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Circulating microRNAs as a potential diagnostic marker in chronic pancreatitis, pancreatic cancer and colorectal cancer

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ABSTRACT

Introduction. We evaluated the expression of selected circulating microRNAs (miRNAs) in chronic pancreatitis (CP), pancreatic ductal adenocarcinoma (PDAC), and colorectal cancer (CRC) patients and healthy volunteers to test for differences in their levels and potential use as biomarkers.

Material and methods. A study of plasma miRNAs expression was performed in 88 patients: 40 (45%) CP patients, 20 (23%) PDAC patients, and 28 (32%) CRC patients. Expression of miRNA-17-5p, miRNA-93-5p, miRNA-320a-5p, miRNA-519d-3p, miRNA-526b-3p, and miRNA-5590-3p was assessed by the qRT-PCR method.

Results. Higher expression of miRNA-93-5p was observed in patients with PDAC ($p = 0.02$) and CRC ($p = 0.005$) compared to healthy individuals. Lower expression of miRNA-519d-3p was found in PC ($p = 0.01$) and PDAC ($p = 0.02$) compared to healthy volunteers. Higher expression of miRNA-93-5p was observed in patients with CP who had a higher concentration of CA-19-9 compared to patients with a low level or unknown status of this marker ($p = 0.03$). Examination of miRNA-519-3p expression distinguished patients with CP from healthy volunteers with sensitivity and specificity of 60% and 80%, respectively. Testing miRNA-93-5p and miRNA-519 expression distinguished PDAC patients and healthy participants with sensitivity and specificity of 60% and 77% (for miRNA-93-5p examination), as well as 59% and 79% (for miRNA-519-3p examination). Examination of miRNA-17 and miRNA-93-5p distinguished CRC patients and healthy donors. Sensitivity and specificity of this test were 78% and 50% for miRNA-17 examination, as well as 78% and 80% for miRNA-93-5p examination.

Conclusions. Our data indicate that miRNA-93, miRNA-17, and miRNA-519 demonstrate potential as biomarker molecules in the diagnosis of CP, PDAC, and CRC.

Key words: biomarkers, chronic pancreatitis, colorectal cancer, microRNA, pancreatic cancer

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Introduction

Cancer has become a global health problem resulting in a shortened life and lowering its quality. Among all gastrointestinal cancers, two of them come to the fore: colorectal cancer, due to high incidence concerning environmental factors, and pancreatic ductal adenocarcinoma (PDAC) because of poor prognosis [1]. The last one may be confused with chronic pancreatitis (CP) due to similarity in the clinical course and imaging studies.

Chronic pancreatitis is a disease that, due to its slow oligosymptomatic course in an early phase, causes many diagnostic problems. In 2016, a new mechanistic definition of CP was proposed, which was accepted by the majority of international gastroenterological societies. According to this definition, CP is a pathologic fibro-inflammatory syndrome of the pancreas with genetic, environmental, and/or other risk factors. It leads to parenchymal injury or stress. As a consequence of injury, exocrine and, in the latest stage, endocrine insufficiency develops [2].

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Chronic pancreatitis is also associated with the risk of pancreatic ductal adenocarcinoma (PDAC), higher than in the general population. Patients with CP have a nearly 8-fold increased risk of developing pancreatic cancer five years after diagnosis [3]. Until now little is known about this relationship although some *in vivo* studies indicated a significant role of interleukin-22 (IL-22) in the promotion of PDAC development [4].

Pancreatic ductal adenocarcinoma is a highly aggressive disease with a poor prognosis and rising incidence and with an average 5-year survival rate of less than 10% [5]. Enlargement of the pancreas (tumor-like mass) with inflammation in the course of CP may mimic PDAC at imaging, which precludes pre-operative diagnosis and may lead to unnecessary surgical intervention [6]. Moreover, these two pathologies show similar biochemical parameters and clinical manifestations [7]. For these reasons, there is an urgent need to identify non-invasive markers that help distinguish PDAC from CP because all available serological and imaging examinations are non-specific for these diseases.

Colorectal cancer (CRC) is now the third most common cancer in the western world. According to the World Health Organization, 1.8 million new cases of CRC in 2018 were diagnosed, and 862 000 patients died from CRC [8]. Although we have screening tools, most notably colonoscopy, many colorectal cancers are diagnosed in advanced stages. A better understanding of pathological and molecular mechanisms of CRC may provide new perspectives for cancer prevention and care.

The non-coding microRNAs (miRNAs) seem to be promising and valuable markers of cancer development. These molecular players, 18–25 nucleotides in length, are involved in various biological processes, including cell proliferation, apoptosis, differentiation, and metabolism. MicroRNAs function is post-transcriptional regulation of gene expression complementary linkage to sequences within mRNA molecules (most often to untranslated regions). As a result, these mRNAs are silenced, and the expression of the proteins they encode decreases. Therefore, miRNAs could act as oncogenes if they reduce the expression of tumor suppressor genes or as tumor suppressors if they reduce the expression of oncogenes. Moreover, the same miRNAs can have a dual function, having the ability to bind to different mRNA molecules [9]. MiRNAs are present in stable forms in body fluids, such as plasma or serum, so their expression profiles are tightly related to the pathological conditions inside the cells [10, 11].

