MicroRNA in pancreatic cancer

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MicroRNAs, (miRNAs, miRs) represent a biologically important class of small non-coding RNAs closely associated with the post-transcriptional control of gene expression. miRNAs are single-stranded molecules containing approximately 18-24 nucleotides, and are responsible for regulating the expression of nearly 60% of all protein-coding genes. Pancreatic cancer is an aggressive tumor, diagnosed at late stage. It is characterized by a high mortality rate and limited treatment strategy. This makes microRNAs a very useful biomarker, prognostic factor and target for gene therapies.

microRNA

microRNAs are involved in the epigenetic regulation of the amount of protein synthesised in cells. The synthesis of miRNAs takes place in the cell nucleus. It is estimated that up to 44% of these molecules are formed during the splicing process from spliced introns, 7% from protein-coding exons and about 6% from 3' UTR or 5' UTR fragments of protein-coding genes. About 42% of miRs are derived from sequences of independent genes coding only miRNAs.

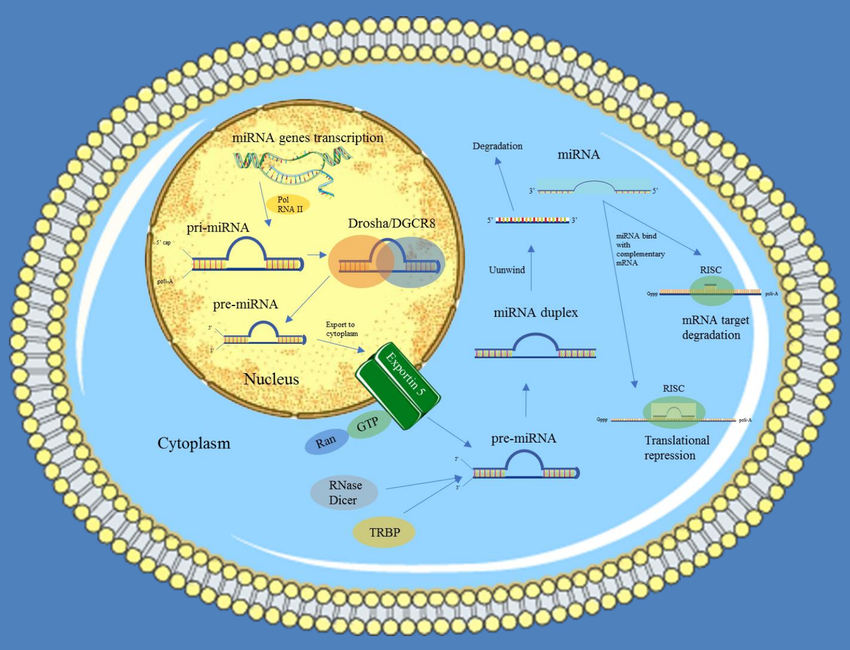

Fig.1 Synthesis of miRNA. [4] The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license

More than 2,500 different miRNAs have already been recognised in humans. A schematic of miRNA synthesis is shown in Figure 1. [1-3] MicroRNAs can be transported actively into the extracellular space and into the blood in exosomes due to their small size. Quantitative changes or the appearance of miRs that are physiologically absent in a given tissue can be detected in all diseases. Dysregulation of miRNAs is implicated in defective insulin secretion, glucose secretion, diabetic nephritis and heart disease. miRNAs are subject to transcriptional regulation depending on intracellular and extracellular signals. Thus, it is possible that they play a role in modulating signalling pathways, translating into specific effectors such as changes in metabolism, cell cycle, proliferation or cell death. Preliminarily identified signalling pathways dysregulated in pancreatic cancer, based on the literature, include: KRAS, PI3K/AKT, JAK/STAT, WNT/B-Catenin, TGF-B. [2-5]

Pancreatic cancer

Pancreatic cancer is one of the most aggressive cancers. The number of new pancreatic cancer cases in the European Union (including the UK) was 59,000 cases in 1990, 109,000 cases in 2019 and projected to be 147,000 in 2039. No gender differences are observed in incidence and mortality. These cancers show a similar age-related trend, with a gradual increase after 30 years of age, reaching the highest burden after 80 years of age. The epidemiological burden is related to socio-demographic status. The highest burden of pancreatic cancer is observed in the Asia-Pacific region, while the lowest in the Middle East and North Africa. The high mortality rate of cancer is caused, among other things, by late diagnosis; patients often do not show any symptoms until they develop an advanced stage of the disease. Approximately 90% of pancreatic cancers are PDAC (Pancreatic Ductal Adenocarcinoma), classified as exocrine tumors.

