

Novel Biomarkers in Pancreatic Cancer

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1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) cancer is one of the most aggressive human cancers, and the fifth most frequent cause of cancer-related mortality in Western society. Pancreatic cancer is well known for high metastatic potential, early local invasion and poor outcome. The overall 5-year survival rate is less than 5%, respectively 10-30% for R0 resection (Huang et al., 2010). Less than 10% of newly diagnosed pancreatic cancers could be detected in early-stage (Takayama et al., 2010).

Clinical research in the field of cancer biomarkers is essential in understanding the biology and the heterogeneity of cancer disease. The factors involved in early PDAC development remain unknown. The detection of pancreatic cancer at early stages, the prediction of the potential resectability, or response to therapy are the current major challenges in improving the clinical outcome of PDAC. Therefore, predictive markers of responsiveness to adjuvant therapy would allow patients selection to appropriate treatment (Duffy et al., 2007). The aim of the postoperative surveillance following curative surgery for PDAC is to detect recurrences or metastases as early as possible.

Currently, there are only few studies that have identified cancer biomarkers with clinical significance.

Molecular biological factors are important tools for early diagnosis, prognosis, not only for therapeutic strategy but also for novel and more efficient therapeutic agents' identification. On the other hand a biomarker must be easily quantified, in order to minimize the invasiveness of therapeutically interventions. Recent finding in the molecular biology field of pancreatic cancer have assisted in translational research, giving hope for individualized therapy and better disease management.

2. Identification of new potential tumor tissue biomarkers

Much effort goes into finding new accurate prognostic, diagnostic single or combined tumor biomarkers. Nowadays, the research in this field is focused not only on finding biomarkers that could discriminate between pathological pancreas conditions (disease related biomarkers), but also to evaluate the aggressiveness grade of PDAC and to determine the therapy response (drug related biomarkers).

Presently, screening for pancreatic cancer is based on state-of-the-art imaging or even invasive diagnostic methods (Balasenthil et al., 2011). Serum and other body fluids, such as urine, pancreatic juice represents sources available by less invasive methods for biomarkers screening.

Several techniques like immunohistochemistry, fluorescence and chromogenic *in situ* hybridization, expression profiling- performed by microarray or quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR), and mutation analysis are used in identification of new biomarkers. Among these techniques, immunohistochemical tests remain the most widely used in routine practice and, importantly, in the assessment of biomarkers in translational research.

In a recent study, Wachter *et. al* investigated the expression level of an oncofetal protein, insulin-like growth factor II messenger ribonucleic acid-binding protein 3 (IMP3), which represent a marker for tumor aggressiveness in many different tumors (Wachter et al., 2011; Ozdemir et al., 2011).

The investigation was conducted on tissue biopsies from PDAC, chronic sclerosing pancreatitis, PDAC metastases cases in order to determine IMP3 expression. For IMP3 expression evaluation large tissue sections were used in the immunohistochemical analysis. The results obtained showed that PDAC were positive for IMP3 expression in a high percentage (88.4%) of cases, whereas normal or inflammatory pancreatic tissue was weakly positive (23.1%). A strong IMP3 expression was found in PDAC metastases (94.4%). The sensitivity and specificity of IMP3 expression test to discriminate between PDAC and chronic sclerosing pancreatitis using core needle biopsies were found to be 88.4% and 94.6%, respectively. The authors consider that IMP3 might be an easy to use and potentially new immunohistochemical marker for the diagnosis of PDAC in core needle biopsies (Asioli et al., 2010; Walter et al., 2009).

Another prognostic biomarker in PDAC which characterize the tumor aggressiveness recently studied was vimentin. Vimentin protein is a marker of mesenchymal differentiation, being correlated especially with aggressive carcinomas. In a high percentage primary pancreatic adenocarcinomas contain neoplastic cells that express vimentin, and the presence of this protein was correlated with poor histological differentiation and predicts a shorter postsurgical survival (Li et al., 2009; Handra-Luca et al., 2011).

Several technical strategies (SDS-PAGE, mass spectrometry, immunoblot) are used to find candidate biomarkers for the presurgical management of malignant and premalignant pancreatic conditions. Pancreatic cystic neoplasms represent 10–15% of primary cystic masses of the pancreas and are detected with an increasing frequency due to the use of advanced imaging modalities in clinical practice. On the other hand, the diagnosis of pancreatic cystic neoplasms remains a challenge because available diagnostic techniques are

not so specific. The analysis of pancreatic cyst fluids obtained from various cystic lesions showed that specific histological lesions are associated with distinct protein patterns. Two important factors, olfactomedin-4 (antiapoptotic protein that promotes tumor growth) and mucin-18 (melanoma cell adhesion molecule) were proposed as biomarkers of pancreatic cancer (Cuoghi et al., 2011).