This study is focused on miRNA-17-5p, miR-NA-93--5p, miRNA-320a, miRNA-519d-3p, miR-NA-526b-3p, and miRNA-5590-3p, which according to the reviewed literature, are associated with inflammation and carcinogenesis. MiRNA-17-5p was up-regulated in pancreatic adenocarcinoma and directly targeted retinoblastoma-like protein 2 (RBL2). High levels of miR-17-5p and low levels of RBL2 protein are associated with poor prognosis [12]. The summary of molecular targets for miRNAs is in Table 1 [12–25]. Moreover, miRNA-93-5p is involved in gemcitabine resistance in pancreatic cancer via targeting the PTEN-mediated PI3K/Akt signaling pathway [13]. There is an indication that miRNA-320a takes part in promoting 5-fluorouracil (5-FU) resistance of human pancreatic cancer cells by targeting the programmed cell death 4 (PDCD4) transcript and is involved in proliferation, invasion, metastasis, drug-resistance characteristics, and the epithelial-to-mesenchymal transition of pancreatic cancer [14]. Moreover, the expression of miR-320a is considered a predictive marker for chemotherapy in pancreatic cancer patients [14]. The miRNA-519d-3p is a suppressor molecule whose downregulation in pancreatic cancer was observed with the simultaneously high level of ribosomal protein S15a (RPS15A), a gene that regulates expression of β-catenin and activity of the Wnt signaling pathway [15] Up-regulation of miRNA-519d-3p could suppress proliferation of pancreatic cancer cells and activity of Wnt/β-catenin, imitating the impact of *RPS15A* silencing [15]. MiRNA-526b-3p is considered a tumor suppressor. MiRNA-526b-3p directly targets the 3'UTR (untranslated region) of E2F transcription factor 1 (E2F1), decreasing its expression. Overexpression of miRNA-526b-3p inhibited the proliferation of CRC cells by reducing the level of E2F1 [16]. In glioma, miRNA-526b-3p regulates the tumor process through WEE1 (WEE1 G2 checkpoint kinase), and it is reported as a prognostic factor for this neoplasm [17]. MiRNA-5590 is considered a tumor suppressor molecule that prevents excessive cell proliferation and migration; its importance has been evaluated in human gastric cancer and breast cancer [18, 19].

Table 1. Summarized table of molecular targets for miRNAs

Molecule	Role	Target genes	Citation
miR-17-5p	Oncogene	*RBL2*	[12]
miR-93-5p	Oncogene	*MDR1, PTEN, CDKN1A*	[13, 20]
miR-320a	Oncogene	*PDCD4*	[14]
miR-519d-3p	Tumor suppressor	*RPS15A, BCL6, CCND1, BCL-W, HIF-1a*	[15, 21–25]
miR-526b-3p	Tumor suppressor	*E2F1, WEE1*	[16, 17]
miR-5590	Tumor suppressor	*TGFβ-R1, TGFβ-R2, SMAD3, SMAD4*	[18, 19]

In this pilot study, we have examined the expression of the above-mentioned miRNAs in the plasma of CP, PDAC, and CRC patients and correlated them with available clinical and demographic data and with markers of carcinogenesis: CA19-9 and carcinoembryonic antigen (CEA).

Our study aimed to investigate the potential of selected molecules as diagnostic biomarkers. Additionally, we checked whether the examination of miRNA expression could help differentiate between CP and PDAC.

Materials and methods

Studied group

The study was approved by the Research Ethics Committee of the Medical University of Lublin (approval no. KE 0254-/54/2015) and conducted in conformity with the Declaration of Helsinki.

The study of plasma miRNA expression was performed in 88 patients. Blood samples were taken at the moment of diagnosis. The study population included 40 (45%) patients with chronic pancreatitis, 20 (23%) patients with pancreatic cancer (PC), and 28 (32%) patients with colorectal cancer (CRC). Fifty-nine (67%) male and 29 (33%) female patients were in the examined group [median age and standard deviations (SD): 63.5 ± 16.2 years, range 27–96 years]. The clinical and demographic data are presented in Table 2. The control group consisted of 31 healthy participants (median age and SD: 45 ± 11.8 years, range 29–67 years). The control group did not differ significantly in terms of age and sex from the examined group.

MicroRNAs isolation

Blood was collected in EDTA (ethylenediaminetetraacetic acid) tubes and then centrifuged (2000 × g, for 10 min.) to obtain plasma. Plasma was stored at –80°C until miRNA isolation.

Isolation of total RNA with miRNAs fraction from plasma was performed using miRNeasy Serum/Plasma Kit (Qiagen, Germany). The amount and purity of RNA were assessed using an Eppendorf BioPhotometer (Eppendorf, Germany). RNA was stored at –80°C until the reverse transcription reaction was performed.

Reverse transcription reaction

The TaqMan™ Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, USA) was used to transcribe the miRNAs into complementary DNA (cDNA) according to the manufacturer's instructions. RT (Reverse transcription) was performed in a TPersonal Biometra thermocycler (Analytik-Jena Company, Germany). cDNA was stored at –20°C until quantitative polymerase chain reaction (qPCR) was performed.

Quantitative polymerase chain reaction

The expression of six microRNAs, which are attributed to properties of oncogenes or tumor suppressors (miRNA-17-5p, miRNA-93-5p, miRNA-320a-5p, miRNA-519d-3p, miRNA-526b-3p, miRNA-5590-3p), was assessed. Expression was examined by the qPCR method on the Illumina Eco (Illumina Inc, USA) device. The 20 microliter PCR mix for assessing miRNAs expression consisted of 10 μL TaqMan Fast Advanced Master Mix (Applied Biosystems, USA), 1 μL TaqMan Advanced miRNA Assay (separate reaction for each miRNA), 4 μL RNase-free water and 5 μL cDNA. Quantitative polymerase chain reaction was carried out under the following conditions: 95°C for 30 sec. and then 40 cycles: 95°C for 3 sec. and 62°C for 30 sec. miRNA-191-5p and cel-miR-39-3p were used as an internal control and spike-in control, respectively. Commercial sets of TaqMan primers and probes were used for each of the microRNAs and the internal control (Applied Biosystems, USA)

The $2^{-\Delta Ct}$ method was used for the calculation of the expression.

