

Emerging roles of microRNAs in pancreatic cancer diagnosis, therapy and prognosis (Review)

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Received March 31, 2015; Accepted July 8, 2015

DOI: 10.3892/ijo.2015.3129

Abstract. Pancreatic cancer is one of the leading causes of cancer related death. Increasing incidence and mortality indicates a lack of detection and post diagnostic management of this disease. Recent evidences suggest that, miRNAs are very attractive target molecules that can serve as biomarkers for predicting development and progression of pancreatic cancer. Furthermore, miRNAs are also promising therapeutic targets for pancreatic cancer. The objective of the present review is to discuss the significance of miRNA in pancreatic cancer development, diagnosis, therapy and prognosis. We extracted and compiled the useful information from PubMed database, which satisfied our criteria for analysis of miRNAs in pancreatic cancer diagnosis, therapy and prognosis. A summary of the most important miRNAs known to regulate pancreatic tumorigenesis is provided. The review also provides a collection of evidence that show miRNA profiles of biofluids hold much promise for use as biomarkers to predict and detect development of pancreatic cancer in its early stages. Identification of key miRNA networks in pancreatic cancer will provide long-awaited diagnostic/therapeutic/prognostic tools for early detection, better treatment options, and extended life expectancy and quality of life in PDAC patients.

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1. Introduction

Pancreatic cancer is the tenth most common malignancy in the world (1). In particular, pancreatic ductal adenocarcinoma (PDAC) is the most common type (90%) of pancreatic cancer diagnosed in the exocrine component of the pancreas (2). It is frequently diagnosed in developed countries and is more common in men than in women (3). Patients with pancreatic cancer have a very low survival rate of ~1% at 5 years, which is mainly due to difficulty in detecting pancreatic cancer at early stages (4). Approximately 280,000 new pancreatic cancer cases are diagnosed globally each year. The incidence of pancreatic cancer is expected to increase by 55% between 2010 and 2030. The most common risk factors for developing pancreatic cancer are smoking, diabetes, hereditary pancreatitis, multiple endocrine neoplasia type 1 syndrome, hereditary nonpolyposis colon cancer, von Hippel-Lindau syndrome, ataxia telangiectasia, and the familial atypical multiple mole melanoma syndrome (FAMMM) (3).

Like several other cancers, early detection of pancreatic cancer increases the probability of a better prognosis. However, pancreatic cancer is difficult to detect and diagnose because it does not exhibit any specific, detectable symptoms and is hidden behind other large abdominal organs (5). Moreover, pancreatic cancer causes symptoms that are characteristic of other common illnesses, such as jaundice, back/abdominal pain, loss of appetite, weight loss and fatigue (6). In addition to the lack of unique symptoms, pancreatic cancer is highly invasive and metastatic and highly resistant to chemotherapy (7,8). The standard treatment options available for patients with pancreatic cancer are surgery, radiation, chemotherapy [5-FU, gemcitabine and cetuximab] (9), chemoradiation, and some marginally effective targeted therapies [inhibitors for VEGF,

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Abbreviations: miRNA, microRNA; PDAC, pancreatic ductal adenocarcinoma

Key words: biomarker, prognosis, pancreatic ductal adenocarcinoma, microRNA, diagnosis, therapeutics

