Genes and surgery in pancreatic cancer
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SUMMARY AND CONCLUSIONS
SUMMARY
Periampullary adenocarcinoma consisting of pancreatic, distal bile duct or ampullary carcinoma, is an aggressive disease with a high mortality rate. Pancreatic carcinoma is the most common periampullary malignancy and the fourth leading cause of cancer-related death in western countries. Because potentially curative surgery is restricted to small tumors without evidence of local ingrowth or distant metastases, early detection is a sine qua non. The growing understanding of pancreatic carcinogenesis and the concomitant accumulation of genetic changes enable the use of molecular markers in early detection and management.

Given the limited number of candidates for resection, minimization of surgical risks is imperative. Around 80% of patients have unresectable disease at presentation and need to get optimal palliation by means of surgical or non-surgical treatment. When patients unexpectedly have unresectable disease at laparotomy, the best surgical palliative procedure has to be chosen in order to optimize the quality of the remaining lifetime.

This thesis consists of two parts: Part I entitled Molecular Markers and Part II entitled Management. The nine studies described in Part I investigated various clinical applications of some potential genes as molecular markers encountered in pancreatic carcinogenesis. The five studies described in Part II evaluated the optimal management in patients with resectable and unresectable periampullary cancer.

Part I – Molecular Markers
A majority of patients undergoing resection for periampullary cancer will eventually suffer from locoregional relapse or distant metastases. The latter ultimately depend on dissemination of malignant cells, which has possibly already taken place before primary surgery. The ability to detect these so-called micrometastases would improve preoperative patient selection and could guide future multimodality treatment. In Chapter 1 micrometastases were detected in bone marrow by immunocytochemical analysis in 12 of the 31 (39%) patients with a pancreatic or ampullary carcinoma. After a median follow-up of 17 months 15 of the 31 (48%) patients had died: 7 of the 10 (70%) with and 8 of the 21 (38%) without micrometastases, indicating a significantly shorter survival in the presence of bone marrow micrometastases. However, clinical use of cytokeratin markers cannot be recommended at present, because positive staining was seen in two patients who turned out to have pancreatitis without malignancy.

Fine-needle aspiration (FNA) is a useful technique in the diagnostic process of a pancreatic mass. While most aspirates are diagnostic of a malignant or benign condition, a substantial minority is atypical and cannot adequately rule out cancer. Chapter 2 shows that a molecular panel including the K-ras, p53, and DPC4 genes correlates with conventional cytology and is able to support the diagnosis of adenocarcinoma in 6 (32%) of 19 aspirates originally diagnosed as atypical by cytology
alone. Its use may lead to earlier and less invasive diagnosis, and may be helpful in the differentiation between pancreatic cancer and pancreatitis.

Standard staging procedures of periampullary tumors do not identify all patients with an adverse outcome despite apparently successful resection. Cytology and K-ras mutation analysis of perioperative peritoneal washings in patients with periampullary cancer might identify a subset of patients with a worse prognosis. In Chapter 3 positive cytology for malignancy in peritoneal washings obtained during exploratory laparotomy for a periampullary tumor was found in 8 of the 134 (6%) patients. Although the incidence is low, when present it does convey a bad prognosis. Six of the eight (75%) patients with a positive peritoneal cytology were found to have unresectable cancer, and the other two (25%) patients did poorly with a median survival of 6 and 14 months. Patients with a positive cytology had a significant shorter median survival than patients with an atypical or negative cytology. The rate of K-ras mutations in peritoneal cytology was even lower. These data suggest little role for standard conventional cytology or K-ras mutation analysis of peritoneal fluid in patients with potentially resectable periampullary cancer.

