Clin. Lab. 2017;63:XXX-XXX

REVIEW ARTICLE

Circulating microRNAs Expressions as Genetic Biomarkers in Pancreatic Cancer Patients Continuous Non-Invasive Monitoring

Mihai Sandesc [1], Anca Dinu [1], Alexandru F. Rogobete [1, 2], Ovidiu H. Bedreag [1, 2], Dorel Sandesc [1, 2], Marius Papurica [1, 2], Lavinia M. Bratu [3], Silvius Negoita [4], Corina Vernic [1], Sonia E. Popovici [1], Dan Corneci [4]

[1] *Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania*
[2] *Clinic of Anesthesia and Intensive Care, Emergency County Hospital "Pius Brinzeu", Timisoara, Romania*
[3] *Faculty of Pharmacy, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania*
[4] *Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

SUMMARY

Background: **Pancreatic cancer is one of the most important causes of death worldwide. The main cause is late detection. Also, an important factor playing a role in altering the clinical status of these patients is the lack of methods for the evaluation of therapeutic response. A marker that can be useful, both in early diagnosis and in evaluating and monitoring non-invasive treatment response, is analyzing the expression of miRNAs. In this paper, we summarize genetic and epigenetic aspects of miRNAs in pancreatic cancer. Moreover, we want to emphasize potential miRNAs expressions that can be used as biomarkers for the management of patients with pancreatic cancer.**

Methods: **Studies available in scientific databases, such as PubMed and Scopus, were analyzed for conducting the present study. The keywords "miRNAs expression", "pancreatic cancer", and "genetic biomarkers" were used in the search engine.**

Results: **Following the searches, 187 primary scientific articles were analyzed. After rigorous analysis 40 articles were selected for the study. A high percentage of papers highlight the importance of using microRNAs as modern, non-invasive, and accurate biomarkers, designed for the early diagnosis and continuous monitoring of both the clinical outcome and treatment response of the patient.**

Conclusions: **The expression of miRNAs can be successfully used for the evaluation and non-invasive monitoring of patients with pancreatic cancer.**

(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170608)

Correspondence:
Alexandru Florin Rogobete, MSc, PhDs
Clinical Researcher
Faculty of Medicine
"Victor Babes" University of
Medicine and Pharmacy
Pta Eftimie Murgu Nr. 2
Timisoara
Romania
Email: alexandru.rogoebete@umft.ro

Review Article accepted June 21, 2017

KEY WORDS

miRNA expression, genetic biomarkers, pancreatic cancer, epigenetic miRNAs

INTRODUCTION

Pancreatic cancer has a high mortality rate, especially because it is diagnosed at late stage. Another important aspect regarding the high mortality rates among patients with pancreatic cancer is the resistance to radiotherapy and chemotherapy. In terms of pathology, the most common form of pancreatic cancer arises from the cells

of the exocrine pancreas and is known as pancreatic ductal adenocarcinoma (PDAC). According to statistics, the incidence of this subtype is over 90%. The American Cancer Society (www.cancer.org) reported an estimated 48960 new cases of pancreatic cancer in 2015. Moreover, it reports a mortality rate of over 40,000 cases [1]. Regarding biochemical and metabolic pathways of developing pancreatic cancer, a number of features were highlighted. One of the most important is given by the high rate of glucose intake. It also highlights an increased rate of glycolysis called the Warburg effect. Compared with healthy pancreatic cells, the cancer cells show an increased expression of amino acid glutamine. Moreover, PDAC is characterized by increased expression of autophagy processes which alter a number of biochemical and genetic mechanisms, leading to the augmentation of cancer cells' proliferation. One of the most discussed genetic mutations in pancreatic cancer is represented by the KRAS mutation. This has direct implications on lipid metabolism, studies being focused on the metabolic disaster. Recent studies have revealed a significant increase in the expression of enzymes responsible for lipogenesis and lipolysis. In terms of the biochemical mechanism, the focus is on the increased expression of the enzyme fatty acid synthase (FASN). Moreover, the citric acid cycle and acetyl-CoA alterations also come into discussion in relation to these mechanisms [1].