Statistical analysis

Statistical analysis was performed using Statistica 13 software (Tibco Software, USA). The Mann-Whitney U-test was used to assess the differences in expression of particular miRNAs between individual groups. Receiver operating characteristic (ROC) curves with area under the curve (AUC) analyzes were used to assess the diagnostic utility of miRNAs in distinguishing patients from healthy participants, as well as CP and PTAC patients. A p-value below 0.05 was considered significant.

Results

Comparison of microRNA expression in patients and healthy donors

MiRNA-17 expression was higher in patients with colorectal cancer compared to healthy participants ($p = 0.05$). Moreover, significantly higher expression of miRNA-93 was observed in patients with pancreatic cancer and colorectal cancer compared to healthy individuals ($p = 0.02$ and $p = 0.005$, respectively). Further, significantly lower expression of microRNA-519 was found in chronic pancreatitis and pancreatic cancer compared to healthy subjects ($p = 0.01$ and $p = 0.02$, respectively) (Fig. 1).

MiRNAs expression in patients

Significantly higher expression of miRNA-93 was observed in patients with CP who had a higher concentration of CA-19-9 compared to patients with a low level or unknown status of this marker ($p = 0.03$). Significantly lower expression of miRNA-519 was found in

Table 2.Clinical and demographic data of the patients included in the study

Features, n = 88 (100%)	CP (n = 40)	PDAC (n = 20)	CRC (28)
Age	Median age: 56 years (SD = 14.0, range: 27–87 years)	Median age: 64 years (SD = 13.3, range: 37–96 years)	Median age: 78 years (SD = 14.8, range: 40–91 years)
Age below the median; n = 43 (49%)	20 (50%)	9 (45)	14 (50%)
Age above the median; n = 45 (51%)	20 (50%)	11 (55)	14 (50%)
Sex			
Male; n = 59 (67%)	31 (77.5%)	10 (50%)	18 (64%)
Female; n = 29 (33%)	9 (22.5%)	10 (50%)	10 (36%)
Diabetes			
No; n = 69 (78%)	29 (72.5)	13 (65%)	27 (96%)
Yes; n = 19 (22%)	11 (27.5)	7 (35%)	1 (4%)
Acute pancreatitis in the past			
No; n = 71 (81%)	24 (60%)	19 (95%)	28 (100%)
Yes; n = 17 (19%)	16 (40%)	1 (5%)	0 (0%)
Metabolic syndrome			
No; n = 85 (96.5%)	39 (97.5)	20 (100%)	26 (93%)
Yes; n = 3 (3.5%)	1 (2.5)	0 (0%)	2 (7%)
Diet			
Light diet; n = 38 (43%)	13 (32.5%)	9 (45%)	16 (57%)
Does not follow the diet; n = 16 (18%)	13 (32.5)	3 (15%)	0 (0%)
Diabetic; n = 15 (17%)	7 (17.5%)	4 (20%)	4 (14%)
Fat-free and peptic ulcer diet; n = 2 (2%)	1 (2.5%)	0 (0%)	1 (4%)
No data; n = 17 (19%)	6 (15%)	4 (20%)	7 (25%)
Exposure to carcinogens			
No; n = 49 (56%)	12 (30%)	12 (60%)	25 (89%)
Yes (smoking, alcohol); n = 39 (44%)	28 (70%)	8 (40%)	3 (11%)
CEA			
< 4 U/mL; n = 63 (72%)	38 (95%)	9 (45%)	16 (57%)
≥ 4 U/mL; n = 15 (17%)	1 (2.5%)	5 (25%)	9 (32%)
No data; n = 10 (11%)	1 (2.5%)	6 (30%)	3 (11%)
CA-19-9			
< 37 U/mL; n = 28 (32%)	24 (60%)	4 (20%)	0 (0%)
≥ 37 U/mL; n = 28 (32%)	10 (25%)	15 (75%)	3 (11%)
No data; n = 32 (36%)	6 (15%)	1 (5%)	25 (89%)

CP — chronic pancreatitis; PDAC — pancreatic ductal adenocarcinoma; CRC — colorectal cancer; CEA — carcinoembryonic antigen

patients with chronic pancreatitis who were diagnosed with diabetes compared to patients without this disease (p = 0.02). Moreover, significantly higher expression of miRNA-320 was noticed in female in comparison to male patients with chronic pancreatitis (p = 0.004) (Fig. 2). No differences were found in the relative expression of the examined miRNAs between CP and PDAC patients.

The diagnostic value of miRNAs expression assessment

We found three molecules that differentiated CP, PC, and CRC from healthy subjects.

Examination of miRNA-519 expression distinguished patients with chronic pancreatitis from healthy volunteers with sensitivity and specificity of the diagnostic test at 60% and 80%, respectively [AUC = 0.68; 95% confidence interval (CI) 0.55–0.80; p = 0.006] (Fig. 3A).

Examination of miRNA-93 and miRNA-519 expression distinguished pancreatic cancer patients from healthy participants. The sensitivity and specificity of the diagnostic test for assessment of miRNA-93 expression were 60% and 77%, respectively (AUC = 0.69; 95% CI 0.53–0.85; p = 0.002). While the sensitivity and specificity of the diagnostic test for miRNA-519 were 59% and 79% (AUC = 0.69; 95% CI 0.53–0.85; p = 0.002) (Fig. 3B).

