



Prognostic and Predictive Values of MicroRNAs in Pancreatic Cancer

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Abstract

MicroRNAs (miRNAs) are small and non-coding RNA that has been shown to be involved in multiple cellular processes including cell proliferation, apoptosis, survival, invasion, metastasis, and chemotherapeutic resistance of pancreatic cancer. There is growing body of evidence showing the dysregulation of some miRNA in PDAC, suggesting their values as prognostic and predictive biomarker. In particular it has been shown that miR-486-5p and miR-938 can discriminate PDAC patients from healthy individuals and those with chronic pancreatitis. Moreover, they suggested the diagnostic value of miR-486-5p for the detection of PDAC patients, compared to CA 19-9. In turn several miRNAs have emerged as therapeutic targets, including let-7, miR-29a, miR-17-5p, miR-365, miR-181b, miR-21, miR-221 and miR-96, which have been reported to be involved in chemotherapy resistance. The current review focuses on recent advances with respect to the roles of miRNAs in PDAC as diagnostic and prognostic marker in PDAC.

Keywords: MicroRNAs; PDAC; Targets; Prognostic and predictive biomarker

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancers, and is in the fourth leading cause of cancer death [1,2]. PDAC is often diagnosed at late stages and is characterized by its aggressive phenotypes, high metastatic potential, and resistance to chemo-/radio-therapies. Therefore, there is greatly needed to identify new diagnostic and/or prognostic biomarkers to identify patients at earlier stages and improve the outcome of PDAC patients.

MicroRNAs are highly conserved, short noncoding, 17–25 nucleotide long RNA products that act as post-transcriptional regulators of gene expression through partial complementarity binding to messenger RNA, leading to mRNA degradation or inhibition of mRNA translation. Due to important role of miRNAs in tumorigenesis it has become a global challenge to use them as a diagnosis tools in pancreatic cancer [3]. It has been shown that microRNA are stable and detectable in blood samples, suggesting several studies have been conducted to assess the role of miRNAs in early diagnosis of PDAC, from which many have focused on miRNA expression in cell cultures and cancerous cell line and a few studies have been focused on circulating miRNAs that can be easily measured in blood samples without the need of biopsy. This review focuses on recent advances of miRNA research in PDAC and their potential value as diagnostic/prognostic marker in PDAC.

MicroRNAs biogenesis and function

Genes that encode miRNAs are much longer than the mature miRNA molecule. Some of the miRNAs genes reside in introns and share a same expression profile with their host genes. Other miRNA genes are transcribed from their own promoters. MiRNAs are initially transcribed by RNA polymerase II into a large RNA precursor, called pri-miRNA that is comprised of a 5' cap and a poly-A tail. Pri-miRNA is then processed into nucleus by a complex of RNase III enzyme, called Drosha, and Pasha/DGCR8 [4-6]. Pre-miRNAs export into cytoplasm by export in 5 and Ran (RAS-related nuclear protein)-GTP complex [7]. Pre-miRNA is further processed by Dicer, forming a double-stranded nucleotides, from which only one strand is integrated into RNA-induced silencing

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Received Date: 15 Jul 2016

Accepted Date: 17 Jul 2016

Published Date: 08 Sep 2016

Citation:

Ghasemi F, Shahid Sales S, Hassanian SM, Avan A. Prognostic and Predictive Values of MicroRNAs in Pancreatic Cancer. *Clin Oncol.* 2016; 1: 1077.

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complex (RISC). [8,9]. One strand of the ds-miRNA integrate into RISC complex and bind to 3' untranslated regions (UTRs) of their target mRNAs [10]. MiRNA gene inhibition can be described by three processes: cleavage; mRNA degradation; inhibition of translation. The first process is seldom used in mammals whereas the other 2 are more common, however the exact mechanism of action of miRNA is still debated [11]. Even though it was thought that miRNAs recognize 3'-UTR of their target mRNA, some studies suggest that they can also bind to 5'-UTR of mRNAs [12,13]. Additionally miRNAs are linked to translation upregulation upon growth arrest [14].

miRNAs can be detected by several methods, including microRNA cloning, northern blotting, real-time RT-PCR, microRNA arrays, and In situ hybridization (ISH) [15].