Regarding prognosis, the increased rate of mortality is due mainly to late detection of the disease. Furthermore, in the case of these diagnosed patients the low accuracy of monitoring and treatment methods also come into question. Thus, numerous studies were carried out related to the evaluation of patients with pancreatic cancer, the expression of miRNAs being one of the most promising methods.

In this updated paper, we present significant differences of miRNAs that are specific for pancreatic cancer. Moreover, we summarize a range of miRNA species that can be used as biomarkers for future evaluation and monitoring of patients with pancreatic cancer.

Review of changes in miRNA expressions in pancreatic cancer

miRNAs are species whose genetic synthesis begins in the cell nucleus (Figure 1) [2]. Under different circumstances, they are excreted from the cell in various forms, being detectable in most body fluids.

Hao et al. showed changes in miRNA-483-3p expression in pancreatic cancer cells. Moreover, they showed strong correlations between the expression of miRNA-483-3p and the activity of the genetic mutation DPC4/SMAD4 [3].

Mucin MUC1 presents an increased expression in most epithelial cancers. Studies have shown a significant increase in MUC1 expression in the case of PDAC. MUC1 also interacts with several other biochemical and genetic systems, the clinical course of these patients being significantly altered. Trehoux et al. revealed a change in the functionality of miRNA-29a and miRNA-330-5p regarding interaction with MUC1. They also reported that MUC1 is responsible for indirectly modulating the expression of miRNA-183, miRNA-200a, miRNA-876-3p, and miRNA-939 [4].

A similar study conducted by Zhang et al. showed an increase in expression of miRNA-160a, miRNA-190, miRNA-186, miRNA-221, miRNA-222, miRNA-200b, miRNA-15b, and miRNA-95 in pancreatic cancer [5]. Habbe et al. also highlights an increased expression of miRNA-155 [6] and Lee et al. an important decrease in the expression of miRNA-345, miRNA-142-p, and miRNA-139 [7].

In a similar study, Roldo et al. identified a significant increase in the expressions of miRNA-17, miRNA-20, miRNA-92-1, miRNA-99a, miRNA-99b, miRNA-100, miRNA-103, miRNA-107, miRNA-125a, miRNA-125b-1, miRNA-125b-2, miRNA-130a, miRNA-132, miRNA-204, miRNA-211, and miRNA-342 [8]. Volinia et al. also highlights an increased expression of miRNA-21, miRNA-17-5p, miRNA-191, miRNA-29b-2, miRNA-223, miRNA-128b, miRNA-199a-1, miRNA-24-1, miRNA-24-2, miRNA-146, miRNA-17-5p, miRNA-191, miRNA-29b-2, miRNA-223, miRNA-128b, miRNA-199a-1, miRNA-24-1, and miRNA-24-2 [9].

Another form of pancreatic cancer is represented by the intraductal papillary mucinous neoplasm (IPMN). Current diagnosis methods for IPMN do not allow early detection, the incidence of pancreatic cancer being the highest in this case. Wang et al. conducted a study on the expression of miRNAs in the case of IPMN. They showed increased expression for miRNA-138, miRNA-195, miRNA-204, miRNA-216a, miRNA-217, miRNA-218, miRNA-802, miRNA-155, miRNA-214, miRNA-26, miRNA-30b, miRNA-31, and miRNA-125, and a decreasing the expression of miRNA-451a and miRNA-428b. Moreover, they found strong correlations between the incidence of IPMN and KRAS as well as DPC4/SMAD4 and TP53 mutations [10]. A similar study was conducted by Yang et al., who reported aberrant modifications in the expression of miRNA-940. They also showed strong correlations between the altered expression of miRNA-940 and GSK3β and the SFRP1 genetic mutations [11]. The main cause of death in patients with pancreatic cancer is represented by a poor response to treatment. Dhaya et al. reported statistical correlations regarding miRNA-21, miRNA-99a, miRNA-100, and miRNA-210 expressions and poor response to chemotherapy. These results can therefore be useful in predicting response to chemotherapy, as well as in optimizing therapy for each individual patient [12]. Zhao et al., in a similar study, showed a decrease in the expression of miRNA-217 in pancreatic cancer. They also showed a strong correlation between altered miRNA-217 expression and the KRAS genetic mutation. Alterations of miRNA-217 expression were also highlighted through studies conducted by Yu et al. [13].