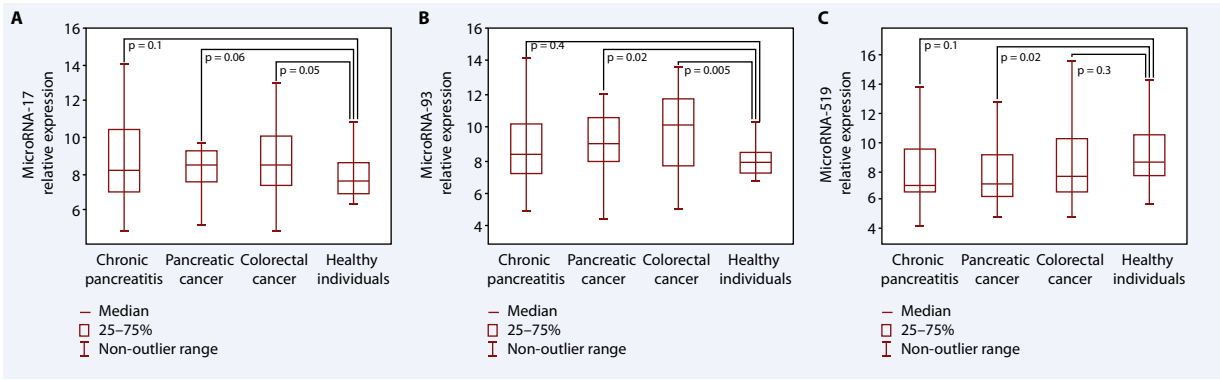

Figure 1. Comparison of the expression of selected miRNAs in patients with chronic pancreatitis (CP), pancreatic ductal adenocarcinoma (PDAC), and colorectal cancer (CRC), and healthy volunteers

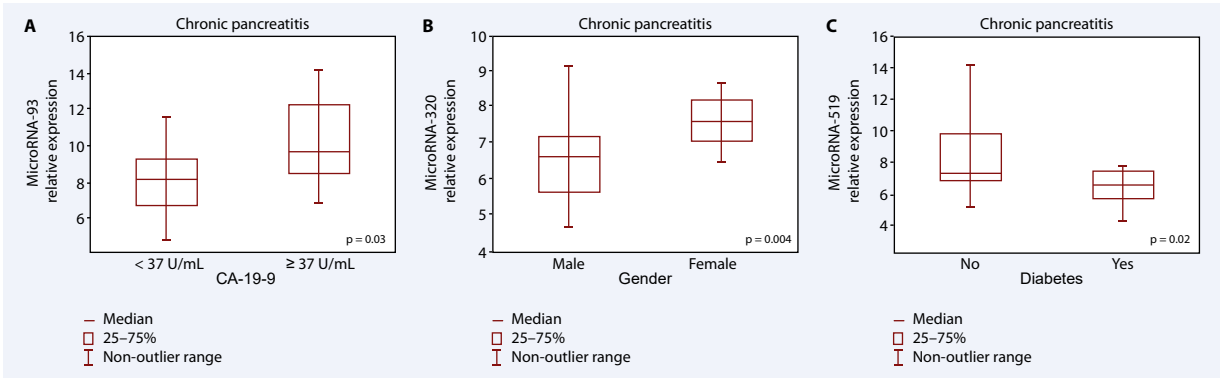

Figure 2. Expression of selected microRNAs in patients with chronic pancreatitis (CP) depending on CA 19-9 concentration, sex, and diabetes coexistence

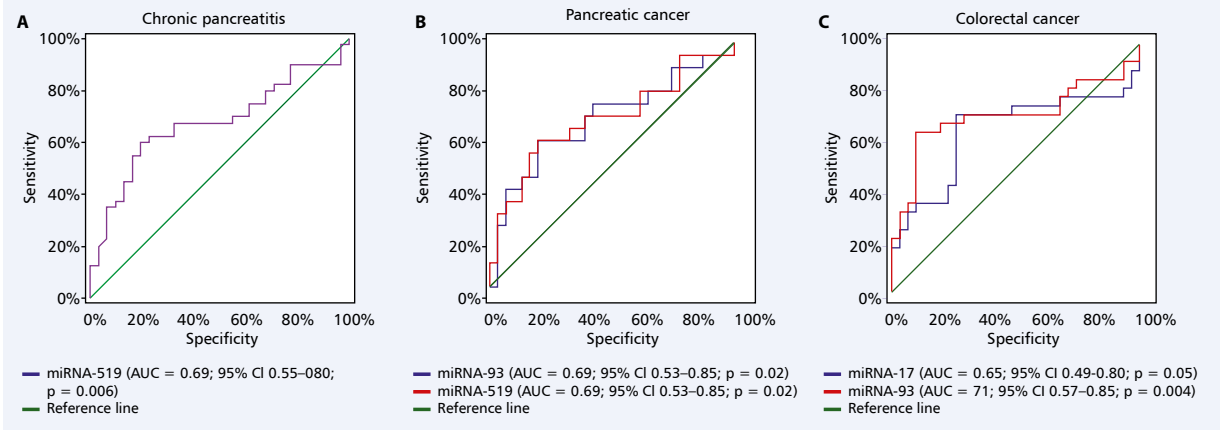

Figure 3. Sensitivity of the tests assessing the expression of selected miRNAs in distinguishing patients with chronic pancreatitis (CP), pancreatic ductal adenocarcinoma (PDAC), and colorectal cancer (CRC) from healthy participants; AUC — area under the curve; CI — confidence interval

Examination of miRNA-17 and miRNA-93 expression distinguished patients with colorectal cancer from healthy participants. The sensitivity and specificity of the diagnostic test for examination of miRNA-17 expression were 78% and 50% (AUC = 0.65; 95% CI 0.49–0.80; p = 0.05). Test for miRNA-93 expression had 78% sensitivity and 80% specificity (AUC = 0.71; 95% CI 0.57–0.85; p = 0.004) (Fig. 3C).

