

WJG 20th Anniversary Special Issues (14): Pancreatic cancer**MicroRNAs as emerging biomarkers and therapeutic targets for pancreatic cancer**

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Abstract

Despite tremendous efforts from scientists and clinicians worldwide, pancreatic adenocarcinoma (PDAC) remains a deadly disease due to the lack of early diagnostic tools and reliable therapeutic approaches. Consequently, a majority of patients (80%) display an advanced disease that results in a low resection rate leading to an overall median survival of less than 6 months. Accordingly, robust markers for the early diagnosis and prognosis of pancreatic cancer, or markers indicative of survival and/or metastatic disease are des-

perately needed to help alleviate the dismal prognosis of this cancer. In addition, the discovery of new therapeutic targets is mandatory to design effective treatments. In this review, we will highlight the translational studies demonstrating that microRNAs may soon translate into clinical applications as long-awaited screening tools and therapeutic targets for PDAC.

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Key words: MicroRNAs; Biomarkers; Pancreatic cancer; Therapeutic targets; Precancerous lesions

Core tip: Robust biomarkers and reliable treatments are needed to help alleviate the dismal prognosis of pancreatic cancer. In this review, we will highlight the translational studies demonstrating that microRNAs may soon translate into clinical applications as long-awaited screening tools and therapeutic targets for this cancer.

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PANCREATIC CANCER

There are currently no means for the reliable diagnosis of early stages of pancreatic cancer (PDAC) and the curative treatment of late stages. Consequently, the vast majority

of patients (80%) display an advanced disease that results in a low resection rate leading to a dismal overall median survival of less than 6 mo^[1]. The estimated 5-year survival rate is lower than 2%. While PDAC is not among the most common tumors, it is one of the most frequent causes of cancer-related death with approximately 40000 death/year in the United States and in Europe. Thus, there is an urgent need to discover diagnostic as well as prognostic molecular markers together with reliable therapeutics to improve pancreatic cancer management.

PDAC is a highly heterogeneous disease^[2] defined by numerous alterations in multiple signaling pathways^[3]. Additionally, specific cellular clones for primary tumors and metastasis have been identified^[4]. Interestingly, the type and number of genomic rearrangements in DNA vary considerably between patients, and occur early during tumor development^[5]. On the other end, pioneering studies using genome-wide profiling showed that microRNAs (miRNA) expression can discriminate cancers with high efficacy^[6]. In this review, we will focus on the use of miRNAs as promising biomarkers and therapeutic targets for pancreatic cancer (Tables 1-3).

GENERAL CONCEPT OF MIRNAS AND CANCER

miRNAs are small RNA molecules that functions as translation inhibitors of messenger RNA by their binding to 3'-untranslated region^[7-9]. These molecules are tightly involved in the regulation of many physiological processes such as development, proliferation, invasion, and apoptosis among others. Interestingly, their expression is profoundly altered in cancer and/or is strongly modulated during carcinogenesis. Thus, the activation of tumor-suppressive miRNAs and the inhibition of oncogenic miRNAs by small molecules or gene transfer may have the potential to provide a fundamentally new approach for the development of cancer therapeutics. Probably the most important advantage in comparison with current approaches targeting single genes is the ability to modulate many different pathways "at once" taking into account that one miRNA can regulate hundreds of genes, frequently in the context of a cell-specific network.

MIRNAS AS DIAGNOSTIC MARKERS FOR PANCREATIC CANCER

To date, many strategies based on high-throughput screening are used to discover relevant clinical biomarkers. For PDAC in particular and pancreatic tissue in general, these protocols are often hindered by the intrinsic high levels of many nucleases. Consequently, the high stability of miRNAs in tissues and fluids is a key advantage over protein and mRNA. In addition, miRNAs can be quantified in very low amounts of material and in highly degraded samples, such as small biopsies and fine needle aspirates. This is mandatory to support the use of miRNAs as biomarkers for PDAC at the clinical level. In the

next sections, we will update the excellent reviews^[2,10-15] and meta-analysis^[16] from other groups, and reviews and book chapters we recently published^[17-19] on the use of miRNAs as biomarkers in PDAC (Tables 1 and 2).