Strong correlations between the downregulation of

Table 1. miRNA expressions in pancreatic cancer.

miRNA	Observations	Expression modification	References
miRNA-18a	Blood sampling	Increased expression	[33]
miRNA-223	Blood sampling	Increased expression	[33]
miRNA-148a	Tissue sampling; Strong statistical association with PDAC	Decreased expression	[34]
miRNA-127	Tissue sampling; Strong statistical association with PDAC	Decreased expression	[34]
miRNA-21	Tissue sampling; Strong statistical association with PDAC	Increased expression	[34,35]
miRNA-10b	Tissue sampling; Strong statistical association with PDAC	Increased expression	[34]
miRNA-210	Blood sample	Increased expression	[35]
miRNA-155	Blood sample	Increased expression	[35]
miRNA-196	Blood sample	Increased expression	[35]
miRNA-203	Poor prognosis	Increased expression	[36]
miRNA-1914	Poor prognosis	Increased expression	[37]
miRNA-1274a	Poor prognosis	Increased expression	[37]
miRNA-1249	Poor prognosis	Increased expression	[37]
miRNA-1207-3p	Poor prognosis	Increased expression	[37]
miRNA-466	Poor prognosis	Increased expression	[37]
miRNA-1290	Poor prognosis	Increased expression	[37]
miRNA-31	Poor prognosis	Increased expression	[37]
miRNA-218	Associated with high risk of recurrence	Decreased expression	[37]
miRNA-148a	Associated with high risk of recurrence	Decreased expression	[38]
miRNA-187	Associated with high risk of recurrence	Decreased expression	[38]
miRNA-192	Increased expression in patients with pancreatic ductal adenocarcinoma in contrast to healthy patients	Increased expression	[39]
miRNA-196	Increased expression in patients with pancreatic ductal adenocarcinoma in contrast to healthy patients	Increased expression	[39]
miRNA-200	Increased expression in patients with pancreatic ductal adenocarcinoma in contrast to healthy patients	Increased expression	[39]
miRNA-30	Increased expression in patients with pancreatic ductal adenocarcinoma in contrast to healthy patients	Increased expression	[39]
miRNA-423	Increased expression in patients with pancreatic ductal adenocarcinoma in contrast to healthy patients	Increased expression	[39]
miRNA-222	Poor outcome	Increased expression	[40]
miRNA-31	Poor outcome	Increased expression	[40]
miRNA-130b	Poor outcome	Decreased expression	[40]
miRNA-217	Poor outcome	Decreased expression	[40]
miRNA-375	Poor outcome	Decreased expression	[40]

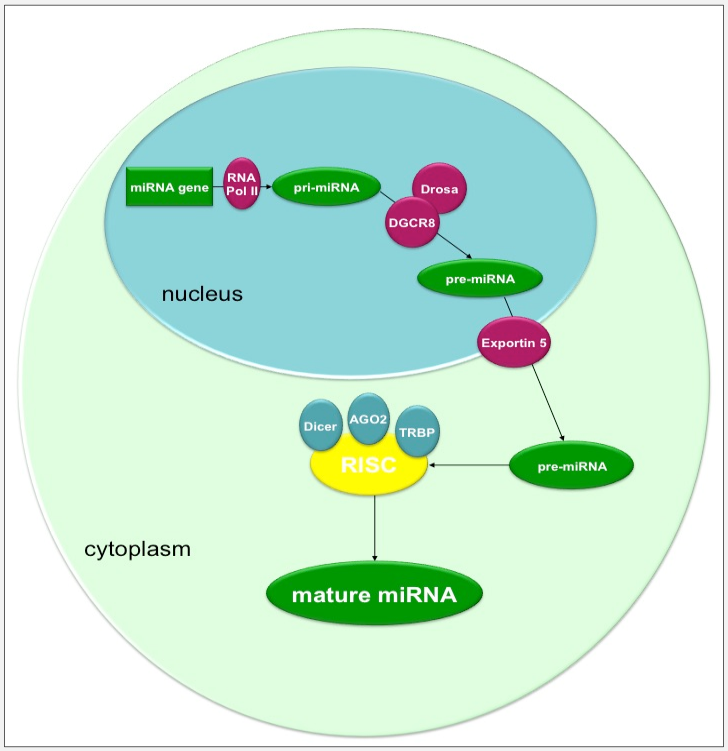

Figure 1. Biogenesis mechanism for microRNAs (miRNAs).

