Biliopancreatic malignancy: Screening the at risk patient with molecular markers

C. Caldas
University of Cambridge, Department of Oncology and Cambridge Institute for Medical Research, Cambridge, UK

Summary

Biliopancreatic malignancy is one of the leading causes of cancer death in the Western world. Defining at risk groups has been difficult. Diabetes mellitus and pancreatitis increase the risk of pancreatic carcinoma, and inflammatory bowel disease and associated sclerosing cholangitis increase the risk of biliary tract malignancy. Pancreatic carcinoma has also been described in pedigrees with inherited cancer predisposition. Extensive molecular profiling of pancreatic carcinomas has been accomplished over the past few years, but similar knowledge in other biliopancreatic malignancies is lacking. In almost all pancreas cancers at least one alteration will occur out of a combination of K-ras mutations and inactivation of the tumor suppressor genes p16/MTSl/ink4a, p53 and DPC4/Smad4. Mutations of K-ras and p16 have been described in hyperplastic and dysplastic pancreatic ductal lesions believed to be the non-malignant precursors of pancreatic carcinoma. Detection of K-ras mutations in clinical samples (biliopancreatic secretions, stool, duodenal aspirates, and blood) identical to ones present in primary pancreatic cancers and/or their precursor ductal lesions has been reported in pilot studies. Recently detection of 18q deletions (at the DPC4 locus) in pancreatic secretions from early pancreatic cancers was also reported. These advances raise the possibility that within well defined at risk groups it will be possible to use a combined set of molecular markers to screen clinical samples and detect early pancreatic cancer or even pre-malignant lesions. The fulfillment of this promise will depend on proving the role of molecular screening in decreasing morbidity and mortality, which will require well designed clinical studies.

Key words: biliary carcinoma, biliopancreatic malignancy, K-ras, molecular detection, molecular markers, p16, pancreatic carcinoma, risk factors, screening

Introduction

The success of any cancer screening strategy depends on the clear identification of an at risk population ("target population"), the availability of screening methodology with high sensitivity and specificity to identify the affected individuals, and access to prophylactic and/or therapeutic measures that have high efficacy, i.e. eradicate the cancer or preneoplastic lesion, with reduced morbidity and mortality. Biliopancreatic malignancy as defined for the purposes of these review (pancreatic adenocarcinoma and carcinomas of the extrahepatic biliary tract) is one of the leading causes of cancer death [1]. The overall 5 year survival in these patients is less than 5% and the yearly incidence and mortality are virtually identical. Despite this dismal prognosis in highly selected groups of patients, which in total represent less than 10% of cases, with localized resectable disease the 5 year survival is 20% and in patients with no involvement of lymph nodes it is as high as 40% [2]. This appears to suggest that with earlier diagnosis one could hope to have a significant impact in overall survival since patients would be eligible to undergo curative surgical resection. Improvements in imaging and serological tumor markers (CEA and CA19-9) have not translated in significant improvements in early diagnosis [1]. Over the past few years great strides have been made in describing the somatic genetic alterations underlying human pancreatic cancers. Because these alterations are clonal markers of cancer cells they have the potential to be used to design tests aiming at detecting small numbers of cancer cells [3], or even "pre-malignant" cells from precursor ductal lesions. This manuscript deals with screening the at risk patient with molecular markers and several of the topics will also be elaborated on other papers in this issue. I will define what are at risk groups for biliopancreatic malignancy, briefly review the molecular genetics of these carcinomas and describe the putative preneoplastic lesions and their associated genetic alterations, and finally review the pilot studies describing the use of molecular markers for screening.