Endoscopic retrograde cholangiopancreatography (ERCP) is an important diagnostic tool in patients with a biliary stenosis. During ERCP cells can be collected to differentiate between a malignant and a benign cause. In a previous study, brush cytology specimens from extrahepatic biliary stenoses were obtained during ERCP in 312 consecutive patients. Mutations in the K-ras oncogene were detected by an enriched polymerase chain reaction (PCR) and an allele-specific oligonucleotide (ASO) hybridization assay. Chapter 4 is a follow-up study of the eight patients with a K-ras mutation and a clinically benign extrahepatic biliary stenosis. After a median follow-up of 65 months, six of the eight patients were alive without evidence of malignancy and two died of other causes than pancreatic cancer. Therefore the K-ras mutations in all these eight patients had to be considered as confirmed false-positives. Nevertheless, we think that patients with a positive K-ras mutation in biliary cytology require careful follow-up, because a false-positive result is infrequent.

The specificity of K-ras mutation analysis in brush cytology using an ASO assay remains a concern because the consequences of unnecessary surgical resection are unacceptable. In Chapter 5 the prevalence of K-ras mutations in brush cytology and bile in the general population was estimated using the “epidemiologic necropsy” study design. K-ras mutations were detected in 78 of the 317 (25%) brush cytology specimens from autopsy patients, in 18 of the 317 (6%) bile specimens, and in pancreatic tissue carrying PanIN-1A and PanIN-1B lesions. The prevalence of K-ras mutations in the general population could be estimated at 14% in brush cytology and 3% in bile. Therefore, it was concluded that K-ras mutation analysis using an ASO assay should not be used as a screening test in the general population, i.e. subjects not at risk for pancreatic cancer.

The same brush samples from the 312 consecutive patients with an extrahepatic biliary stenosis
mentioned earlier were used to perform the K-ras mutation analysis with the novel amplification refractory mutation system (ARMS) assay described in Chapter 6. The results were compared to both conventional cytology and K-ras mutation analysis using the ASO assay, and evaluated in view of the final diagnosis. The test characteristics of the ARMS and ASO assays were largely in agreement with a sensitivity for detecting a malignancy of 49% and 42%, respectively. The specificity of the quantitative ARMS assay could be increased to 100% by setting limits for the false positives. This reduced the sensitivity of ARMS from 49% to 43%. It is felt that the ARMS assay deserves consideration as an adjunct in the surveillance of pre-symptomatic patients at risk for hereditary pancreatic cancer.

Despite the advances made in the understanding of pancreatic carcinogenesis, little is known about the initiating genetic events in the pancreatic duct epithelium that facilitates its progression to cancer. Telomere shortening can cause chromosomal instability. In order to elucidate the onset of this phenomenon in the non-invasive precursor lesions, we examined in Chapter 7 the telomere repeat lengths in a series of 82 PanIN lesions of all histological grades. Telomeres were strikingly shortened in 79 of 82 (96%) PanINs, including 21 of 23 (91%) PanIN-1A lesions, compared to adjacent normal structures. Telomere shortening in PanIN lesions did not correlate with proliferation measured by quantitative Ki-67-labeling index. Shortening of telomeres in PanINs predisposes to the accumulation of chromosomal abnormalities and to the subsequent development of invasive carcinoma. Telomere shortening can be regarded as the most common early genetic abnormality in pancreatic cancer recognized to date.

Genes differentially expressed in pancreatic cancer were evaluated in Chapter 8 by using GeneChip arrays and serial analysis of gene expression (SAGE). Ninety-seven genes were identified as having fivefold or higher expression levels in pancreatic cancer as compared to normal tissue. The expression profiles of 12 genes were confirmed by immunohistochemical labeling, in situ hybridization, or RT-PCR. Sixty-nine genes were not implicated in pancreatic cancer before and therefore have potential as novel tumor markers.