MicroRNAs as a diagnostic and prognosis marker in PDAC

PDAC has a dismal outcome because of the late onset of symptoms related to the disease. Recently many miRNAs are shown to have aberrant expressions in PDAC. In particular, Carlson and colleagues measured 847 miRNAs and found that only 7 of them (miR-375, 141, 548b-5p, 513a-3p, 222, 221 and 92a) were significantly dysregulated in pancreatic cancer patients in contrast to controls. They observed that miR-375, miR-141, miR-548b-5p and miR-513a-3p were upregulated and miR-222, miR-221 and miR-92a were downregulated mostly in chronic pancreatitis. Receiver operating characteristic (ROC) analysis showed that the area under curve (AUC) for CA19-9 was 0.85 and AUC for miR-375 was 0.72, suggesting that miR-375 was not superior to CA19-9 in case of PDAC diagnosis [16]. Another study suggested that the plasma level of miR-182 was significantly higher in PDAC patients, compared to chronic pancreatitis cases and healthy controls. This study showed that miR-182 with 64.1 % sensitivity and 82.6 specificity was more specific and sensitive than CA19-9. The combined use of CA19-9 and miR-182 had a sensitivity of 84.68 % and specificity of 86.77 % [17]. Ho et al. [18] reported that circulating miR-210 was stable as a hypoxia induced biomarker in plasma, and overexpressed in PDAC patients with unresectable T4 stage tumor [18]. Similarly Liu et al in 2012 evaluated the expression of some other microRNAs in newly diagnosed pancreatic cancer patients, control and patients with chronic pancreatitis (CP). They showed that the level of miR-16, miR-21, miR-155, miR-181a, miR-181b, miR-196a and miR-210 were significantly higher in cancer patients than both CP and healthy controls. It was also shown that the combination of miR-16, miR-196a and CA19-9 had more sensitivity and specificity for diagnosis of PDAC than CA19-9 alone. The sensitivity and specificity of the combination for discrimination of PDAC from normal group were 92.0% and 95.6% and for discrimination PDAC from CP were 88.4% and 96.3%, respectively. However this study showed no significant improvement in assessing the outcome using microRNAs alone or in combination with CA19-9. [19] Another study evaluated the expression of circulating miR-221 and miR-375 in Japanese patients. This study showed the higher level of miR-221 in plasma of PDAC patients compared to control. AUC for miR-221 alone was 0.743 whereas AUC for the ratio of miR-221/miR-375 was 0.762 [20]. Another study on seven miRNAs (miR-21, 155, 196a, 181a, 181b, 221, and 222) showed a significant association between miR-21 and PDAC, and capability of miR-21 to distinguish PDAC from chronic pancreatitis and healthy people [21]. Ho et al. [18] reported that miR-210 levels could be used as a prognostic tool to predict the behavior of pancreatic cancers, because of its value as a hypoxic marker and the fact that hypoxia makes the outcome of a malignancy dismal [18]. In

Chen et al. [19] study higher levels of miR-182 were associated with lower disease free survival and overall survival; its significance was shown by both a univariate and multivariate analysis, and function of miR-182 as a novel biomarker for prognosis of pancreatic cancer was implied [17]. Kawaguchi et al reported the association of plasma miR-221 with outcome of pancreatic cancer. Patients with higher miR-221 levels were more likely to develop distant metastasis (P value=0.0417) and non-resectable tumors (P value=0.0218). Surprisingly level of miR-221 could distinguish non-resectable patients from resectable patients in cases of borderline report of imaging studies [20].

Although miR-196a could only distinguish sick (PDAC and chronic pancreatitis) from healthy sera, it demonstrated a higher profile in patients with unresectable tumors; moreover higher levels of miR-196a was associated with lower median survival time (6.1 months) of PDAC patients and vice versa (low levels of miR196a predicted survival time of 12 months). (P value = 0.007) [21].

