K-ras and p53 in cancer of the pancreas and extrahepatic biliary tract

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General Introduction

Because cancer of the pancreas and extrahepatic bile duct include cancer of the bile duct, gall bladder and ampulla of Vater, since many clinicians, they learn to some extent be identified as an entity. Both organs develop from the most cranial part of the embryonic foregut, which suggests a common histogenesis, and the spectrum of histologic types of carcinoma arising from the two distinct duct systems is very similar [1]. The majority of these cancers are distal adenocarcinomas [3-5]. Differences also exist regarding molecular genetic alterations [1]. Finally, because of the anatomic location, patients with resection of both organs often have a comparable clinical presentation and the same diagnostic work-up techniques and the same radiologic imaging.
The distinction between cancer and benign diseases of the pancreas and extrahepatic biliary tract can be difficult because of similar symptomatology and overlapping radiological findings. In certain cases a tissue diagnosis is needed to reach a definitive diagnosis. This is important for optimal patient management. For this purpose cells from the bile ducts can be collected during endoscopic procedures. However, histopathological and cytopathological findings are not always unequivocal and the sensitivity of conventional light microscopic cytology is low. Therefore, we assessed the diagnostic value of the detection in (cyto)pathological specimens of alterations in the K-ras oncogene and p53 tumor suppressor gene, two common molecular changes in cancer of the pancreas and extrahepatic biliary tract. K-ras and p53 alterations were also used to address questions concerning epidemiology and tumor pathogenesis.

Because cancer of the pancreas and extrahepatic biliary tract including cancer of the bile duct, gall bladder and ampulla of Vater, share many features, they can to some extent be looked upon as an entity. Both organs develop from the most caudal part of the embryonic foregut, which suggests a common histogenesis, and the spectrum of histologic types of carcinomas arising from the two distinct ductal systems is very similar [1]. The majority of these cancers are ductal adenocarcinomas [2-5]. Similarities also exist regarding molecular genetic alterations [1]. Finally, because of the anatomic location, patients with tumors of both organs often have a comparable clinical presentation and the same diagnostic work up, including cytopathological sampling, is followed [6-9]. The etiology of these carcinomas is largely unclear, although some risk factors have been identified [10-20].

The incidence of these cancers is low. The incidence of pancreatic cancer in the United States (US) is 6 per 100,000 every year [21,22]. In contrast, these cancers are very lethal. Pancreatic cancer is the sixth leading cause of cancer deaths in The Netherlands following cancer of the lung, breast, colon, prostate, and stomach [23].

Surgery is the therapy of choice and the only option for cure. Morbidity and mortality of the surgical procedures have decreased over time with improved surgical techniques and when performed in centers with much experience [24]. Unfortunately, at the time of diagnosis, carcinoma of the pancreas and biliary tract have often already spread locally or to distant sites and thus the resectability rate is low [3,25,26].

The advanced disease stage at the time of diagnosis results in low survival rates even after surgical treatment. The overall 5-year survival rate is about 10%. Depending on the radicality of the resection, surgical treatment can improve 5-year survival to more than 20% in patients with pancreatic carcinoma and bile duct cancer [3,21,26-29].

Most patients' initial symptoms are non-specific, i.e. weight-loss, abdominal pain, anorexia and malaise [30,31]. In many cases obstructive jaundice is the presenting symptom, but this is also caused by benign diseases in the pancreatic head region [32,33].

A clinical suspicion of malignant disease of the pancreas or biliary tract is often further examined by diagnostic imaging techniques. With ultra-soundography and computed tomography a mass can be seen in the pancreas and liver hilus region in many patients with cancer. The presence and the level of bile duct stenosis can be visualized with endoscopic retrograde cholangiopancreatography (ERCP) [34,40]. Although specific radiological criteria have been described as an indication for malignant disease, none of the imaging methods can distinguish malignant disease from benign disease with certainty.

In certain cases, histopathology or cytopathology is necessary for a definitive confirmation of malignant disease. Without an unequivocal diagnosis, a number of patients without malignant disease will
undergo procedures for malignant disease inappropriately, and vice versa. Especially when a non-surgical treatment is chosen, it is important to know the nature of the disease to make proper decisions. The absence of a certain diagnosis is distressing to the patient and precludes adequate predictions on prognosis. Also, it will result in delayed or unnecessary additional diagnostic interventions.