CD99 (cell surface glycoprotein involved in leukocyte migration), a useful diagnostic marker for Ewing sarcoma/primitive neuroectodermal tumor (Rocchi et al., 2010) was proposed for differentiation of solid-pseudopapillary neoplasm from other pancreatic tumor. The tissues positivity for CD99 was investigated in a recent study (Guo et al., 2011) using immunohistochemical staining technique. The solid-pseudopapillary neoplasm cells tumors exhibited paranuclear dot-like immunoreactivity for CD99. In contrast, in pancreatic neuroendocrine tumors a small percent of PDAC stained positive for CD99 at cytoplasmatic and membrane level. Pancreatic solid-pseudopapillary neoplasm exhibits a unique dot-like staining pattern for CD99 and could provide a definitive diagnosis of solid-pseudopapillary neoplasm and differentiation from other pancreatic tumors.

Immunohistochemical analysis of pancreatic cancer tissue provided also several candidate biomarkers for survival estimation. CDCP1 (CUB domain containing protein 1) determines anchorage- independent growth and migration of cancer cells. A higher expression level of this factor is correlated with the overall survival of pancreatic cancer patients (Miyazawa et al., 2010). L1-CAM (L1-cell adhesion molecule) expression was also correlated with perineural invasion of pancreatic cancer cells and poor survival (Ben at al., 2010). Higher expression of B7-H3, a co-stimulatory immune molecule, plays a critical role in the T cell-mediated immune response and presents a positive correlation with pancreatic cancer prognosis.

KOC (K homology domain containing protein) gene encodes a protein that contains several KH domains, which are important in RNA binding and are known to be involved in RNA synthesis and metabolism. This protein was found to be overexpressed in pancreatic cancer. Immunohistochemical analysis showed strong staining for KOC protein in invasive pancreatic tissue carcinomas versus normal pancreatic tissue. It was proposed to be a molecular marker with a high sensitivity and specificity in discriminating PDAC from benign ductal epithelium (Toll et al., 2009).

3. Genome candidate biomarkers

Most of the adenocarcinomas develop gradually from precursor lesions PanINs (Pancreatic intraepithelial neoplasias). These events are accompanied by genetic modifications. Most genetic abnormalities reported in pancreatic cancer are represented by deletions and duplications of specific chromosomal loci, mutations/deletions of oncogenes and tumor-suppressor genes (KRAS, CDKN1A/p16, TP53, MADH4/SMAD4/DPC4 and BRCA2) [www.cancer-genetics.org].

3.1 Gene expression studies and potential factors involved in pancreas oncogenesis

Quantification of target mRNA gene levels represents a new tool for genome function analysis. High-throughput technologies like gene expression profiling using microarray and sequencing become important investigation methods for normal physiological and

pathological processes. Therefore, studies based on quantification of gene expression levels revealed new potential factors involved in pancreas oncogenesis. Using Affymetrix and cDNA microarrays, nearly 1,100 molecules have been reported to be overexpressed in PanIN and IPMN lesions. A large majority of these molecules showed elevated mRNA levels expression in PDAC and in precursor lesions. Molecules such as S100P, MMP7, MUC4, FSCN1, and MUC5AC are found to be overexpressed in all type of lesions PanIN, IPMN and PDAC (Harsha et al., 2009).

Badea et al. using microarray study identified a number of genes whose over-expression appears to be inversely correlated with patient survival: keratin 7, laminin gamma 2, stratifin, platelet phosphofruktokinase, annexin A2, MAP4K4 and OACT2 (MBOAT2), which are specifically upregulated in the neoplastic epithelium (Badea et al., 2008).

The attention was focused especially on the calcium-binding protein- S100P. This protein was found to be expressed in pancreatic precursor lesions PanIN 2 or PanIN 3 and in pancreatic tumor creating the possibility to use the quantification of its expression level for earlier detection (Downen et al., 2005). Expression levels of *S100P* mRNA were found to be higher in pancreatic juice from patients with pancreatic cancer and IPMN (Ohucida et al., 2006). On the other hand, three members of S100A family (S100A2, S100A4, and S100A6) were found to be associated with poor prognostic. S100A family members were involved in cell cycle regulation and cell invasion (Tanase et al., 2010).

The STAT3 transcription factor was found to be constitutively activated in PDAC. This is an important factor in stem cell self-renewal process, cancer cell survival, and inflammation. A close correlation between the levels of tyrosine-phosphorylated STAT3 and of the gp130 receptor was found. An upregulation of the IL6/LIF-gp130 pathway was also showed to be involved in STAT3 activation in pancreatic cancer (Corcoran et al., 2011). The same study asserts that STAT3 is required for the development of the precursor pancreatic lesions, acinar-to-ductal metaplasia (ADM) and PanIN, therefore evaluation of gp130 and phospho-STAT3 expression may be an effective biomarker.

3.2 Angiogenesis factors

With the increasing use of antiangiogenic agents for the treatment of cancers, is necessary the identification of candidate biomarkers for evaluation of the response and resistance. Is also, important to identify new biomarkers and to eliminate the risk for antiangiogenic therapies failure (Duda et al., 2010).

EGF (Epidermal Growth Factor), VEGF (Vascular Endothelial, Growth Factor) , heparanase, thrombospondin, cathepsins were the most important angiogenic factor specific for pancreatic cancer involved in cell growth promoting. The overexpression of EGF and his receptor EGFR were linked to tumor staging, but no clear data regarding the overall survival are available at this moment (Heidemann et al.,2006).

