Review

MicroRNA-based Targeted Therapeutics in Pancreatic Cancer

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Abstract. *The discovery during the last decade of microRNAs (miRs, miRNA) and their role in regulating normal physiological processes as well as in the pathogenesis of human tumors has been a revolutionary development in molecular oncology. miRNAs activating or inhibiting oncogenic molecular pathways that are involved in tumorigenesis, cell progression, invasion, angiogenesis and metastasis are now considered of major impact in many cancer types. miRNA-based therapeutics that inhibit the levels of oncogenic miRNAs (oncomiRs) or elevate tumor suppressor miRs have enormous potential as molecular therapeutic targets. Thus, the development of new targeted cancer therapies based on miRNAs promise to revolutionize cancer treatment due to their increased efficacy compared to conventional chemoradiation-based therapies and hopefully to lower levels of adverse effects.*

A revolutionary development in molecular oncology during the last decade has been the discovery of the role of micro RNAs (miRNA) in the pathogenesis of tumors (1). miRNAs are small (18-25 nucleotides in length) non-coding RNA molecules that act as post-transcriptional regulators of gene expression by binding to the 3'-UTR of target genes (2). miRNAs are produced in the nucleus by the cleavage of 85 nucleotide-long hair-pin shaped pre-miRNAs by RNase III Dicer followed by their transportation to the cytoplasm by

Exportin-5, pre-miRNAs are also cleaved from a larger precursor called pri-miRNA by the enzyme RNase III in the nucleus (2). miRNAs, together with the Argonaute family proteins, form the RNA-induced silencing complex (RISC), which suppresses the translation of mRNAs in the cytoplasm. The miRNAs are not specific to a single mRNA sequence and can regulate a set of different mRNAs, thus each mRNA can be regulated by a set of different miRNAs (3). miRNAs are secreted by cancer cells into the extracellular fluids and systemic circulation through exosomes, small 40-100 nm nano-sized cell-derived vesicles that are released from most cells can modulate the function of neighboring and distant cells and play an important role in intercellular communication process in tumor microenvironment (4).

Recent studies show that miRNAs regulate the expression of genes involved in many cellular processes including cell proliferation, differentiation, invasion, angiogenesis, drug resistance and apoptosis, all of which contribute to tumor growth and progression (2-4). miRNAs may function as oncogenes (oncomiRs) or tumor suppressors and their expression is often dysregulated in tumors. OncomiRs promote cancer development by negatively regulating tumor suppressor genes that control cell proliferation, migration/invasion or key mediators related to molecular mechanisms in cancer promotion (2, 3). Tumor suppressor miRNAs often inhibit the expression of oncogenes and genes that promote cell proliferation, invasion and tumorigenesis. Studies show that the expression of tumor suppressors often reduces, ceases/or is lost, while oncomiRs expression increases in cancer cell lines as well as within patients' tumors (3). Taken together, these data suggest that the expression profiles of certain miRNA molecules may become useful biomarkers in cancer diagnostics (4). In addition, miRNA-based therapies inhibiting oncomiRs or activating of tumor suppressor miRs, could be a powerful tool for cancer prevention and therapy (5-8).

Pancreatic cancer is one of the deadliest types of cancer, with approximately 2% of patient survivors within 5-years following their diagnosis (9). Because of that late-stage

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Table I. miRNAs differentially expressed in PDAC samples with a potential to be used as biomarkers for PDAC.

Pancreatic cancer	
Up-regulated miRs	Down-regulated miRs
miR10b, miR-21, miR23a, miR-31, miR-143, miR-145, miR-146a, miR-150, miR-155, miR-181a/b/c, miR-196a/b, miR-21, miR-210, miR-221, miR-222, miR-223, miR-376a and miR-301	miR-29c, miR -30a, miR-34a, miR -96, -130b, miR -141, miR -148a, miR-148b, miR-216, miR-217, miR-375 and miR -494

diagnosis and the limited chemotherapeutic opportunities, the prognosis of pancreatic cancer is extremely poor and median survival in the US and EU is about 6 months (9). Pancreatic ductal adenocarcinomas (PDAC) derived from the exocrine pancreas account for 95% of pancreatic cancers. Current chemotherapy of PDAC is frequently insufficient, leading to 6 months of patient survival on average (10). Therefore, there is an urgent need for a better understanding of the molecular mechanisms behind the role of miRNAs in the behavior of pancreatic cancer that can lead to the identification of novel molecular targets and development of novel targeted therapies to improve patient survival.