Risk factors for biliopancreatic malignancy

Epidemiological studies and previous medical history

Age and sex could be defined as "risk factors", since biliopancreatic malignancy is a disease of the elderly and more common in males. The potential "target population for screening" is therefore enormous! Several studies have looked at dietary factors and cigarette smoking as risk factors for pancreatic cancer (reviewed in refs. 1,4) and have found statistically significant relative risks (RR). One is not able to extract from these studies the lifetime risk of pancreatic cancer and therefore a history of these risk factors is not useful per se in devising screening strategies, or even in concluding that screening would be justified at all. In a recent meta-analysis an overall RR of 2.1 of pancreatic cancer associated with a history of diabetes mellitus was determined [5]. A similar RR has been
determined for pancreatic cancer in association with a previous history of pancreatitis [6,7]. This association is very strong and translates into high standardized incidence ratios and an overall incidence of pancreatic cancer in patients with chronic pancreatitis of about 3% [8]. These numbers could potentially justify a screening strategy if one, for example, compares this incidence with the prevalence of particular “founder-type” germline mutations in breast cancer predisposition genes in certain populations where screening for such germline mutations (for example the BRCA2 6174delT) has been advocated by some. A history of previous partial gastrectomy may also be a risk factor for pancreatic carcinoma [4]. Inflammatory bowel disease and associated sclerosing colangitis are known risk factors for biliary tract malignancy [9,10].

**Familial and genetic risk factors: Familial pancreatic adenocarcinoma**

Several case series of families with aggregation of cases of pancreatic adenocarcinoma inherited in a pattern suggestive of a autosomal dominant trait have been described (reviewed in ref.4). To date no predisposing gene that is mutated in the germline of affected individuals has been identified. Individual risk assessments are therefore more difficult until one or more such genes are identified but in appropriate pedigrees one might tell an individual that there is a 50% chance he inherited a copy of the mutant gene. In such individuals , and in mutant-gene carriers in the future, screening strategies for early detection would be probably appropriate.

**Inherited cancer syndromes in which pancreatic cancer is a feature: Familial atypical mole-multiple melanoma (FAMMM)**

Pancreatic carcinoma is the second commonest malignancy in FAMMM families. However the excess of pancreatic cancers appears to occur almost exclusively in certain FAMMM families with germline mutations of p16 that result in impairment of the protein function in *in vitro* assays [11,12]. This “genotype-phenotype” correlation would therefore identify FAMMM families where screening could be indicated.

**Familial breast and ovarian cancer syndromes**

To date two breast and ovarian cancer susceptibility genes have been cloned: *BRCA1* and *BRCA2*. Pancreatic adenocarcinoma is seen in some of these families in persons who have inherited the at risk haplotype (reviewed in ref. 4). Interestingly the Hopkins group has described germline *BRCA2* mutations in 7.3% of patients with apparently sporadic pancreatic carcinomas [13]. Subsequently germline *BRCA2* 6174delT mutations were reported in Ashkenazi Jewish pancreatic cancer patients [14]. In carriers of this mutation the risk of pancreatic cancer is estimated to be 7% by the age of 75 [14].

**Other inherited cancer syndromes**

Pancreatic adenocarcinoma is also featured in the tumor spectrum of several other inherited cancer syndromes, although often it is not clear if this is a result of chance finding (reviewed in ref. 4). These include hereditary non-polyposis colorectal cancer, Peutz-Jeghers syndrome, Li-Fraumeni syndrome, familial adenomatous polyposis and ataxia telangiectasia.

**Molecular genetics of biliopancreatic malignancies**

This item is extensively reviewed elsewhere and I will only summarize the findings that are relevant for the potential use of genetic alterations as clonal molecular markers in the clinical context of screening. The most common somatic genetic alterations in pancreatic carcinoma include activating mutations of K-ras in up to 90% of tumors [15] and inactivation of the tumor suppressor genes p53, p16 and DPC4 in 50-75%, 85-95%, and 45% of carcinomas, respectively [16-19]. If one were to screen the 4 genes in all pancreatic cancers an alteration in at least one would be found in close to 100% of cases. This just illustrates that probably a combination of markers will have to be used in the clinical setting to increase sensitivity.