It is characterized by high invasive and metastases to lymph nodes, liver, lungs and intestines. The 5-year survival of patients with PDAC is 3-6%. Tumors derived from endocrine cells are rare and are called neuroendocrine tumors. [6-8]

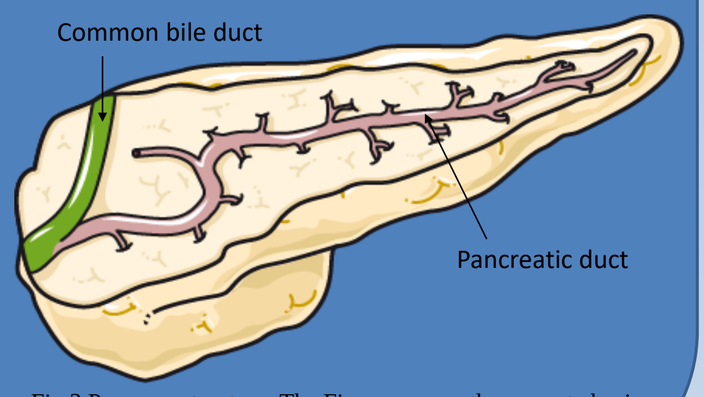

Fig.3 Pancreas structure. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license

Role of microRNAs in pancreatic cancer

It was shown that a large number of miRNA molecules were variably expressed in patients with PDAC/acute pancreatitis compared to healthy controls. Changes in miR profile also appeared in the blood of patients (Table 1). The most frequent recurring relationship between the different studies was an increase in miR-21, miR-155 and miR-221 expression, while miR-34 and miR-145 were most frequently down-regulated. During the cell cycle and proliferation, miRNA plays a role in the regulation of suppressor genes (by downregulating their expression) and oncogenes (by overexpressing them) leading to uncontrolled cell proliferation (Table 2) MiR also influences the control of Cyclin-Dependent Kinases (CDKs), cyclins, p27 protein controlling cell cycle progression. MicroRNA is involved in the induction and inhibition of apoptosis, which is important in the gene acquisition to avoid apoptosis in cancer cells (Table 3). That includes controls the apoptosis activation pathway dependent on the p53 and the Bcl-2 family of proteins (Figure 2). MiRs are also involved in tumour progression to mesenchyme cells and subsequent further invasion and migration to distant organs. [2-5]