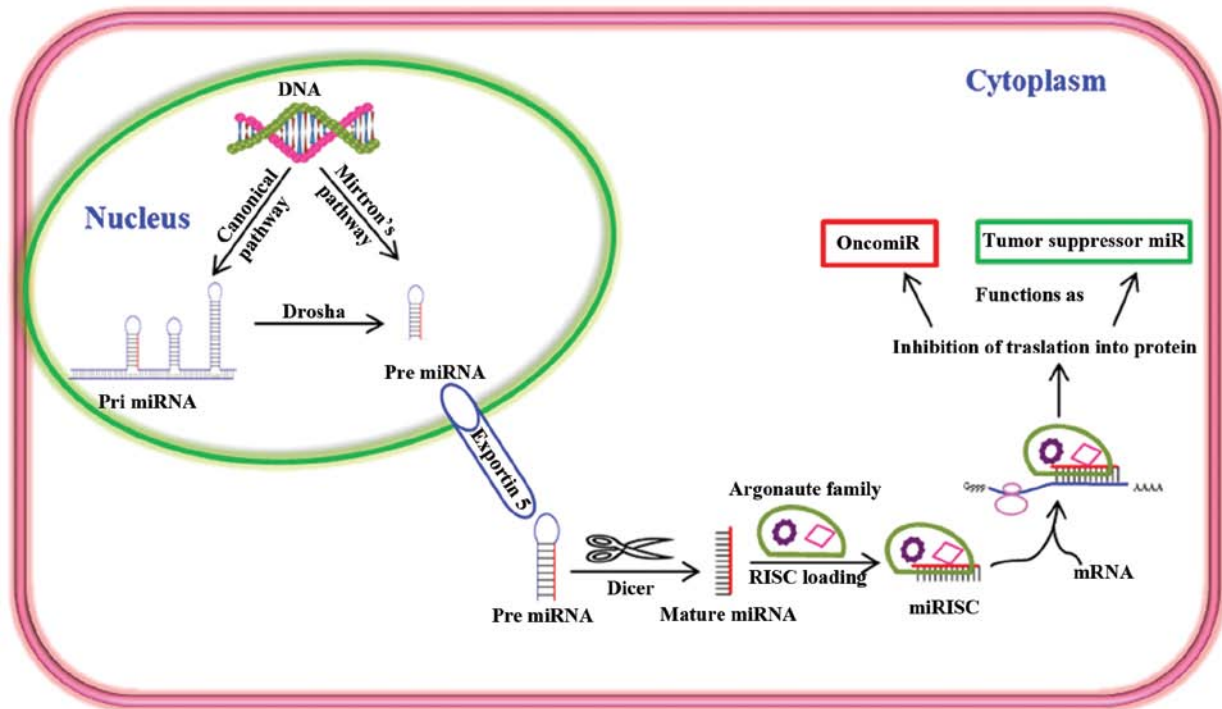


Figure 1. Biogenesis and function of miRNA in cancer cells. Pre miRNAs are produced in the nucleus through canonical and mirtron's pathway. Dicer processes pre miRNAs as they enter the cytoplasm via active exportin 5. miRNA-induced silencing complex (miRISC) is formed by integration of argonaute family proteins that act as guides and allow recognition of the complementary sequences in target mRNA. miRISC subsequently inhibits the translation of target mRNA or degrades the target mRNA. Various miRNAs can function as tumor suppressors or oncomiRs.

EGFR (9,10) mTOR (11)]. Despite remarkable global research efforts by clinicians and cancer scientists, pancreatic cancer remains fatal. Even though the incidence of pancreatic cancer is lower relative to other cancers, it is the fourth leading cause of cancer-related death in the U.S. According to the Pancreatic Cancer Action Network pancreatic cancer is expected to become the 2nd leading cause of cancer-related death by the year 2020. Because there is not an effective treatment for this deadly cancer (12), there is an immediate need to improve our understanding of pancreatic cancer. The better understanding of mechanism of disease pathogenesis will facilitate early detection and development of effective treatments. The focus of the present review is to summarize current knowledge regarding the roles of miRNAs in pancreatic cancer diagnosis, treatment and prognosis.

2. microRNA (miRNA or miR)

The importance of small non-coding microRNA (miRNA) molecules in the regulation of gene and protein expression was discovered and documented in early 90s. During the last decade, remarkable amount of research efforts have been dedicated towards understanding the role of miRNA regulatory networks in mammalian genetics and human diseases (12,13).