Parallel to Chapter 8, we identified in Chapter 9 the genes differentially expressed in ampullary carcinoma compared to normal duodenum by using global gene expression profiling. We identified 235 fragments expressed at least fivefold higher in ampullary carcinoma than in normal duodenum. The expression profiles of eight candidate genes were confirmed by immunohistochemistry or in situ hybridization on tissue micro arrays containing 54 ampullary cancers. The osteopontin gene was expressed 27-fold higher in ampullary cancers compared to normal duodenum. Mean pre-operative serum osteopontin levels measured by competitive ELISA were around 900 ng/ml in patients with ampullary carcinoma or ampullary adenoma, around 300 ng/ml in patients with a non-neoplastic periampullary disease, and around 200 ng/ml in age-matched healthy controls (p<0.001). Measurement of markers of ampullary carcinoma such as osteopontin might aid in
the early detection and differential diagnosis of patients with ampullary lesions.

Part II – Management

Considering all periampullary carcinomas, ampullary carcinoma has the best 5-year survival after resection compared to pancreatic and bile duct carcinoma. Controversy currently exists over which tumors should be treated by local resection. In Chapter 10 short-term results and long-term survival after local and radical resection of ampullary adenomas and adenocarcinomas were analyzed in 145 patients. Local resection was adequate in 16 of the 25 (64%) patients, 12 patients with an adenoma and 4 patients with stage 1a adenocarcinoma. In the remaining 9 patients subsequent pancreaticoduodenectomy (PD) was needed for non-radical resection margins. The other 120 patients underwent an elective PD for adenoma (n=7), in situ carcinoma (n=1) or ampullary carcinoma (n=112). The 5-year survival rate in patients with an ampullary adenoma was 92% after local resection and 86% after PD. The results of this study show that local resection is an adequate surgical treatment for patients with an ampullary adenoma but should not be advised in patients with an ampullary carcinoma.

Given the small number of long-term survivors among patients with pancreatic cancer, minimization of surgery-related mortality and morbidity is imperative. Nevertheless, centralization of pancreatic surgery in high-volume centers is still under debate. In Chapter 11 a systematic review was performed to retrieve the best available evidence on the effect of centralization of pancreatic surgery on hospital mortality as primary outcome. The relative risk of postoperative mortality in a high volume hospital compared to a low volume hospital for the volume cut-off values of 2, 5, 10, and 20 pancreatic resections per year was between 0.25 and 1.10, 0.29 and 0.76, 0.21 and 0.62, and 0.07 and 0.15, respectively. Eleven of the 12 included studies showed a lower postoperative mortality rate in higher volume hospitals, irrespective of the arbitrary cut-off value. Therefore it was concluded that convincing evidence of an inverse relation between hospital volume and mortality after pancreatic resection does exist and that the plea for centralization should be enforced.

Following previous chapter, the effect of this plea for centralization in the Netherlands, one of the few countries with an independent nationwide registry, was analyzed in Chapter 12. For this purpose the changes in mortality after PD as well as the changes in referral pattern were evaluated. Since the start of the independent registry in 1994, the available data on the volume-outcome relationship were published and presented, always followed by profound discussions on the consequences of centralization. However, during the nine-year lasting plea for centralization of PD among the surgical community no significant change was seen in either mortality or referral pattern. The mortality rate in hospitals performing less than 5 PDs per year compared to the hospitals performing more than 24 PDs per year was 16.1% versus 1.5% in 1994 and did not
change significantly to 14.8% versus 2.5% in 2002. The Dutch surgical community failed to reduce the overall mortality after PD and failed to change the referral pattern.

The median survival rate of patients with an unresectable periampullary carcinoma varies between 6 and 12 months. During this time frame palliation should focus on major symptoms such as obstructive jaundice, duodenal obstruction and pain. In Chapter 13 current recommendations on the palliative treatment of patients with unresectable periampullary cancer are summarized. Patients with a life expectancy of less than 6 months should be palliated non-surgically, and those with a life expectancy of more than 6 months should be palliated surgically. From all surgical bypasses described, the choledochojunostomy is generally preferred. Pain is a frequent symptom related to poor survival and should be treated adequately. Pain management by means of a celiac plexus blockade has proven to be effective.

