Molecular diagnosis of pancreatic cancer: where do we stand?

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1. ABSTRACT

Pancreatic cancer remains a disease with a dismal prognosis due mostly to its late diagnosis. An early diagnosis would have a significant impact on the prognosis and, eventually, on the incidence of the disease itself. Many progresses have been made in the molecular diagnosis of pancreatic cancer. High risk patients would likely benefit from biologic screening, before the general population. Most of the markers remain limited to phase I and II studies. The challenges include the lack of specificity of some of the markers, as well as the lack of standardization within the laboratories. Further research is necessary prior to the application of the currently known biomarkers for the diagnosis of pancreatic cancer.

2. INTRODUCTION

Pancreatic cancer is an insidious disease, and, although it accounts for only 2-3% of all malignancies, it is the tenth most common cancer in Europe, and the forth cause of cancer related death in the Western hemisphere (1), (2). Of the approximately 40,000 new cases of pancreatic cancer in the United States, only 15% are diagnosed at a surgically resectable stage, 18% will survive more than 1 year after the diagnosis, and 4% after 5 years (3-5). This means that more than 34,000 deaths from pancreatic cancer are expected to occur annually (3). This dismal prognosis is mostly dependent on the subtle clinical presentation. The disease has far exceeded the early stages, when it manifests clinically (6). It is obvious then, how in
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Table 1. Risk factors for pancreatic cancer

<table>
<thead>
<tr>
<th>Sporadic</th>
<th>Genetic</th>
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<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>BRCA-2 mutation</td>
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<tr>
<td>Non-hereditary pancreatitis</td>
<td>P16 mutation</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>Hereditary pancreatitis</td>
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<tr>
<td>Hereditary non polyposis colorectal cancer</td>
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spite of the advances in the traditional therapeutic modalities (i.e. surgery, radiation therapy and chemotherapy), the prognosis remains so poor. The research on this field, as in other malignancy, has then been focusing in achieving an early diagnosis.

The capacity of tumor growth and ability to spread has already been demonstrated, and it is linked to a particular subset of cells (tumor stem cells) (7). The presence of stem cells in hematological disease has been known for some time, and currently they have been recognized not only for pancreatic cancer, but also for other solid organs cancers (i.e. breast, brain, prostate, and lung) (8-14). The understanding and the recognition of this small subgroup of cells could have a tremendous therapeutic impact. In spite of the difficulty in identifying these cells as a result of the lack of specific cell surface markers, the identification of these cells has significantly expanded in the last decade. This is mainly because of the development of newer diagnostic methodologies. In particular it has been found that in mice models the pancreatic adenocarcinoma sub cell line Panc-1 that expresses both CD44 and CD 24, has a significant higher tumorigenic potential (8). The identified tumor stem cell would be responsible not only for initiating the disease de novo, but also for promoting regrowth after the initial response to therapy. This can be translated into clinical application by targeting this particular sub population with new drugs. Regardless of the growing number of molecular markers now identified, their application in clinical practice remains very limited. This factor is not only due to the lack of standardization of diagnostic parameters, but also in the lack of specificity, and in some cases, sensitivities of these particular markers.

3. RISK FACTORS FOR PANCREATIC CANCER

The principles of a successful screening program have been highlighted by Klapman et al. (15). First, a reasonable yield is expected, especially when expensive techniques are required. The screening has to lead to an improvement in the overall survival and the benefits of the screening have to outweigh the risks. The relative rarity of the disease does not justify extensive and costly screening of the general population, unless it could lead to at least a 16% cancer detection rate, as demonstrated by Rulyak et al. (16). The key is then to identify the population at risk based on several know factors.

Canto et al. have demonstrated how a selective screening protocol applied to the high risk patients is justifiable since it can yield to a significant percentage of diagnosis of otherwise asymptomatic individuals (10% in their study) (17). The advantage of this approach is also enhanced by the low complication rate (2%) of the methodology utilized for the screening (i.e. EUS with FNA). At this time it is limited to high risk individuals, and until some of the molecular marker studies are validated, it remains the most practical approach to the diagnosis of this deadly disease.