miRNAs are a class of post-transcriptional regulators, which mainly repress expression of genes and proteins. miRNAs form a part of the cellular regulatory network that governs various important biological functions, such as cell growth, proliferation, differentiation, development and apoptosis (14). In the recent past, miRNAs have become a central focus in cancer research because many miRNAs function

as either tumor suppressors or oncogenes (Fig. 1). In addition, miRNAs are very promising diagnostic and prognostic biomarkers for human diseases, including pancreatic cancer. They are found in most biological fluids [e.g., blood, amniotic fluid, breast milk, bronchial lavage, cerebrospinal fluid (CSF), colostrum, peritoneal fluid, pleural fluid, saliva, seminal fluid, tears and urine] (15) and are more stable than protein and mRNA (16). Identification of biomarkers in biological fluids is particularly attractive because this represents a quick, non-invasive, and relatively inexpensive method for detecting and diagnosing disease (17). Identification of a specific miRNA profile in body fluids would be invaluable for early diagnosis, therapy and prognosis of pancreatic cancer. Several miRNAs have been shown to play a vital part in regulating pancreatic cancer by influencing pancreatic cancer growth, progression, invasion, metastasis and resistance to therapy (Table I) (18-28). The miRNAs that are specifically involved in pancreatic cancer and their target genes are shown in Table II (29-39).

3. miRNAs as biomarkers for pancreatic cancer diagnosis

The overall sensitivity for the detection of pancreatic cancer by established gastrointestinal tumor markers, such as carcinoembryonic antigen (CEA) and CA 19-9 varies depending on tumor grade. However, the sensitivity and specificity of CA 19-9 for accurate diagnosis of pancreatic cancer using serum is debatable. Therefore, conventional serum antigen markers are inappropriate tools for diagnosing pancreatic cancer (40,41). There is a great need to develop better diagnostic markers for pancreatic cancer. Some progress has been made towards delineating the roles of miRNAs in the patho-

Table I. The reported oncogenic miRNA (OncomiR) and tumor suppressor miRNAs in human pancreatic cancer.

miRNA	Function	Reported activity for pancreatic cancer	Refs.
miR 21	OncomiR	Expressed in early PDA	(18)
miR181b	OncomiR	miRNA-181b sensitizes PDAC cells to gemcitabine by targeting BCL-2	(19)
miR-221	OncomiR	Plasma miR-221 concentrations were significantly higher in PCa patients. Useful biomarker for cancer detection, monitoring tumor dynamics and predicting malignant outcomes	(20)
miR-27a-3p	OncomiR	Used as a biomarkers for PC diagnosis	(21)
miR-100	OncomiR	Regulates IGF1-receptor expression in metastatic pancreatic cancer cells	(22)
miR-34 family	Tumor suppressor miR	Targeting the anti-apoptotic mRNAs such as BCL2, MYCN	(23)
miR-124	Tumor suppressor miR	miR-124 is downregulated and epigenetically silenced in pancreatic cancer	(24)
miR-217	Tumor suppressor miR	Frequently downregulated miR-217 can regulate KRAS and function as a tumor suppressor in PDAC. Useful therapeutic agent for miRNA-based PDAC therapy	(25)
miR-148a	Tumor suppressor miR	An inappropriate therapeutic Tool for PC	(26)
miR-138	Tumor suppressor miR	Regulates IGF1-receptor expression in metastatic pancreatic cancer cells	(22)
miR-15a-3p	Tumor suppressor miR	Induces apoptosis	(27)
miR-216a	Tumor suppressor miR	Downregulated expression of miR-216a suggests the involvement of miR-216a in the tumorigenesis and development of pancreatic cancer. Potentially serve as a novel tumor marker and also a prognostic factor for pancreatic cancer	(28)

genesis of pancreatic cancer. Blood is one of the easiest and most preferable types of biofluid for diagnosis and detection of most diseases. Plasma levels of miRNA-16 and miRNA196a in combination with CA 19-9 has been shown to work very efficiently for screening early pancreatic cancer (85.2%) (42). In addition, Wang *et al* (43) studied the expression levels of miR-21, miR-210, miR-155 and miR-196a in plasma from PDAC patients in comparison to healthy individuals, which showed that miR-155 overexpression was an early biomarker for pancreatic neoplasia, while miR-196a expression correlated with the progression of PDAC (43). In another study, overexpression of miR-155 was found in 80% of early pancreatic lesions (stage II) in microdissected panIN tissues (44). In addition, blood samples collected from pancreatic cancer patients had higher expression levels of miR-200a, 200b and 210 (15). Furthermore, the combination of miR-196a and miR-217 expression patterns differentiated PDAC from healthy controls and chronic pancreatitis cases (45). Using a similar approach, another group of researchers also observed much higher levels of circulating miR-18a in the plasma of 36 pancreatic cancer patients with compared to 30 healthy volunteers (46). These reports indicate the importance of miRNAs as potential biomarkers for the diagnosis of pancreatic cancer.