The synthesis of microRNAs begins in the nucleus with the action of RNA polymerase II on protein-coding. This forms a first species, called pri-microRNA. Through successive reactions of polyadenylation, catalyzed by DGCR8 and Drosa, the precursor for the microRNA species, called pre-microRNA is obtained. pre-microRNA thus formed is transported into the cytoplasm through Exportin 5. In the cytoplasm, the Dicer complex, TRBP, and AGO2 act on the pre-microRNA, forming double stranded mature microRNA (19 - 24 nucleotides). The Figure was reproduced according to Papurica et al. [2].

miRNA-126 and the KRAS genetic mutations were proven by Frampton et al. as well. Last but not least, they showed a statistical correlation between the CRK and ADAM9 mutations and the altered miRNA-126 [14]. A similar study was conducted by Kent et al., who reported strong correlations between the KRAS genetic mutation and the altered expression of miRNA-143 and miRNA-145 [15].

Ma et al. reported an increase in the expression of miRNA-21 and miRNA-31, as well as a decrease in the expression of miRNA-375 in patients with pancreatic cancer [16].

Regarding the statistical correlation between miRNA expressions and patient prognosis in case of pancreatic cancer, Greither et al. highlighted statistically significant correlations between decreased expression of miRNA-155 ($p = 0.005$), miRNA-203 ($p = 0.017$), miRNA-210 ($p = 0.005$), and miRNA-222 ($p = 0.035$) and poor outcome [17]. Regarding the mechanisms involved in oncogenes' proliferation, numerous studies have reported several changes in the expression and functionality of an important number of miRNAs. Li et al., in a study on the expression of miRNAs regarding neoplastic proliferation mechanisms, revealed significant implications for miRNA-146a [18]. Another study revealed an increased expression of miRNA-16, miRNA-21, miRNA-24, miRNA-26, and let-7 family and a decrease in the expression of miRNA-29c, miRNA-30a-3p, miRNA-96, miRNA-130b, miRNA-141, miRNA-148a, miRNA-148b, miRNA-216, miRNA-217, miRNA-375 and miRNA-494, and an increase in the expression of miRNA-31, miRNA-143, miRNA-145, miRNA-146a, miRNA-150, miRNA-155, miRNA-196, miRNA-196b, miRNA-210, miRNA-222, and miRNA-223 [19–22]. A similar study was conducted by Zhang et al., which showed an increased methylation for miRNA-124-1, miRNA-124-2, and miRNA-124-3 in patients with PDAC [5]. Ovyang et al. also showed an increase in the expression of miRNA-10b in case of neoplastic pancreatic processes. They reveal a

strong link with the fat-interacting protein 30 (TIP30) [23]. Wang et al. also showed that miRNA-182 presents an increased expression in patients with pancreatic cancer [24]. Yuan et al., in a similar study, showed an important modification for the expression of miRNA-20, miRNA-21, miRNA-25, miRNA-155, miRNA-196a, and miRNA-210. Numerous studies have shown an increased concentration of CA19-9 protein in patients with pancreatic cancer. Moreover, the expression of MIC-1/GDF15 is part of the family transforming growth factor beta (TGF-β) and reported significant increases in cases of pancreatic cancer. Yuan et al. showed significant changes regarding the genetic profile for macrophage inhibitory cytokine-1 (MIC-1) and protein CA19-9 [25].

Another epigenetic marker that can bring important answers on the evolution of patients with pancreatic cancer is miRNA-183-5p. Miao et al. conducted a study on epigenetic changes in patients with pancreatic cancer. After analyzing it through quantitative polymerase chain reaction (qPCR) they reported an increase for miRNA-183-5p. They also revealed implications of the inhibition suppressor of cytokine signaling 6 (SOCS-6) [26].