Historically, Pr Schmittgen's group was the first to report the expression profiles of miRNAs in PDAC. They identified miRNAs specifically over expressed in PDAC (miR-376a, miR-301) or in other tumors (miR-155, miR-21, miR-221 and miR-222)^[20]. Two additional miRNAs (miR-132 and miR-212) were recently reported to be over expressed in PDAC as compared to normal or benign adjacent pancreas to the tumor^[21]. Another study by Pr Shao's group yielded conflicting results as they demonstrated that miR-132 was down regulated in cancer *vs* normal benign normal tissues^[22]. Other miRNAs, such as miR-96^[23], miR-34a^[24] and miR-21^[25], have been reported to be altered in PDAC as compared to normal adjacent tissue. miRNAs expression may also help to discriminate PDAC from chronic pancreatitis. This is of particular importance to prevent from unnecessary and possibly debilitating surgery, or to delay tumor treatment, respectively. Historically, Pr Bloomston's group reported that 21 miRNAs with increased expression and 4 underexpressed miRNAs differentiated PDAC from normal tissue in 90% of samples and from pancreatitis with 93% accuracy^[26]. Twenty additional miRNAs were discovered by Szafranska *et al*^[27] to discriminate between PDAC, chronic pancreatic and normal pancreas. Later, expression of miR-203^[28], miR-148a^[29], miR-196b^[29], miR-196a^[29] and miR-205^[29] were demonstrated to be altered in PDAC *vs* chronic pancreatitis. Alternatively, miRNA expression profiles have been recently used to distinguish PDAC from cholangiocarcinoma, two virtually indistinguishable cancers using conventional histopathological and clinical characteristics^[30].

Endoscopic ultrasound-guided fine needle aspirations (FNA) material allows for the screening of the vast majority (> 85%) of PDAC patients that are not eligible for surgery, and, as consequence, may provide new insights for the diagnosis and prognosis of PDAC. Pr Szafranska's group was the first to demonstrate that the expression of miR-196a and miR-217 in FNA material can classify PDAC from benign lesion^[31]. This pioneering study led to the development of the first molecular test for the identification of PDAC^[32]. Hence, we demonstrated that *let-7* miRNA expression is repressed in PDAC FNAs^[33], and that the measurement of hypermethylation of miR-148a encoding DNA region is potentially useful to differentiate PDAC and pseudo-tumor forms of chronic pancreatitis^[34].

MIRNAS AS PROGNOSTIC AND PREDICTIVE MARKERS FOR PANCREATIC CANCER

MiRNAs are also scrutinized for their ability to predict cancer prognosis and/or response to treatment. Bloomston *et al*^[26] were the first to report that miR-452,