4. miRNAs as therapeutic targets in pancreatic cancer

The widely used chemotherapeutic treatment for pancreatic cancer is gemcitabine, which shows a moderate tumor suppres-

sion response rate of ~12% (12). Therefore, the development of new and improved therapies for the treatment of pancreatic cancer is crucial. Clinical studies have demonstrated the efficacy of miRNA as a therapeutic tool in the management of PDAC (12,47). Tremendous efforts have been made *in vitro* and *in vivo* using preclinical models of cancer, to inhibit oncogenic miRNAs with antagomiRs (48,49). AntagomiRs exhibit great potential as miRNA-based therapeutics for cancer treatment. However, optimization and execution of miRNA-based therapeutics lag behind other current therapies (48,49). Additional research is needed in order for miRNA-based therapy to become a standard anticancer therapy. Based on current data, intense research efforts are required to improve outcome for successful pancreatic cancer treatment.

The role of potential target, hsa-miR-155, which is upregulated in PDAC was reported to be a regulator of the putative tumor suppressor SEL1L. According to this report, inhibition of this aberrantly upregulated miRNA in human pancreatic ductal adenocarcinoma would serve as a potential therapeutic strategy for PDAC by increasing expression of SEL1L (50). In another report, the therapeutic efficacy of miR-34b was demonstrated using pancreatic tissues from 64 pancreatic cancer patients. miR-34b was shown to act as a tumor metastasis suppressor through negative modulation of oncogenic SMAD3 (51). Another miRNA with possible therapeutic potential in pancreatic cancer is miR-142-3p. Triptolide, a diterpene triepoxide isolated from the Chinese herb *Tripterygium wilfordii* inhibits the proliferation of pancreatic cancer cells by

Table II. The deregulated miRNAs and target genes in PDAC.

miRNA	Expression profile in tumors	Target genes of miRNA in PDAC	Refs.
miR-126, 217, 96, let-7, 143/145	Downregulated	K-RAS, CRK, RREB1, Notch-1, AKT and β -catenin	(29,30)
miR-21	Upregulated	PTEN, HNRPK, PDCD4, K-RAS, BTG2, TGFB2, MARCKS, pellino-1, TM1, SPRY2, TPM1, Maspin, PPAR α , RECK, NFIB, CDC25A, LRRFIP1, BCL2, RTN4 and TAp63	(31-35)
miR-210	Upregulated	NPTX1, EFNA3, AcvR1b, ISCU, COX10, E2F3, RAD52, ACVR1B, MNT, FGFRL1, HOXA1 and HOXA9	(31,33,34,36,37)
miR-155	Upregulated	LDOC1, TP53INP1, HIF-1a, VEZF1, FOXP3, FOXO3a, CYR61, RHOA, SMAD1, SMAD5, HIVEP2, CEBPB, RUNX2, MYO10, MyD88, SHIP1, C/EBP β , IFN-gammaR α , JARID2, BACH1, ZIC3, ZNF652, ARID2, GTR1, AT1R, RIPK1, FADD, AID, JARID2 and RHOA	(31-34,36,37)
miR-196a	Upregulated	HOXA1, HOXA5, HOXB6, S100A9, KRT5, SPRR2C, HOXA7, HOXB8, HOXC8, HOXD8, HMGA2 and ANXA1	(31,34,36,37)
miR-200b	Upregulated	EP300 and RND3	(34,35)
miR-221	Upregulated	p27 Kip1, p57 Kip 2, KIT, ER- α , PUMA, PTEN, MDM2, PI3K, ICAM-1, Bmf and Bim	(32-36)
miR-222	Upregulated	P ⁵⁷ , TP53INP1, PUMA, PTEN, p27, Bim, MMP1, SOD2 and STAT5A	(33,34,36)
miR-196a	Upregulated	HOXA1, HOXA5, HOXB6, S100A9, KRT5, SPRR2C, HOXA7, HOXB8, HOXC8, HOXD8, HMGA2 and ANXA1	(31,34,36,37)
miR-15b	Upregulated	HOXB8, ZEB1, SIP1, Arl2 and BCL2	(32,34,38)
miR-186	Upregulated	P2X7, HOXB8, ZEB1 and SIP1	(34)
miR-190	Upregulated	NeuroD and TP53INP1	(31,34,39)