Another important factor that acts on angiogenesis process is VEGF. His effects in PDAC are increased by interaction with MMP-9, a cellular matrix remodeling factor. The therapy against both MMP-9 and VEGF in pancreatic cancer resulted in a significant decrease in PDAC growth and microvessel density versus a single target treatment (Tanase-Pistol et al.,2008).

Heparanases are endoglycosidases that cleave the heparan sulfate side chain of heparan sulfate proteoglycans (major membrane components) inducing extracellular matrix remodelling. On the other hand, heparanase increased growth factor bFGF (fibroblast growth factor) release, stimulating the angiogenesis process (Rohloff et al., 2002).

Thrombospondin (TSP-1) expression level was linked to a good prognostic for PDAC development. TSP-1 protein was found abundantly in stroma surrounding tumor cells and its expression is inversely correlated with microvessel density (Tobita et al., 2002).

The role of cathepsins in pancreatic cancer development and progression is still controverted. Cathepsin b (CTSB) and cathepsin l CTSL was found to be overexpressed in PDAC. A correlation between CTSB expression level and perineural invasion concludes the role of cathepsins in local tumor invasion (Niedergethmann et al., 2000).

3.3 ZIP3 (Zinc/iron regulated transporter-related protein 3) a possible tumor suppressor

Zinc is the most abundant trace element in cells. For example, about 25% of the total zinc present is found within the cell nuclei, being a component of chromatin. Zinc is an important factor in cellular processes, including cell division and proliferation, immune function, and defense against free radicals; zinc deficiency may be associated with an increased risk of cancer (Christudoss et al., 2011; Prasad et al., 2002). Zinc is found in over 300 enzymes, including copper/zinc superoxide dismutase, which is an important antioxidant enzyme, involved in cellular protection components from oxidation and damage.

In a current study, *Costello et al.* found a major loss of zinc in ductal and acinar epithelium in adenocarcinoma compared to the normal epithelium (Costello et al., 2011). The decrease in zinc quantity is a characteristic not only for pancreatic cancer, but also for precursor lesions. The mentioned group showed that the gene expression of ZIP3 (basilar membrane zinc uptake transporter) is present in normal ductal/acinar epithelium and absent in adenocarcinoma. The decreased expression of ZIP3 determines the loss of zinc in early and progressing malignancy. RREB1 transcription factor was found to be down regulated along with ZIP3 and might be the silencing cause of ZIP3 gene. Zinc treatment exhibited cytotoxic effect on Panc1 cell line. ZIP3 and RREB-1 expression level changes represent early events in the development of adenocarcinoma suggesting that ZIP3 might be a tumor suppressor gene.

3.4 Other important factors

FAP (fibroblast activation protein) is involved in the control of fibroblast growth or epithelial-mesenchymal interactions during development, tissue repair, and epithelial carcinogenesis. It is highly expressed in PDAC and is considered to be related to a poor prognostic. Targeting FAP factors are considered to be a promising and a new therapeutical road (Cohen et al., 2008).

Maspin (Serp1B5- mammary serine protease inhibitor) is a tumor suppressor gene. Maspin induces apoptosis in neoplastic cells and expression of maspin are suppressed as the carcinoma progresses in breast and prostatic carcinoma (Jiang et al., 2002). The pattern of maspin gene expression is dependent of the disease stage. For example, highly expression

was observed in intraductal papillary mucinous neoplasms, mucinous cystic neoplasms, and in carcinomas, whereas in adenomas is lower. It was also observed a significant decrease in maspin expression when intraductal papillary mucinous neoplasms progresses to invasion. However, the group with high maspin expression presented microinvasion, whereas low expression maspin group showed extensive invasion (Kashima et al., 2008). These results conclude that maspin could be a good prognostic factor for invasion.

4. Cancer stem cell biomarkers

Stem cell markers are a promising group of new biomarkers. Solid tumors contain small proportions of cells that are capable of proliferation, self-renewal, and differentiation into various cell types. These types of cells (cancer stem cells) are characterized by treatment resistance, especially to ionizing radiation. Therefore, it is a very challenging situation in order to identify these cells, to understand the mechanisms of resistance, and to evaluate the patient therapy outcome regarding the response to treatment. Identifying the markers that characterize cancer stem cells is the main research and a specific pattern regarding cell surface markers is emerging. In breast cancer, stem cells presented a characteristic antigenic pattern, whereas in high-grade gliomas, expression of CD133 on the cell surface selects a population of treatment resistant cells (Woodward & Sulman, 2008).

In pancreatic cancer, several surface markers have been identified for a subpopulation of the tumor cells with stem cell characteristics. These cancer stem cells were identified by expression of the cell surface markers CD44, CD24, and ESA (Li et al., 2007). When injected in the pancreas of immunocompromised mice these human cells are able to self-renew and generate differentiated progeny, to recapitulate the phenotype of the tumor from which they were derived.

Another subpopulation of cancer stem cells highly tumorigenic was isolated from patients with PDAC. These cell types were CD133+ and were able to induce tumor formation in athymic mice. This subpopulation of migrating cancer stem cells are characterized also by expression of the CXCR4 receptor and are involved in tumor metastasis Hermann et al., 2007.