Discussion

Despite advancement of research into early detection of malignant neoplasms, many cancers are still detected too late, especially pancreatic cancer, which is characterized by an aggressive course and a poor prognosis. Another problem is that the image on CT (computed tomography) or MRI (magnetic resonance imaging) is similar in pancreatic cancer and CP patients, so there is an urgent need to find markers allowing for differentiation of these two diseases. Nowadays, CA 19-9 is believed to be a blood marker in the early detection of PDAC, but on the other hand, it has a reduced diagnostic value because of false positive and false negative results [26]. This means that CA 19-9 level is elevated not only in PDAC but also in the biliary tract, stomach, colorectal, lung, or thyroid tumors and also in non-malignant pathologies, including pancreatitis, diabetes mellitus, as well as other pulmonary, thyroidal, and gynecologic diseases [27]. Moreover, another limitation of CA 19-9 examination is the fact that as a sialylated Lewis blood group antigen, also CA 19-9 is not detected in people who lack the expression of fucosyltransferase, an enzyme required for the production of both CA 19-9 and Lewis antigen [28]. The above problems do not allow the detection of PDAC in early stages and prevent effective surgical treatment [28].

We have much greater possibilities in the early detection of colorectal cancer thanks to the wide availability of screening tests, including colonoscopy. However, due to the reluctance (aversion) of patients to this examination, the need for tiring preparation and high costs, markers are necessary to facilitate identification of the best candidates for this study. The CEA tumor marker has fallen short of expectations and is not recommended for screening. Also in our study, the majority of CRC patients had normal or slightly raised CEA level at the moment of diagnosis.

Aberrant miRNA expression profiles have been studied in many types of cancers, including PDAC and colorectal cancer [29]. MiRNAs may be interesting blood-based biomarkers in clinical practice because they are stable in circulation, not degraded by endogenous RNases, non-invasive, and simple to collect [30]. Chronic inflammation regulates carcinogenesis on different levels, starting from tumor initiation, through proliferation and progression, ending up in metastasis; miRNAs are involved in this process [31]. Depending on the type of tumor and immune cells involved in this process, mediators produced by inflammatory cells increase mutagenesis and activate epigenetic machinery, including histone modifications, long non-coding RNA and miRNAs that modulate gene expression and promotor gene methylation [32]. Accumulating evidence indicates that miRNAs are frequently dysregulated in human cancers, and alterations of miRNAs expression in CRC have been well documented [33].

As miRNAs are mediators in carcinogenesis and inflammation, we have selected a group of miRNAs potentially involved in CRC, PDAC, and CP pathophysiology. We chose also some miRNAs known for having a potential diagnostic and prognostic role in patients with other cancers, especially lung cancer. In the next part of the discussion, we will look at those miRNAs that were associated with CP, PDAC, or CRC in our study: miRNA-17-5p, miRNA-93-5p, and miRNA-519d-3p.

The examination of the miRNA-93-5p molecule appears to have a diagnostic, predictive, and therapeutic potential. The test based on the evaluation of the level of this molecule in the serum or plasma was very promising; however, some limitations of this microRNA should be pointed out. In our study, high expression of miRNA-93-5p occurred in colorectal-cancer and pancreatic-cancer patients, with no significant difference between these cancers. That is, this molecule is not tumor-specific and, arguably, cannot be used as a stand-alone diagnostic or prognostic/predictive factor. However, it can be a valuable ancillary parameter for cancer screening, early cancer diagnosis, or the likelihood of resistance to treatment. Shao et al. indicated that a test based on a combined analysis of the miR-93-5p and miR-18a expression in serum (miR-93-5p+miR--18a marker) has a better value potential for diagnosis and prognosis in non-small cell lung cancer (NSCLC) patients than the examination of single markers [34]. Likewise, Vila-Navarro et al. indicated that multiple miRNAs assessed simultaneously in one test provided much better information (in terms of sensitivity and specificity) in the identification of PDAC. Moreover, they showed that CA19.9 increased the diagnostic potential of test-examined miRNAs signatures. The test combining miRNAs and CA19.9 (miR-33a-3p+miR--320a+CA19.9) achieved an AUC of 0.95 (93% sensitivity and 85% specificity) [35]. In our study, we observed significantly higher expression of the miR-93 molecule in the group of patients suffering from chronic pancreatitis, with a concentration of this marker above 37 U/mL. Nevertheless, we did not observe such a relationship in pancreatic cancer patients, and we found no differences in the expression of this miRNA between CP and PDAC. We think it is worthwhile to expand our study to an enlarged group of CP and PDAC patients and include protein tumor markers and other microRNAs that have the possibility of differentiating these two diseases.

The miR-93-5p molecule is considered to be oncogenic, whereas in our study we also observed a significant decrease in expression of the tumor suppressor miR-519d-3p in patients with both chronic pancreatitis and pancreatic cancer. Furthermore, we found that the expression of this molecule is significantly reduced

in patients with chronic pancreatitis who were also diagnosed with diabetes. We tentatively suggest that this microRNA could be disease-tissue-specific. However, there is limited research on pancreatic diseases involving this molecule. Table 3 [20–25, 34–42] contains information on the miRNA-17-5p, miRNA-93, and miRNA-519d-3p molecules and their role in cancer in relation to the results obtained in this study.

Table 3. Description of miRNA-17-5p, miRNA-93, and miRNA-519d-3p roles in cancer in relation to the results obtained in this study