So, in order to develop an effective screening protocol for early diagnosis of pancreatic cancer, it is important, then, to identify patients at higher risk of developing the disease. Although the direct etiologic factors involved in the carcinogenesis of pancreatic neoplasia remain poorly understood, several risk factors have been elucidated. They can be divided in sporadic and genetic (Table 1).

4. SPORADIC RISK FACTORS

Among the acquired risk factors, certainly cigarette smoking is considered one of the more relevant, since it can double the risk of developing pancreatic cancer (18, 19).

Diabetes mellitus seems to represent not only an early clinical expression of the neoplastic process, but it is in itself a predisposing factor. In addition, it carries up to a two fold risk for developing pancreatic cancer, for unclear reasons (20-22). Non-hereditary pancreatitis has been correlated to the onset of pancreatic cancer (23).

5. GENETIC RISK FACTORS

Besides sporadic forms of pancreatic cancers, up to 8% of them express a familial predisposition (24). One of the most relevant risk factors in this category is the positive family history for the disease. As previously shown by the National Familial Pancreatic Tumor Registry, first degree relatives of patients affected by pancreatic cancer have up to 18-fold increased risk of developing the disease themselves(25, 26). This risk increases exponentially when multiple family members are affected, with a 57-fold increase when more than 3 relatives have a history of pancreatic cancer (26). Specific genetic alterations also carry a higher likelihood of developing pancreatic cancer. Of these, the mutations BRCA2 and p16 are the better described (27, 28). BRCA2 has been more tightly associated with breast and ovarian cancers, but up to 19% of patients with a family history of pancreatic cancer will have BRCA2 (27, 29). Familial atypical multiple mole melanoma (FAMMM) is characterized by the development of multiple melanomas. The germ line mutation has been identified at the level of the p16 gene and carries a 22-fold increase risk of cancer (28, 30). The Peutz-Jeghers syndrome, which carries the characteristic features of multiple hamartomatous polyps, carries up to a 36% risk of developing pancreatic carcinoma (31). The individuals with the hereditary form of pancreatitis have up to a 50-fold relative risk of developing pancreatic cancer (23). Finally patients with hereditary non polyposis colorectal cancer (HNPPC) carry an increased risk of developing pancreatic cancer (32, 33).
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Table 2. Correlation between tumor progression and genetic alterations

<table>
<thead>
<tr>
<th>Precursor</th>
<th>PanIN-1A</th>
<th>PanIN-1B</th>
<th>PanIN-2</th>
<th>PanIN-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetics</td>
<td>Her-2/neu (86%)</td>
<td>Her-2/neu (86%)</td>
<td>Her-2/neu (100%)</td>
<td>Her-2/neu (100%)</td>
</tr>
<tr>
<td></td>
<td>K-ras (35%)</td>
<td>K-ras (43%)</td>
<td>K-ras</td>
<td>K-ras (86%)</td>
</tr>
<tr>
<td></td>
<td>P16 (30%)</td>
<td>P16 (55%)</td>
<td>P16 (71%)</td>
<td>P53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DCP4</td>
<td>BRCA-2</td>
</tr>
<tr>
<td>Histology</td>
<td>flat columnar epithelial lesions</td>
<td>papillary architecture</td>
<td>presence of dysplasia</td>
<td>presence of atypia</td>
</tr>
</tbody>
</table>

6. TUMOR PROGRESSION MODEL

As previously described in colorectal cancer, the tumor progression for pancreatic cancer seems to occur in a similar stepwise fashion. The progression from normal ductal cells to precursor lesions such as intraductal neoplasia of various degree, intraductal pancreatic mucinous neoplasm, and mucinous cystic neoplasm has been well established (15). The first model of cancer progression for pancreatic adenocarcinoma has been described by Sommers et al. (34). It was only in 1999 that the current nomenclature was decided upon by the Pancreas Cancer Think Tank (35). During that assembly, several expert pancreas pathologists adopted the standard PanIN (pancreatic intraepithelial neoplasia) classification system currently used. The normal cuboidal pancreatic epithelium changes from flat mucinous lesions to hyperplastic papillary duct lesions without atypia (PanIN1), then progresses to the presence of dysplasia (PanIN2), then evolves into atypia (PanIN3, carcinoma in situ), and lastly, to infiltrating carcinoma (35, 36).

