

Chapter 11

Modulating MicroRNA Expression for the Therapy of Pancreatic Cancer

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1 Introduction

Pancreatic cancer is the fourth leading cause of death by cancer worldwide with an increasing incidence and a very poor prognosis [1]. The estimated 5-year survival is lower than 5 %. Patients' median survival following diagnosis is approximately 6 months. Nowadays, there is no curative treatment excepting surgery for 15 % of patients. Nevertheless palliative chemotherapy (gemcitabine) can be applied. Development of pancreatic cancer is very slow and involves many actors from which microRNAs.

MicroRNAs (miRNAs, miRs) derive from endogenous genes (from intergenic or intragenic genomic regions) transcribed for the most part by RNA polymerase II. They follow a complex maturation process implicating key enzymes such as DROSHA, DGCR8 and DICER [2]. They are small non coding RNA that functions as translation inhibitors of messenger RNA mainly following binding to 3'-untranslated region [3–5]. This mechanism is conserved from plants to humans.

Because they regulate more than 30 % of mammalian gene products, microRNAs are tightly involved in the regulation of many physiological processes including development, proliferation, cell signaling and apoptosis. In addition, microRNAs play important roles in many diseases, including cancer, cardiovascular disease, and immune disorders. In oncology, two main families of microRNAs can be defined: oncomiRs (such as miR-21 and miR-155) which target messenger RNAs from tumor suppressor genes and tumor suppressor microRNAs (tsmiR) (let7, miR-34a and miR-146a) which target oncogenic mRNAs. More recently, another class of microRNA implicated in cell metastasis has been described

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(MetastmiR). Indeed, Ma et al. described that the over expression of miR-10b in non invasive breast cancer cell line alone confer metastatic potential. MicroRNAs are involved in many oncogenic pathways [2] for example miR-34a and miR-146a are induced by p53 and NF-kB, respectively [6] while miR-21 which inhibits the p53 network [7] is induced by many oncogenic pathways including activated KRAS and EGF receptor among other [8].

The alteration of microRNA expression in cancer has been described for the first time by Calin and colleagues in 2002 [9]. Several mechanisms are implicated in this deregulation such chromosomal aberrations, transcriptional control by oncogenic transcription factors (such as MYC) [10], environmental factors, polymorphisms [11], epigenetics [12] and altered expression or function of proteins involved in microRNAs maturation [13]. Recently, Dicer and Drosha were found decreased in 60 % and 51 % of ovarian-cancer specimens, respectively [14]. As a consequence, microRNA profiling permits the differential diagnosis between normal *vs* cancerous tissue and to indentify tissues of origin for metastases [2]. In pancreatic cancer, Bloomston et al. originally published that 21 upregulated and 4 downregulated microRNAs could differentiate pancreatic tumors from benign pancreatic tissue in 90 % of their samples [15].

2 MicroRNAs as Emerging Therapeutic Targets

Single microRNA are demonstrated to control the expression of hundreds of genes, and represent a new class of therapeutic targets to modulate many pathways simultaneously and to reduce the emergence of resistant cellular clones that remains a major concern in oncology [16]. In addition, recent publications demonstrate that altering the level of expression of the entire population of cellular microRNAs by targeting microRNA processing alters tumor progression in a disease-specific manner [17].

MicroRNAs are also established as key players in cancer cell resistance to treatment. MiR-21, one of the most cited oncomiR, is implicated in the resistance to chemotherapy of many types of cancer including breast and pancreatic cancer among others [18, 19]. In the later example, miR-21 targeting in combination with gemcitabine treatment induces tumor regression. Other microRNAs are implicated in pancreatic cancer cells chemoresistance such as miR-17-5p [20] and miR-181b [21]. MicroRNAs are also implicated in cancer cell resistance to radiotherapy. Indeed, Di Francesco and colleagues demonstrated that DNA damage response is affected by miR-27a in lung adenocarcinoma-derived cell lines by a direct interaction between miR-27a and the 3'UTR region of the ATM kinase (Ataxia-Telangiectasia Mutated) [22]. ATM regulates H2AX phosphorylation and the activation of check point and cell cycle arrest following DNA damages. Taken together, these studies demonstrate the importance of microRNA in carcinogenesis but also in response to treatment making microRNAs very appealing therapeutic targets.

3 MicroRNA Targeting in Cancer

microRNA can be considered as emerging targets for the treatment of cancer including pancreatic cancer either following restoration of the expression of tumor suppressor microRNAs (let-7, miR-143-145, miR-34) or the targeting of pro-oncogenic microRNA (miR-21, miR-155, miR-27). Many strategies have been developed to achieve this goal (antisens, microRNA decoys. . .). Interestingly, some approaches allow the synchronized targeting of several microRNAs by using so called ‘Tough Decoys’ (TuDs) [23]. Consequently, many microRNAs carriers are needed to deliver these moieties and to avoid the different biological barriers [24]. Later in the chapter we will suggest which vector could be used for the specific targeting of a diseased cell.

