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REVIEWS

Review on Non-Invasive Diagnosis of Pancreatic Cancer

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Abstract

The pancreatic cancer has the worst prognosis among gastrointestinal cancers, with a mortality rate close to incidence. The analysis on the globe carried out by GLOBOCAN in 2012 places the pancreatic cancer on the 13th place in terms of incidence and on the 8th place in terms of mortality of all cancers and in relation with digestive cancers it occupies the 6th place for both epidemiological indices. The high mortality is justified by the paucity of symptoms, since it becomes clinically manifest upon the onset of secondary determinations and the lack of response to treatment. 80-85% of the patients come in the stage of non-resecability. The lack of sensitive tumoral markers specific to the early diagnosis of the pancreatic cancer has a major contribution to the poor prognosis.
The goal of this article is to present all the discoveries that have been made so far in the field of biomarkers involved in the pancreatic cancer, namely CEA carcinoembryonic antigen, CA19-9 antigen and microRNA.

Rezumat

Cancerul pancreatic are cel mai infaust prognostic dintre cancerele gastrointestinale, cu o mortalitate apropiată cu incidența. Analiza pe glob, realizata de GLOBOCAN în 2012, situează cancerul pancreatic pe locul al 13-lea ca incidență și pe locul opt ca mortalitatea din totalitatea cancerelor și raportat la cancerele digestive, acesta ocupă locul 6 la ambii indici epidemiologici. Mortalitatea crescută se justifică prin paucitatea simptomelor, devenind manifest clinic în momentul apariției determinărilor secundare cat şi prin lipsa de răspuns la tratament. 80-85% dintre pacienți se prezintă în stadiu de nerezecabilitate. Lipsa markerilor tumorali sensibili și specifici pentru diagnosticul precoce al cancerului pancreatic, are o contributie majora la prognosticul infaust.
Scopul acestui articol este de a trece in revista toate descoperirile facute până acum în domeniul biomarkerilor implicati in cancerului pancreatic, respectiv antigenul carioembrionar CEA, antigenul CA19-9 și microRNA.

INTRODUCTION

The pancreatic cancer has the worst prognosis among gastrointestinal cancers with a mortality rate close to incidence. The analysis on the globe carried out by GLOBOCAN in 2012 places the pancreatic cancer on the 13th place in terms of incidence and on the 8th place in terms of mortality of all cancers and in relation with digestive cancers it occupies the 6th place for both epidemiological indices[1].

Due to the lack of mesenterium, of the intimate contact with the common biliary duct and other retroperitoneal structures and to the position of vicinity with the stomach, duodenum and colon, most clinical manifestations represent the late consequence of the invasion or compression of these structures[2]. For this reason, 80-85% of the patients come in the phase of non-excisability[3].

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To diagnose the pancreatic cancer, the *European Society of Medical Oncology* (ESMO) recommends abdominal ultrasound as an initial investigation; *Endoscopic Retrograde Cholangio-Pancreatography* (ERCP) for biliary obstructions, and endoscopic ultrasound, MD-CT (contrast-enhanced multi-detector), MRI, MRCP as additional investigations. Despite the fact that it has a sensitivity superior to CT (PET 87%, CT 53%), PET is used only to detect metastases or to investigate the uncertain results obtained by CT[4].

The abdominal ultrasound is the first intention diagnostic test for pancreatic cancer as it has a sensitivity of 90% and a specificity of 95% in case of tumors larger than 3 cm. Despite all these, this is an operator-dependent method and it cannot discriminate between cancer and chronic or autoimmune pancreatitis[5].

Endoscopic ultrasound (EUS) was initially used for the confirmation and staging of the solid focal tumors seen by other imagistic methods (trans-abdominal ultrasound, CT or MR). At present, it represents the nonsurgical method having the highest sensitivity (98%) in detecting benign or malignant pancreatic formations (superior to conventional computer tomography with a sensitivity of 86%)[6].