Interestingly the morphologic continuum correlates very closely with specific genetic alterations (Table 2). In particular the mutations of K-ras oncogene and the overexpression of HER-2/neu are present with increase frequencies in the PanIN atypias. In fact, 35% of PanIN-1A lesions express K-ras mutations, 43% of PanIN-1B and 86% of PanIN-3 express mutations (37-39). In a similar fashion, HER-2/neu overexpression has been discovered in 86% of PanIN-1A lesions and 100% of PanIN-3 ones (40). The overexpression of HER-2/neu is more prevalent in precursors of pancreatic adenocarcinomas (PanIN), than in the invasive form itself (40). Also tumor suppression genes follow a similar pattern in the progression of the pancreatic cancer evolution. The tumor suppressor gene p16 is inactive or absent in all infiltrating adenocarcinomas. The rate of its inactivation follows a sequential increase from 30% in PanIN-1A lesions, to 55% in PanIN-1B and PanIN-2, to 71% in PanIN-3 (41). The inactivation of other tumor-suppressor genes such as p53, DCP4 (deleted in pancreatic carcinoma locus 4), and BRCA-2 seems to occur later in the progression model (PanIN-3), sparing the low grade lesions (PanIN-1) (42).

In summary, K-ras mutations tend to occur early in the disease process, whereas alterations of p16 are intermediate findings (43). Late in the neoplastic progression, changes in p53, DCP4 and telomerase usually occur (43). In general k-ras and telomerase activity are more useful in the diagnosis of pancreatic carcinoma, whereas p53 and p16 have more of a prognostic role (43).

7. CANCER BIOMARKERS

The possibility of discovering a reliable and reproducible marker for the early diagnosis of pancreatic cancer is very attractive. Annual fecal occult blood testing in patients older than 50 years of age, can decrease the mortality for colorectal carcinoma by as much as 33% (44). Similarly, it would be ideal to find a marker for pancreatic cancer that can be easily measured in a non-invasively obtained specimen. The ideal marker obviously should be identified in a specimen obtainable with the lowest risk and discomfort for the patient, such as serum. Because of the intrinsic characteristics of the pancreatic neoplastic precursor (PanINs and IPMNs), especially their size and the lack of invasion, it is unlikely that any serum markers will reach a significant systemic amount, hence the biomarkers are unlikely helpful for an early diagnosis (45). The next step then will be to collect and measure markers levels from additional sources, namely pancreatic juice. Pancreatic juice seems to be an ideal source of biomarkers. Potentially the progression to acinar adenocarcinoma can be preannounced by the secretion of different markers into the pancreatic lumen. Several authors have tested a panel of different markers (Cyclin D2, FOXE1, NPTX2, ppENK, and TFP12) that could provide a diagnostic assay (46). At the moment none of these combinations has been validated enough to be applicable into the clinical arena.

In alternative tumor-related molecules (biomarkers) can be measured in the cancer itself. Obviously the cancer has to be of a certain mass prior to sampling the tissue. Some of the major advantages of the biomarkers are in increasing the accuracy of other standard cytological diagnosis and in the assessment of prognosis and response to treatment.

Even in the presence of identifiable image abnormalities, the definite diagnosis of a malignant or premalignant process is challenging. EUS and FNA certainly provide a significant addition to the diagnostic armamentarium, but often even the latter modality can be inconclusive. This is the area where molecular markers can make the difference and improving the yield of cytology. On the other hand, not every individual who harbors low-grade pancreatic intraepithelial neoplasia (PanIN) will evolve into invasive disease. This is another limitation of the histological evaluation alone. Once again molecular markers could distinguish among the different degrees of PanIN, selecting out the individuals with high grade features and more significant potential to develop neoplastic disease in the future. Most of the potential markers identified are still in phase I and II studies (47).
The addition of protein identification, should then improve sensitive enough to accurately diagnose pancreatic cancers.