All the miRNAs and their targeting genes are involved in the following major 11 signaling pathways of PDAC such as K-Ras signaling, Notch signaling, angiogenesis signaling, cell cycle signaling, apoptosis signaling, invasion and metastasis signaling, Her2/neu signaling, PTEN signaling, insulin receptor signaling, PI3K/AKT signaling, Hedgehog signaling, glioblastoma multiforme signaling and prostate cancer signaling (29,30,39).

upregulating miR-142-3p which negatively regulates HSP70 expression in PDAC cell lines (52). Similarly, Qazi *et al* (9) showed that enforced expression of miR-101, enhanced the expression of E-cadherin levels and reduced the pancreatic tumor growth rate in SCID mouse xenograft model. Thus, miR-101 has demonstrated a potential therapeutic target of PDAC (9).

However, despite these promising studies, it is important to consider that not all patients respond the same way to a given anticancer therapy. Therefore, the best treatment approach is the one in which the therapy can be tailored to each individual patient. miRNAs provide the foundation for developing tailored and targeted treatment strategies against pancreatic cancer, because both specific miRNAs and antagomiRs can be identified easily and quickly in blood or other bodily fluids to determine the best treatment strategy. Thus, instead of concentrating on one miRNA or antagomiR, a more effective approach would be a combination of therapies, in which a panel

of miRNAs/antagomiRs in conjunction with chemotherapy is tailored to meet the needs of each patient.

5. Role of miRNAs in the prognosis of pancreatic cancer

Global miRNA microarray profiling may discriminate miRNA expression in normal vs. pancreatic cancer tissues and serve as a potential prognostic predictor of disease. High expression of miR-452, miR-105, miR-127, miR-518a-2, miR-187, and miR-30a-3p correlated with increased survival rates of more than two years (53). Notably, deregulated levels of miRNAs, miR-21, miR-155, and miR-196a in plasma, and miR-141 in the sera were observed in pancreatic cancer patients who had a poor overall survival rate (54). Furthermore, another study also reported that the levels of miR-196a were shown to be elevated in sera of the PDAC patients (55) in correlation with poor survival and advanced disease stage (55,56). In addition, it has been suggested that miR-196a expression is a more

Table III. MicroRNAs predicted to be involved in pancreatic cancer patient survival.

miR in pancreatic cancer	Survival status	Expression status	Refs
miR-10b, miR-219, miR-196a and 196a-2p	Poor survival	High expression	(33,55,64)
miR-21	Worse survival	High expression	(65)
miR-142-5p, 204 and miR-200c	Improved survival	High expression	(66,67)
miR-34a	Better survival	High expression	(67)
miR-216 and -217	Poor survival	Reduced expression	(68)

specific indicator of PDAC progression (45). Overexpression of miR-196a-2 and miR-219 are also associated with reduced survival. The median survival for patients with miR-196a-2 overexpression was 14.3 months compared with 26.5 months for those with low expression (57). The median survival for those with miR-219 overexpression was 13.6 months compared with 23.8 for those with low expression (58).