CT scan is mainly used for pancreatic cancer staging[7]. MRI is recommended for patients with CT contraindications (nephropathy, pregnancy, and allergy to the contract medium) or when the CT result is uncertain and has a sensitivity of 81-99% and specificity of 70-93%[8].

Biopsy using endoscopic ultrasound is recommended only if the pancreatic lesions are ambiguous during the imagistic examination. Metastases may be subject to biopsy percutaneously under CT guidance, echographically or during endoscopic ultrasound. It has been established that EUS-FNA is the most sensitive (75-90%), specific (94-100%) and lacked of complications (below 1%) method used in the histological diagnosis of pancreatic tumors[9].

Despite the development of the diagnosis manners, the surgical techniques and the chemotherapeutic treatment, the survival rate has not improved in the past decades.

Thus, after diagnosis, the one year survival is rated to 24%, and 5-year survival to 5%[10].

The latest researches have shown that there is a latency period of 10 years since the onset of the first tumoral modifications until the instauration of the first symptoms[11], a period where the existence of screening biomarkers might change the current prognosticated values in the sense of patients' early diagnostication. During the three past decades, more markers have been proposed for the pancreatic cancer, but no marker has been implemented in the screening strategy. Among these there was CA19-9 carbohydrate antigen and carcinoembryonic antigen (CEA). CEA is a glycoprotein used in the clinic as a tumoral marker for the diagnosis of breast cancer, stomach cancer, colorectal cancer and pancreatic cancer, where it has a specificity of 79% and a sensitivity of only 54%. For this reason, it may be tested in combination with CA19-9, specificity and sensitivity in diagnosis increasing to 86%[12]. Despite all these, the European Group on Tumor Markers (EGTM) does not recommend it due to the falsely positive results in certain cases of non-malignant jaundice[13]. Markers such as MIC1 (macrophage inhibitory cytokine 1), osteopontin, tissue inhibitor of matrix metalloproteinase-1, and mesothelin genes have not demonstrated their superiority towards CA19-9, in the diagnostication of the pancreatic cancer[14].

Previous studies showed that microRNA plays an important role in oncogenesis and metastatic spreading of the pancreatic cancer[1].

MicroRNA are non-coding RNA fragments having a length of 20-22 nucleotides whose essential role resides in the post-transcriptional regulation of gene expression through the degradation or repression of translation of certain specific types of RNA messenger ("target"). They determine a reduction of the quantity and activity of proteins involved in cellular processes essential for the normal functioning of the cell, such as apoptosis, differentiation and cellular cycle[15].

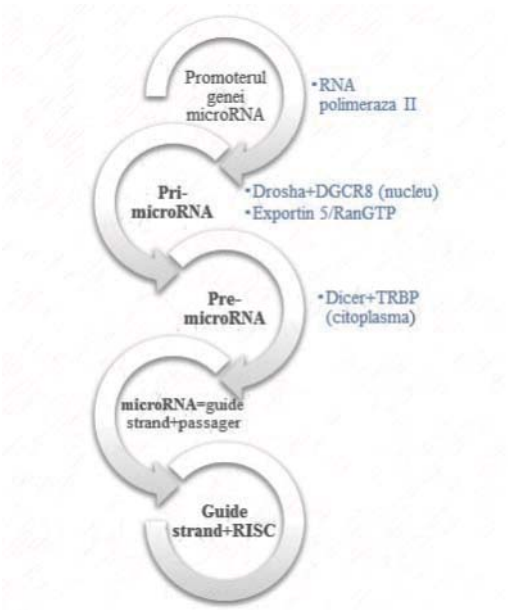
Abnormal levels of microRNA have been encountered in cancer, autoimmune diseases, viral infections or sepsis.

It has been noticed that certain types of microRNA regulate the level of proto-oncogenes or tumoral suppression genes, their expression being often modified in diverse tumoral tissues and, consequently, they might be used as tumoral markers.

Thus, certain micro-RNA (mir 34a, mir124, mir 143, mir 203, mir 200, mir 146a) act as proto-oncogene inhibitors having the role of a tumor suppressor, and they will appear in small quantities in the tumoral tissues, whereas other types of microRNA (21,221,192,155,10a) inhibit the tumoral suppression genes having an increased expression at tumoral level[16].