8. PROTEINS

Because of the frequent posttranslational mutations, the solely RNA quantification, might not be sensitive enough to accurately diagnose pancreatic cancers. The addition of protein identification, should then improve the sensitivity of the biomarker. Along with electrophoretic techniques for the identification of proteins, mass spectrometry, electrophoresis and, more recently, surface-enhanced laser desorption ionization (SELDI), a form of mass spectrometry analysis, and matrix associated laser desorption ionization (MALDI) have become popular (48, 49).

Limitations of these methodologies include not only the reproducibility in different laboratories, but also the presence of large amount of these proteins in normal individuals (50).

In order to overcome some of these limitations, it is necessary to develop new assays based on quantitative measurements. These methods include LigAmp, which combines DNA ligation with PCR amplification, and BEAMing (Beads, Emulsion, Amplification, and Magnetics) (51, 52).

Protein based methodologies are the most commonly used in tumor markers. The blood sample is among the easiest specimen to obtain with minimal risk for the patient. CA19-9 is certainly the better known of the serum markers. Just like other markers, this is almost exclusively used to monitor the therapeutic response, and to aid in the diagnosis of recurrences. CA19-9 was first described by Kaprowski et al in 1979 (53). It is now the most frequently utilized marker in pancreatic cancer. It is derived from lacto-N-fucopenteose II, and as such approximately 10% of the population does not express Lewis anomalies (54). In addition its sensitivity tends to be low with positive predictive values of only 0.5% (59). Its limitation in the diagnosis comes from the high incidence of false-positive (low specificity) in the presence of benign pancreatic anomalies (54). In addition its sensitivity tends to be low since up to 10% of the population does not express Lewis antigens, hence not expressing detectable levels of the CA 19-9 antigen, as previously noted (60).

Among the serum markers, besides the already cited CA19-9, additional markers that target antigens of MUC-1 have been described. Among the latter, PAM4 seems to detect MUC-1 proteins in pancreatic cancer more specifically than in other neoplasias, and also more specifically than CA19-9 (61). The development of new microarray platforms for gene expression characterization has opened the doors to new markers identification (62). Some examples of these markers include mesothelin, macrophage inhibitory cytokine 1 (MIC-1), and osteopontin (63). Unfortunately, some of these markers (MIC-1), although more sensitive than CA19-9, and not influenced by the stage of the disease, lack of specificity (64, 65).

Additional markers have been studied by themselves, or in combination. Newly described biomarkers brought more excitement to the field. CEACAM1 is a member of the human carcinoembryonic antigen (CEA) family. CEACAM1 is normally expressed in several epithelia and, as such, can be found in bile. The specific function of CEACAM1 in pancreatic cancer is not understood. In general CEACAM1 functions as an adhesion molecule with angiogenic properties, directly promoting tumor spread (66). It also inhibits natural killer cells function, indirectly promoting cancer progression (67).

Although the expression of CEACAM1 is significantly lower in certain tumors, such as breast and colon, its level increases in others, such as lung and melanoma (68-71). The levels of CEACAM1 appear higher in pancreatic neoplasias, than in benign pancreatic diseases (72). The presence of increased levels of CEACAM1 in pancreatic intraductal neoplasia 3 also implies an expression of the gene early in the disease process. Simeone et al. developed a comparative ELISA analysis, through which they were able to compare CEACAM1 levels in both serum and tissue samples of patient affected by pancreatic cancer and in patients with chronic pancreatitis (73). Furthermore, these authors demonstrated, on one hand the superior specificity and sensitivity of