MicroRNAs as therapeutic target in PDAC

Pancreatic cancer treatment is mainly surgical, however few clinical trials suggested that patients could benefit from adjuvant chemotherapy with 5-fluorouracil (5-FU) or gemcitabine [22-24]. The main treatment for metastasizing PDAC is chemotherapy and PDAC is extremely resistant to chemotherapeutic agents; this resistance mainly occur because of increased drug efflux, DNA repair, alterations in drug target, apoptosis evasion or cell cycle regulation [25,26]. Aberrant expression of miRNAs affect many target mRNAs, therefore multiple proteins, resulting in emersion of potential resistance to certain drugs via various cellular processes. Higher levels of miR-21 in formalin-fixed paraffin embedded specimens of resected PDAC was associated with lower response to adjuvant chemotherapy with 5-fluorouracil following tumor resection and patients with lower levels of miR-21 expected lower overall survival time. Hwang et al. [27] also reported that use of anti-miR-21 in pancreatic cancer cells increased sensitivity to adjuvant therapy in vitro. Donahue et al. determined miR-21 in pancreatic tumor cells or cancer associated fibroblasts using in situ hybridization and concluded that higher level of miR-21 expression was associated with lower overall survival in patients on 5-FU [28]. miR-21 expression in cancer cell lines correlated with gemcitabine resistance. Moreover, Pre-miR-21 transfection into cancerous cells significantly decreased gemcitabine anti-proliferative effects and apoptosis induction and increased matrix metalloproteinase and vascular endothelial growth factor expression [29]. Data from Yan et al. research showed that pancreatic cancer cells transfected with miR-17-5p inhibitor, exhibited increased chemosensitivity to gemcitabine alongside growth inhibition, spontaneous apoptosis and higher caspase-3 activation [30]. Lower levels of miR-10b in cancer cells were associated with better response to gemcitabine-based chemo radiotherapy and overall survival. Patients expressing low levels of miR-10b in fine needle aspirate samples were predicted to have greater than 50% survival rate after 2 years, whereas 5-year survival rate of PDAC patients treated with surgery or adjuvant chemotherapy is less than 30% despite complete resection [31]. miR-141 also plays a role in chemosensitivity of pancreas cancer, as a study reported pancreatic cancer cells (PANC-1 cells) transfected with miR-141, exhibited higher sensitivity to both 5-FU and gemcitabine [32]. In an in vitro study done by Li et al miR-99a antagonism was transfected into pancreas cancer cell line (AsPC-1); decreased miR-99a expression resulted in higher cell proliferation, migration and invasion of AsPC-1 cells, therefore it was suggested that miR-99a may provide as an effective therapy in pancreatic

cancer [33]. Another study showed the role of miR-150 as a tumor suppressor in pancreatic cancer and studied the effect of nanoparticle delivery system of miR-150 on pancreatic cancer in vitro. The results shown lower growth, motility, clonogenicity and invasion upon delivery of miR-150 into cancer cells [34]. miR-146 is downregulated in pancreatic cancer cell lines, Li et al. [35] transfected cancer cells with miR-146 and reported inhibition of epithelial growth factor receptor (EGFR) and NFκB leading to decreased invasive capability and metastasis of cancer cells. Furthermore, pancreatic cancer cells were treated with 3,3'-diinodolymethane (DIM) or isoflavone, which increased miR-146a expression and inhibited pancreatic cancer cell invasion. [35] miR-96 targets KRAS oncogene and acts as a tumor suppressor in PDAC. In vitro and in vivo assays reported that miR-96 downregulated KRAS, ergo it decreased cancer cell invasion and migration and tumor growth, these findings suggest a novel therapeutic role for miR-96 in PDAC and other KRAS associated cancers [36].

Zhao et al. [32] showed that miR-141 is downregulated in pancreatic cancer and transfected cancer cell lines with miR-141, resulting in inhibition of cell proliferation, colony formation and invasion and induction of apoptosis. Interestingly, their *in vivo* study revealed that miR-141 can be used to suppress pancreatic xenograft tumor growth in nude mice [32].

Conclusions and Future Perspectives

Despite extensive efforts in diagnosis of PDAC patients at earlier, this malignancy is still remains as a major unsolved health problem with poor prognosis. Thus there is an urgent need to identify new prognostic and predictive biomarker. Recently microRNAs are emerged novel biomarker to prognostic the risk of developing or progression of this disease. Aberrant expressions of these markers have been reported, suggesting their values as an ideal diagnostic and prognostic marker. On the other hand, the restoration of the function of miRNAs, which act as tumor suppressor or downregulating the expression of miRNAs that act as oncogene, could provide a valuable therapeutic option for treatment of PDAC. Furthermore, since it has been shown that this small non coding RNAs can be circulated in body fluid, they might be used as an ideal none invasive biomarkers for early detection of this malignancy. However, further studies are warranted to explore the clinical utility of this marker in large and multicenter setting as prognostic, predictive and therapeutic target in PDAC.

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