One of the most important features of using miRNAs as biomarkers are represented by the fact that they are non-invasive and can be identified in various biological fluids. Recent studies have identified altered miRNA expressions in urine samples from patients with cancer. Debernardi et al. conducted a study on the expression of miRNAs in this type of bodily fluid. In the study they showed an increase in the expression of miRNA-143, miRNA-223, miRNA-204, and miRNA-30e that did not appear in healthy patients [27].

Saliva is another fluid where different expressions of miRNAs have been identified. Xie et al. showed changes in the expression of miRNA-433-5p, miRNA-4665-3p, miRNA-940, miRNA-1273g-3p, miRNA-3676-5p, miRNA-3679-5p, miRNA-3940-5p, miRNA-4327, miRNA-4442, and miRNA-5100 in saliva from patients with pancreatic cancer [28]. A similar study regarding miRNA expressions in saliva was performed by Humean et al., who reported an up-regulation for miRNA-21, miRNA-23a, miRNA-23b, and miRNA-29c. Moreover, they reported alterations for miRNA-210 and let-7c in patients with pancreatitis [29].

An important biochemical factor in the proliferation and poor outcome of patients with pancreatic cancer is chronic inflammation. Thus, the carcinogenic mechanisms and biochemical pathways of pro-inflammatory cytokines play an essential role in altering the clinical status of these patients. An important factor involved in the modulation of pro-inflammatory pathways is represented by nuclear transcription factor kappa B (NF-kB). Numerous studies have highlighted a number of statistically valid and strong links between NF-kB activity and the altered expression of miRNAs [20]. He et al. studied the expression of miRNA-371-5p used for qRT-PCR in patients with pancreatic cancer. They reported a strong correlation between altered miRNA-371-5p expression and poor outcome [30].

Namjung et al. conducted a study regarding miRNA expressions in pancreatic ductal adenocarcinoma cancer, reporting significant changes for miRNA-106b-star, miRNA-324-3p, miRNA-615, miRNA-324, miRNA-145-5, miRNA-263-5p, and miRNA-574-3p. Moreover, alterations regarding expression of miRNAs and COX2 pathway were also highlighted [31].

CONCLUSION

Patients with pancreatic cancer have high mortality rates, mainly due to the late detection of the disease. Another important cause contributing to reduced survival rates is the absence of accurate markers able to assess the response to the treatment. Using miRNA expressions as biomarkers for the detection and evaluation of patients with pancreatic cancer can significantly reduce mortality rates. Thus, we can state that miRNAs can be considered biomarkers of the future in terms of management of patients with pancreatic cancer.

Declaration of Interest:
Nothing to declare.

References:

1. Falasca M, Kim M, Casari I. Pancreatic cancer: Current research and future directions. Biochim Biophys Acta 2016;1865(2):123-32 (PMID: 26794394).

2. Papurica M, Rogobete AF, Sandesc D, et al. Redox Changes Induced by General Anesthesia in Critically Ill Patients with Multiple Traumas. Mol Biol Int 2015;2015:238586 (PMID: 26693352).

3. Hao J, Zhang S, Zhou Y, Hu X, Shao C. MicroRNA 483-3p suppresses the expression of DPC4/Smad4 in pancreatic cancer. FEBS Lett 2011;585(1):207-13 (PMID: 21112326).

4. Tréhoux S, Lahdaoui F, Delpu Y, et al. Micro-RNAs miR-29a and miR-330-5p function as tumor suppressors by targeting the MUC1 mucin in pancreatic cancer cells. Biochim Biophys Acta 2015;1853(10):2392-403 (PMID: 26036346).

5. Zhang Y, Li M, Wang H, et al. Profiling of 95 MicroRNAs in Pancreatic Cancer Cell Lines and Surgical Specimens by Real-Time PCR Analysis. World J Surg 2008;33(4):698-709 (PMID: 19030927).