Comparative analysis of 98 normal and 88 PDAC patients revealed miR-21 and miR-155 as good biomarkers for predicting tumor stage and prognosis (58). Low expression of miR-21 was associated with increased survival. On the contrary, high expression of miR-34a and miR-30d was correlated with increased survival in patients who did not receive surgical tumor resection. Patients who underwent surgery for PDAC are expected to have reduced overall survival if they have high expression of miR-212 and miR-675 and low expression of miR-148a, miR-187 and let-7g (59). Compared with ductal epithelia from normal pancreatic tissues of organ donors, expression of miR-21 was upregulated by >1000-fold in microdissected pancreatic cancer tumors (60). Overexpression of miR-21 was associated with worse pancreatic cancer progression in gemcitabine-treated PDAC patients (60). miR-10b is another important miRNA marker that could predict successful treatment response and suitability for surgery in PDAC patients (56). In a study, 106 patient samples examined for the levels of expression of miR-10b, which revealed low levels of miR-10b in epithelial cells from benign lesions compared to those in pancreatic cancer lesions. The authors proposed that decreased miR-10b could improve the response to multimodality neoadjuvant therapy (61,62).

Two independent sets of samples obtained from patients with resected PDAC (with subsets of 19 and 60 patients) were subjected to miRNA microarray analysis. The analysis confirmed that miR-211 expression was the most predictive biomarker for treatment outcome in PDAC patients with significant associations with both overall survival and disease-free survival (63). Schultz and others (59) studied the expression levels of miRNAs in tissue samples from 225 patients. The results strongly suggested that miRNAs, miR-212 and miR-675 were upregulated and miR-148a, miR-187 and let-7g were downregulated and these miRNAs could function as independent predictors for reduced overall survival in PDAC patients (59). Thus, altered expression of miRNAs are strongly associated with poor survival, decreased response to treatment, and increased metastatic disease in PDAC (Table III) (16,64-68).

6. miRNA induces chemoresistance in pancreatic cancer

Chemotherapy is the most common treatment option for many cancers, including pancreatic cancer. However, it offers little therapeutic value in many cases due to rapid development of chemoresistance. Pancreatic cancer is a notoriously chemoresistant cancer, which renders chemotherapy highly ineffective in a majority of patients. Various miRNAs, notably oncomiR-21, miR-34a, miR-196a, miR-221 and miR-214, have been implicated in the induction of chemoresistance in PDAC (56,69). miR-10b, miR-34a and members of the miR-200 family were reported to have roles in gemcitabine chemoresistance. Upregulation of miR-21 was implicated in the induction of chemoresistance in PANC-1 cells (70). In contrast, downregulation of miR-21 increased the sensitivity of PANC-1 cells to gemcitabine and indole-3-carbinol combination therapy. Similarly, miR-10b has been shown to be responsible for chemoresistance in many tumor types. Cancer stem cells (CSCs) are highly resistance to chemotherapeutic drugs; miRNAs are shown to be involved in developing resistance to chemotherapy by CSCs (71). In this regard, the roles of miRNAs and stem cells in the development of PDAC chemoresistance need to be examined. Pancreatic cancer stem cells sorted from the BxPC3 cell line exhibited much higher chemoresistance upon holoclone formation, which coincided with high levels of miR-214, miR-21, miR-221, miR-222 and miR-155 and lower levels of Let-7a, miR-30c, miR-30b and miR-30a. Holoclone-forming stem cells may contribute to pancreatic cancer chemoresistance. Therefore, the stem cell holoclones represent a useful model for studying pancreatic cancer chemoresistance and for designing treatment strategies to effectively combat the development of chemoresistance (72).