Another candidate cell marker investigated was aldehyde dehydrogenase 1A1 (ALDH1A1), which has been identified to label cancer stem cells in breast cancer (Ginestier et al., 2007), colon cancer (Huang et al., 2009), lung cancer (Jiang et al., 2009) and head and neck squamous cancer (Chen et al., 2009). ALDH1 is a member of the aldehyde dehydrogenase gene (ALDH) superfamily playing an important role in the metabolism of endogenous and exogenous aldehydes. This NAD(P)(+)-dependent enzyme is also involved in the formation of molecules that are important in cellular processes, like retinoic acid (acting like modulator of gene regulation and cell differentiation), betaine and gamma-aminobutyric acid and exhibits additional, non-enzymatic functions, being able to bind to some hormones (Yoshida et al., 1998). The ALDH1 gene expression is ubiquitous in many human tissues; the protein is found localized mainly in the cellular cytoplasm.

It was also shown that ALDH1 has the capacity to detoxify aldophosphamide, conferring chemoresistance against cyclophosphamide to overexpressing cell (Hilton, 1984). Data regarding the effect of ALDH1A1 overexpression are controversial. Increased expression of

ALDH1 in ovarian cancer correlates with a favourable prognostic. In contrast, pancreatic cancer increased expression of ALDH1A1 was correlated with poor survival (Rasheed et al., 2010).

ALDH1+ breast tumors are negative for estrogen receptor and progesterone receptor overexpression, but present a high level of Ki67 expression (a nuclear protein that is associated with cellular proliferation) (Morimoto et al., 2009). Therefore, Kahlert et al. showed that a high expression of ALDH1A1 is significantly correlated with the proliferation rate of pancreatic tumour cells (Kahlert et al., 2011).

5. Drug-related biomarkers

Most of the pancreatic cancer patients have inoperable disease due to distant metastases or locally advanced tumor and the main therapeutic decision in this case is systemic chemotherapy or chemoradiation. Therefore, understanding the mechanisms that govern drug-related resistance and the factors involved in this process are a mandatory condition for therapeutic management success.

5.1 Metabolic prognosis factors for gemcitabine resistance

Patients with advanced or metastatic pancreatic cancer are treated with gemcitabine as a first line chemotherapeutic agent. Gemcitabine is a 2',2'-difluoro-2-deoxycytidine analogue that inhibits DNA replication and repair. Gemcitabine possesses radiosensitizing properties and its administration must be combined with radiotherapy. The sensitivity to gemcitabine and its efficiency were correlated with several factors. One of the factors that correlate with gemcitabine sensitivity is hENT1 (human equilibrative nucleoside transporter 1) (Nakano et al., 2007).

Pancreatic cancer cells that highly express hENT1 are sensitive to gemcitabine by uptaking this therapeutic agent in cancer cells. The absence hENT1 expression in metastatic pancreatic adenocarcinoma patients treated with gemcitabine was associated with a poor prognostic compared with patients whom tumor cancer cells presented hENT1 expression (Hamada & Shimosegawa, 2011).

At the cellular level, gemcitabine is phosphorylated to its active metabolites by dCK (deoxycytidine kinase). Another group reported that not only hENT1 higher expression is a possible prognostic factor, but also dCK expression level and its activity correlates with gemcitabine activity (Ashida et al., 2009). High dCK enzyme activity was linked with gemcitabine sensitivity in experimental models (Kroep et al., 2002) and biopsy samples analysis (Sebastiani et al., 2006).

Gemcitabine inactivation is performed through an enzymatic deamination process. There are three important enzymes involved in gemcitabine deamination (deoxycytidylate deaminase-DCD, cytidine deaminase-CDA and 5'-nucleotidase -5'-NT). The increased activity of such enzymes could induce the resistance to gemcitabine (Kroep et al., 2002; Giovannetti et al., 2006; Nakano et al., 2007) Gemcitabine resistance mechanism is also realized by high expression of RRM1 and RRM2, which are the two subunits (large and small) of ribonucleotide reductase. This enzyme regulates the rate of DNA synthesis, and is also known to convert ribonucleotides to deoxyribonucleotides. Gemcitabine exerts its

cytotoxicity by inhibiting ribonucleotide reductase (Davidson et al., 2004; Bergman et al., 2005; Nakahira et al., 2006)

6. Epigenetic biomarkers

Cancer initiation and progression is traditionally characterized as a genetic alteration, but recent years crystallized in a new direction regarding epigenetic mechanisms involvement in oncogenesis. Epigenetic mechanisms are essential for normal development and for maintaining of a tissue specific pattern of gene expression. Epigenetic modifications lead to an abnormal genetic expression and further to malign transformation. The research in epigenetic field has demonstrated the involvement of an intensive reprogramming of epigenetic machinery (DNA methylation, histone modifications, microRNA expression). The reverse nature of epigenetic abnormalities permitted the development of epigenetic therapy field.

6.1 Gene promoter methylation status and pancreatic oncogenesis

The most known epigenetic modification in oncogenesis is DNA hypermethylation. This event is accompanied by genetic silencing. Identification and characterization of epigenetically silenced genes is important in order to understand the roles of such epigenetic modification in oncogenesis and to discover new tumor markers. It was reported that in pancreas cancer some tumor suppressor genes presented aberrant CpG islands hypermethylation at gene promoter level.

