 cells were enriched by tumor sphere forming assay. These tumor-initiating cells showed high expression levels of Notch and Bcl-2 with loss of miR-34 expression. Impressively, miR-34 restoration led to 87% reduction of the tumor-initiating cell population, and significant tumor sphere growth inhibition occurred in vitro as tumor formation inhibition occurred in vivo [64].

Clinical survival and miRNAs

Poor survival is a characteristic feature of PC. Several miRNAs have been suggested to be predictors of survival. As mentioned above, miR-21 appears to confer chemoresistance in PC cell lines; a high level of miR-21 also predicted limited survival in lymph node-negative PC patients [15]. miR-196a-2 also may be a negative survival predictor; median survival for pancreatic cancer patients has been shown to be 14.3 versus 26.5 months, depending on whether miR-196a-2 expression is high or low, respectively [38]. High expression of miR-196a-2 also resulted in shorter 2-year survival of 17%, as compared to 64% in patients with low miR-196a-2 expression [11]. In addition, increased expression of miR-155, miR-203, miR-210, and miR-222 has been reported to be associated with poor survival in PC patients [38].

miR-200c is a member of the miR-200 family, and its expression in PC shows a wide range. Some PC cell lines, such as CAPAN-1, SW1990, CFPAC-1, and H48 N, express higher levels of miR-200c than HPDE; meanwhile, ASPC-1 and CAPAN-2 express similar levels of miR-200c to HPDE, and BxPC-3, NOR-P1, KP-1 N, KP-2, KP-3, Hs766T, SUIT-2, PANC-1, and Mia PaCa-2 express lower levels of miR-200c than HPDE [65]. Yu et al. [65] demonstrated that PC patients with high levels of miR-200c had a better survival rate than those with low levels of miR-200c, suggesting that miR-200c may be a valuable prognostic marker for PC. Specifically, the median survival times and five-year survival rates were 42 months and 33.5% in the high miR-200c expression group, and 19 months and 11.2% in the low miR-200c expression group.

Biomarkers for PC diagnosis

Poor survival of PC, especially PDAC, is attributed to the advanced stage of the cancer at diagnosis. Therefore, the development of minimally invasive biomarker assays for early detection and effective clinical management is needed urgently. In an analysis of potential miRNA bio-markers for early detection of pancreatic neoplasia, Habbe et al. [17] found miR-155 and miR-21 are overexpressed significantly in tissue from intraductal papillary mucinous neoplasms (IPMNs), noninvasive precursor lesions of PC. Because both miR-155 and miR-21 have been reported to be overexpressed in invasive PC, these two markers may not be useful for early detection of PC.

Meanwhile, it has been reported that miR-196a combined with miR-217 can be used to classify benign versus malignant pancreatic tissues in pancreatic fine-needle aspirates [66]. Although this approach is unlikely to be used routinely for early detection of or screening for PC, this assay may be useful in screening high-risk individuals or monitoring the response to treatment. A recent investigation showed that the expression levels of four miRNAs in plasma, miR-21, miR-210, miR-155, and miR-196a, was significantly higher in patients with pancreatic adenocarcinoma than in a healthy control group [67]. This work

again demonstrated the feasibility of using miRNAs as biomarkers for early and minimally invasive detection of pancreatic adenocarcinoma.

The detection of biomarkers in the sera of patients with PC offers the possibility of earlier diagnosis. miR-210 is a marker of hypoxia whose expression can be induced by conditions of low oxygen. miR-210 also has been detected in the serum of PC patients, and its expression levels were fourfold higher in these cancer patients than in normal controls [68]. Meanwhile, miR-200a and miR-200b are not only overexpressed in most PC tissues but also have been detected in sera in patients with PC. Li et al. [56] found that both miR-200a and miR-200b were elevated significantly in the sera of patients with PC compared with healthy controls. However, the authors also found that the serum levels of miR-200a and miR-200b were not significantly different from those observed in patients with pancreatitis, indicating that circulating miR-200a and miR-200b are not biomarkers specific for PC.

Finally, epigenetic features of miRNAs may provide valuable information leading to the development of new biomarkers. Hypermethylation is one molecular mechanism that can inactivate gene expression. Hanoun et al. [69] found that hypermethylation of the DNA region encoding miR-148a led to the inhibition of its gene expression in preneoplastic pancreatic intraepithelial neoplasia (PanIN). Importantly, this phenomenon of hypermethylation can differentiate PDAC from chronic pancreatitis; this is significant because some miRNA biomarkers, such as miR-200a and miR-200b, cannot discriminate between PC and pancreatitis, as described above [56]. Exploring hypermethylation of many other miRNAs whose levels are low in PC may yield meaningful biomarkers.