The value of mutations in genes for screening will also depend on their presence in early tumors, and preferably in pre-invasive lesions. The precursor lesions of pancreatic cancer are believed to be ductal lesions, recently described as pancreatic intraductal neoplasia (PIN) [20]. The percentage of PIN that progress and the rate at which progression of PIN occurs is unknown and a controversial issue. The frequent finding of K-ras mutations in most PIN lesions [21,22] and in a subset of PIN lesions p16 mutations or loss of expression [23,24] appear to lend support to the contention that they are indeed clonal and neoplastic, and raises the possibility of using mutations as clonal markers of pre-invasive PIN. Unfortunately K-ras mutations are detected in PIN in most chronic pancreatitis and in otherwise normal pancreata [22,25,26]. The situation is very similar to what has been described in colorectal cancer (CRC) tumorigenesis: aberrant crypts are believed to be the precursor lesions and have frequently K-ras mutations [27]. APC is the gatekeeper of the colorectal mucosa, and only aberrant crypts with APC mutations appear to progress to CRC [27]. Until such a “gatekeeper” gene is identified in pancreatic ducts it is unclear what “set” of genes will identify the PIN lesions destined to progress to pancreatic carcinoma.

**Molecular detection of pancreatic carcinoma in clinical samples**

The value of mutations as molecular markers for cancer diagnosis was first demonstrated for CRC and bladder cancer [28,29]. The high prevalence of K-ras mutations in pancreatic carcinoma immediately alerted for the possibility of its clinical use and that potential was
confirmed when mutations were identified in FNAs\(^1\) of pancreatic masses [30]. Since then K-ras mutations identical to the ones present in the primary tumor have been identified in blood, pancreatic secretions, stool, duodenal aspirates and biliary secretions of pancreatic cancer patients [21,31-33]. The identification of K-ras mutations in the stool of pancreatic carcinoma and ductal pancreatic hyperplasia patients is particularly noteworthy because of the potential for widespread application and non-invasiveness. The initial study identified mutations in 5 of 11 patients with pancreatic carcinoma and also in 1 of 3 patients with chronic pancreatitis and 2 of 3 patients with cholangiocarcinoma [21]. A subsequent study by another group using a different method confirmed these findings [34]. Combining the two studies the finding of K-ras mutations appeared to have a sensitivity of 40%. Clearly the importance of these two studies is the demonstration that cells/DNA from pancreatic ducts can be identified in the stool using molecular methods. The use of other molecular markers, for example p16, and improved methods to isolate intact cells from stool samples would suggest that increased sensitivity and specificity can be achieved.

Blood samples are also easy to obtain and cancer cell-derived DNA can be isolated from the plasma or serum collected. To date K-ras mutations have been identified in pancreatic cancer patients with bulk disease [35,36] but potentially other markers could be used to identify cancer at an earlier stage. K-ras mutations clearly increase the sensitivity of detection of malignant cells in biliopancreatic secretions and can complement cytology [37,38]. The specificity is compromised for the same reason that K-ras mutations are also found in the absence of cancer [39]. Duodenal aspirates are easier to obtain and associated with significant less morbidity and therefore with more potential to use in screening. To date K-ras mutations have been detected in the duodenal secretions of patients with pancreatic cancer, and also patients with chronic pancreatitis [25,32,40,41].

In a recent report loss of chromosome 18q was detected by FISH\(^2\) in cells from pancreatic juice of patients with early pancreatic cancer and intraductal papillary-mucinous tumors [42].

In summary, all these reports confirm that in pilot studies gene mutations can be used as molecular markers to detect cancer cells in clinical samples. Improved technology and better definition of genetic alterations that characterize pre-invasive lesions “destined” to become malignant will result in improved sensitivity and specificity and therefore potential for application in screening trials.

**Outstanding research questions**

I believe that the feasibility of using molecular markers for screening of biliopancreatic malignancies has been clearly demonstrated. We are now at the stage where well designed trials should be planned to:

1. prospectively study “at risk groups” such as patients with chronic pancreatitis, carriers of germline BRCA2 mutations, and individuals from FAMMM kindred with germline p16 mutations;
2. exhaustively characterize the molecular profiles in PIN lesions;
3. determine the sensitivity and specificity of molecular markers in duodenal secretions vs biliopancreatic juice vs stool vs blood.

**Acknowledgements**

CC is supported by the Cancer Research Campaign [CRC]

**Notes**

1 FNA- fine needle aspirations; 2FISH- fluorescent in situ hybridization

**References**


Correspondence to:
Carlos Caldas, MD
University of Cambridge
Department of Oncology and Cambridge Institute for Medical Research
Wellcome Trust/MRC Building
Addenbrooke's Hospital
Cambridge CB2 2XY, UK
E-mail: cc234@cam.ac.uk