The first tumor suppressor gene identified to be specific for pancreatic cancer was p16/CDKN1A (Schutte et al., 1997). Subsequently, new hypermethylated genes were associated with pancreatic cancer (hMLH1, E-cadherin, ppENK, CDKN1C, SPARC, TFPI-2, GATA4,5, BNIP3, TSLC1, HHIP, MUC2, reprimin, CXCR4 si SOCS1) (Omura et al., 2009).

Several important epigenetically silenced factors have been identified (hsa-miR-9-1, ZNF415, CNTNAP2 si ELOVL4) (Omura et al 2009; Grady et al.,2008; Lehmann et al.,2008; Fabbri et al., 2007; Moriss et al., 2004).

Using methylated CpG island amplification (MCA) and representational difference analysis (RDA), Ueki *et al* group identified that gene preproenkephalin (*ppENK*) presented a hypermethylated promoter in most pancreatic cancers (Ueki et al., 2001). *ppENK* encodes an opioid peptide which presents growth-suppressor properties.

It was showed that CDKN1C/p57KIP2 gene presented a decrease expression in intraductal papillary mucinous neoplasm. The gene encodes for cyclin-dependent kinase inhibitor, and is a negative regulator of cell proliferation (Sato et al., 2005). Partial methylation of the *CDKN1C* promoter was found in pancreatic cancer cell lines and pancreas cancer.

SPARC (secreted protein acidic and rich in cysteine, or osteonectin/BM40) is a matrix-associated protein; calcium binding, that inhibits cell-cycle progression, and influences the synthesis of extracellular matrix. The gene codifying this protein was found aberrantly methylated in pancreatic cancer by. SPARC is a factor involved in many processes like cell migration, proliferation, matrix cell adhesion (Sato et al., 2003, Gao et al., 2010).

Tissue factor pathway inhibitor 2 (TFPI-2) is a Kunitz-type serine proteinase inhibitor, which has been identified as a putative tumor-suppressor gene. Aberrant methylation of *TFPI-2*

was found in pancreatic cancer xenografts and primary pancreatic adenocarcinomas. Re-expression of the *TFPI-2* gene led in the proliferation, migration and invasion of cancer cells (Sato et al., 2005).

GATA gene family members were also epigenetic silenced in pancreatic cancer. For example, *GATA-5* was frequently methylated in pancreatic cancers, whereas *GATA-4* was hypomethylated (Fu et al., 2007).

Another gene commonly silenced epigenetically in pancreatic cancer is *BNIP3*. The protein encoded by this gene contains a BH3 domain and a transmembrane domain associated with pro-apoptotic function. *BNIP3* gene silencing induced a drug resistance mechanism in pancreatic cancer cells, as a potential drug resistance mechanism (Okami et al., 2004)

Shimizu et al. using a novel method called "microarray coupled with methyl-CpG targeted transcriptional activation" (MeTA-array for short), identified 16 genes hypermethylated in three representative pancreatic cancer cell lines, AsPC-1, MIA PaCa-2 and PANC-1. Among these 16 genes several presented higher methylation level (*CSMD2*, *SLC32A1*, *TMEM204* and *TRH*). *CSMD2*, *SLC32A1* and *TRH* genes presented also a hypermethylated pattern in primary pancreatic cancers (Shimizu et al., 2011).

In contrast, a great number of genes are overexpressed in pancreatic cancer. These genes presented hypomethylation of the promoter in pancreatic cancer versus normal pancreatic tissue.

DNA hypomethylation of promoter CpGs were identified in genes with overexpression pattern including *claudin4*, *lipocalin2*, *14-3-3sigma*/ *stratifin*, *trefoil factor 2*, *S100A4*, *mesothelin*, *PSCA*, *S100P* and *maspin* (Sato et al., 2003).

6.2 Histone modifications hallmarks for oncogenesis process

Covalent histone modifications are important regulatory elements in many biological processes. These modifications control the chromatin status by electrostatic interaction changes and non-histonic protein recruitment. Histones suffer specific N-terminal end post-translational changes represented by acetylation, methylation, phosphorylation, sumoylation, ubiquitination and ADP-ribosylation. These modifications alter DNA-histones interaction having a major impact on chromatin structure (Strahl et al., 2000).

Certain histone modifications influence gene transcription level. The interplay between histone modifications led to 'histone code hypothesis'. For example, lysine acetylation neutralizes the charge between DNA and histone tails and correlates with a more transcription permissive status of chromatin (Jenuwein et al., 2001).