It is often difficult to obtain tissue from the biliary tree. In case of a tumoral mass, material can be collected by fine needle biopsy or aspiration under CT guidance. However, seeding metastases have been described following this procedure [41]. During the relative non-invasive ERCP procedure, duodenal fluid, pancreatic juice and brushings from the bile ducts can be collected for cytological analysis. Unfortunately, although the specificity of cytology is virtually 100%, the sensitivity is only 30-40% [6,7]. The limited diagnostic yield is caused by the low number of malignant cells that are collected during the procedure, the resemblance of malignant cells and reactive changed epithelial cells, and the difficulty to distinguish normal ductal cells from cells derived from very well differentiated adenocarcinomas.

New promising diagnostic tumor markers, detectable in easily obtained patient material like biliary cytology specimens, may come from genetic cancer research. These sensitive molecular markers may improve the diagnosis of malignancy and may lead to early diagnosis of patients resulting in a better prognosis.

Genetic cancer research has expanded enormously over the last decades because of the accumulation of molecular techniques, initiated by the invention of the polymerase chain reaction (PCR). The PCR made it possible to multiply specific parts of the DNA genome, making it accessible for investigation. The proceeding knowledge of cancer genetics has now postulated that abnormalities in several classes of genes underlie oncogenesis. The clinical application of this knowledge for diagnostic, therapeutic, prognostic, and epidemiological purposes is currently being investigated and researchers are looking assiduously for new, genome aberrations in cancer.

The frequency and type of genetic alterations differ among neoplasms, but within a certain type of tumor the frequency and type of alterations are relatively constant. In some tumors the frequency of a particular alteration is very high. Such a frequent genetic alteration may be an attractive tumor marker that may be utilized for diagnostic purposes. Ideally, such a tumor marker must be detectable with relatively easy, quick and inexpensive methods in material easily obtainable from the patient, e.g. blood, stools, urine. The relatively constant mutational pattern within certain types of tumors and the finding that carcinogens can induce specific genetic alterations make these molecular genetic changes also an attractive target for epidemiological studies [42,46].

Currently, cancer-causing genes can be divided in three broad classes: oncogenes, tumor suppressor genes, and genome maintenance genes.

Oncogenes encode for proteins that are important in controlling cell growth and/or differentiation. Alterations in an oncogene can lead to an increased activity of its protein and thereby to an increased proliferation of the cell with the altered gene, rendering a clone of genetically altered cells, i.e. a neoplasm. Among the most common genetic alterations in human carcinomas are activating point mutations in the ras oncogenes [47]. There are three ras genes encoding for almost homologous 21 kD proteins. The ras protein is a membrane associated GTP-binding protein that plays a key role in the mitogenic pathway from cell-surface receptors. In all three, the activating point mutations are in codons 12, 13 and 61. The mutations lead to inactivation of the intrinsic GTP-ase function of the protein, which means that the protein remains in the active GTP-bound state, thereby promoting cell growth.

The K-ras oncogene is located on
the long arm of chromosome 12. K-ras is one of the most extensively investigated oncogenes and the most frequently mutated oncogene in epithelial cancers [48]. About 90% of pancreatic carcinomas and 50% of cancers of the extrahepatic biliary tract contain K-ras mutations and virtually all these mutations are in codon 12 [49-54]. This restriction of the mutations to one codon greatly simplifies their detection with PCR-based techniques.

Tumor suppressor genes are also involved in the cell-cycle control. In contrast to oncogenes, which need to be (over)activated to get a neoplastic potential, tumor suppressor genes need to be inactivated and they are therefore considered recessive. Both alleles need to be affected to obtain a relative growth advantage. Often, one allele of the gene is inactivated by mutation while the other allele is lost by deletion.

The p53 tumor suppressor gene has been extensively investigated in cancer genetics over the past years and it is the most frequently altered gene known in human cancer [55]. The gene is located on the short arm of chromosome 17 and encodes for a 53 kD phosphoprotein. The gene is activated in case of DNA damage. The protein has a specific DNA binding site and acts as a transcription factor in the nucleus for other genes through which it performs its tumor suppressive function [56]. The p53 protein can promote cell cycle arrest in the G1 phase just before DNA replication to allow repair of the damaged DNA [57]. It can also promote programmed cell death, i.e. apoptosis, and is directly involved in DNA repair itself [58]. The p53 protein will thus prevent DNA aberrations with or without neoplastic properties to be anchored in the genome of cells.