3.1 *In the Absence of Carriers*

Nowadays, microRNAs upregulation (tsmiR) is done by the use of microRNA mimics contrary to downregulation of oncomiR that is achieved using antisense oligonucleotide (ASO or antagomiR) or microRNA sponges (with repeated miRNA antisense sequence). These strategies take advantage of small RNAs (19–22 nt) that are by definition very sensitive to nuclease degradation. Consequently, it is mandatory to conjugate cholesterol with 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-O-MOE) or 2'-fluoro substitutions. These substitutions improve microRNA modulators stability and effectiveness of microRNA inhibition *in vivo* [25].

3.2 *Non Viral Nanovectors*

MicroRNA modulators have a small size (7–20 kDa) so they undergo kidney filtration [2]. In addition, these non endogenous modulators should avoid phagocytic immune cells (macrophages and monocytes) in the bloodstream. So it is necessary to combine them with a carrier. There are different nanovectors which can be used to protect microRNA modulators, to improve targeting and to improve the cellular uptake of the modulator. Lipid-based nanovectors (liposomes) can be toxic for cells, are non specific and can induce immune response [26]. Accordingly, they must be modified to serve as microRNA carriers. Pramanik and colleagues demonstrated that the systemic injection of miR-34a and the miR-143/145 clusters (two main tsmiR lost in pancreatic cancer) using lipid-based nanovectors in orthotopic xenografts model induce tumor growth inhibition with increasing apoptosis and decreasing proliferation [27]. Interestingly, tumor cells can be targeted with modified liposomes. Polycationic liposome-hyaluronic acid (LPH) are used because hyaluronic acid is a targeting agent due to its cell surface receptor CD44

which is overexpressed on various tumors. LPH could be combined with the tumor targeting GC4 single-chain antibody fragment (scFv-LPH) or with an integrin-binding tripeptide (cRGD-LPH) for targeting integrin receptors on tumor vasculature. Many other possibilities of lipid-based nanovectors combination are described by Dr Leone's group [28]. Polyethyleneimines (PEI) is commonly used due to its global positive charge which ensures a strong interaction with the negatively charged plasma membrane. Polyurethane-short branch polyethylenimine (PU-PEI) is not cytotoxic and has high transfection efficiency as described by Chiou *and al* for the delivery of miR-145 to treat lung adenocarcinoma *in vivo* [29]. Nowadays it is possible to modify PEI nanovectors with rabies virus glycoprotein (RVG) to allow PEI-microRNA modulator system to cross through the blood-brain barrier. For example miR-124a (neuron specific microRNA) delivery in brain promotes neurogenesis [30]. Atelocollagen that derives from type I collagen can also be used as a microRNA carrier. MicroRNA modulators-atelocollagen complex have a high delivery efficiency and limited immunogenicity. Matsuyama and colleagues described that the local administration of miR-135b inhibitors with atelocollagen suppressed the growth of subcutaneous Karpas 299 tumors in a xenograft model [31]. Last, Calin's team has recently described nanovector inspired from endogenous intracellular transport of microRNA. Indeed, microRNA-protein complex composed by Argonaute 2 protein or lipoproteins (HDL) are actively secreted or can be part of cell-derived membrane vesicles such as exosomes or apoptotic bodies [32]. Recently, Ohno et al., demonstrated the feasibility of targeting EGFR-expressing cancerous tissues after systemic injection in a RAG 2^{-/-} mice of let-7a microRNA in a modified exosomes by the GE11 peptide (specific ligand of EGFR less mitogenic than EGF). Their results suggest that exosomes can be used therapeutically as a nanovector delivery system for microRNAs [33].

3.3 Viral Vectors

Viral vectors are very efficient for gene transfer and can be easily targeted to diseased cells. MicroRNA replacement or inhibition using lentivectors, adenovectors or adeno-associated vectors (AAV) have been shown to inhibit tumor growth in experimental models of lung, prostate, breast and liver cancer. Pr Tyler Jacks's team demonstrated that let-7 g overexpression using lentiviral vector in both murine and human non-small cell lung tumors induced significant growth reduction [34]. In another study, miR-145 overexpression using adenoviral vector in combination with 5-FU treatment in orthotopic breast cancer mice *in vivo* significantly showed anti-tumor effects as compared to chemotherapy alone [35]. Last, Dr Mendell's team described that the systemic injection of miR-26a in a mouse model of hepatocellular carcinoma (HCC) using AAV, inhibits cancer cell proliferation and induces tumor-specific apoptosis without toxicity. This study is a proof of concept that expression of a microRNA lost in cancer using a dedicated delivery system is well tolerated [36].

3.4 Route of Administration

There are three main routes of administration depending on the type of microRNA delivery systems used [2]. MicroRNA can be injected systemically in the absence of carrier (antagomiR, LNA and modified oligos), while non viral and viral vectors permit systemic, locally or intranasal delivery. Importantly, local injection can help minimize microRNA modulators exposure to nuclease degradation in body fluids and decrease unspecific uptake in non target tissues. Accordingly, Dr Slack's group described that the intranasal injection of *let-7*-encoding adenovector reduces tumor growth in mouse models of lung cancer due to the capability of this class of vector to have a unique cell surface receptor and to transduce epithelial cells [37]. To finish, systemic or local injection can also be done for nanoparticles carrying microRNAs [2].