Biogenesis and maturation of microRNA molecules

MicroRNA is initially transcribed from the genome by means of RNA polymerase II that connects to the promoter of microRNA gene being in the proximity of DNA sequence to be decoded resulting primary microRNA or pre-microRNA.



Pre-microRNA maturation requires the action of two ribonucleases III: Drosha and Dicer. The first ribonuclease, Drosha together with Pasha protein or DGCR8 are located in the nucleus. They process the primary microRNA into a precursor of about 70 nucleotides of microRNA called pre-microRNA, which is then transported from the nucleus into the cytoplasm by means of a protein from karyopherin family, Exportin 5/RanGTP protein. The transport into cytoplasm mediated by Exportin5 protein is dependent on energy (it needs GTP).

Arrived into the cytoplasm, pre-microRNA is then cleaved by the second endoribonuclease Dicer together with TRBP protein (trans-activator RNA binding protein) into a sequence of about 20 nucleotides, a double helix practically representing the final form of microRNA. Only one of the components of the double helix, called guide strand will be selected to continue the process, the other strand being subsequently degraded.

The following stage consists in the introduction of guide strand component into RISC (RNA-induced silencing complex), a complex containing several proteins that help to the recognition of RNA messenger sequence complementary to the microRNA sequence and subsequently the degradation or repression of the translation. This process takes place in 3'UTR ("untranslated region") region of RNA messenger. Argonaut proteins are key elements of the RISC complex and they act as endonucleases oriented towards the complex made up of RNA messenger coupled with a microRNA sequence, thus resulting a reduction of translated proteins and of their activity.

Another mechanism by which microRNA may cause the quantitative and qualitative reduction of the synthesis of a protein is represented by the fast destruction of the target RNA messenger by deadenylation. The interaction between 5'-3' ends of RNA molecule is interrupted, which results in the repression of translation initiation.

Deadenylation and translation repression are two mechanisms acting independently and they may function as alternative safety mechanisms in case one of them does not inhibit translation efficiently[17].

Signaling pathways used by micro-RNA

Numerous studies have shown that about 5-10% of the malignant pancreatic tumors have genetic causes. The most frequent modifications include mutations of K-ras oncogene (90%), p53 (85%), SMAD4/DPC4 (50%), and p16 (85% mutant and 15% silent epigenetically) that are accompanied by genomic and transcriptomic modifications facilitating the impairment of the cellular cycle and cellular survival, and favoring invasion and metastases.

The progression from minimum epithelial dysplasia (pancreatic intraepithelial neoplasia of 1A and 1B level) to more severe dysplasia (pancreatic intraepithelial neoplasia of 2 and 3 level) and finally to the invasive carcinoma occurs in parallel with the successive accumulation of mutations that include the activation of KRAS2 oncogene, the inactivation of CDKN2A tumor suppressor gene (codifying the inhibitor of cyclin-dependent kinase 4), and finally the inactivation of TP53 and DPC4 (SMAD4) tumor suppressor genes[18].

a) K-ras

One of the most frequent (in proportion of 25%) oncogene activated in human cancers is K-ras, the human cellular homologue of the oncogene isolated from Kirsten virus of rat sarcoma[15]. All RAS genes have a similar intron-exon structure, and the codified proteins are GTP-ase (G proteins linking GDP/GTP and acting as transducers of the intracellular signal) with an important role in the processes of cellular proliferation, differentiation and apoptosis. Normally, GAP proteins promote GTP hydrolysis and reverse the RAS activation. But during the process of tumoral transformation, the mutations occurring at the level of RAS lead to its activation and it can no longer be deactivated by GAP proteins.

Kras mutations may be identified in initial stages in the pancreatic juice and they will become positive in the blood when the tumor no longer observes the excisability criteria[19].