Table 1 MicroRNAs as diagnostic markers for pancreatic cancer

miRNA	Biopsies	FNA	Circulating	Ref.
Let-7a	X (↓)	X (↓)		[33]
miR-34a	X (↓)			[24]
miR-96	X (↓)			[23]
miR-99a			X (↑)	[55]
miR-101	X (↑)			[67]
miR-132	X (↑/↓)			[21,22]
miR-141	X (↓)			[27]
miR-143	X (↑)			[27]
miR-145	X (↑)			[27]
miR-146a	X (↑)			[27]
miR-148a	X (↓)			[27,29]
miR-148b	X (↓)	X (↓)		[27,34]
miR-150	X (↑)			[27]
miR155	X (↑)		X (↑)	[26,27,53,62,67,68]
miR-16			X (↑)	[57]
miR-181a	X (↑)			[26]
miR-181b	X (↑)			[26]
miR-181d	X (↑)			[26]
miR-185			X (↑)	[59]
miR-191			X (↑)	[55,59]
miR-196a	X (↑)	X (↑)	X (↑)	[27,29,32,53,57]
miR-196b	X (↑)			[27,29]
miR-20a			X (↑)	[55,59]
miR-200a			X (↑)	[56]
miR-200b			X (↑)	[56]
miR-203	X (↑)			[28]
miR-210	X (↑)		X (↑)	[29,54]
miR-212	X (↑)			[20]
miR-216	X (↓)			[27]
miR-217	X (↓)	X (↓)		[27,31]
miR-21	X (↑)	X (↑)	X (↑)	[20,26,25,51,53,55,60,64,67-69]
miR-210	X (↑)		X (↑)	[27,53]
miR-221	X (↑)		X (↑)	[20,26,58,69]
miR-222	X (↑)			[20,26,27]
miR-223	X (↑)			[27]
miR-24			X (↑)	[55]
miR-27a-3p			X (↑)	[59]
miR-29c	X (↓)			[27]
miR-30a-3p	X (↓)			[27]
miR-301	X (↑)			[20]
miR-31	X (↑)			[27]
miR-375	X (↓)			[27]
miR-376a	X (↑)			[20]
miR-494	X (↓)			[27]
miR-1290	X (↑)			[61]

miRNAs: MicroRNAs; Biopsies: Resected tumors; FNA: Fine needle aspiration; ↑: Upregulated; ↓: Downregulated.

miR-105, miR-127, miR-518a-2, miR-187, and miR-30a-3p are over-expressed in the tumors of patients with survival greater than 2 years. Moreover, tumors with high expression of miR-196a-2 or miR-219 have a lower median survival compared with those with low expression. In addition, over expression of miR-155^[35], miR-200^[35], miR-203^[35], miR-205^[35], miR-200c^[36], miR-21^[37], miR-212^[38] and miR-675^[38] and reduced expression of miR-34a^[37], miR-30d^[37], miR-148a^[38], miR-187^[38], miR-130b^[39] and *let-7g*^[38] in PDAC are associated with poorer survival rate. Last, low miR-211 expression was demonstrated as an independent factor of poor prognosis in

resected PDAC^[40].

Gemcitabine is broadly used as a first-line chemotherapeutic treatment for patients with unresectable locally advanced or metastatic pancreatic cancer^[41]. However, the 5-year survival rate is only 2%^[42], with 1-year survival rates ranging from 17% to 23%^[41]. Recently, phase II and III trials exploring gemcitabine-based combinations with erlotinib^[43], FOLFIRINOX^[44] or nab-Paclitaxel^[45] were found to improve overall survival of patients. However, the moderate activity of standard gemcitabine and gemcitabine-based regimens still encourages the discovery of robust biomarkers that may help to stratify PDAC patients for tailored therapy. Gemcitabine requires transporter proteins to cross cell membranes. Low expression of human equilibrative nucleoside transporter-1 (hENT1) may result in gemcitabine resistance in PDAC. Recent studies have revealed that high levels of hENT1 in PDAC predict longer survival times in patients treated with adjuvant gemcitabine^[46]. In another study, CO-101, a lipid-drug conjugate of gemcitabine, was designed to enter cells independently of hENT1^[47]. However, CO-101 was found not superior to gemcitabine in patients with metastatic PDAC and low tumor hENT1. In addition, metastasis hENT1 expression doesn't predict gemcitabine outcome. Interestingly, Giovannetti *et al*^[48] found that high miR-21 expression in tumors is associated with shorter overall survival both in the metastatic and in the adjuvant setting, while patients with low miR-21 expression may benefit from gemcitabine treatment^[49]. Gemcitabine resistance is also associated with the cellular over expression of miR-146 and the reduced expression of miR-205 and miR-7^[50]. Last but not least, Pr Korc's group recently demonstrated that miR-10b is a novel and powerful diagnostic biomarker for PDAC^[51]. Like miR-21, miR-10b is over expressed in the FNA material from PDAC patients. Additionally, reduced expression of miR-10b is associated with improved response to multimodality neoadjuvant therapy, likelihood of surgical resection, delayed time to metastasis, and increased survival. Thus, miR-10b is likely to be a novel marker to diagnose PDAC, but may also serve as a biomarker for response to gemcitabine-based neoadjuvant therapy, and be predictive of early metastasis formation. In experimental models, miR-10b was demonstrated to promote PDAC-derived cells proliferation and invasion by suppressing TIP30, which enhances EGFR signaling, facilitates EGF-TGF-β cross-talk together with the expression of epithelial-mesenchymal transition-promoting genes^[52].