A point mutation of the gene often results in a conformational change of the protein. Because of this change it cannot bind to DNA and carry out its function. Mutations in the p53 tumor suppressor gene occur throughout the gene with a predilection for exons 5, 6, 7, and 8. This makes the detection of p53 mutations an energy- and time-consuming, cumbersome process. However, the conformational change of the mutant p53 protein product also leads to stabilization of the protein and therefore a prolonged half-life [59]. For this reason, in contrast to the normal wild-type p53 protein with a very short half-life, the mutant p53 product can be detected with standard immunohistochemical methods and positive p53 immunostaining strongly correlates with p53 mutations [60]. The frequency of p53 mutations in cancer of the pancreas and extrahepatic biliary tract is between 50% and 70% [61-63].

The third class of genes are the genome maintenance genes coding for proteins which repair small DNA aberrations that occur during DNA replication [64-69]. The absence of this cellular repair mechanism leads to an accumulation of mutations to other cancer-causing genes. Mutations also occur in small non-coding DNA regions, the microsatellite repeats, which results in changes in their length. These differences in length can be detected as microsatellite instability that is thus indicative for inactivated genome maintenance genes. These genes also act in a recessive manner. Mutations in genome maintenance genes rarely occur in carcinoma of the pancreas and the extrahepatic biliary tree [64].

Clinical applications of both alterations in the K-ras oncogene and p53 tumor suppressor gene are attractive to investigate in pancreatic cancer and cancer of the extrahepatic biliary tract for the following reasons. 1. Both genes are well-established cancer-causing genes. 2. The prevalences of K-ras and p53 alterations in these carcinomas are high. 3. The detection methods are very sensitive and potentially are able to detect small numbers of malignant cells in the abundance of cells without the alteration. 4. Alterations in both genes can be detected with relatively easy methods. 5. The results can be obtained within 48 hours making the tests suitable for routine clinical purposes.
Outline of the thesis

As described in the introduction, the distinction between malignant and benign disease of the pancreas and extrahepatic biliary tract may be difficult with conventional clinical and pathological diagnostics, which will preclude or delay appropriate clinical management. In chapter 1 through 4 the detection of alterations in the K-ras oncogene and the p53 tumor suppressor gene in biliary cytology specimens was evaluated for this differential.

In chapter 1 and 2 the potential diagnostic use of detection of K-ras codon 12 mutations in duodenal fluid and endobiliary brush cytology was determined, respectively, in an in vitro setting i.e. duodenal fluid and brush cytology was collected from postsurgical resection specimens. In this way we were able to compare the findings in the primary lesions with the findings in the secondary sources. This would give us insight in the limitations of the test as a diagnostic tool in a clinical setting. To further investigate this, we performed a discrepancy analysis in chapter 2 to determine the causes of inconsistent outcomes in the primary lesion and secondary source. In the same way the potential diagnostic use of p53 immunostaining of endobiliary brush cytology was determined in chapter 2.

The actual diagnostic value of K-ras and p53 was investigated in chapter 3 and 4. Both tests were evaluated on prospectively collected endobiliary brush cytology obtained during ERCP from a large series of consecutive patients with extrahepatic bile duct stenosis.

Peroperatively during laparotomy and preoperatively during laparoscopic staging for tumors of the pancreatic head region, lesions on the liver surface are frequently seen. Macroscopically and microscopically it can be difficult to determine the nature of these lesions. In chapter 5 we investigated whether the K-ras mutational analysis could contribute to the distinction between benign liver lesions and liver metastases of carcinomas of the region of the head of the pancreas which is of great importance for further treatment. Generally, in the presence of liver metastases, the patient is excluded from curative resection.

In chapter 6 cholangiocarcinomas of patients from a high-incidence area and a random group of 'conventional' cholangiocarcinomas were compared regarding K-ras and p53 alterations. Differences in type or frequency of mutations would indicate a different etiology of the carcinomas in both groups, and potentially point to specific carcinogens in the high-incidence area.

A rare neoplasm that occurs in both the pancreas and the biliary tract is the osteoclast-like giant cell tumor. There is controversy whether the 2 components in these tumors, the ductal component and the osteoclast-like giant cells, are derived from a common precursor cell, from different precursor cells or have a distinct origin. In chapter 7, the different components of osteoclast-like giant cell tumors were examined separately for the presence of K-ras codon 12 mutations, p53 alterations, and other immunohistochemical markers to elucidate their connection. Insight into tumor pathogenesis may have clinical implications.

The thesis includes a summary in English and Dutch.

References

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