4 MicroRNA Targeting in Pancreatic Cancer

Concerning pancreatic cancer, there are several studies of microRNA targeting using different carriers. The most recent studies of microRNA targeting are described below. First of all, intravenous injections of miR-34 or miR143/145 lipid-based nanoparticles in pancreatic cancer xenografts induced tumor growth reduction and apoptosis [27]. However, while this strategy may permit the targeting of distant metastasis, the transfection efficacy of this approach was not mentioned. In a similar work, Hu and colleagues developed a nanovector-based miR-34a delivery system combined with CC9 peptide that increases the targeting and penetrating capability in pancreatic cancer-derived cells. Interestingly, systemic administration of this complex inhibits tumor growth and induces pancreatic cancer cell apoptosis in a murine model of PANC-1 subcutaneous xenografts [38]. Again, in vivo transfection efficacy of this approach is not quantified. In addition, both strategies used subcutaneous models of pancreatic tumor growth that greatly diverges from orthotopic tumors. On the other hand, miR-21 is barely expressed in normal cells and participates in many oncogenic pathways. This particular miRNA is most frequently associated with poor outcome in cancer including pancreatic neoplasia. Our group recently asked whether targeting miR-21 could impair tumor growth and sensitize pancreatic tumors to chemotherapy. We used lentiviral vectors encoding for miR-21 decoys that efficiently silence miR-21 in cancer cells. Intratumoral injection of miR-21 decoys in an orthotopic human pancreatic cancer xenograft model inhibits tumor progression. We next combined miR-21 targeting vector with repeated intra peritoneal gemcitabine injection. We demonstrate that miR-21 alone is more efficient than the standard of care chemotherapy to inhibit tumor progression and, more importantly, that combining miR-21 targeting with chemotherapy induced tumor regression in a very aggressive model

of pancreatic cancer [19]. Thus, microRNAs such as miR-21, are promising targets for pancreatic cancer therapy.

Nevertheless, few reports demonstrated that microRNAs modulators are ineffective to inhibit cancer growth. In these studies; the delivery systems do not appear to be faulty, but the enforced expression of the candidate microRNA may not result in the antitumoral effect expected. For instance, Delpu and colleagues analyzed the potential role of miR-148a over-expression in PDAC using lentiviral vector carriers. While this microRNA is lost during pancreatic carcinogenesis [12], they demonstrated that miR-148a expression *in vivo* using lentiviral vectors does not impede tumor growth [39]. In another example, restoring Let-7 expression using lentiviral vectors in pancreatic cancer derived cell lines strongly inhibits cell proliferation but fails to impede tumor growth [40]. Thus, it is mandatory to perform *in vivo* studies to demonstrate the antitumoral activity of microRNA-based therapeutics before further (pre)clinical evaluation.

5 MicroRNA and Clinical Trials for Cancer

Nowadays, microRNAs are commonly associated with clinical trials and can be used as robust and reliable biomarkers for different diseases. Nevertheless there is no clinical trial to date using microRNA as a therapeutic target in cancer. Indeed, MiR-122 is the only microRNA that has been implicated in clinical trials (phase 2 with 36 patients) for patients with chronic hepatitis C viral infection. This microRNA is liver-specific and *de rigueur* for hepatitis C virus replication. Repeated weekly subcutaneously injection of different doses of miravirsen (LNA-antimiR-122) have been performed. Miravirsen efficiently inhibits miR-122 in HCV patients. Interestingly, miravirsen is safe and well tolerated and provoke a dose dependent reduction in HCV RNA levels [41].

6 Conclusion

Despite these very encouraging results, it is important to question why microRNAs are not widely used in cancer clinical trials. Importantly, most of the studies have been performed in immunosuppressed experimental models and in very few immune-competent animals. As microRNAs have been associated with the regulation of TLRs [42], further experiments are needed to demonstrate the safety of such approach. In addition long term studies in model organisms must be performed, to identify unexpected serious adverse events linked to microRNA modulators administration. Along with, targeting tumor cells using delivery vehicles remains a challenge in the gene therapy field of research. Last but not least, the specificity of microRNA modulators must be scrutinized because lead off-target effects (i.e. silencing of non targeted genes) have been already described for other RNA

interference strategies using siRNA [43]. In conclusion, the potential benefits for basic cancer research, medicine and public health of using microRNAs as therapeutic targets are numerous. As existing treatment offer little benefit, targeting microRNAs may give therapeutic perspectives for the treatment of pancreatic cancer or other human solid tumors. Such challenges notwithstanding, this strategy represents a welcome and refreshing set of new considerations to ponder in a disease that has too often been met with frustration and nihilism in the past.

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