CIRCULATING MIRNAS AS BIOMARKERS FOR PANCREATIC CANCER

The recent discovery of miRNAs in serum or plasma opens up the possibility of using non coding RNAs as circulating biomarkers of disease. Wang *et al*^[53] were the first to report the detection of miRNA in the blood of PDAC patients. They demonstrated that plasmatic miR-21, miR-210, miR-196a and miR-155 reveal a sensi-

Table 2 MicroRNAs as prognostic and predictive markers for pancreatic cancer

miRNA	Biopsies	FNA	Prognostic	Predictive of treatment efficacy	Ref.
miR-105, miR-127, miR-187, miR-30a-3p, miR-452, miR-518a-2	X (↑)		+		[26]
miR-155, miR-200, miR-203, miR-205	X (↑)		+		[35]
miR-21 (↑), miR-34a (↓), miR-30d (↓)	X		-		[37]
miR-212 (↑), miR-675 (↑), miR-148a (↓), miR-187 (↓), let-7g (↓)	X		-		[38]
miR-146 (↑), miR-205 (↓), miR-7 (↓)	X		-	-	[50]
miR-10b		X (↑)		-	[51,52]
miR-196a	X (↑)		-		[26]
miR-219	X (↑)		-		[36]
miR-200c	X (↑)		+		[36]
miR-21	X (↑)	X (↑)	-	-	[40,41,51]

miRNAs: MicroRNAs; Biopsies: Resected tumors; FNA: Fine needle aspiration; ↑: Upregulated; ↓: Downregulated; +: Good prognosis/response to treatment; -: Bad prognosis/response to treatment.

Table 3 MicroRNAs as therapeutic targets in pancreatic cancer

miRNA	Expression	Known target(s)	Function	Ref.
Let-7	↓	KRAS	Inhibition of cell proliferation, chemosensitization	[33,85,86]
miR-10b	↑	TIP30	Increased cell proliferation and invasion	[52]
miR-21	↑		Inhibition of cell proliferation, invasion, tumor growth, chemoresistance and inhibition of apoptosis	[95]
miR-23b			Radioresistance	[91]
miR-29a		Wnt/β-catenin	Chemosensitization to gemcitabine	[88]
miR-34a	↓	Smad3	Inhibition of cell proliferation and invasion, induction of apoptosis	[82,83,94]
miR-96	↓	KRAS	Inhibition of cell proliferation, invasion, tumor growth and induction of apoptosis	[23,70]
miR-99b		mTOR	Radioresistance	[90]
miR-132	↑↓	Rb1	Alteration of cell proliferation	[31,32]
miR-138		lipocalin	Inhibition of tumorigenicity	[93]
miR-141	↓	MAP4K4	Inhibition of cell proliferation and invasion, chemosensitization	[92]
miR-142-3p			Inhibition of cell proliferation	[72]
miR-148a	↓		None	[78]
miR-148b	↓	AMPKα1	Inhibition of cell proliferation, invasion and chemosensitization	[79]
miR-150*		IGF-1R	Induction of apoptosis	[73]
miR-181b	↑	NFKB	Chemosensitization to gemcitabine	[89]
miR-197			Induction of EMT	[75]
miR-198	↓	MSLN, OCT-2, PBX-1, VCP	Inhibition of cell proliferation, invasion, tumor growth and induction of apoptosis	[71]
miR-212	↑	Rb1	Increased cell proliferation	[21]
miR-218	↓		Inhibition of cell proliferation and tumor growth and metastasis	[84]
miR-221	↑		Increased migration, proliferation and EMT	[77]
miR-320c		SMARCC1	Chemosensitization to gemcitabine	[77]
miR-373			Increased tumor growth	[74]
miR-630		IGF-1R	Induction of apoptosis	[73]
miR-655			Inhibition of EMT	[76]