Hypermethylated CpG islands of silenced tumor-suppressor genes are correlated with deacetylation of histones H3 and H4, methylation of H3 lysine 9 (H3K9), H3 lysine 27 (H3K27), and H4 lysine 20 (H4K20), and demethylation of H3 lysine 4 (Rosenfeld et al., 2009; Barski et al., 2007). Methylation of histone H3 lysine 4 (H3K4) and H3 lysine 36 is associated with relaxed chromatin status (Benevolenskaya et al., 2007). Histone modifications recruit effector proteins. Acetylated lysines are recognized by bromodomains within nucleosome remodeling complexes, methylated H3K4 and the helicase Chd1 chromodomain recruits activating complexes of chromatin (Daniel et al., 2005). In contrast, methylated H3K9 and

H3K27 interacts with heterochromatin protein 1 (HP 1) and Polycomb-group (PcG) proteins leading to chromatin compaction (Fischle et al., 2003). PcG proteins function as transcriptional repressors, but the molecular mechanisms of Polycomb repressive complex (PRC)-mediated repression is not clear (Sparmann et al., 2006). PcG proteins recruits DNMTs (DNA methyl transferase) involved in the hypermethylation of tumor suppressor genes (Vire et al., 2006).

Only few studies have examined genes that are regulated by histone modifications in pancreatic cancers. For example, mucin family gene underwent histone alterations in pancreatic cancers in association with gene overexpression. The 5' region of MUC1 gene transcriptional start site is enriched in tri/dimethylated H3K9 and methylated DNA in non-tumor cells (Yamada et al., 2008). Transcriptional start site of MUC2 is highly enriched in di- and tri-methylated H3K4, acetylated H3K9, and acetylated H3K27 in pancreatic cancer cells. Vincent *et al* demonstrated that MUC4 transcription activity is affected by many factors (DNMT3A, DNMT3B, HDAC1 and HDAC3, DNA methylation, histone modification (Vincent et al., 2008).

7. Model organisms studies provide potential biomarkers in pancreas oncogenesis

Model organisms are widely used to explore potential causes and treatments for human disease. This strategy is made possible by the conservation of metabolic and developmental pathways and genetic material over the course of evolution.

It is known that Enolase 1 (α -enolase or non-neuronal enolase -NNE), is an isoenzyme of enolase, which catalyze the conversion of 2-phosphoglycerate into phosphoenolpyruvate.

Several studies have shown that enolase 1 plays an important role in in tumorigenesis, cancer invasion and metastasis. Proteomic studies reported that expression of enolase 1 is increased in cancers, such as hepatocellular carcinoma (Takashima et al. 2008, Hamaguchi et al., 2008) , non-small lung cancer (He et al., 2007), esophageal adenocarcinoma (Zhao et al., 2007), prostate cancer (van den Bemd et al., 2006), colon cancer (Katayama et al., 2006), oral epithelial and squamous cell carcinoma (Ito et al., 2007).

In pancreatic cancer Mikuriya et al using two-dimensional electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry showed that the expression levels of glycolytic enzymes, including enolase 1, increased in the cancerous pancreatic tissues patients compared with the paired non-cancerous tissues (Mikuriya et al., 2007).

In order to evaluate Enolase 1 expression changes, Lei et al. used chemical induced carcinogenesis in rats. Implantation of 7,12-dimethylbenzanthracene in rat pancreas leads to pancreatic cancer and PanINs. Alpha-enolase was specifically overexpressed in tumors compared with normal and pancreatic tissues (Lei et al., 2011).

The group found several proteins overexpressed in this carcinogenesis model, along with enolase 1 (Tumor protein translationally controlled 1, Expressed in non-metastatic cells 2, Pancreatic elastase 3B , Necdin, Hbp23, Chromodomain helicase DNA-binding protein, Albumin+retinoid X receptor-interacting protein, Heterogeneous nuclear ribonucleoprotein A2/B1-hnRNP A2/B1).

The hnRNP A2/B1 protein plays an important role in the biogenesis and transport of mRNA. Abnormal expression of this protein leads to alteration of normal transcription. In concordance with this study a previous work found high levels of hnRNP A2/B1 expression in a limited number of human pancreatic adenocarcinomas from smokers and two pancreatic tumor cell lines, HPAF-11 and SU 86 (Shen et al., 2004). In contrast, carboxyl ester lipase (CEL pancreatic exocrine enzyme) expression level progressively decreased with DMBA-induced disease severity.

DMBA implantation into the rat pancreas is an effective method to induce PanINs and pancreas cancer in order to determine which the first change in proteins expression pattern is, and to identify markers for pancreas lesions progression.

8. Novel biomarkers for the non-invasive diagnosis of pancreatic cancer

Actually, diagnostic methods for pancreatic cancer include invasive procedures (tissue sampling by endoscopy), involving risks and causing complications. The necessity for less invasive diagnostic methods is increasing; therefore the development of non-invasive biomarkers in pancreatic cancer is mandatory.

8.1 Plasma biomarkers

The major directions of proteomic range from basic research to discovery, validation and use of clinical applications. Protein profiling methods include high resolution two-dimensional gels, two-dimensional differential in-gel electrophoresis, LC-MS and LC-MS/MS using accurate mass tags, and protein identifications using mass spectrometry methods. These methods were used in many studies for identification of prognostic and/or predictive biomarkers that may help stratify patients.

The only clinically available serum biomarker for PDAC is CA 19-9, which is useful for the follow-up of pancreatic cancer patients receiving treatment, but has not been recommended for cancer screening (Goggins et al., 2000; Locker et al., 2006). The American Society of Clinical Oncology (ASCO) 2006 guidelines for the use of tumor markers do not recommend CA19-9 as a screening test for pancreatic cancer (Rosty et al., 2002; Liang et al., 2009). Several other serum markers have been proposed for pancreatic cancer.