miRNA	Characteristic	Source
miRNA-17-5p	Expression is higher in patients with colorectal cancer compared to healthy participants with sensitivity and specificity 78% and 50%, respectively	The study presented here
	Cancer patients with high expression of miR-17-5p have a worse prognosis than those with low expression. This molecule may be involved in the progression of lymphatic metastasis and vein invasion in cancer	Kong et al. [36]
	Metastasis suppression function	Fan et al. [37]
miRNA-93	Distinguished patients with colorectal cancer from healthy donors with 78% sensitivity and 80% specificity	The study presented here
	Distinguished patients with PC from healthy volunteers (higher expression in PC) with 60% and 77% of sensitivity and specificity, respectively	The study presented here
	High circulating miR-93 expression could discriminate between pancreatic cancer patients and healthy people, with AUC = 0.80	Vila-Navarro et al. [35]
	The 3-year survival rate of NSCLC patients is significantly lower in the group of patients with low miR-93-5p serum expression than in the group of patients with high expression of this molecule (log-rank: p = 0.0442); has a diagnostic potential with AUC = 0.7926	Shao et al. [34]
	An increased expression of urinary exosomes (UEs) derived miRNA-93 has a diagnostic potential to discriminate BC patients from the healthy people with AUC = 0.838; high miR-93-5p serum level is significantly associated with early BC recurrence	Lin et al. [38]
	High serum level is a potential prognostic factor for the risk of early disease recurrence in CRLM; expression is significantly higher in CRLM in comparison to the non-metastatic liver tissue	Despotović et al. [39]
	An exosomal cargo responsible for the pro-tumorigenic effects of cancer-associated fibroblasts in colorectal cancer. Cancer-associated fibroblast exosomes contained more miR-93-5p than normal fibroblast exosomes, which increased the proliferation of CRC cells and protected them from radiation-induced apoptosis	Chen et al. [40]
	Is elevated in drug-resistant CRC cells, and downregulation of miR-93-5p expression results in increased sensitivity to chemotherapy; inhibition of miR-93-5p is found to downregulate MDR1 (ATP binding cassette subfamily B member 1, ABCB1) expression, increase intracellular chemotherapeutic concentration, and increase the percentage of cells in the G1 cycle phase by upregulating Cyclin Dependent Kinase Inhibitor 1A (CDKN1A) gene and protein expression	Wang et al. [20]
miRNA-519d-3p	Expression distinguishes patients with chronic pancreatitis from healthy volunteers with sensitivity and specificity of 60% and 80%, respectively while the sensitivity and specificity of this test in distinguishing pancreatic cancer patients from healthy participants is 59% and 79%, respectively	The study presented here
	Functions as a tumor suppressor by targeting and downregulating the expression of B-Cell Lymphoma 6 Protein (BCL6)	Li et al. [21]
	Expression significantly decreased in glioma tissues; regulation of B-Cell Lymphoma 1 Protein (CCND1)	Ma and Li [22], Zhang and Hong [23]
	In OSCC tissues, downregulating miR-519d-3p expression correlated with a higher tumor grade, and upregulating miR-519d-3p expression inhibited OSCC cells viability and proliferation as well as increased cells in G0/G1 cell cycle	Zhang and Hong [23]
	Plasma expression is significantly decreased in NSCLC patients compared to healthy individuals; molecular targets of this molecule: BCL2-like protein 2 (BCL-W) and hypoxia-inducible factor 1 subunit alpha (HIF-1α): miRNA-519 and expression of BCL-W and HIF-1 α mRNA showed an inverse correlation in NSCLC	Choi et al. [24]
	HIF-1A mRNA is negatively correlated with the miR-519d-3p levels in human PDAC tissue samples; miR-519d-3p negatively regulated ribosomal protein S15a (RPS15A) expression in pancreatic cancer cells	Sun et al. [25]
	Expression is significantly decreased in pancreatic cancer tissues, which is involved in the Wnt /β-catenin signaling pathway	Liang et al. [41]
	Levels in pancreatic cancer cells were reduced following hypoxia; transfection with miR-519 mimics inhibited pancreatic cancer cells' invasiveness and induced apoptosis under hypoxic conditions; programmed death ligand 1 (PD-L1) as a target of miR-519 and rescued the miR-519 mimic-attenuated tumorigenesis of pancreatic cancer cells under hypoxic conditions; treatment with miR-519 significantly suppressed the tumor growth of pancreatic cancer cells	Nong et al. [42]

AUC — area under the curve; PC — pancreatic cancer; NSCLC — non-small cell lung cancer; BC — bladder cancer; CRLM — colorectal cancer with liver metastasis; CRC — colorectal cancer; OSCC — oral squamous cell carcinoma; PDAC — pancreatic ductal adenocarcinoma

There is no evidence of the impact of circulating miRNA-519d-3p as a biomarker in chronic pancreatitis. We showed that examination of miRNA-519 expression distinguished patients with chronic pancreatitis from healthy volunteers with sensitivity and specificity of 60% and 80%, respectively, while the sensitivity and specificity of this test in distinguishing pancreatic cancer patients from healthy participants were 59% and 79%, respectively. However, this finding must be confirmed and validated in an independent enlarged study group.

Our studies have some limitations, such as small study groups or lack of data on the stage or localization of possible metastases. However, there may be strong indications to extend the research to an enlarged group of patients and to conduct it using biological tests that would indicate the target transcripts for the studied miRNA molecules.

Conclusions

Our study indicated that microRNA-93 has diagnostic potential in colorectal and pancreatic cancers, but literature data indicated that it cannot be a stand-alone diagnostic/predictive factor. In the case of miRNA-519d-3p, due to limited literature data on serum/plasma studies in pancreatic cancer or chronic pancreatitis, we could draw conclusions based on our own studies, which suggested that this molecule probably has a suppressor function and its expression can be a supportive factor for the diagnosis of PDAC or CP. However, it does not distinguish between these two diseases.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

Authors declare no conflict of interest.