↑: Upregulated; ↓: Downregulated; EMT: Epithelial-mesenchymal transition.

tivity of 64% and a specificity of 89% for PDAC. A recent study further confirmed that circulating miR-210^[54] and miR-21^[55] are elevated in PDAC patients and may potentially serve as a useful biomarker for PDAC diagnosis. In addition, miR-200a^[56], miR-200b^[56], miR-16^[57], miR-196a^[57], miR-20a^[55], miR-24^[55], miR-25^[55], miR-99a^[55], miR-185^[55], miR-221^[58] and miR-191^[55] were described as significantly elevated in the sera of PDAC as compared with controls. Combining miR-16^[57] and miR-196a^[57], or miR-27a-3p^[59] detection with CA 19-9 quantification is even more effective to discriminate PDAC from controls. However, Pr Hoheisel's group recently reported that blood miRNAs profile could not discriminate pancreatitis from PDAC efficiently^[60]. Last, but not least, Pr Goggin's group recently demonstrated that miR-1290 accurately

distinguishes patients with low-stage pancreatic cancer from healthy and disease controls^[61]. Such study paves the way for the non-invasive detection of early PDAC lesions.

OPEN QUESTION: WHAT IS THE SIGNIFICANCE OF MIRNAS IN HIGH RISK PATIENTS FOR DEVELOPING PANCREATIC CANCER?

One of the current avenues of research to improve the management of pancreatic cancer is to better understand the early stages of the disease in order to allow for curative surgery and to prevent the risk of cancer in popula-

tions at risk. Advances in biomedical research have led to recent evidence that pancreatic cancer develops from preneoplastic lesions which can be considered as very effective risk factors. Three types of lesions have been identified so far: pancreatic intraepithelial neoplasia, mucinous cystadenomas, and Intraductal Papillary Mucinous Neoplasia of the pancreas (IPMN). Interestingly, the latter lesions can be readily detected due to the progress and the multiplicity of the imaging devices in the clinical departments. The risk of degeneration of IPMNs varies according to the type of injured duct: it is of the order of 60% for IPMN located in the main duct (or mixed) while this risk is estimated at 15% for branch ducts. IPMN now represent 25% of the diagnosed pancreatic cystic tumors and 20% of resected pancreatic tumors, respectively. Therefore, one of the most promising strategies to improve the dismal prognosis of pancreatic cancer is to identify early indicators of degeneration of IPMNs in populations at high risk of developing this cancer. Interestingly, miRNAs have recently revealed a great potential as reliable early diagnosis biomarkers in IPMNs. Again, miR-21 and miR-155 are highly expressed in IPMN, while miR-155 is elevated in IPMN-associated pancreatic juice as compared to controls^[62]. We demonstrated that miR-205 and miR-21 overexpression precede phenotypic changes in the pancreatic ducts, both in human samples and in transgenic mice developing cancer^[63]. Interestingly, such over expression may occur early in the transformation from normal pancreatic tissue, as benign cystic tumors of low and high malignant potential express high levels of this miRNA^[64]. This strongly suggests that miRNAs such as miR-21 can possibly be used for an early diagnosis of this neoplasm. In a similar experimental model, Yabushita *et al.*^[65] recently reported the over expression of miR-155, miR-21, miR-210, miR-18a, miR-203, miR-30b-5p, miR-31, miR369-5p, miR3-376a and miR-541 in the serum of a human KRAS oncogenic transgenic rat model. More importantly, Matthaeci *et al.*^[66] assessed the diagnostic benefit of using miRNAs as biomarkers in pancreatic cyst fluid in patients, to identify IPMN that require resection and exclude non-mucinous cysts with a sensitivity of 89%, a specificity of 100%, and AUC of 1. This work was further completed by Pr Giovannetti's group who demonstrated that miR-21, miR-155 and miR-101 showed significant differences in invasive *vs* non-invasive IPMNs, with miR-21 described as an independent prognostic biomarker in invasive IPMNs^[67]. Again, miR-21 and miR-155 were recently described as upregulated during the development and progression of IPMN^[68]. MiR-21 in cystic fluid was identified as a candidate biomarker to distinguish between benign, pre-malignant, and malignant cysts^[69], while miR-221 could be used for the identification of more advanced malignant disease^[69]. Last, a work from Pr Maitra's group recently revealed that a 9-miRNA panel quantified in cystic fluid may aid in diagnosis and surgical treatment decisions for patients with pancreatic cystic lesions, such as high-grade IPMNs^[66]. Thus, miRNAs may reveal as non-invasive indicators of degeneration in a population at high risk of