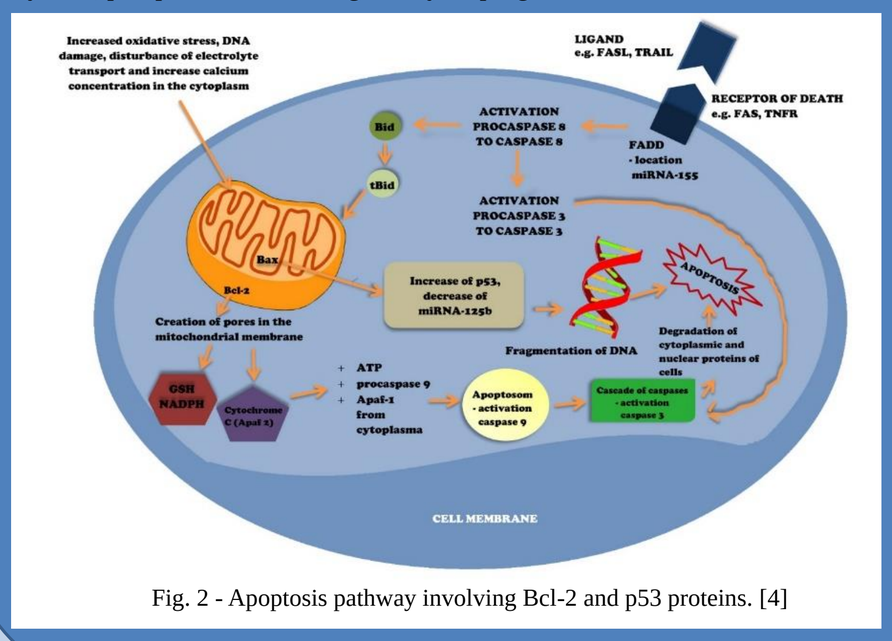

Fig. 2 - Apoptosis pathway involving Bcl-2 and p53 proteins. [4]

Table 1 - Expression of various miRNAs in PDAC tissue compared to healthy pancreatic tissue and PDAC patients blood [1-5]

Sample Type	miRNA's	Expression
PDAC tissues	miR-196, miR-200a, miR-27a, miR-21, miR-222, miR-210, miR-221, miR-155, miR-212.	↑
	miR-200, miR-96, miR-21, miR-146, miR-245, miR-122, miR-31, miR-34, miR-145.	↓
Blood of PDAC patients	miR-18a, miR-21, miR-22, miR-24, miR-25, miR-99a, miR-155, miR-185, miR-191, miR-196a, miR-642b, miR-885-5p	↑
Serum of PDAC patients	miR-2	↑
Serum of Acute pancreatitis patients	miR-7, miR-9, miR-122, miR-14	↑
PDAC migration and invasion tissues	miR-10a, miR-100, miR-34b, miR-21, miR-4295	↑
	miR-194, miR-429, miR-200b, miR-200c, miR-143, miR-126, miR-146a, miR-31, Let-7	↓

Table 2 - Role of miRNA's as control of cell cycle and proliferation in pancreatic cancer [1-5]

miRNA	Expression	Target/Effect
miR-21	↑	Reduces the expression of PTEN (tumour suppressor gene, inhibits proliferation and controls the number of cell cycle divisions) at mRNA level
miR-424-5p	↑	Downregulation of SOCS6 expression. This leads to increased ERK pathway activity and an increase in cell proliferation and migration.
miR-124	↓	Negatively regulates the expression of the Rac1 oncogene (MKK4-JNK-C-JUN pathway, responsible for inhibiting tumour cell proliferation). Poor prognostic factor.
miR-203	↓	Induces cells into the G1 phase of the cell cycle, resulting in increased proliferation
miR-27a	↑	Negatively regulates the expression of the Spry 2 suppressor gene
miR-143, miR-126	↑/↓	Regulation of KRAS pathway expression. Causes abnormal proliferation
miR-26a	↑/↓	Regulation of Cyclin E2 expression, associated with the cell's transition from G1 to S phase of the cell cycle

Table 3 - Role of miRNA's as control of apoptosis in pancreatic cancer [1-5]

miRNA	Expression	Target/Effect
miR-34a	↓	Regulation of Bcl-2, Notch 1, Notch 2 protein expression to avoid apoptosis
miR-155	↑	Suppression of the proapoptotic protein p53 (TP53INP1 gene)
miR-203	↓	Overexpression of Survivin, a proteins responsible for inhibition of apoptosis
miR-23a	↑/↓	This miR promotes apoptosis by regulating the factor APAF1, which, following a cascade of cytochrome C-related signals, triggers apoptosis
miR-150 miR-603	↑/↓	Decreased expression of IGF-1R, which inhibits apoptosis
miR-196a miR-214	↑/↓	Act indirectly on TP53 by controlling the expression of ING4 and ING5
miR-24	↑/↓	Regulation of B1M expression, related to the BCL-2 family of proteins