References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer Statistics, 2021. CA Cancer J Clin. 2021; 71(1): 7–33, doi: 10.3322/caac.21654, indexed in Pubmed: 33433946.
2. Whitcomb DC, Frulloni L, Garg P, et al. Chronic pancreatitis: An international draft consensus proposal for a new mechanistic definition. Pancreatology. 2016; 16(2): 218–224, doi: 10.1016/j.pan.2016.02.001, indexed in Pubmed: 26924663.
3. Kirkegård J, Mortensen FV, Cronin-Fenton D. Chronic Pancreatitis and Pancreatic Cancer Risk: A Systematic Review and Meta-analysis. Am J Gastroenterol. 2017; 112(9): 1366–1372, doi: 10.1038/ajg.2017.218, indexed in Pubmed: 28762376.
4. Perusina Lanfranca M, Zhang Y, Girgis A, et al. Interleukin 22 Signaling Regulates Acinar Cell Plasticity to Promote Pancreatic Tumor Development in Mice. Gastroenterology. 2020; 158(5): 1417–1432.e11, doi: 10.1053/j.gastro.2019.12.010, indexed in Pubmed: 31843590.
5. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016; 66(4): 271–289, doi: 10.3322/caac.21349, indexed in Pubmed: 27253694.
6. Wolske KM, Ponnatapura J, Kolokythas O, et al. Chronic Pancreatitis or Pancreatic Tumor? A Problem-solving Approach. Radiographics. 2019; 39(7): 1965–1982, doi: 10.1148/rg.2019190011, indexed in Pubmed: 31584860.
7. Zheng Z, Chen Y, Tan C, et al. Risk of pancreatic cancer in patients undergoing surgery for chronic pancreatitis. BMC Surg. 2019; 19(1): 83, doi: 10.1186/s12893-019-0537-1, indexed in Pubmed: 31286902.
8. https://www.who.int/news-room.
9. Filipów S, Łaczmański Ł. Blood Circulating miRNAs as Cancer Biomarkers for Diagnosis and Surgical Treatment Response. Front Genet. 2019; 10: 169, doi: 10.3389/fgene.2019.00169, indexed in Pubmed: 30915102.
10. Ortiz-Quintero B. Cell-free microRNAs in blood and other body fluids, as cancer biomarkers. Cell Prolif. 2016; 49(3): 281–303, doi: 10.1111/cpr.12262, indexed in Pubmed: 27218664.
11. O'Brien J, Hayder H, Zayed Y, et al. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne). 2018; 9: 402, doi: 10.3389/fendo.2018.00402, indexed in Pubmed: 30123182.
12. Zhu Y, Gu J, Li Y, et al. MiR-17-5p enhances pancreatic cancer proliferation by altering cell cycle profiles via disruption of RBL2/E2F4--repressing complexes. Cancer Lett. 2018; 412: 59–68, doi: 10.1016/j.canlet.2017.09.044, indexed in Pubmed: 28987387.
13. Wu Y, Xu W, Yang Y, et al. miRNA-93-5p Promotes Gemcitabine Resistance in Pancreatic Cancer Cells by Targeting the PTEN-Mediated PI3K/Akt Signaling Pathway. Ann Clin Lab Sci. 2021; 51(3): 310–320, indexed in Pubmed: 34162560.
14. Wang W, Zhao L, Wei X, et al. MicroRNA-320a promotes 5-FU resistance in human pancreatic cancer cells. Sci Rep. 2016; 6: 27641, doi: 10.1038/srep27641, indexed in Pubmed: 27279541.
15. Liang J, Liu Y, Zhang L, et al. Overexpression of microRNA-519d-3p suppressed the growth of pancreatic cancer cells by inhibiting ribosomal protein S15A-mediated Wnt/β-catenin signaling. Chem Biol Interact. 2019; 304: 1–9, doi: 10.1016/j.cbi.2019.02.026, indexed in Pubmed: 30831090.
16. Fang Z, Yang H, Chen D, et al. YY1 promotes colorectal cancer proliferation through the /E2F1 axis. Am J Cancer Res. 2019; 9(12): 2679–2692, indexed in Pubmed: 31911854.
17. Wu M, Li X, Liu Q, et al. miR-526b-3p serves as a prognostic factor and regulates the proliferation, invasion, and migration of glioma through targeting WEE1. Cancer Manag Res. 2019; 11: 3099–3110, doi: 10.2147/CMAR.S192361, indexed in Pubmed: 31114353.
18. Abedini Bakhshmand E, Soltani BM. Regulatory effect of hsa-miR-5590-3P on TGFβ signaling through targeting of TGFβ-R1, TGFβ-R2, SMAD3 and SMAD4 transcripts. Biol Chem. 2019; 400(5): 677–685, doi: 10.1515/hsz-2018-0264, indexed in Pubmed: 30391930.
19. Wu N, Han Y, Liu H, et al. miR-5590-3p inhibited tumor growth in gastric cancer by targeting DDX5/AKT/m-TOR pathway. Biochem Biophys Res Commun. 2018; 503(3): 1491–1497, doi: 10.1016/j.bbrc.2018.07.068, indexed in Pubmed: 30029874.
20. Wang SJ, Cao YF, Yang ZQ, et al. MicroRNA-93-5p increases multidrug resistance in human colorectal carcinoma cells by downregulating cyclin dependent kinase inhibitor 1A gene expression. Oncol Lett. 2017; 13(2): 722–730, doi: 10.3892/ol.2016.5463, indexed in Pubmed: 28356951.
21. Li YY, Shao JP, Zhang SP, et al. miR-519d-3p Inhibits Cell Proliferation and Invasion of Gastric Cancer by Downregulating B-Cell Lymphoma 6. Cytogenet Genome Res. 2018; 154(1): 12–19, doi: 10.1159/000487372, indexed in Pubmed: 29510377.
22. Ma L, Li J. MicroRNA-519d-3p inhibits cell proliferation and cell cycle G1/S transition in glioma by targeting CCND1. Biosci Biotechnol Biochem. 2020; 84(2): 297–304, doi: 10.1080/09168451.2019.1682510, indexed in Pubmed: 31661371.
23. Zhang W, Hong W. Upregulation of miR-519d-3p Inhibits Viability, Proliferation, and G1/S Cell Cycle Transition of Oral Squamous Cell Carcinoma Cells Through Targeting CCND1. Cancer Biother Radiopharm. 2020 [Epub ahead of print], doi: 10.1089/cbr.2020.3984, indexed in Pubmed: 33052706.
24. Choi JY, Seok HJ, Kim RK, et al. miR-519d-3p suppresses tumorigenicity and metastasis by inhibiting Bcl-w and HIF-1α in NSCLC. Mol Ther Oncolytics. 2021; 22: 368–379, doi: 10.1016/j.omto.2021.06.015, indexed in Pubmed: 34553025.