<table>
<thead>
<tr>
<th>DNA Based</th>
<th>Genetic</th>
<th>Oncogenes</th>
<th>K-ras</th>
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<tr>
<td>Proteins</td>
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<tr>
<td>RNA Based</td>
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<td>Proteins</td>
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<th>Table 3. Biomarkers and their biochemical targets</th>
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<tr>
<td>DNA Based</td>
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<td>Proteins</td>
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The different biomarkers can be found at different biochemical target levels: DNA, RNA and proteins (Table 3).
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CEACAM1 at the tissue level, over both CA19-9 and CEA (sensitivity of 85% and specificity of 98%), and the added benefit of measuring all three biomarkers, on the other (73). In spite of the promising findings at the tissue level, CEACAM1 in the serum was not specific enough to distinguish neoplastic processes from chronic inflammatory states (73).

Many additional markers have been investigated in the past, but unfortunately they were not found to be superior to CA19-9. Among these are CEA, Amylin, DUPAN2, CAM 17.7, CA 195 (74-78). As previously described, the combination of two or more tumor markers increases the accuracy of the markers measured individually. Although the idea of combining several biomarkers together in order to increase the sensitivity and specificity is attractive, this has not been proven to be the case. In fact Koopman et al. tested five different markers together (MIC-1, CA19-9, TIMP-1, osteopontin, and PAP). He found MIC-1 to be a more sensitive tumor marker than CA19-9 alone, but the combination with the other markers did not increase the diagnostic yield further (64). Furthermore, the same authors showed how elevated levels of MIC1 are present since the early stages of pancreatic cancer, making its determination an attractive adjunct to early detection of the disease (64). This is particularly helpful in high risk subjects and can be used in addition to the traditional more invasive diagnostic tools such as endoscopic ultrasound and computed tomography. Unfortunately, similarly to other markers, MIC 1 levels are significantly increased in inflammatory processes of the pancreas, and in patient with alteration of the macrophage activation process (64). This entails a lack of specificity of the tumor marker.

Recently, Marchesi et al. have demonstrated the importance of chemokine receptor CX3CR1 in the dissemination of pancreatic adenocarcinoma (79). The dissemination of tumors via perineural pathways is grossly understudied. Although several neoplasias have been proven to utilize this pathway, the above cited study links it with pancreatic adenocarcinoma. The rich presence of neural tissue within the gland, as well as the adjacent retroperitoneal neural plexus, makes this form of tumor invasion very prominent (80). The tumor cells upregulate the CX3CR1 and can then infiltrate peripheral nerves. The latter factor, not only can explain the frequent association with pain in advanced pancreatic cancers, but also how the nerve reservoir might constitute a source for local recurrence (81). The determination of CX3CR1, then, could separate out a subgroup of patients that are more susceptible to local invasion and relapse, and future therapy aimed specifically to the receptors could improve the prognosis (79).

The significant limitation in applying these scientific advances in the daily clinical practice come from the lack of statistically significant reference sets from individuals with early cancer outside of specific specialized laboratories. Additional research is then necessary prior to the application of this promising marker as an early diagnostic tool. The problem is that none of them is specific enough to distinguish malignancy from chronic pancreatitis.

9. GENETIC ALTERATIONS

As previously mentioned, several genomic DNA alterations have been identified in pancreatic cancer. The highly prevalent mutations as well as the relative uniformity of these mutations, has been subject of extensive research. Also a higher mutation frequency has been linked to a more advanced progression phase (82). The most consistently associated with pancreatic adenocarcinoma and, probably, with the precursor lesions are K-ras, p16, p53, and DCP4. Three different types of genes take part in the development of pancreatic cancer: oncogenes, tumor suppressor genes and DNA mismatch repair genes. Among the different oncogenes, K-ras mutations are certainly the most prevalent in pancreatic cancers (up to 90%) (83, 84). The clinical application of these genetic variances is more challenging than it could appear. In fact, even the more consistent mutations (such as K-ras) do not carry enough specificity to be applied in large scale. In spite of the relatively easy detection of the mutations from non invasive samples (such as stool), their presence in benign conditions such as chronic pancreatitis, and in chronic smokers, prevaricate their use without large prospective trials (82, 85). In other words identifying K-ras mutations does not identify the lesion that will progress into carcinoma (37). Potentially adding other more specific markers such as telomerase could make the detection more clinically valuable.