developing incurable cancer. Once identified, patients will be stratified and will benefit from early surgical management that will greatly improve their survival and prognosis. Finally, this approach is likely to strengthen the surveillance protocol and to reduce the costs associated with patients care.

ROLE OF MIRNAS IN PANCREATIC CANCER

miRNAs are broadly involved in pancreatic carcinogenesis

Many miRNAs have been reported to alter cancer proliferation and/or migration, both *in vitro* and *in vivo*. miR-132 and miR-212 were recently reported to be over expressed in pancreatic cancer as compared to normal or benign adjacent pancreas to the tumor^[21]. Interestingly, these miRNAs target the retinoblastoma tumor suppressor 1 (Rb1) to favor cancer cell proliferation^[21]. Another study by Pr Shao's group yielded conflicting results as they demonstrated that miR-132 was down regulated in cancer *vs* normal benign normal tissues^[22]. In the later study, enforced expression of miR-132 in cell lines derived from PDAC led to proliferation and colony formation inhibition^[22]. Yu *et al.*^[23] reported that miR-96 is downregulated in PDAC as compared to normal tissues and targets KRAS. Consequently, restoring miR-96 expression strongly inhibited *in vitro* cell proliferation, invasion, induced apoptosis and reduced tumor growth. This was further confirmed in a recent study linking ecotropic viral integration site 1 oncoprotein-mediated inhibition of miR-96 to promote KRAS expression during early pancreatic carcinogenesis^[70]. MiR-198 acts as a central tumor suppressor in PDAC and modulates the expression of many oncogenic factors such as MSLN, OCT-2, PBX-1, and VCP^[71]. Very interestingly, low miR-198 expression prognosticates poor patient outcome, while high miR-198 may disrupt this oncogenic network and predict better prognosis and increased survival.

Epigenetic regulation of miRNAs involved in pancreatic cancer progression

MiR-148 family members may have distinct effects on PDAC-derived cells proliferation. While miR-148a expression is lost during PDAC carcinogenesis following methylation of its DNA sequence^[34], we recently demonstrated that enforced expression of this miRNA didn't impaired PDAC-derived cells cell proliferation nor tumor growth in experimental models^[72]. On the other hand, recent results described that miR-148b can inhibit cell proliferation, invasion, and enhance chemosensitivity of PDAC by targeting AMPK α 1^[73]. MiR-124 is also silenced by aberrant methylation in PDAC; consequently, tumor progression and metastasis are enhanced due to the lack of Rac1 targeting^[74]. MiR-34a miRNA, which is directly regulated by p53, is also subjected to epigenetic silencing in numerous neoplasms, including PDAC^[75]. Strikingly, this miRNA plays a pivotal role in PDAC stem cell self-

renewal and may hold significant promise as novel target for PDAC^[24]. In addition, the natural compound genistein up-regulates this miRNA to suppress cell proliferation and induce cell death by apoptosis of PDAC-derived cell lines^[76]. MiR-34a was also recently reported as a tumor metastasis suppressor by negatively modulating Smad3^[77]. Last, Li *et al.*^[78] recently demonstrated that the histone methyltransferase enhancer of zeste homolog 2 inhibits miR-218 expression, that prevents proliferation of PDAC cells in culture, and tumor growth and metastasis in nude mice.