More recent studies have identified a series of specific micro-RNA that use Kras signaling way in pancreatic oncogenesis. Among them, Mir96 acts by means of KRAS having the role to inhibit the cellular proliferation and invasiveness, and to induce apoptosis, thus reducing the growth of the tumoral cell[20]. Consequently, it may be found in small quantities in the pancreatic cancer (Table 1).

b) PI3K–AKT are protein kinases with an important role in the cellular survival, proliferation, and di-

Table 1. MicroRNA in pancreatic cancer

miRNA	expression status	target genes	biomarker for diagnosis	Prognosis value	chemosensitivity	potential target for treatment
let 7	Downregulation	KRAS,MAPK				+
mir 10a	Upregulation	HOXA1		Poor		+
mir 16	Upregulation	FOXP3	+			
mir 15b	Upregulation	CCNE1		Poor		
mir 20a	Downregulation	STAT3	+			+
mir23a	Upregulation	AGT12,APAF 1		Poor	+	
mir 24	Downregulation	ACVR2B, GFRA1, and MTHFR	+			
mir 25	Upregulation	PTEN,E2F1	+			
mir 21	Upregulation	PTEN,PDCD4,TPM1,TIMP3	+++	Poor	+	+
mir 27a	Upregulation	SPRY2	mir27a-3p	Poor		+
mir31	Upregulation	MiaPaCa2				+
mir30a-3p	Downregulation	MUC4,MUC16		Good		
mir 34	Downregulation	p53, CDK6			+	
mir 96	Downregulation	KRAS				+
mir 99a	Downregulation	mTOR	+			
mir105	Downregulation	MUC4,MUC16		Good		
mir 124	Downregulation	Rac1		Poor		
mir 127	Downregulation	MUC4,MUC16		Good		
mir 128	Upregulation			Poor		
mir 130b	Downregulation	STAT3,Hedgehog		Poor		
mir 146a	Downregulation	TRAF6,IRAK1,STAT1			+	+
mir 148a,b	Downregulation	DNMT3b,MTIF,CCKBR,BCL2	+			
mir 155	Upregulation	tp53inp1,sel1l	+++	Poor		
mir 181	Upregulation	TIMP3,TCL1			+	
mir 185	Upregulation	DNA methyltransferases 1	+			
mir 187	Downregulation	MUC4,MUC16		Good		
mir 191	Upregulation	USP10	+			
mir 196a	Upregulation	HOXB8,ANXA1,HMGA2	+++	Poor		
mir 198	Downregulation	MSLN,PBX-1,VCP		Poor		+
mir 200c	Downregulation	MUC4,MUC16		Good	+	+
mir 203	Upregulation	TP531NPI,ELAVL2	+	Poor		
mir 204	Downregulation	MCl-1				+
mir 205	Downregulation	TUBB3			+	
mir 210	Upregulation	HOXA1,FGFRL1,HOXA9	+++	Poor		
mir 217-219	Downregulation	AKT,Kras		Poor		
mir 221/222	Upregulation	CDKN1b(P27),puma,pten	+	Poor		+
mir320c	Upregulation	SMARCC1			+	
mir 365	Downregulation	SHC1, BAX			+	
mir 373	Upregulation	TP53INP1,LATS2,CD44	+			
mir 375	Downregulation	PDK1,14-3-3ZETA	+			
mir 424-5p	Upregulation	SOCS6		Poor		
mir 452	Downregulation	MUC4,MUC16		Good		
mir 1290	Upregulation	FoxA1	+			
mir 3548	Downregulation	MiaPaCa2				+

fferentiation, in chemotaxis as well as in glucose homeostasis.

PI3K way is a major effector of Kras signaling way and it is necessary for the survival of the tumoral cells. One of the PI3K targets is Rac, a G protein that may be activated by phosphatidyl 3,4,5 – triphosphate (PIP3). Rac protein by means of the nuclear factor (NF)-κB regulates anti-apoptotic ways thus playing an important role in the genesis of pancreatic tumor[15]. It has been showed that the increased expression of mir301a suppresses NKRF expression thus leading to the increase of NFKB activation by promoting the initiation and progression of the pancreatic cancer[21] (Table 1).

c) Notch pathway

Notch proteins belong to the family of membrane proteins and function as receptors for membrane ligands. They use the method of lateral inhibition by which a single cell is programmed to differentiate, whereas its neighboring cells remain undifferentiated (a procedure used by the pancreas during the embrionary development).