Contrary to the oncogenes, the tumor-suppressor genes participation to the oncogenic phases is by a loss of function. The most common mutation in this category of genes is p-53, which is found in up to 70% of pancreatic carcinomas (86). Normally p-53 binds to specific sequences of DNA and regulates gene transcription. Among the potential alteration of p-53, it appears that the intragenetic deletions are more prevalent in pancreatic cancer than in other form of cancers (87). The clinical relevance of this observation remains unknown, but it could be traced to specific environmental mutagens. Since K-ras and p53 alterations are common in pancreatic neoplasms, and also appear at e relatively early stage of the disease, they represent, not only a valuable addition to the diagnostic armamentarium, but also a potential invaluable screening tool. In fact, K-ras has been detected not only in pancreatic tissue, but also in a variety of alternative sources such us duodenal and pancreatic fluid, stool and blood (82, 83). The limitation of the application of this methodology for screening comes from the low specificity of K-ras for pancreatic carcinoma, as it is expressed in other malignancies such as colorectal cancer (86).

The other very prevalent mutation of a tumor suppressor gene is p16 (also called CDK2 or MTS1). The mutation of p16 has been found in nearly 100% of pancreatic cancers (88). All of the above mentioned proteins (K-ras, p16, p53) control the mitotic cycle through additional regulating proteins (kinases, kinases inhibitors and cyclins). Furthermore it seems that a combined action
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of lack of tumor-suppression by p16 and p53 and overexpression of K-ras is responsible for the disregulation of the cell division process responsible for the development of pancreatic carcinoma (89).

A more recent addition to the pool of tumor suppressor genes associated with pancreatic adenocarcinoma is the DCP4 (deleted in pancreatic carcinoma, locus 4). The latter seems to be strongly associated with the familial forms of pancreatic adenocarcinoma, but also could have a role in the sporadic form of the disease in up to 50% of the cases (86). The mechanism through which the tumor suppressor gene works seems to be mediated by TGFβ-like pathway.

The role of mismatch repair genes inactivation is somewhat less well defined than the two other categories (86).

The natural progression in biomarkers would be the isolation of DNA and RNA markers from blood samples in order to recognize tumor cells. Unfortunately, aside of some isolated encouraging results (90), the overall feasibility of these techniques needs to be studied extensively.

The recent development of microarray for cDNA and oligonucleotide, allows for rapid and simultaneous detection of thousand of genes, and open the door to future research (91).

10. RNA

The development of pancreatic cancer is dependent from alteration in the DNA itself (genetic component), but also from changes in the phenotype unrelated to genetic alteration of the DNA (epigenetic component). Most of the identified epigenetic alterations include aberrant methylation of several genes (p16, DAB1, Cyclin D2), that can be detected by polymerase chain reaction (PCR) (92). The methylation of these genes is almost exclusively present in neoplastic processes, and could represent an important tool for early diagnosis (93-95). The more recently described chip methodologies are capable of diagnose mitochondrial mutations (96). The obvious advantage in being able to identify mutations at the mitochondrial level instead of at the DNA level is the abundance of mitochondrial genome (97, 98). Unfortunately they express a high polymorphism, making the isolation process of tumor specific mutations, challenging (98).

Although certainly interesting, the latter modality requires further investigation prior to its wide spread clinical application.

The development of new techniques such as cDNA microarrays and serial analysis of gene expression (SAGE) has allowed for quicker and more comprehensive screening of overexpressed mRNA’s in tumor cells, resulting in the acquisition of new potential biomarkers. The well established gel electrophoresis and mass spectrometry, or immunohistochemical techniques are still in use for the identification of the protein markers.

