Review

Role of tumor markers and mutations in cells and pancreatic juice in the diagnosis of pancreatic cancer


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Summary

Background: Unresectability at the time of presentation is the most important reason for the poor survival rate of pancreatic carcinoma. Molecular-based tests might improve the early detection of pancreatic cancer at a time when surgical resection is still an option for cure.

Methods: The literature was reviewed concerning the role of molecular-based tests applied to sources other than pancreatic tissue itself, including ERCP-samples, blood and stool, with emphasis on the detection of K-ras mutations and mutant p53 gene product.

Results: K-ras mutations have been successfully detected in ERCP brush samples, leading to an increase of the sensitivity and improvement of the diagnostic yield. When pancreatic juice and duodenal fluid are tested for K-ras mutations, the yield is less. K-ras mutations can also be detected in the blood, especially in patients with larger tumors. The presence of K-ras mutations proved also to be useful in discriminating benign and malignant liver nodules, i.e. when during surgery there is suspicion of liver metastases of pancreatic cancer. The accumulation of p53 gene product to immunochemically detectable levels in ERCP brush samples also increases the sensitivity of conventional light microscopy. Other molecular markers such as telomerase and TIMP-1 may prove to be useful too, but await more extensive evaluation.

Conclusion: Molecular-based tests may be of value in the early detection of pancreatic cancer and might therefore contribute to a better patient survival rate.

Key words: diagnosis, K-ras, molecular markers, pancreas cancer, p53, screening

Introduction

Pancreatic cancer is the fifth leading cause of cancer-related death. This is mainly because pancreatic cancer usually is diagnosed late in the course of the disease, when the tumor has spread locally or metastasized, and curable resection is no longer possible. The overall 5-yr survival for patients with pancreatic cancer is only 3% [1,2]. However, after a successful pancreaticoduodenectomy the 5-yr survival approaches 20% overall, and patients with a small tumor (<3cm), negative lymph nodes and negative resection margins have a 40% chance to survive 5 years [3-4]. Thus, early detection of pancreatic cancer, when resection is still an option, is crucial for a better patient outcome.

By the time a tumor is clinically detectable, an accumulation of generalized and specific molecular genetic alterations has already taken place in the tumor. Molecular-based tests to identify these genetic alterations can be used to enhance the early detection of pancreatic cancer.

Pancreatic cancer development

Pancreatic ducts and ductules adjacent to infiltrating cancers show hyperplasias and pancreatic intraepithelial neoplasias which are the precursor lesions to pancreatic cancers [5-7]. Similar to the tumor progression model described for colorectal cancer, in the pancreas the normal cuboidal epithelium undergoes stepwise changes from flat mucinous duct lesions, to papillary duct lesions without atypia, to papillary duct lesions with atypia, and finally to infiltrating carcinoma [5-8]. These early tumor stages are already accompanied by an accumulation of molecular genetic alterations. The specific genes associated with the development of pancreatic cancer can be divided into three gene classes: oncogenes, tumor suppressor genes and DNA mismatch repair genes.

Oncogenes

Activating point mutation in the K-ras oncogene is by far the most common genetic alteration in pancreatic cancer [9]. Mutation of the K-ras oncogene impairs the intrinsic GTPase activity of the K-ras gene product leading to a protein that is constitutively active in signal transduction, which results in transformation of the cell. The vast majority (~90%) of the pancreatic cancers harbour point mutations in the K-ras oncogene [9-12].

Tumor suppressor genes

The p53 tumor suppressor gene is inactivated in 50-70% of the pancreatic carcinomas [13-16]. In most cases this gene is inactivated by loss of one allele accompanied by an intragenic mutation of the other allele. This results in the loss of important controls of cell growth: cell cycle
regulation and induction of apoptosis [17,18]. The Deleted in Pancreatic Carcinoma 4 (DPC4) tumor suppressor gene is inactivated in 50% of pancreatic carcinomas [16,19,20]. The gene has homology to a group of genes known as the “Smad” genes, which play a role in the TGF-β receptor II signal transduction pathway [21-23]. The p16 (Multiple Tumor Suppressor 1) gene is inactivated in approximately 95% of pancreatic cancers [24-26]. The p16 gene product is an inhibitor of the cyclin-dependent kinase (CDK) 4 that promotes progression of the cell division cycle [27]. Inactivation occurs in 15% of the cancers through methylation of its promoter region. The remaining cases show homozygous deletions (40%) or deletions accompanied by intragenic mutations in the other allele (40%) [28]. Other tumor suppressor genes which have been described, such as BRCA2, Rh, M KK4 and STK11 are inactivated at a much lower frequency in pancreatic cancer than p16, p53 and DPC4 [29-34].