When unresectability is detected during explorative laparotomy with the intention to perform a resection, surgical palliation is preferable to non-surgical palliation. In Chapter 14 a randomized trial is described evaluating the role of prophylactic gastrojejunostomy for unresectable periampullary cancer found during laparotomy. Clinical symptoms of gastric outlet obstruction were found in 2 of the 36 patients (5.5%) with a double bypass, and in 12 of the 29 patients (41.4%) with a single bypass (p=0.001). In the double bypass group the absolute risk reduction (ARR) for a relaparotomy by means of a (re-)gastrojejunostomy was 18%, and the numbers needed to treat (NNT) were 6. Postoperative morbidity including delayed gastric emptying and length of hospital stay, as well as survival and quality of life were not different in both groups. It was concluded that a double bypass consisting of a hepatocjejunostomy and a prophylactic gastrojejunostomy is preferable to a single bypass consisting of only a hepatocjejunostomy in patients undergoing surgical palliation for unresectable periampullary carcinoma.

The studies described in this thesis show that molecular markers may have a role in early detection of periampullary carcinoma and the identification of patients predisposed to this disease. The standard diagnostic procedures used for differentiation between malignant and benign periampullary tumors may be supported by different molecular techniques.

A molecular panel including K-ras, p53 and DPC4 has proved to be useful in clarifying the diagnosis in patients with atypical fine needle aspirates (FNA). K-ras mutation analysis is also of value in increasing the sensitivity of brush cytology from a biliary stenosis obtained during endoscopic retrograde cholangiopancreatography (ERCP).

Unfortunately, its use is hampered by the fact that the specificity of K-ras mutation analysis in brush cytology using the ASO assay is not a 100%. Although this apparently indicates that benign lesions with a K-ras mutation should be considered as precursor lesions, their natural history is as...
yet mostly unknown. Moreover, in our “epidemiologic necropsy” study, the prevalence of K-ras mutations in brush and bile from the general population using the ASO assay could be estimated on 14% and 3%, respectively, making it unsuitable as a screening test. However, by using the novel quantitative ARMS assay in a large consecutive series of 312 brush cytologies, the specificity of K-ras mutation analysis could be increased to 100%. This finding opens up new perspectives for the clinical use of K-ras mutation analysis; our next study will be to determine the prevalence of K-ras mutations in brush and bile from necropsies using the ARMS assay.

Novel potential tumor markers of periampullary cancer have been identified by the use of gene expression profiling. Immediate translation of these results into routine daily practice is still limited. Their suitability as marker for early detection of cancer has to be determined in future studies.

The finding of telomere shortening as the earliest genetic abnormality in pancreatic carcinogenesis suggests an adjustment of the pancreatic cancer progression model (figure 1).

Local resection is an adequate procedure for patients with an ampullary adenoma. Resection by means of a PD is generally the only curative option for periampullary malignancies including pancreatic, ampullary and distal bile duct carcinoma. PD is a high-risk surgical procedure with considerable morbidity and mortality, but can be performed safely in specialized centers. The systematic review reveals that there is a correlation between hospital volume and hospital mortality. Despite a continuous plea for centralization of PD in the Netherlands, no significant change in either hospital mortality or referral pattern has been observed. Since centralization encounters emotional and logistic problems in the Netherlands, a joint solution has to be found for the persisting high hospital mortality rate after PD at low-volume hospitals.

Most patients present with unresectable disease and therefore palliation features largely in the treatment of pancreatic cancer. The choice between endoscopic or surgical palliative procedures

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**Figure 1** Proposed new progression model for pancreatic carcinoma
should be based on the remaining life expectancy. In patients who are expected to live longer than 6 months it is generally recommended to perform a surgical bypass for biliary and duodenal obstruction. As the techniques for endoscopic palliation are improving, recommendations may change over the years.

Surgical palliation is also preferred when a periampullary carcinoma is found to be unresectable during explorative laparotomy. The randomized controlled trial described in this thesis reveals that a prophylactic gastrojejunostomy should be performed routinely in these patients.