Although immunohistochemistry is commonly used to measure the expression of biomarkers in the sampled tissue, miRNA analysis can be more easily quantified by PCR methodologies. An example of the latter concept applied to the clinical practice is the work by Giovannetti et al (99). These authors measured levels of human equilibrative nucleoside transporter-1 (hENT1), which is involved in the uptake of gemcitabine, in frozen sections of pancreatic cancer tissue. Its level seemed to correlate with the clinical outcome, indicating the possibility of predicting the individual tumor response to chemotherapeutic agents, such as gemcitabine. MicroRNAs (miRNAs) molecules have been recently examined in an effort to detect cancer development (100, 101). Although the studies prove the increment of these particular molecules in pancreatic cancer, the possibility of miRNAs to add benefit to the spectrum of biomarkers remains undefined. Finally, RNA based markers, in the form of portion of RNA that regulate gene expression (microRNA), have been studied, but their clinical application remains to be proven (45).

Of the RNA markers, telomerase is the one that has been the most promising. . Telomerase is a ribonucleoprotein enzyme that catalyzes the addition of a specific nucleotide sequence to the ends of chromosomal DNA. The enzyme telomerase is constituted by several components. Its role is to maintain the length of telomer, which generally decrease with the cell aging. This activity is ordinarily lost in the somatic cells, except in tissue with high turnover, such as lymphocytes. Typically cancer cells express telomerase in a high percentage of cases (90%) (102). Since telomerase is present in lymphocytes, it is expected that telomerase activity will be present in inflammatory cells, in particular when lymphocytes CD25 are present (103). This also applies in chronic pancreatitis, as shown by the frequent presence of lymphocytes CD 25 in the pancreatic fluid of these patients (103). In order, then to use telomerase as an oncologic screening marker, the fluid has to be deprived of lymphocytes CD 25.

11. CURRENT DIAGNOSTIC METHODS

The traditional diagnosis of pancreatic malignancy is obtained by imaging studies (CT or MR), often obtained for either unrelated reasons or because of vague abdominal symptoms. Once a pancreatic lesion has been identified, the addition of markers such as CA19-9 or CEA does not provide any aid in making the final diagnosis due to the lack of sensitivity and specificity, and further work up with more invasive procedure assists in finalizing the diagnosis. More recently the addition of endoscopic ultrasound (EUS), with the ability to obtain cytological specimen, has become the diagnostic modality of choice (104). Although EUS-FNA is an accurate modality for diagnosis of pancreatic cancer with quoted sensitivities and specificity of 85% and 100%, pancreatic cysts and chronic pancreatitis constitute two categories difficult to diagnose (105). This is due to the lack of cellular material from the
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cyst aspirate and the inability to distinguish between benign and malignant disease in the setting of chronic pancreatitis (106). In order to increase the diagnostic yield of cytology, molecular markers detection has been added. Among the molecular markers, telomerase expression has been proposed. As previously noted, its activity is almost absent in somatic cells, whereas its expression is significantly up regulated in neoplastic cells. The expression is not specific to pancreatic cancer, since has been found in 85% of solid tumor including lung, breast, colon, prostate, ovary and skin (107). When used in combination with cytology, telomerase assays increases the sensitivity of cytology from 85% to 98%, maintaining 100% specificity (105). So, the addition of telomerase activity supports the diagnosis of malignancy, even if the cytology is non-conclusive or negative. In order to increase the accuracy of molecular diagnosis of pancreatic cancer, a combination of several markers in a panel has been adopted (108). In particular the combination of K-ras, p16 and p53, seems the most promising. The rate limiting step at this point appears to be the limitation in the detection of these mutations at low concentration.

Some of the cancer associated molecules have also been evaluated for radiolabeling, in order to improve the sensitivity of imaging modalities. Hanaoka et al., in fact, radiolabeled a peptide specific for the CXCR4 chemokine receptor (109). Unfortunately the uptake of the peptide is much more significant in metabolically active organs such as liver and spleen, masking any potential tumor sites.