Mismatch repair genes
Mismatch repair genes (hMSH2, hMLH1, hPMS1, hPMS2, hMSH6/GTBP and hMSH3) repair single base pair changes and small insertions/deletions that occur during DNA replication [35-38]. Inactivation of one of these genes leads to accumulation of mutations in microsatellite repeats, a phenotype called microsatellite instability. In hereditary non-polyposis colorectal cancer (HNPCC) germline mutations occur in one of the mismatch repair genes [38-40]. Microsatellite instability has been shown in a variety of cancers including endometrium, stomach and lung [39-43]. In a recent study microsatellite instability has also been reported in pancreatic cancer [35]. Of the 82 pancreatic carcinomas analyzed three showed microsatellite instability. Interestingly, these three carcinomas had a specific “medullary” histologic appearance, very similar to the one reported in colon cancers with microsatellite instability [35,40].

Genetic alterations as a diagnostic tool
Mutations of the K-ras oncogene and p53 alterations are the most widely studied neoplastic markers in the pancreas. Because these alterations occur in a relatively early stage of pancreatic carcinogenesis, at a high frequency and most importantly, because they can be detected by easy and sensitive methods.
K-ras oncogene:
Activating point mutations in codon 12 of K-ras are an attractive target, since they are present in almost all cases of pancreatic cancer [9-12]. Importantly, most of the alterations are restricted to one codon, [9] which greatly facilitates their detection. Furthermore, mutations in K-ras codon 12 are an early event in pancreatic carcinogenesis; K-ras mutations have been described in the early pancreatic precursor lesions [44-46].
Molecular detection of K-ras mutations:
The technique for detecting K-ras mutations has improved dramatically since Almoguera et al. (1988) first described K-ras mutations in pancreatic carcinomas [47]. In particular, hybridization with allele-specific oligonucleotide (ASO) and enrichment of PCR products for mutant sequences (mutant-enriched PCR) by digesting the wild-type sequences after introducing an artificially created restriction site, have made it possible to detect small numbers of cells with mutant K-ras even when they are admixed with an excess of cells containing wild type K-ras [9,48-50].
In addition to the pancreatic tissue itself, K-ras mutations have been detected in several secondary sources including pancreatic juice, endoscopic retrograde choanalangiopancreatography (ERCP) brush samples, duodenal fluid; stool and blood [10,12,51-56]. The identification of mutations in ERCP brush samples in combination with conventional cytology improves the sensitivity of diagnosing cancer over cytology alone, in our studies from 76 to 89% (in a pilot study of 17 patients) and in a large group of 312 consecutive patients from 36% to 62% [54,55].
Detection of K-ras mutations in duodenal fluid, stool and blood might also be attractive as a screening method since the collection of these materials, especially stool and blood, is less invasive than the ERCP procedure. Yamada et al. (1998) could detect K-ras mutations in the plasma in 60% (9 of 15) of the patients with pancreatic carcinomas harboring K-ras gene mutations. They also found a significant correlation between the presence of detectable K-ras mutation in the plasma and tumor size. When blood samples were positive for K-ras mutations, tumors were less likely to be resectable [56]. In postoperatively obtained duodenal fluid, K-ras mutations were detectable in 25% of the patients with periampullary cancer, but the negative predictive value was low (22%): 32 of 41 patients with wild-type duodenal fluids had cancer [51]. Iguchi et al. (1996) examined preoperatively obtained duodenal fluid of patients who underwent a secretin test for stimulation and found K-ras mutations in 63% of the patients with pancreatic cancer. Only 1 of the 41 patients with a benign pancreatic disorder had a K-ras mutation in the duodenal fluid [58]. Caldas et al. (1994) reported that K-ras mutations can also be detected in the stool; in 6 of 11 patients (55%) with pancreatic adenocarcinoma K-ras mutations were detected [10].
K-ras mutation analysis also proved to be useful in distinguishing between benign bile duct proliferations and pancreatic cancer metastases in the liver, when during laparotomy a suspicious lesion in the liver was seen [57]. In short, K-ras mutations detected in secondary sources may contribute to early detection and diagnostic accuracy by differentiating pancreatic cancer from benign lesions.
P53
Another attractive target for the detection of pancreatic cancer is the p53 tumor suppressor gene. This gene is also frequently altered in pancreatic adenocarcinomas [13-15]. In contrast with the K-ras oncogene, p53 mutations are not concentrated at one locus [59], which makes mutation analysis laborious. The use of indirect markers helps to overcome the technical difficulty in searching for p53
mutations. Immunochemical staining for the p53 protein as a detection method for mutant p53 is a feasible alternative, since a p53 mutation often results in a more stable protein than the wild type p53 protein. Mutant p53 gene product is therefore more likely to accumulate to immunohistochemically detectable levels [59-61]. Although p53 mutations occur in pancreatic carcinoma in situ, p53 mutations are more common in invasive cancer [61,62].