The excessive expression of Notch way is correlated with the excessive expression of the vascular endothelial growth factor (VEFG). On the other hand, the concomitant inhibition of the epidermal growth factor (EGF) and the notch way leads to the reduction of the cellular proliferation and an increase of apoptosis, but also to a reduction of NF–κB activity, thus playing a major role in the oncogenesis of the pancreatic cancer[2].

More studies have shown that Notch proteins are regulated by micro RNA during pancreatic oncogenesis. Preclinical studies have shown that p53 targets Notch proteins by means of miR – 34 and, consequently, it plays a role in the maintaining and survival of the initial tumoral pancreatic cells. On the other hand, the reactivation of miR - 34 inhibits the invasion and proliferation of the tumoral pancreatic cells finally leading to apoptosis[22]. Mir144 is another example of microRNA acting by means of Notch proteins (Table 1).

d) Angiogenesis is the formation of new blood vessels around an inside the tumor through the proliferation and migration of the endothelial cells.

Angioblasts proliferate under the influence of VEGF and FGF, followed by the formation of some lumens delimitated by the immature endothelial cells, after which it develops vascular channels delimitated by mature endothelial cells. Neovascularization has a double effect: it provides the nutritive contribution and oxygen and the endothelial cells formed stimulate the tumoral growth factors by secreting polypeptides such as insulin like growth factor, PDGF, GM-CSF, IL-1. Angiogenesis is necessary for the tumoral growth and metastases being biologically correlated to the metastasis spreading.

Cellular levels of mir 15b and mir-16 decrease in hypoxia conditions. Thus, since the inhibiting activity they exercise on VEGF is reduced, they promote tumoral angiogenesis[23]. Hypoxia is involved in this way by HIF factor which finally leads to an increased expression of VEGF. Other types of micro RNA involved in this signaling way are miR- 203 and miR – 222[24]. *In vitro* studies have shown that mir- 222 affects c - kit expression and, consequently, controls angiogenesis (Table 1).

e) The cellular cycle

The regulation of the cellular cycle is crucial for the cellular survival. A series of cyclin-dependent kinases have been identified as being involved in the signaling ways that lead to the progression of the cellular cycle.

P16 is a central regulator of G1-S phase of the cellular cycle by inhibiting the phosphorylation of retinoblastoma protein (pRb), and by blocking E2F release. It has been noticed that 95% of pancreatic carcinomas exhibit a loss of p16 function. This gene is inactivated in the tumoral cells due to the deletes or hypermethylation processes at the promoter level. The locus codifying p16 protein also codifies other proteins as well such as p27 and p57[15].

The increased expression of miR221 in the pancreatic cancer affects the translation of p27 (CDKN1B gene), links p27 proteins and prevent the activation of E cyclin complex with CDK2 or CDK4, thus controlling the progression of the cellular cycle in G1 phase.

On the other hand, mir-124, expressed in a small quantity in the pancreatic cancer, inhibits the proliferation, invasion and metastasis spreading by the direct action that it has on Rac1. Rac1 is a protein from GTP family which is involved not only in the control of the cellular cycle, but also in the cytoskeletal reorganization, cellular adhesion, the activation of protein-kinases, and epithelial differentiation[1].

f) Hedgehog

Hedgehog protein is produced as a precursor which by autocatalysis is divided and attached to the cellular surface. These proteins are capable to signal autocrine and paracrine. If the cell receives signals from hedgehog proteins it expresses the membrane proteins: Smoothened and Patch. In the absence of hedgehog signaling, the patch receptor suppresses the activity of trans-membrane receptor, Smoothened. Despite all

these, in the presence of hedgehog signaling, the receptor is stimulated leading to the activation of a protein which arrived at the level of the nucleus will activate the gene transcription. The Hedgehog signaling way appears precociously in the progression of intraepithelial pancreatic neoplasia. PATCH1, a member of the hedgehog signaling pathway is the direct target of mir 212[25].