12. IPMN

Intraductal papillary mucinous neoplasm (IPMN) is a distinct entity from pancreatic ductal carcinoma. Histologically it is characterized by papillary proliferations of epithelial cells and cystic dilatation of the pancreatic duct with excess production of mucin. Their behavior varies from purely benign with no invasion potential, to invasive carcinoma. Based on the site of the tumor development, these tumors can be divided into main duct and branch duct types. Main duct types are more frequently associated with malignancy, whereas most of the branch duct types are benign (110). The prediction of malignant potential influences the treatment. In fact a more selective approach has replaced the previously adopted aggressive one. The indication for surgical resection is now limited to frank or suspected malignancy and symptomatic cases (110). Therefore the prediction for malignant potential becomes paramount. Currently the main indicators of potential for malignancy include symptomatic patients, the presence of diabetes or jaundice, tumor size (>3-5 cm), the main duct type, dilatation of the main pancreatic duct, and mural nodes (111-113). Also several imaging techniques have been utilized to predict the histological behavior if these tumors. Ultrasonography, computed tomography, magnetic resonance cholangiopancreatography (MRCP) and the more invasive, endoscopic retrograde cholangiopancreatography (ERCP) can all define the anatomic features of the pancreatic duct. The addition of endoscopic ultrasound, further characterize the anatomic variations. Furthermore pancreatic fluid sampling during the ERCP can provide cytological proof of the diagnosis. As in pancreatic adenocarcinoma, pancreatic fluid cytology carries significant limitations. As previously mentioned, the treatment of IPMN is now selective. Patients with non-resected IPMN should be followed very closely with imaging studying such as CT and/or MRCP. Molecular markers could play a role in differentiating IPMN with potential for malignant progression from the purely benign adenomas. Unfortunately such a marker has not been identified yet. Furthermore, because of the initial non invasive nature of IPMN, it is unlikely that a protein or DNA component is released systemically in the serum at an early stage. It is then more likely than a pancreatic fluid sampling has a higher diagnostic yield. A potentially useful marker is the pancreatic secretory trypsin inhibitor (PSTI). When measured in the pancreatic juice of individuals affected by IPMN, it was found to be present in significantly higher levels than in controls (114). In addition, IPMN has been associated with other extrapancreatic malignancies such as gastric and colorectal cancers in up to 30% of the cases (115). A correct diagnosis of IPMN could lead to early screening and diagnosis of other occult malignancies.

13. NEUROENDOCRINE TUMORS

The diagnosis of neuroendocrine tumors of the pancreas is closely related to the measurements of the specific markers. Furthermore the marker levels can be accurately utilized to monitor the response to the treatments and to diagnose recurrences and metastasis (116). Although the majority of the neoplasias of the endocrine pancreas present even more insidiously than the respective exocrine part, specific syndromes can lead to an early diagnosis. Besides the neuroendocrine product of the pancreas, specific proteins, chromogranins, co-exist within the endocrine cells of the pancreas. Of the different chromogranins, chromogranin A is considered to be the best of the generic markers for neuroendocrine tumors (117). Furthermore quantitative changes of chromogranin usually indicate progression of the disease (118). In fact chromogranin A has been correlated with tumor mass (119). Direct measurement of the specific peptide secreted is generally diagnostic. Traditionally some of these products are more easily detected by their by products (5HIAA from serotonin) in the urine, than with more complex serum assays (120). In the particular case of carcinoid, the secretion of the product tends to exclude the hindgut as a location for the carcinoid.

14. CONCLUSIONS

Pancreatic cancer remains a disease with a dismal prognosis due mostly to its late diagnosis. An early diagnosis would have a significant impact on the prognosis and, eventually, on the incidence of the disease itself.

Many progresses have been made in the molecular diagnosis of pancreatic cancer. High risk patients would likely benefits from biologic screening, before the general population. Most of the markers remain limited to
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phase I and II studies. The challenges include the lack of specificity of some of the markers, as well as the lack of standardization within the laboratories. Further research is necessary prior to the application of the currently known biomarkers for the diagnosis of pancreatic cancer.

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16. REFERENCES


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