Up to now, no immunohistochemical detection of p53 is described in normal pancreatic tissue [49,61,62]. We found that immunocytochemical staining for p53 mutations of cytology samples in cytologic analysis increases the sensitivity of identifying cancer [53,54]. In a recent study the diagnostic value of p53 immunocytochemistry was evaluated on endobiliary brush cytology specimens of 53 patients; the sensitivity increased from 28% for cytology alone to 43% for cytology in combination with p53 immunocytochemistry [63], particularly for distal bile duct carcinomas. Using cell specimens from selective pancreatic duct brushings, which is a laborious technique, p53 immunocytochemistry increased the sensitivity from about 60% for Papanicolaou staining alone to 90% for the combination of Papanicolaou staining and p53 immunocytochemistry [64,65].

**P16 gene**

Recently, Wilentz et al. (1998) immunohistochemically stained a large group of pancreatic precursor lesions and showed that loss of p16 gene product is important in the progression of pancreatic cancer [66]. Despite the high frequency of inactivation of p16 and the development of immunocytochemistry against the p16 gene product, the interpretation of the immunocytochemical results is difficult, since negative immunostaining means that the cells have inactivated the gene [65]. Especially the interpretation of cytology samples is difficult, since in contrast to tissue samples, there are often not enough malignant cells to distinguish between positive, negative and background staining. However, Belinsky et al. (1998) showed that the aberrant methylation of the p16 promoter region can be used for detection of this change in secondary sources- [67]. Although hypermethylation of the p16 gene occurs in only 15% of pancreatic cancer, it is a potential target for detecting pancreatic cancer cells.

**Mismatch repair genes**

Microsatellite instability has been used in other organs on cellular specimens for the detection of cancer, such as bladder cancer, but the frequency of this alteration in pancreatic cancers (~4%) is presumably too low to use it for diagnostic purposes [35,68].

**Conclusion**

In the future molecular-based tests will play an increasingly important role in the clinical setting. As shown above the assessment of molecular changes in human tissues can be useful in consolidating a diagnosis in those cases in which morphology alone can not give conclusive information. Using current techniques, molecular analysis of genetic changes in secondary sources including blood, stool and pancreatic juice can be used to detect a neoplasm in a preclinical stage. Importantly, individually sub-optimal markers can be combined to yield higher sensitivity and specificity for cancer [69].

However, the molecular-based tests should be interpreted with caution, since genetic alterations occurring very early in the pancreatic carcinogenesis do not necessarily prove that a patient will develop invasive malignancy. Furthermore, the genetic changes found in sources other than the pancreas itself (blood, stool) should be evaluated prudently, since, for example, K-ras mutations are also frequently encountered in other carcinomas, like colorectal carcinoma. Therefore, the origin of the cells containing the genetic alteration should be carefully determined.

New molecular markers such as “tissue inhibitor of metalloproteinase type 1” (TIMP-1) and telomerase are currently under investigation and may prove to be useful in the early detection of pancreatic cancer [69,70].

In conclusion, the determination of molecular alterations can serve as a valuable supplement in the diagnosis of pancreatic cancer and can improve the early diagnosis of pancreatic cancer, which is crucial for a better patient survival rate.

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