7. Importance of model selection for miRNA research in pancreatic cancer

During the past several decades, a great deal of effort and resources have been utilized but only a few promising advances have been made towards more effective prevention and treatment strategies against pancreatic cancer. A major limitation has been a lack of animal models that mimic the human disease. Several chemotherapeutic drugs for pancreatic cancer may be highly effective in two-dimensional (2D) *in vitro* cell culture models, while many of these drugs fail to show the same effect in clinical trials. A recent study by

Longati *et al* (8) reported the use of three-dimensional (3D) culture models, in which significant increases in chemo- and radiation therapy resistance were observed in comparison with 2D cell culture models. These 3D models were associated with increased expression of matrix proteins, stromal markers, and various miRNAs, which are known to be involved in the multidrug-resistant phenotypes of many pancreatic cancers. Consequently, most drugs tested in 3D cultures showed limited effects in contrast to 2D cultures (8). Thus, the 3D culture system is a more suitable model for predicting responsiveness to a potential drug. Traditional cell culture-based assays and xenograft models consume huge amounts of financial resources but do not necessarily improve the clinical outcome of patients with PDAC. Genetically engineered mouse models (GEMM) are suitable for identifying early genetic alterations in pancreatic precursor lesions and for studying early diagnosis and treatment. A major advantage of using GEMM is the conditional strategy to activate or inactivate expression of therapeutic or pathogenic genes, respectively, temporarily in specific tissues. GEMM also reflect intratumoral genetic heterogeneity, which is one of the hallmarks of PDAC. A lack of intratumoral genetic heterogeneity in xenografts and the cell lines may produce results that are not a true representation of human pancreatic cancer (73). Another approach is a patient-derived xenograft (PDX) model, a key model to translate the effective miRNAs into clinics as potential biomarkers or therapeutic targets for PDAC. PDX is increasingly used to characterize and validate several diagnostic and therapeutic strategies. The resemblance of intra and intertumoral genetic heterogeneity along with complexity of the microenvironment of PDX mimics more accurately the human PDAC. Direct representation of parental tumor with realistic genetic integrity is an advantage of PDX over the cell line-derived xenograft model. Another advantage of clinically relevant PDX is the similar stromal compartments of the parental tumor at the early passages. The key features of orthotopic and subcutaneous PDX are accurate predictability of drug response, identification of tumor specific treatment regimens, and high correlation between pre-clinical and clinical outcome in terms of relative sensitivity, resistance and therapeutic efficacy of the drug with molecular insights (74,75). Overall, PDX seems to be an appropriate and sufficient model to recapitulate both genetic and morphological alterations that leads to the development of PDAC. Together, these findings suggest that advanced models are required to test and validate miRNAs that would be effective in early diagnosis and development of suitable treatment for pancreatic cancer.

8. Future directions

Future research on the roles of miRNAs in the pathogenesis of pancreatic cancer will unfold the mechanisms of this deadly disease. Studies on the biogenesis of miRNAs will help in understanding the regulatory machinery of the overall metabolic state of the tumor. The identification of the key metabolic switches that favor expression of a particular set of miRNAs should help improve therapeutic odds of success in pancreatic cancer.

According to the current scenario, many key oncogenic and tumor suppressor miRNAs and downstream molecular

targets have been identified in PDAC. Identification and targeting of pancreatic cancer associated miRNA will have a tremendous impact on non/minimally invasive diagnostics, that may open new doors to improve the rate of curative outcome. The discovery of pancreatic cancer related miRNA as a therapeutic agent is warranted to bring a major breakthrough in many aspects of pancreatic cancer therapy. The development of state-of-the art microRNA delivery methods may increase the potential of miRNAs as therapeutic agents of choice in pancreatic cancer. Future analysis of miRNAs in a larger patient cohort is warranted to verify if this is a useful biomarker for early detection, prognosis, and therapy in a majority of pancreatic cancer patients.

9. Conclusions

Recent studies demonstrate the diverse and significant roles of non-protein-coding genomic regions (miRNAs) in pancreatic cancer progression and metastasis. Therefore, future research efforts that focus on miRNAs must be just as vigorous as the efforts made towards understanding the contributions of the coding genome (individual genes). A great deal of research in this field is required before we can effectively translate this into the clinicals to treat pancreatic cancer. Understanding the roles of miRNAs and tapping into the excellent potential of miRNAs will have a great beneficial impact on the early detection, diagnosis, treatment and prognosis of pancreatic cancer.

Acknowledgements

We thank Dr Rebecca Lopez-Valdez for her professional editing of the manuscript.

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