Role of microRNA in the management of pancreatic adenocarcinoma

As we have mentioned before, at present, there are no efficient paraclinical methods for the early diagnosis of the pancreatic adenocarcinoma. The imagistic methods allow the visualization of cancer only when this is in advanced stage. For this purpose, they try to identify certain tumoral markers (miRNA having the most promising results) that will be used for the screening of the patients at risk.

The key for the successful analysis of miRNA in the pancreatic cancer is represented by the quantity, quality and type of sample/material harvested. Thus, the serum samples are the most adequate for the clinic diagnosis and the determination of prognosis, but the main problem in this case is the limited quantity of miRNA and the more reduced specificity. The samples from frozen surgical resections or included in paraffin blocks usually offer a large quantity of material both for histology and for molecular tests. The high content of malignant cells in the examined material is necessary for the quantification of the genes associated to cancer. But many patients are not subject to surgical interventions, a situation when the pancreatic tissue may be harvested only by puncture - fine needle biopsy (FNB) under endoscopic ultrasound guidance (EUS). The disadvantage in this case is represented by the small quantity of product obtained. Once the sample has been obtained, the total quantity of RNA (and microRNA) is extracted that will reversely transcribed in DNAc. Subsequently, microRNA may be analysed either by PCR amplification or by hybridization.

a) response to treatment in non-excisable cases[26]

The most frequent causes to develop resistance to drugs are represented by the inhibition of the efflux pumps, the lack of sensitivity to the apoptosis induced by the drug and the development of some mechanisms for drug elimination.

The microclimate of the tumoral cell (the interaction between integrins and the components of the extracellular matrix) is responsible for the inborn resistance[27].

The tumoral cells having a high expression of mir-21 are resistant to the gemcitabine treatment by the decrease of PTEN expression and the increase of the activity of PI3K/Akt/mTOR signaling way. The modulation of apoptosis, the AKT phosphorylation and the expression of the genes involved in the invasive behaviour may contribute to the inducing of resistance to treatment in this case. Wang et colab have noticed that an increase of FasL expression after the treatment with gemcitabine leads to the apoptosis of the tumor cells, this phenomenon not occurring any more if the tissue exhibits an ectopic increase of mir-216.

At the same time, the researchers demonstrated that this resistance may be prevented through the administration of PI3K and mTOR inhibitors (mammalian target of rapamycin).

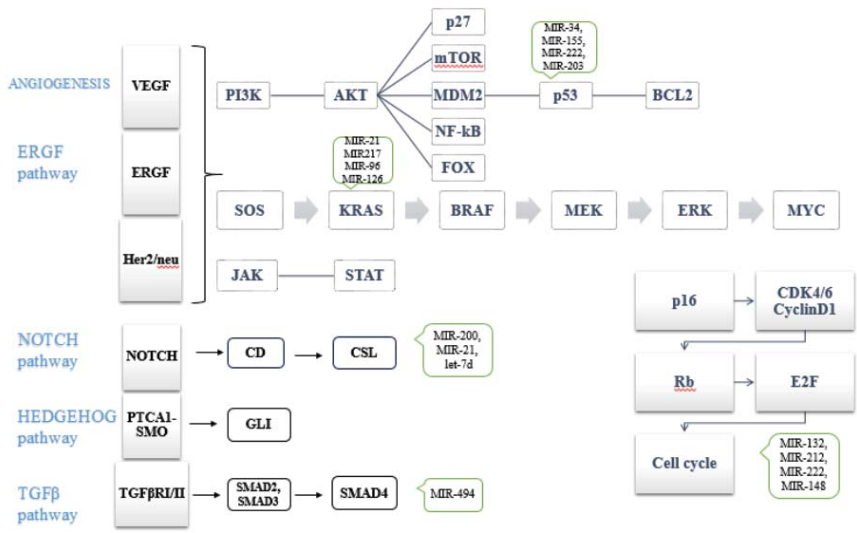


Figure 3. Tumoral signaling pathways.

