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# miRNAs in pancreatic cancer: Therapeutic potential, delivery challenges and strategies☆

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a severe pancreatic malignancy and is predicted to victimize 1.5% of men and women during their lifetime (Cancer statistics: SEER stat fact sheet, National Cancer Institute, 2014). miRNAs have emerged as a promising prognostic, diagnostic and therapeutic tool to fight against pancreatic cancer. miRNAs could modulate gene expression by imperfect base-pairing with target mRNA and hence provide means to fine-tune multiple genes simultaneously and alter various signaling pathways associated with the disease. This exceptional miRNA feature has provided a paradigm shift from the conventional one drug one target concept to one drug multiple target theory. However, in vivo miRNA delivery is not fully realized due to challenges posed by this special class of therapeutic molecules, which involves thorough understanding of the biogenesis and physicochemical properties of miRNA and delivery carriers along with the pathophysiology of the PDAC. This review highlights the delivery strategies of miRNA modulators (mimic/inhibitor) in cancer with special emphasis on PDAC since successful delivery of miRNA in vivo constitutes the major challenge in clinical translation of this promising class of therapeutics.



**Contents**



*Abbreviations:* Ago, Argonaute; ASO, Antisense oligonucleotide; bFGF, Basic fibroblast growth factor; CPP, Cell-penetrating peptide; CSCs, Cancer stem cells; cRGD, Cyclo Arg-Gly-Asp; DNA, Deoxyribonucleic acid; ECM, Extracellular matrix; EGFR, Epidermal growth factor receptor; EMT, Epithelial to mesenchymal transition; EPR, Enhanced permeability and retention; FGF, Fibroblast growth factor; 5-FU, 5-Fluorouracil; GAG, Glycosaminoglycan; GLI, Glioma-associated oncogene; HCV, Hepatitis C virus; Hh, Hedgehog; HUVECs, Human umbilical vein endothelial cells; LNA, Locked nucleic acids; miRISC, miRNA-induced silencing complex; miRNA/miR, microRNA; MMP, Matrix metalloproteinase; mRNA, Messenger RNA; NPs, Nanoparticles; ODNs, Oligonucleotides; PCC, Pancreatic cancer cells; PDAC, Pancreatic ductal adenocarcinoma; PDGF, Platelet derived growth factor; PEI, Polyethyleneimine; PLL, Poly(l-lysine); PLGA, Poly(lactic-co-glycolic acid); PMO, Phosphorodiamide morpholino oligomer; PNA, Peptide nucleic acids; Pol II, Polymerase II; Pre-miRNA, Precursor miRNA; Pri-miRNA, Primary miRNA; PS, Phosphothioate; PSC, Pancreatic stellate cells; PTCH, Patched; Rb, Retinoblastoma; RISC, RNA induced silencing complex; RNA, Ribonucleic acid; RNAi, RNA interference; Shh, Sonic hedgehog; shRNA, Small hairpin RNA; siRNA, Small interfering RNA; SMO, Smoothened; SNALP, Stable nucleic acid lipid particle; TFO, Triplex forming oligonucleotide; TGF-β, Transforming growth factor-beta; VEGF, Vascular endothelial growth factor; ZEB-1, Zinc finger E-box-binding homeobox 1.

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## 1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) accounts for 90% of the pancreatic cancers and is considered to be the most fatal malignancy causing 6.8% of all cancer related deaths with 46,420 estimated new cases and 39,590 estimated deaths in 2014 and a reported 5 year survival rate of 6.7% [1]. Different treatment modalities exist for PDAC, among which surgery is the mainstay of treatment. However, greater than 80% of the patients when diagnosed with PDAC show symptoms of local invasion and distant metastasis making them inoperable [2,3]. This necessitates the use of chemotherapeutics and/or radiation therapies which have also shown only a modest improvement in reducing the tumor growth. Considering the limited effectiveness of the current treatment strategies, extensive efforts are being made to improve the treatment outcome of PDAC including exploring drug combination as well as targeted drug therapies. Among the various chemotherapeutic agents used for PDAC, gemcitabine is considered as the first-line drug for PDAC. It has generated a lot of interest and several of its combinations with other chemotherapeutic drugs such as albumin-bound (nab)-paclitaxel [4–7], erlotinib [8–10], sunitinib [11–13] everolimus and cisplatin [14] are being investigated. Apart from gemcitabine, another four drug combination, FLOFRINOX (fluorouracil, leucovorin, irinotecan, and oxaliplatin) has shown a significant increase in survival (11.1 months versus 6.8 months) than the single agent gemcitabine in phase III clinical trials of metastatic pancreatic cancer [15]. However, the benefit observed from these combination therapies is only moderate and not complete which might be attributed to the emergence of chemoresistance and desmoplasia in PDAC.