Therapeutic implication

Our growing knowledge of the roles of miRNAs in PC development and progression suggests new directions for therapeutic intervention. Specifically, inactivation of upregulated miRNA oncogenes or reintroduction of lost miRNA tumor suppressors in PC cells could be achieved. Many miRNAs have been tested for their tumor-suppression function in PC cell lines. For example, as already mentioned, miR-96 directly targets the KRAS oncogene, and ectopic expression of miR-96 can reduce pancreatic cell proliferation, migration, and invasion, indicating its potential therapeutic role in PC [34]. Other miRNAs with tumor suppressor function, including miR-20a, miR-34, and miR-200, could be used as novel therapeutic agents for PC [32, 59, 64]. Meanwhile, oncogenic miRNAs, such as miR-21 and miR-27 [30, 31] could be effective therapeutic targets with the use of antisense technology and other specific inhibitors. Finally, it has been shown that inhibition of oncogenic miRNAs such as miR-21 or re-expression of tumor suppressive miRNAs such as miR-34 can sensitize PC cells to chemotherapy and radiotherapy [20, 31, 64].

Conclusions and perspectives

Pancreatic cancer is a devastating disease with very poor prognosis. Discovery of miRNAs provides a new opportunity to study the tumorigenesis and metastatic mechanisms of PC. Although some miRNAs have been found to be associated with proliferation, invasion, and poor survival of PC, the precise mechanisms controlling the above processes remains unclear. Key questions to guide further investigation include how these miRNA become dysregulated and what their targets are. To elucidate diagnostic strategies, all identified miRNA biomarkers must be tested in more clinical serum samples, including those from patients with PC, those with pancreatitis, and normal controls. With respect to the therapeutic applications of miRNAs, it is important to develop systemic delivery systems for miRNAs in order to treat metastatic PC.

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Table 1

Important miRNAs that are aberrantly expressed in pancreatic cancer

miRNA	Expression	Function	Target genes	Reference
miR-21	Upregulation	Increases proliferation, invasion, and chemoresistance	PTEN, PDCD4, TPM1, TIMP3	15, 20, 31, 36
miR-155	Upregulation	Increases proliferation and tumorigenesis	TP53INP1	9, 17, 38, 40
miR-196a	Upregulation	Poor survival marker	HOXB8, ANXA1, HMGA2	10, 11, 14, 23, 33
miR-27a	Upregulation	Increases growth, colony formation, and migration	Spry2	30
miR-210	Upregulation	Hypoxia-inducible miRNA	HOXA1, FGFR1, HOXA9	10, 19
miR-200b	Upregulation	Tumor suppressor	ZEB1, ZEB2	12, 25, 53
miR-221/222	Upregulation	Increased proliferation	CDKN1B (p27), PUMA, PTEN	9, 10, 11, 12, 29, 35, 42
miR-15b	Upregulation	Cell cycle progression	CCNE1	9, 12, 28
miR-181	Upregulation	Tumor suppressor	TIMP3, TCL1	9, 11, 26, 41
miR-100	Upregulation	Tumor suppressor miRNA	Plk1, FRAP1/mTOR	9, 24, 37
miR-148a,b	Downregulation	Not known	DNMT3b, Mtf	11, 16, 18
miR-146a	Downregulation	Reduces invasion	TRAF6, IRAK1, Stat1	21, 22, 49, 51
miR-20a	Downregulation	Reduces proliferation and invasion	Stat3	32
miR-96	Downregulation	Induces apoptosis and reduces proliferation and invasion	KRAS	10, 34
miR-375	Downregulation	Tumor suppressor miRNA	PDK1, 14-3-3zeta	10, 11, 27

PTEN phosphatase and tensin homolog, *PDCD4* programmed cell death 4, *TPM1* tropomyosin 1, *TIMP3* tissue inhibitor of metalloproteinases 3, *TP53INP1* tumor protein 53-induced nuclear protein 1, *HOXB8* Homeobox B8, *ANXA1* annexin A1, *HMGA2* high-mobility group AT-hook 2, *Spry2* Sprouty homolog 2, *HOXA1* Homeobox A1, *FGFR1* fibroblast growth factor receptor-like 1, *HOXA9* Homeobox A9, *ZEB1* zinc finger E-box-binding homeobox 1, *ZEB2* zinc finger E-box-binding Homeobox 2, *CDKN1B (p27)* cyclin-dependent kinase inhibitor 1B, *PUMA* p53 upregulated modulator of apoptosis, *CCNE1* cyclin E1, *TCL1* T cell leukemia/lymphoma 1, *Plk1* polo like kinase 1, *FRAP1/mTOR* FK506 binding protein 12-rapamycin associated protein 1/mammalian target of rapamycin, *DNMT3b* DNA methyltransferase 3b, *Mtf* microphthalmia associated transcription factor, *TRAF6* TNF receptor-associated factor 6, *IRAK1* interleukin-1 receptor-associated kinase 1, *Stat1* signal transducer and activator of transcription 1, *Stat3* signal transducer and activator of transcription 3, *PDK1* 3-phosphoinositide dependent protein kinase-1