Lentiviral vectors were used on miRNAs which may express antagonists of miRNAs and we obtained the interruption of tumoral proliferation and the induction of in vitro and in vivo apoptosis. Mir-365 is another representative inducing the drug resistance through the action that exercises on SHC1 (adaptor protein Src homology 2 domain containing 1) and on the protein promoting apoptosis (BAX)[28].

Mir-320c provides resistance to gemcitabines by means of SMARCC1, a subunit of the remodeling complex of SWI/SNF chromatin. Subsequent examinations confirmed that only the patients with a positive SMARCC1 had good results following the treatment in terms of survival and lack of recurrence[29].

More studies also found out that miRNAs may increase the sensitivity to treatment.

The genetic transfer of miR - 205 has led restoration of chemosensitivity to gemcitabine with the decrease of expression of the stem cell markers Oct3 / 4 and CD44 and of b chemoresistance markers – class 3 tubulins (TUBB3)[30].

Mir-34 expression is mediated normally by p53 suppressor gene, but it may be inactivated in cancer by the aberrant methylation of CpG. Studies have shown that the restoration of mir-34 expression stops the cellular cycle in G1 phase and induces apoptosis in some cancers[5].

To make sure that the treatment functions, it is recommended to monitor the patient's evolution by means of CA199.

b) differential diagnostic between cancer, pancreatitis and a normal pancreas

High levels of miR-21, miR-155, miR-196a, miR-221 and miR-222 have been reported most frequently in the pancreatic cancer[27].

- Mir 196a, mir 217 extracted from the tissue sample diagnoses the cancer have 100% specificity and 90%sensitivity[31].
- Mir 21, mir 210, mir 196a, mir 155 have 89% specificity and 64% sensitivity in PDAC diagnosis from plasma[32].

MiR-198 and miR-650 may be found in high quantities both in the adenocarcinoma and in chronic pancreatitis as compared to the normal pancreatic tissue, whereas MiR-130b, miR-141, miR-194 and miR-219-1-3p are expressed in low quantities in pancreatitis and pancreatic cancer.

Mir 200a, mir 200b, mir 221 harvested from patients' serum have high values in case of patients with pancreatitis and pancreatic adenocarcinoma. Mir 27a-3p has high values in the mononuclear cells of the peripheral blood.

The evaluation of miR 16, mir 196a, mir 203, mir 210 in plasma is used for the differential diagnosis between PDAC and pancreatitis/normal[33].

Mir 20a, mir 21, mir 24, mir 25, mir 99a, mir 185, mir 191, mir 1290[34] in serum correctly determined PDAC in proportion of 83.6%.

To increase sensitivity, we may determine CA19.9 in parallel according to the following schemes: CA 19.9+ mir 27a-3p or Ca19.9+mir16, MIR 196a in plasma with 95.6% specificity and 92% sensitivity[35].

c) patient's reserved prognosis

The quantification of the microRNA level may be used to determine patients' prognosis. Thus, the increase of MIR-203, mir 196-a2 expression reduces patients' survival down to 14.3 months (as compared to 26.5l), mir219 (13.6 months as compared to 23.8 months)[36]. At the same time, the decrease of mir 124, 128, 375 expressions37 announces a short survival.

The associations between increased mir 212, 675 + low mir 148a, mir187+leg 738 or increased mir21 + low mir34a, mir 30d were identified in case of the patients having a reserved prognosis.

A high level of survival (>2 years) has been identified in case of mir 452, mir 105, mir 127, mir 158-a2, mir 187, mir 30a-3p, mir 200c expression (through the reduction of MUC4.16 expression)[39].

Mir 21, mir 10b, mir 217, mir 155[32], mir 196 are constantly high in PDAC and they are an indicator for a bad prognosis or they may be used to assess the response to treatment.