Recent papers published new promising biomarkers, which can potentially detect early stage pancreatic cancer (Chen et al., 2011).

Roberts *et al.* analyzed serum samples from patients with locally advanced or metastatic adenocarcinoma of the pancreas. Patient group was selected based on length of survival and type of therapy, and serum was subjected to liquid chromatography coupled to tandem mass spectrometry analysis (LC-MS-MS) (Roberts et al., 2011). The proteins presenting important changes in expression levels were validated by enzyme-linked immunosorbent assay (ELISA). After the data were analyzed, the authors selected 1 putative prognostic protein, alpha 1-antichymotrypsin (AACT), and 2 putative predictive proteins, histidine-rich glycoprotein (HRG) and complement factor H (CFH). AACT was found to be negatively correlated with overall survival, whereas CFH was found to have no predictive value as prognostic factor for overall survival. AACT may be a useful prognostic marker in patients with advanced stage pancreatic carcinoma, although additional validation studies are needed.

Another study involving quantification in patient serum of tumor cell metabolites, or secreted factor was conducted by He *et al.*. This study focused on DJ-1 oncoprotein secreted by cancer cells (He *et al.*, 2011). The study group involved patients diagnosed with pancreatic cancer and chronic pancreatitis, along with healthy subjects. DJ-1 serum level and the conventional tumor marker carbohydrate antigen 19-9 (CA 19-9) were measured in order to establish the diagnostic and prognostic value of DJ-1. Serum DJ-1 level was increased in patients with pancreas cancer compared with chronic pancreatitis and healthy individuals. Serum DJ-1 levels were higher than CA 19-9, and combined the two biomarkers provided a sensitivity of 87.5%. After resection DJ-1 levels decreased and patient with lower value of this factor had a better prognosis. This study provides a potential clinical biomarker, easy to quantify from serum, in order to establish a rapid diagnosis and to evaluate prognosis in patients with pancreas cancer.

8.2 Pancreatic juice biomarkers

Analysis of protein expression profiles of pancreatic juice samples harvested from the pancreatic duct has the potential to identify markers that could serve for diagnosis triage of benign from malignant pancreatic lesions and to discriminate between different stages of PDAC.

The research performed by Vareed *et al.* has shown that 56 proteins were found to be elevated in pancreatic juice of PDAC patients compared to benign controls (Vareed *et al.*, 2011).

Protein profiles studies revealed an unique presence of proteins associated with Parkinson's disease namely: aSyn and PARK7 (Bonifati *et al.*, 2003; Singleton *et al.*, 2003).

Increased expression of aSyn has been also identified in melanoma (Matsuo *et al.*, 2010), while its isoform gamma-synuclein (cSyn) has been shown to be elevated in tumors of breast, uterine, colorectal and pancreas (Ye *et al.*, 2009; Hibi *et al.*, 2009; Ahmad *et al.*, 2007; Li *et al.*, 2004; Gupta *et al.*, 2003; Jia *et al.*, 1999; Morgan *et al.*, 2009). Moreover, due to the structural omology between cSyn and aSyn, these factors potentiate invasion in these tumors.

Interestingly, tissue arrays demonstrated a strong staining for aSyn in tumors, and this protein expressed in a subset of PDAC patients was identified in its aggregated form similar to Lewy Bodies seen in Parkinson's disease (Polymeropoulos *et al.*, 1997). Another interesting coincidence is the presence of increased levels of Parkinson's disease associated protein PARK7 (DJ1) (van Duijin *et al.*, 2001) in pancreatic ductal juice of adenocarcinomas. The mechanism by which the protein PARK-7 exerts its oncogenic effect remains unclear. It is presumed the involvement of p38 mitogen activated-protein kinase signaling (Mo *et al.*, 2010).

On the other hand PDAC-associated secretory proteome analysis also revealed increased levels of several metabolic enzymes. The most important metabolic factor was Purine NucleosidePhosphorylase (NP), enzyme involved in salvage pathway of purines, which is operational during inflammation and neoplastic progression, (Bantia *et al.*, 2010). NP activity has been reported to be high in cancer sera (Roberts *et al.*, 2004) and NP expression has also been used to determine the clinical severity of various types of cancers, in combination with another factor adenosine deaminase (ADA) (Mesarosova *et al.*, 1993).

This strong association between NP with inflammation determined to evaluate the expression and activity of this protein in PDAC, specifically in patients with antecedent inflammatory conditions like chronic pancreatitis, and pancreatic intraepithelial neoplasia (PanIN) (Rebours et al., 2010). Therefore, this correlation makes NP levels quantification to be a useful marker for surveillance progression from inflammation to PDAC.

8.3 Saliva biomarkers

Saliva is a body fluid that can be easily obtained without using a special technique. Recent reports suggested the possible utility of saliva in quantification of specific factors to discriminate between pancreatic cancer patients and patients with normal or chronic pancreatitis.