miRNAs regulates the epithelial-mesenchymal transition in pancreatic cancer

Besides the miR-200 family members (reviewed elsewhere), miRNAs such as miR-197 and miR-655 have been recently involved in the epithelial-mesenchymal transition in PDAC cells, by targeting p120 catenin^[79] and ZEB1 and TGFBR2, respectively^[80]. In addition, microRNA-221 participates in the effects of PDGF-BB on migration, proliferation, and to the epithelial-mesenchymal transition in these cells^[81].

miRNAs are key players in drug-mediated inhibition of pancreatic cancer growth

Recently, different molecules were found to alter miRNA expression in PDAC to inhibit cell proliferation and/or tumor growth. Triptolide that downregulates HSP70, a molecular chaperone upregulated in several tumor types, was recently shown to upregulate miR-142-3p in PDAC cells, to inhibit cell proliferation^[82]. More importantly, Minnelide, a water-soluble prodrug of triptolide, induces the expression of miR-142-3p *in vivo*. In addition, the adamantyl retinoid-related molecule 3-Cl-AHPC was recently demonstrated to induce miR-150* and miR-630 miRNAs expression to target IGF-1R and promote apoptosis in PDAC cells^[83]. Inappropriate regulation of intracellular zinc levels may also plays an important role in PDAC. Recently, increased zinc influx mediated by the zinc importer ZIP4 was demonstrated to induce miR-373 expression in pancreatic cancer to promote tumor growth^[84].

Besides miR-148b, *Let-7* is also involved in the chemosensitization of PDAC-derived cell lines. Indeed, reduced expression of the *let-7* miRNAs family members was identified in gemcitabine-resistant PDAC cell lines^[85]. This was correlated with a higher expression of ribonucleotide reductase subunit M2 (RRM2), a key protein involved in gemcitabine resistance. In this work, the authors nicely demonstrated that *Let-7* can regulate RRM2 expression, but also that *Let-7* biogenesis was severely impaired in PDAC cells^[85]. The latter effect seems to be recurrent in PDAC as nuclear TRAILR2 was recently demonstrated to inhibit maturation of *Let-7* in PDAC cell lines to increase their proliferation^[86]. Additionally, miR-320c, miR-29a and miR-181b were found to regulate the resistance of PDAC cells to gemcitabine through SMARCC1^[87], the Wnt/ β -catenin^[88] and the

NF- κ B^[89] signaling pathways, respectively. In a recent report, miR-141 was found to target MAP4K4 to inhibit cell proliferation, clonogenicity and invasion, induce G1 arrest and apoptosis, and enhance chemosensitivity^[90]. Alternatively, radiation resistance of PDAC-derived cell lines has also been linked to miRNAs, such as miR-99b^[91]. In a very interesting study by Wang *et al.*^[92], miR-23b was found to regulate autophagy associated with radioresistance of PDAC cells.

MIRNAS AS NEW THERAPEUTIC TARGETS FOR PANCREATIC CANCER MANAGEMENT

As stated in the previous sections, miRNA expression is profoundly altered in pancreatic cancer and/or is strongly modulated during carcinogenesis. Thus, the activation of tumor-suppressive miRNAs and the inhibition of oncogenic miRNAs may have the potential to provide a fundamentally new approach for the development of therapeutics for many cancers including PDAC. Probably the most important advantage in comparison with current approaches targeting single genes is the ability to modulate many different pathways “at once” taking into account that one miRNA can regulate hundreds of genes, frequently in the context of a cell-specific network. In this section, we will update our recent book chapters on the use of miRNAs as therapeutic tools to control PDAC progression^[17,18] (Table 3).