Low serum levels of mir 218 foretell an unfavorable prognosis with a 5 year survival rate of 7.5%, its values correlating to TNM classification, distance metastasis spreading and the degree of tumoral differentiation[40].

d) potential of metastasis spreading

The invasiveness of the tumor cells and the metastatic processes play an important role in the tumoral progression. An essential stage of cancer spreading is the transformation of epithelial tumor cells in mesenchymal cells which are capable to cross the basal membrane and to reach the blood flow. This transformation results from reduced expression of the transmembrane protein for E-cadherin cell adhesion. In the pancreatic cancer, it has been noticed that this transmembrane protein is inhibited by ZEB1 (zinc finger E-box-binding homeobox 1) and SIP1 (Smad-interacting protein 1, ZEB2, and SMADIP1) by means of the members of mir-200 family (miR-200a, b, c, miR-141 and miR-429), mir-203, miR-208.

Mir-143 has proved its implication in the invasiveness of the tumor cells by the reduction of Rho GT-

Pases activity. These are G proteins controlling many processes associated to metastasis spreading, such as the intercellular contact and cellular migration.

At the same time, miR - 10a supports the capacity of metastasis spreading of the pancreatic cells by suppressing HOXA1,2,3 transcription factors[1].

The induction of mir-21 expression increases the proliferation and spreading of the pancreatic tumor cells by the targeted inhibition of PTEN (phosphatase and tensin homolog), PDCD4 (programmed cell death 4), the expression of tropomyosin 1 (TPM1), and the tissular inhibition of metalloproteinase 3 (TIMP3) indirectly inducing the expression of metalloproteinase matrix 2 and 9, as well as of the vascular endothelial growth factors (VEGF)[41].

It is also known that EP300, a histone regulating the transcription by chromatin remodeling, plays an important role in cellular growth. This enzyme has a low expression in the cancerous cells having a high potential of metastasis spreading and, consequently, it is considered as a suppressor of metastasis spreading. By the direct action a group of microRNA made up of miR-194, miR-200b, miR-200c, and miR-429 exercises on EP300 the susceptibility for cancer spreading is increased. Similarly, mir-224 and mir-486 target CD40 which is a member of the family of receptors of the tumoral necrosis factors. It has a major role in the immune anti-tumoral response and plays the role of metastasis mediator when it is inhibited by the two types of micro-RNA.

On the other hand, the ectopic expression of miR-146a inhibits the expression of NF-KB kinase, EGFR, and (IRAK - 1) what leads to the inhibition of the spreading of these pancreatic cellular lines.

Mir- 20, which is a member of 17-92 –miR family, has a metastatic suppression effect by STAT3 inhibition interrupting the proliferation and invasiveness of the cancerous cells[2].

It has been demonstrated that miR - 10a promotes the metastatic behaviour of the pancreatic cells, its expression being regulated by retinoids. The use of antagonists of retinoic acid receptor inhibits 10a expression and stops the metastasis of the pancreatic cells.

In exchange, mir - 146a suppresses the tumoral invasion, but its expression is low in the pancreatic cancer as compared to the normal pancreatic tissue. Thus, by the use of isoflavones or DIM (3,3'-diinodolylmethane), we may increase miR - 146a expression, thus blocking invasiveness and metastasis spreading[1].

CONCLUSIONS

The main issue in the management of the pancreatic cancer is the lack of a set of biomarkers for an early diagnosis. This is extremely important knowing that survival and prognosis depend on the tumor stage at the moment of diagnosis. The early diagnosis accompanied by small size tumoral resection is usually associated to the best prognosis.

According to the numerous studies on this topic, we may state that by the large implication in the cellular mechanisms for regulation of the cellular cycle, in the DNA repair, the control of apoptosis and in the mechanisms of cancer spreading, miRNAs may be used as potential biomarkers for the clinical management of the pancreatic cancer.

A better understanding of the principles and complex mechanisms of genes expression associated to miRNA may lead to new therapy opportunities for the pancreatic cancer.

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