25. Sun J, Zhang P, Yin T, et al. Upregulation of LncRNA PVT1 Facilitates Pancreatic Ductal Adenocarcinoma Cell Progression and Glycolysis by Regulating MiR-519d-3p and HIF-1A. J Cancer. 2020; 11(9): 2572–2579, doi: 10.7150/jca.37959, indexed in Pubmed: 32201527.
26. Azizian A, Rühlmann F, Krause T, et al. CA19-9 for detecting recurrence of pancreatic cancer. Sci Rep. 2020; 10(1): 1332, doi: 10.1038/s41598-020-57930-x, indexed in Pubmed: 31992753.
27. Kim S, Park BK, Seo JH, et al. Carbohydrate antigen 19-9 elevation without evidence of malignant or pancreatobiliary diseases. Sci Rep. 2020; 10(1): 8820, doi: 10.1038/s41598-020-65720-8, indexed in Pubmed: 32483216.
28. Dasgupta A, Wahed A. Clinical Chemistry, Immunology and Laboratory Quality Control: A Comprehensive Review for Board Preparation, Certification and Clinical Practice. Elsevier 2013.
29. Xin L, Gao J, Wang D, et al. Novel blood-based microRNA biomarker panel for early diagnosis of chronic pancreatitis. Sci Rep. 2017; 7: 40019, doi: 10.1038/srep40019, indexed in Pubmed: 28074846.
30. Condrat CE, Thompson DC, Barbu MG, et al. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells. 2020; 9(2), doi: 10.3390/cells9020276, indexed in Pubmed: 31979244.
31. Michels N, van Aart C, Morisse J, et al. Chronic inflammation towards cancer incidence: A systematic review and meta-analysis of epidemiological studies. Crit Rev Oncol Hematol. 2021; 157: 103177, doi: 10.1016/j.critrevonc.2020.103177, indexed in Pubmed: 33264718.
32. Greten FR, Grivennikov SI. Inflammation and Cancer: Triggers, Mechanisms, and Consequences. Immunity. 2019; 51(1): 27–41, doi: 10.1016/j.immuni.2019.06.025, indexed in Pubmed: 31315034.
33. Gattolliat CH, Uguen A, Pesson M, et al. MicroRNA and targeted mRNA expression profiling analysis in human colorectal adenomas and adenocarcinomas. Eur J Cancer. 2015; 51(3): 409–420, doi: 10.1016/j.ejca.2014.12.007, indexed in Pubmed: 25586944.
34. Shao L, Lu X, Zhou Y, et al. Altered miR-93-5p/miR-18a expression in serum for diagnosing non-small cell lung cancer. Am J Transl Res. 2021; 13(5): 5073–5079, indexed in Pubmed: 34150094.
35. Vila-Navarro E, Duran-Sanchon S, Vila-Casadesús M, et al. Novel Circulating miRNA Signatures for Early Detection of Pancreatic Neoplasia. Clin Transl Gastroenterol. 2019; 10(4): e00029, doi: 10.14309/ctg.0000000000000029, indexed in Pubmed: 31009404.
36. Kong W, Cheng Y, Liang H, et al. Prognostic value of miR-17-5p in cancers: a meta-analysis. Onco Targets Ther. 2018; 11: 3541–3549, doi: 10.2147/OTT.S150340, indexed in Pubmed: 29950859.
37. Fan M, Sethuraman A, Brown M, et al. Systematic analysis of metastasis-associated genes identifies miR-17-5p as a metastatic suppressor of basal-like breast cancer. Breast Cancer Res Treat. 2014; 146(3): 487–502, doi: 10.1007/s10549-014-3040-5, indexed in Pubmed: 25001613.
38. Lin H, Shi X, Li H, et al. Urinary Exosomal miRNAs as biomarkers of bladder Cancer and experimental verification of mechanism of miR-93-5p in bladder Cancer. BMC Cancer. 2021; 21(1): 1293, doi: 10.1186/s12885-021-08926-x, indexed in Pubmed: 34861847.
39. Despotović J, Bogdanović A, Dragičević S, et al. Prognostic potential of circulating miR-93-5p in patients with colorectal cancer liver metastases. Neoplasma. 2022; 69(2): 430–442, doi: 10.4149/neo_2021_210603N749, indexed in Pubmed: 35037761.
40. Chen X, Liu J, Zhang Q, et al. Exosome-mediated transfer of miR-93-5p from cancer-associated fibroblasts confer radioresistance in colorectal cancer cells by downregulating FOXA1 and upregulating TGFB3. J Exp Clin Cancer Res. 2020; 39(1): 65, doi: 10.1186/s13046-019-1507-2, indexed in Pubmed: 32293494.
41. Liang J, Liu Y, Zhang L, et al. Overexpression of microRNA-519d-3p suppressed the growth of pancreatic cancer cells by inhibiting ribosomal protein S15A-mediated Wnt/β-catenin signaling. Chem Biol Interact. 2019; 304: 1–9, doi: 10.1016/j.cbi.2019.02.026, indexed in Pubmed: 30831090.
42. Nong K, Zhang D, Chen C, et al. MicroRNA-519 inhibits hypoxia-induced tumorigenesis of pancreatic cancer by regulating immune checkpoint PD-L1. Oncol Lett. 2020; 19(2): 1427–1433, doi: 10.3892/ol.2019.11234, indexed in Pubmed: 31966071.