Few reports described the use of miRNAs as therapeutic targets to control PDAC tumor progression, *in vivo*. We demonstrated that *let-7* enforced expression strongly inhibits PDAC cell proliferation^[33]. This was achieved either using plasmid-encoding miRNA or lentiviral vectors. However, restoring *let-7* levels in cancer-derived cell lines failed to impede tumor growth progression after intratumoral gene transfer. Using a similar strategy, Lee *et al.*^[93] recently demonstrated that miR-138 transfection of cancer cells *in vivo* reduces tumor formation by targeting neutrophil gelatinase-associated lipocalin. Interestingly, nanoparticles targeted to PDAC-derived cells using bi-functional CC9 peptide successfully delivered miR-34a to inhibit the growth of subcutaneous PANC-1 tumors^[94]. We recently devised a lentiviral vector to target miR-21, one of the most described miRNA in oncology^[95]. Following transduction with this vector, PDAC-derived cells cell proliferation is strongly inhibited, and cancer cells die by apoptosis through the mitochondrial pathway. *In vivo*, a single inoculation of the therapeutic vectors in exponentially growing PDAC tumors stops cancer progression, inhibits cell proliferation and provokes cancer cell death by apoptosis. We found that our approach surpasses the therapeutic efficacy of standard treatments for this disease. Interestingly, miR-21 depletion enhances tumor angiogenesis; consequently, combining miR-21 targeting with gemcitabine eradicate experimental PDAC tumors. During this study, we treated existing tumors with miR-21

antagonists, a paradigm closely related to the clinical scenarios in which such therapies will be employed. While there clearly remains significant work to be done, this work is the first to demonstrate that targeting oncogenic miRNA is very effective to stop the tumor growth of a very aggressive PDAC model. It also emphasizes the central role of miR-21 in this cancer, and paves the way to forthcoming studies to discover the many pathways controlled by this miRNA in PDAC. Because miR-21 is over expressed in most human tumors; therapeutic delivery of miR-21 antagonists may still be beneficial for a large number of cancers for which no cure is available.

CONCLUSION

miRNAs can be detected and quantified not only in frozen tissues, but also in formalin-fixed paraffin-embedded tissues, as well as serum and plasma samples. These tiny but potent molecular markers have proven effective for PDAC classification, prognostic stratification and drug-response prediction. Strikingly, miR-21, and to a lower extent miR-196, miR-217, miR-10b and miR-155, appears to be constantly up regulated in PDAC, and to be indicative of poor survival, response to treatment and/or metastatic disease. PDAC is also frequently associated with a dense stromal reaction that may favor tumor progression and resistance to treatment. Recently, Pr Donahue's group has pointed out that miR-21 expression in PDAC tumor-associated fibroblasts is associated with decreased overall survival and promotes tumor cells invasion^[96]. This work may stem for novel diagnostic and therapeutic strategies for dual targeting of both tumor and stroma in PDAC. Whether this will translate into clinical applications is still highly debated. Above all, circulating miRNAs, in combination with other "omics" approaches such as proteomics, are expected in the future to prove specific and/or sensitive as a long-awaited screening tool for PDAC.

On the other hand, miRNAs are key players in PDAC carcinogenesis, and can be organized in oncogenic networks aimed at inhibiting multiple tumor suppressor genes. They are involved in the regulation in many if not all cancerous pathways such as cell proliferation, dissemination, resistance to apoptosis or chemotherapy. Consequently, the development of miRNA-based therapies have the potential to overcome the limitations of present cancer therapies that often lead to relapse because of the complexity and the redundancy of the targeted signaling pathways. The path from drug discovery to clinical trials is long and still hampered by many challenges. Despite the fact that hundreds of ongoing clinical trials include miRNA as biomarkers, miR-122 is the unique miRNA that as successfully reached clinical trial as targeted therapy to treat HCV infection^[97,98]. Nevertheless, it is our belief that miRNA-based therapeutics (especially to target miR-21) for cancer are not far behind, and that combination of miRNA therapy with targeted or traditional therapies may provoke a synergistic effect for treatment

of cancer in clinical trials in the next few years.

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