A growing body of evidence based on miRNA profiling from *in vitro* models as well as from PDAC patient samples, indicate that a large number of miRNAs is deregulated in pancreatic cancer (11-13). About 439 miRNAs have been found to be differentially expressed in PDAC samples (based on a comparison between 538 PDAC and 206 control samples), indicating some of them as useful biomarkers for PDAC (11) (Table I). The most significant overexpressed miRNAs in pancreatic cancer include miR-10b, miR-21, miR-23a, miR-31, miR-100, miR-143, miR-145, miR-146a, miR-150, miR-155, miR-181a/b/c, miR-196a/b, miR-21, miR-210, miR-221, miR-222, miR-223, miR-376a and miR-301. On the other hand, miR-148a, miR-217, miR-34a and miR-375 are frequently down-regulated in PDAC (11-14).

Currently, miR-based targeted therapeutic strategies in cancer involve the therapeutic administration of: i) tumor suppressor miRNA mimics and ii) inhibitors of miRNAs, known as anti-miRs/antagomiRs, that can block the effect of oncomiRs (5-7). Double-stranded miRNA mimics aim to restore the loss of miRNA expression, whereas anti-miRs, single stranded anti-sense oligonucleotides (complementary to target miRNA molecules), are designed to target mRNAs that bear oncogenic properties, affect drug resistance and cancer stem cell survival and maintenance (5-7). The therapeutic use of miR mimics and anti-miRs/ antagomiRs in cancer clinical trials require an improvement of their binding affinity for the target mRNAs, their stability in the blood circulation, their pharmacokinetics as well as their delivery in the cancer cells/tissue. One of these modifications involves the addition of a methyl or a thiol group in the

passenger strand of nucleotides or in the RNA backbone, to decrease their degradation rate in the circulation. Examples of chemical modifications present in anti-miRs include, the 2'-O-methyl-group-modification and the locked nucleic acid (LNA) modification, which can increase the serum stability to nucleases, improve pharmacological properties, such as increased serum stability and bioavailability as well as better pharmacokinetics profile with enhanced half-life and cellular uptake, leading to effective blocking onco-miRNAs both *in vitro* and *in vivo* tumor models (15-17). Importantly, the 2'-O-methoxyethyl modification also increases the specificity of the miRNA for its targets and it has been successfully used in human clinical trials with good safety records (18).

The specific delivery of RNA intervention therapeutics (siRNA, miR-mimics and anti-miRs/miR-inhibitors) into tumors still remains a major challenge. However, their administration within nano-vehicles/nanoparticles can improve some poor pharmacokinetic features (*i.e.* short half-life) and tumor-cell delivery (19-29).

miRNA-based therapeutics have been shown to be successful in preclinical animal studies (24-31) while some of them have entered human clinical trials for cancer and for other diseases (32,33). The first ever miRNA-based clinical trial was Miravirsen, which has gone on to Phase II with the use of an LNA-based miRNA inhibitor, targeting miR-122 in liver cells infected with Hepatitis C virus (32). Another miRNA mimic drug, MRX34, has been used in a Phase I clinical trial as a lipid-based nanoparticle, to treat solid tumors, such as in the liver, but also for the treatment of hematologic malignancies (33). Unfortunately, this study was recently terminated due to the drug's side effects. Cobomarsen, an anti-miR-155 inhibitor therapy, is ongoing for patients with adult T-cell leukemia/lymphoma and demonstrated greater than five months of clinical response with improvement in objective disease measurements (34). Despite all the successful preclinical studies with the pancreatic cancer models (27-31), miRNA-based gene therapy has not yet been developed to treat pancreatic cancer in humans. In an orthotopic model of pancreatic cancer using MiaPaca-2 cells, the systemic intravenous delivery of lipid nanoparticles carrying miR-34a mimics (a component of the p53 transcriptional network), or a miR-143/145 cluster (an inhibitor of KRAS and RREB1),

has resulted in the decrease of tumor growth, CD44+ cell counts, and in the increase of tumor cell apoptosis (27). In addition to this report, the therapeutic delivery of miR-34a with nanocomplexes with a tumor-targeting and tumor-penetrating, bifunctional CC9 peptide, has been tested in a Panc-1 xenograft model and has revealed increased miR-34a levels and reduced mRNAs of Bcl-2, c-Myc, Cyclin D1 and E2F3 (28). The down-regulation of these major oncogenes has resulted in a significant inhibition of tumor growth, cycle progression, and migration while it has induced apoptosis of PDAC cells in this model (28). The nanoparticle-based delivery system for miR-150-NF, a tumor suppressive miRNA in PDAC, has led to increased intracellular delivery *in vitro* and has inhibited cell proliferation, clonogenicity and invasion of pancreatic cancer cells (29).

In conclusion, miRNA-based therapeutics are promising potential candidates as targeting agents against PDAC. Compared to siRNAs or small molecule inhibitors that target a single gene or protein, miRNAs are expected to be more successful than due to their potential to target multiple genes and several pathways at the same time. However, delivery of the miR-based therapeutics to target cancer cells and tumor tissues is a major challenge for successful, non-toxic and safe clinical applications in pancreatic cancer, as well as in other types cancer.

Conflicts of Interest

The Authors declare no conflict of interests.

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