To overcome the inefficacy of chemotherapeutics, newer molecules are being actively investigated among which microRNAs (miRNA/miR) have emerged out as one of the promising players in PDAC. These are non-coding 20–25 nucleotide endogenous RNA sequences, which modulate gene expression by base pairing with complementary sequences in target mRNAs leading to their silencing and degradation (tumor suppressor miRNAs) or activation and upregulation (oncogenic miRNAs) [16–21]. Different strategies have been developed to either upregulate the expression of a tumor-suppressor gene or to repress the level of oncogenes including antisense oligonucleotides (ASOs), triplex forming oligonucleotides (TFOs), aptamers, ribozymes and DNAzymes, small interfering RNA (siRNA) and miRNAs.

Aptamers are single stranded (ss) RNA or DNA oligonucleotides (5–40 kDa) which bind to their particular molecular target with high specificity and affinity and modulate endogenous gene expression at translation level [22,23]. TFOs bind to the major groove of the double helical DNA where it forms a local triple helix and thus inhibit gene expression at the transcription level [24,25]. Ribozymes are catalytic RNAs, which undergo sequence-specific pairing with RNA, and cleave the phosphodiester backbone at a specified location, thereby functionally inactivating the RNA. While carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other RNA molecules [26]. DNAzymes are analogous deoxynucleotides (DNA) with ribonuclease activity with the advantage of being more amenable to modification and more resistant to degradation [27]. siRNAs and miRNAs share similar physicochemical properties, but exhibit certain differences with respect to their biogenesis and mechanism of action as depicted in Fig. 1.

siRNAs are dsRNAs and are highly sequence specific. These induce cleavage of the target mRNA after getting incorporated into the RNA induced silencing complex (RISC) [28]. Unlike siRNAs, miRNAs are single stranded, occur naturally in plants and animals and lack the exact complementarity with mRNA. In contrast to siRNA, miRNAs interact by imperfect base pairing leading to target mRNA degradation or translational repression. Since miRNAs are not exact complements of target miRNA [29,30], resistance to miRNA therapeutics is less likely to develop since it requires multiple mutations in several genes [31].

First report on miRNA targeting Lin-4 gene in *Caenorhabditis elegans* was published in 1993 by Victor Ambros, Rosalind Lee and Rhonda Feinbaum, since then several advancements in cloning and computation, particularly after early 2000, have taken place identifying their role in several cellular processes including cancer development and its progression [32]. The correlation of miRNA and cancer was first established in 2002 for miR-15 and miR-16-1 which were found to be deleted or downregulated via epigenetic silencing in 69% of the patients of chronic lymphocytic leukemia [33]. Since then several miRNAs have been discovered and extensive attempts have been made to convey these to the target cells. Nonetheless, miRNA delivery still poses several biological challenges including degradation by exogenous RNAs, high molecular weight (~14 kDa) and highly negative charge which makes them impermeable to cellular membranes [34,35]. For effective clinical translation of miRNA therapeutics, safer, non-toxic and clinically viable carrier systems are necessary which can overcome the biological barriers of PDAC before releasing the miRNA cargo into the cytosolic compartment.

Desmoplastic pancreatic tumor microenvironment shows high interstitial pressure and a very dense stroma with vascular dysfunction which impairs drug delivery to the tumor leading to acquired chemoresistance [36]. Further, interaction of the pancreatic stellate cells (PSCs) with the pancreatic cancer cells (PCCs) through paracrine signaling by secreting various growth factors like transforming growth factor-beta 1 (TGF-β1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) make the tumor even more aggressive [37]. Further, the ability of PDAC cells to undergo epithelial to mesenchymal transition (EMT) and chemoresistance add more complexity to the treatment resulting in failure of therapy. Considering the immense therapeutic potential of miRNA in cancers particularly PDAC, this review mainly focuses on the major challenges in miRNA delivery and discusses various delivery strategies used to efficiently deliver the therapeutic miRNAs to the tumor cells by overcoming the barriers in PDAC.

Synthetic miRNA modulators can be delivered to the target tumor by using cationic polymers, such as polyethylenimine (PEI) [38–41], poly(L-lysine) (PLL) [42] and cationic liposomes [43], lipid nanoparticles [44], liposome-polycation-hyaluronic acid (LPH) nanoparticles [45], neutral lipid emulsions [46,47] like MaxSuppressor™ In vivo RNA-LANCEr II [48] and stable nucleic acid lipid particles (SNALPs) [49]. The review discusses the advantages and disadvantages of these delivery systems for miRNA delivery and targeting.

## 2. Molecular pathogenesis and altered signaling pathways in PDAC

Development and progression of PDAC involve a series of molecular aberrations at genetic level resulting in activation of oncogenes, inactivation of tumor-suppressor genes and deregulation of signaling pathways leading to the clinical symptoms (Fig. 2). Comprehensive analysis of 24 pancreatic cancer patients showed an average of 63 genetic alterations defining 12 cellular signaling pathways and processes which were found to be altered in 67–100% of the tumors [50]. Considerable efforts have been made over the past several years towards