Materials and methods

miRNA expression will be tested in PANC-1 cell line as in vitro model. This expression will be tested in comparison to cultured human fibroblasts as a control group. Sample for in vivo miRNA expression studies will be intraoperatively collected pancreatic tumour tissue and whole blood from pancreatic cancer patients. The miR expression results from the blood will be compared with a control group of healthy patients. The next step will be to isolate total RNA and miRNA and transcribe the RNA into cDNA using a reverse transcription reaction. Following this step, I will perform miRNA screening spliced pancreatic cancer tissues collected from patients and blood compare to healthy group of patietns. Screening also will be perform in in PANC-1 and fibroblast cell line. Screening will be done using miRNA expression cards (TaqMan™ Advanced miRNA Human A and B Cards, Applied Biosystems). Among the microRNA's with the highest expression variability according to the screening cards results and based on the literature, we will select those to be used in further studies. Based on the variability of miRNA expression, signalling pathways will be tested by determining the amount of mRNAs encoding the specific proteins of the signalling pathway. Quantitative expression of miRNAs and mRNAs will be investigated by Real-Time PCR.

Summary

Gene expression profiling of miRNAs has resulted in a developing understanding of cancer biology and cancer-related signaling pathways. Currently, most clinical trials use miRNAs as diagnostic biomarkers to assess tumor stage and predictive markers of overall survival time. This is especially important in pancreatic cancer where the only currently used diagnostic marker is CA 19-9, with low sensitivity and specificity. Combined with the therapeutic potential of miR, it is a promising path forward in the diagnosis and treatment of pancreatic cancer.

Bibliography

1. Lopez-Camarillo, Marchat L.A., Arechaga-Ocampo E., Perez-Plasencia C., et.al. MetastamiRs: Non-Coding MicroRNAs Driving Cancer Invasion and Metastasis. International Journal of Molecular Science 2012;13:e1347-e1379. doi: https://doi.org/10.3390/ijms13021347
2. Keiichi Yonemori, Hiroshi Kurahara, Kosei Maemura and Shoji Natsugoe; MicroRNA in pancreatic cancer. Journal of Human Genetics (2017) 62, 33–40; doi:10.1038/jhg.2016.59; published online 2 June 2016
3. Jin Wang, Jinyun Chen, Ping Chang, Aimee LeBlanc, Donghui Li, James L. Abbruzzesse, Marsha L. Frazier, Ann M. Killary and Subrata Sen; MicroRNAs in Plasma of Pancreatic Ductal Adenocarcinoma Patients as Novel Blood-Based Biomarkers of Disease. American Association for Cancer Research. doi:10.1158/1940-6207.CAPR-09-0094
4. Satyanarayana Rachagani , Sushil Kumar, Surinder K. Batra; MicroRNA in pancreatic cancer; Pathological, diagnostic and therapeutic implications. Cancer Letters 292 (2010) 8–16 doi:10.1016/j.canlet.2009.11.010
5. Rawat M, Kadian K, Gupta Y, Kumar A, Chain PSG, Kovbasnjuk O, Kumar S, Parasher G. MicroRNA in Pancreatic Cancer: From Biology to Therapeutic Potential. Genes (Basel). 2019 Sep 25;10(10):752. doi: 10.3390/genes10100752. PMID: 31557962; PMCID: PMC6827136.
6. Giuseppe Lippi , Camilla Mattiuzzi; The global burden of pancreatic cancer. Clinical research Gastroenterology. Arch Med Sci 2020; 16 (4): 820–824 doi: https://doi.org/10.5114/aoms.2020.94845
7. Jingru Yu, Xiaorong Yang, Wei He, Weimin Ye; Burden of pancreatic cancer along with attributable risk factors in Europe between 1990 and 2019, and projections until 2039. International Journal of Cancer Volume 149, Issue 5 p. 993-1001 CANCER EPIDEMIOLOGY. doi: https://doi.org/10.1002/ijc.33617
8. Biancur D.E., Kimmelman A.C. The plasticity of pancreatic cancer metabolism in tumor progression and therapeutic resistance. Biochim. Biophys. Acta Rev. Cancer. 2018;1870:67–75. doi: 10.1016/j.bbcan.2018.04.011.