6. Habbe N, Koorstra JB, Mendell JT, et al. MicroRNA miR-155 is a biomarker of early pancreatic neoplasia. Cancer Biol Ther 2009;8(4):340-6 (PMID: 19106647).

7. Lee EJ, Gusev Y, Jiang J, et al. Expression profiling identifies microRNA signature in pancreatic cancer. Int J Cancer 2009;120 (5):1046–54 (PMID: 17149698).

8. Roldo C, Missiaglia E, Hagan JP, et al. MicroRNA expression abnormalities in pancreatic endocrine and acinar tumors are associated with distinctive pathologic features and clinical behavior. J Clin Oncol 2006;24(29):4677-84 (PMID: 16966691).

9. Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006;103(7):2257-61 (PMID: 16461460).

10. Wang J, Paris PL, Chen J, et al. Next generation sequencing of pancreatic cyst fluid microRNAs from low grade-benign and high grade-invasive lesions. Cancer Lett 2015;356(2):404-9 (PMID: 25304377).

11. Yang H, Liu G, Liu Y, et al. Over-expression of microRNA-940 promotes cell proliferation by targeting GSK3β and sFRP1 in human pancreatic carcinoma. Biomed Pharmacother 2016;83:593-601 (PMID: 27459115).

12. Dhayat SA, Abdeen B, Köhler G, Senninger N, Haier J, Mardin WA. MicroRNA-100 and microRNA-21 as markers of survival and chemotherapy response in pancreatic ductal adenocarcinoma UICC stage II. Clin Epigenetics 2015;7:132 (PMID: 26705427).

13. Yu Y, Liu L, Ma R, Gong H, Xu P, Wang C. MicroRNA-127 is aberrantly downregulated and acted as a functional tumor suppressor in human pancreatic cancer. Tumor Biol 2016;37(10): 14249-57 (PMID: 27571739).

14. Frampton AE, Krell J, Jamieson NB, et al. MicroRNAs with prognostic significance in pancreatic ductal adenocarcinoma: A meta-analysis. Eur J Cancer 2015;51(11):1389-404 (PMID: 260 02251).

15. Kent OA, Fox-Talbot K, Halushka MK. RREB1 repressed miR-143/145 modulates KRAS signaling through downregulation of multiple targets. Oncogene 2013;32(20):2576-85 (PMID: 22751 122).

16. Ma MZ, Kong X, Weng MZ, et al. Candidate microRNA biomarkers of pancreatic ductal adenocarcinoma: meta-analysis, experimental validation and clinical significance. J Exp Clin Cancer Res 2013;32(1):71 (PMID: 24289824).

17. Greither T, Grochola LF, Udelnow A, Lautenschläger C, Würl P, Taubert H. Elevated expression of microRNAs 155, 203, 210 and 222 in pancreatic tumors is associated with poorer survival. Int J Cancer 2010;126(1):73-80 (PMID: 19551852).

18. Li Y, Vandenboom TG 2nd, Wang Z, et al. miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res 2010;70(4):1486-95 (PMID: 20124483).

19. Rachagani S, Kumar S, Batra SK. MicroRNA in pancreatic cancer: Pathological, diagnostic and therapeutic implications. Cancer Lett 2010;292(1):8-16 (PMID: 20004512).

20. Nagaraju GP, Madanraj AS, Aliya S, et al. MicroRNAs as biomarkers and prospective therapeutic targets in colon and pancreatic cancers. Tumor Biol 2016;37(1):97-104 (PMID: 26537581).

21. Ho AS, Huang X, Cao H, et al. Circulating miR-210 as a Novel Hypoxia Marker in Pancreatic Cancer. Transl Oncol 2010;3(2): 109-13 (PMID: 20360935).

22. Gisel A, Valvano M, El Idrissi IG, et al. MiRNAs for the detection of multidrug resistance: Overview and perspectives. Molecules 2014;19(5):5611-23 (PMID: 24786846).

23. Ouyang H, Gore J, Deitz S, Korc M. microRNA-10b enhances pancreatic cancer cell invasion by suppressing TIP30 expression and promoting EGF and TGF-β actions. Oncogene 2014;33(38): 4664-74 (PMID: 24096486).