Using transcriptome profiles, Zhang *et al.* group could differentiate pancreatic cancer patients from healthy subject with a sensitivity of 90.0% and a specificity of 95.0%. The group found 12 mRNA biomarkers specific for pancreatic cancer patients. Seven genes were found to be up-regulated (*MBD3L2*, *KRAS*, *STIM2*, *DMXL2ACRV1*, *DMD*, *CABLES1*), whereas five genes presented a decreased expression *TK2*, *GLTSCR2*, *CDKL3*, *DPM1*, *TPT1* (Zhang et al., 2010).

A similar approach has been made by another group, which evaluated metabolites in saliva using mass spectrometry. Pancreatic cancer cases were successfully detected based on the pancreatic cancer-specific signature (Sugimoto et al., 2010). The levels of ornithine and putrescine were higher in patients with breast or pancreatic cancer, and were markedly higher in patients with oral cancer. The level of tryptophan is also increased in oral and pancreatic cancer, in contrast to arginine level which is decreased several cancers including breast, colonic and pancreatic cancer, which might be due to increased uptake of arginine by tumor tissues with high arginase activity.

8.4 Non- coding RNA as new era biomarkers

A new and important attractive tool is represented by small non-coding RNA. These are regulators of various biological processes like gene expression and are involved in cancer progression. One of the most important players is microRNAs which control many cellular functions, such as migration, invasion and stem cell functions. Abnormal microRNA gene expression was found in many cancers, including pancreatic cancer (Rachangani et al., 2010, Eis et al., 2005, Calin et al., 2005, Yanaihara et al., 2006).

MicroRNA molecules are present in various body fluids (blood, urine, cerebrospinal fluid, pancreatic juice, biliary secretion). Moreover, several microRNAs from sera seemed to be identical for many cancer types. Very important is the fact that miRNAs from sera are stable and exhibit resistance to RN-ase activity, intermittent frosting and defrosting, high temperature values and extreme pH (Mitchell et al., 2008; Albulescu et al., 2011).

Using microarray technology it was established that several microRNAs are highly expressed in pancreatic cancer (miR-21, miR-17-5p, miR-191, miR-29b-2, miR-223, miR-128b, miR-199a-1, miR-24-1, miR-24-2, miR-146, miR-181b-1, miR-20a, miR-107, miR-32, miR-92-2, miR-214, miR-30c, miR-25, miR-221, miR-106) (Volinia et al., 2006). More recently, was established that microRNAs, miR-200a and miR-200b are highly expressed in pancreatic

cancer cell lines, and their expression levels were significantly increased in the sera from pancreatic cancer patients, suggesting that microRNA itself could be a biomarker for pancreatic cancer (Li et al., 2010). The attention was also focused on two pancreas specific miRNAs miR-216 and miR-217. The expression of those miRNAs is decreased or even absent in PADC and in cell lines (Sood et al., 2006). Only miR-217 and miR-196a are able to discriminate between normal pancreas, chronic pancreatitis and tumor PDAC (Szafranska et al., 2008). Furthermore, miR-196a expression is likely specific to PDAC cells and is positively associated with the progression of PDAC.

miR-21 and miR-155 are overexpressed in pancreatic tumor, as compared to tissues from normal pancreas and chronic pancreatitis. Both miR-21 and miR-155 have been suggested to have a proto-oncogene role being overexpressed in several cancers (breast cancer, lung cancer, Burkitt lymphoma, B-cell lymphoma) (Metzler et al., 2004; Yin et al., 2008)

Several studies demonstrated that miR-155 transcription is regulated by transforming growth factor β -TGF β /Smad, nuclear factor- κ B and activator protein-1 family transcription factors through direct interaction with the miR-155/BIC bidirectional promoter. These studies suggest that overexpression of miR-155 in cancer is due to transcriptional activation, involving other cellular deregulated mechanisms (Kong et al., 2008).

9. Conclusions

Asymptomatic pancreatic cancer is hard to detect, but possibly curable. Recent research identified novel biomarkers of pancreatic cancer, but screening for early pancreatic cancer is still challenging. Future work should be addressed to the development of diagnostic techniques with a higher sensitivity to detect even asymptomatic cases. Currently no clinically useful interventions to screen for patients with PDAC are available.

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11. References

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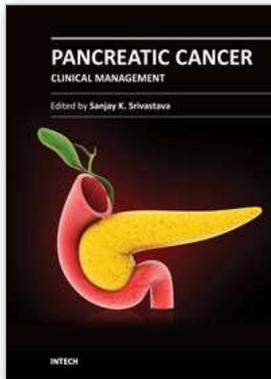
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This book covers pancreatic cancer risk factors, treatment and clinical procedures. It provides an outline of pancreatic cancer genetic risk factors, biomarkers and systems biology for the better understanding of disease. As pancreatic cancer suffers from lack of early diagnosis or prognosis markers, this book encompasses stem cell and genetic markers to identify the disease in early stages. The book uncovers the rationale and effectiveness of monotherapy and combination therapy in combating the devastating disease. As immunotherapy is emerging as an attractive approach to cease pancreatic cancer progression, the present book covers various aspects of immunotherapy including innate, adaptive, active, passive and bacterial approaches. Management of anesthesia during surgery and pain after surgery has been discussed. Book also takes the reader through the role of endoscopy and fine needle guided biopsies in diagnosing and observing the disease progression.

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