24. Wang S, Ji J, Song J, et al. MicroRNA-182 promotes pancreatic cancer cell proliferation and migration by targeting β-TrCP2. Acta Biochim Biophys Sin (Shanghai) 2016;48(12):1085-93 (PMID: 27797718).

25. Yuan W, Tang W, Xie Y, Wang S, Chen Y, Qi J. New combined microRNA and protein plasmatic biomarker panel for pancreatic cancer. Oncotarget 2016;7(48): 80033-45 (PMID: 27713117).

26. Miao F, Zhu J, Chen Y, Tang N, Wang X, Li X. MicroRNA-183-5p promotes the proliferation, invasion and metastasis of human pancreatic adenocarcinoma cells. Oncol Lett 2016;11(1):134-40 (PMCID: PMC4726923).

27. Debernardi S, Massat NJ, Radon TP, et al. Noninvasive urinary miRNA biomarkers for early detection of pancreatic adenocarcinoma. Am J Cancer Res 2015;5(11):3455-66 (PMID: 26807325).

28. Xie Z, Yin X, Gong B, et al. Salivary microRNAs show potential as a noninvasive biomarker for detecting resectable pancreatic cancer. Cancer Prev Res (Phila) 2015;8(2):165-73 (PMID: 25538 087).

29. Humeau M, Vignolle-Vidoni A, Sicard F, et al. Salivary microRNA in pancreatic cancer patients. PLoS One 2015;10(6):e0130 996 (PMID: 26121640).

30. He D, Miao H, Xu Y, et al. MiR-371-5p facilitates pancreatic cancer cell proliferation and decreases patient survival. PLoS One 2014;9(11):e112930 (PMID: 25411783).

31. Namkung J, Kwon W, Choi Y, et al. Molecular subtypes of pancreatic cancer based on miRNA expression profiles have independent prognostic value. J Gastroenterol Hepatol 2016;31(6):1160-7 (PMID: 26644397).

32. David VL, Ercisli MF, Rogobete AF, et al. Early Prediction of Sepsis Incidence in Critically Ill Patients Using Specific Genetic Polymorphisms. Biochem Genet 2016 Epub 2016 Dec 9 DOI: 10. 1007/s10528-016-9785-2 (PMID: 27943002).

33. Tsujiura M, Ichikawa D, Komatsu S, et al. Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer 2010;102(7):1174-9 (PMID: 20234369).

34. Xue Y, Abou Tayoun AN, Abo KM, et al. MicroRNAs as diagnostic markers for pancreatic ductal adenocarcinoma and its precursor, pancreatic intraepithelial neoplasm. Cancer Genet 2016; 206(6):217-21 (PMID: 23933230).

35. Wang J, Chen J, Chang P, et al. MicroRNAs in plasma of pancreatic ductal adenocarcinoma patients as novel blood-based biomarkers of disease. Cancer Prev Res (Phila) 2009;2(9):807-13 (PMID: 19723895).

36. Ikenaga N, Ohuchida K, Mizumoto K, et al. MicroRNA-203 expression as a new prognostic marker of pancreatic adenocarcinoma. Ann Surg Oncol 2010;17(12):3120-8 (PMID: 20652642).

37. Wang S, Zhao Y, Li D, Zhu L, Shen Z. Identification of biomarkers for the prognosis of pancreatic ductal adenocarcinoma with miRNA microarray data. Int J Biol Markers 2015;30(2):e226-33 (PMID: 25791160).

38. Schultz NA, Andersen KK, Roslind A, Willenbrock H, Wojdemann M, Johansen JS. Prognostic microRNAs in cancer tissue from patients operated for pancreatic cancer--five microRNAs in a prognostic index. World J Surg 2012;36(11):2699-707 (PMID: 22851141).

39. Skrha P, Horinek A, Pazourkova E, et al. Serum microRNA-196 and microRNA-200 in pancreatic ductal adenocarcinoma of patients with diabetes mellitus. Pancreatology 2016;16(5):839-43 (PMID: 27267055).

40. Bloomston M, Frankel WL, Petrocca F, et al. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. JAMA 2007;297(17): 19018 (PMID: 17473300).