Detection of c-Ki-ras mutations in bile samples from patients with pancreatic and biliary cancers

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Abstract

Aim—To determine whether c-Ki-ras mutations can be detected in bile from patients with biliary strictures caused by pancreatic cancer and other biliary tumours, with a view to developing bile c-Ki-ras mutations as a non-invasive diagnostic marker of pancreatic cancer.

Methods—Bile was collected from 89 subjects (47 controls (including patients with bile duct stones or benign stricture), 20 patients with pancreatic cancer, 11 with cholangiocarcinoma, five with ampullary cancer, and six with metastatic biliary obstruction) referred for endoscopic retrograde cholangiopancreatography. DNA was extracted from bile and c-Ki-ras codon 12 mutations were detected using PCR and a restriction enzyme digestion method.

Results—c-Ki-ras mutations were detected in 10 (50%) of 20 patients with pancreatic cancer, in one (9%) of 11 with cholangiocarcinoma, and in two (33%) of six patients with metastatic biliary obstruction (primary tumours: colon and prostate). C-Ki-ras mutations were not detected in the controls and patients with ampullary cancer.

Conclusions—The sensitivity of this test is too low at 50% to recommend its use clinically, but with refinement has potential as a diagnostic marker for pancreatic cancer.

Keywords: Bile, c-Ki-ras, pancreatic cancer, biliary cancer.

Most (over 90%) of pancreatic adenocarcinomas harbour mutations in codon 12 of the c-Ki-ras gene. Recent studies have reported the presence of the oncogene in pancreatic juice and pancreatic duct brushings in over 90% of patients with pancreatic cancer, suggesting that the oncogene might be useful as a diagnostic marker of pancreatic cancer. Pancreatic juice is, however, relatively difficult to obtain endoscopically, and performing brushings of the pancreatic duct adds significantly to procedure time of endoscopic retrograde cholangiopancreatography (ERCP). Bile is easy to collect endoscopically, particularly when a stent is inserted. Obstructive jaundice is a frequent presentation of pancreatic cancer, and these patients are generally managed by ERCP, which has the advantage of being a combined diagnostic and therapeutic modality. The aim of our study was to determine whether c-Ki-ras mutations can be detected in bile from patients with biliary strictures caused by pancreatic cancer and other biliary tumours, with a view to developing bile c-Ki-ras mutations as a non-invasive diagnostic marker of pancreatic cancer.
Detection of c-Ki-ras mutations in bile

Results

c-Ki-ras codon 12 mutations were detected in 10 (50%) of the 20 patients with pancreatic cancer, in one (9%) of the 11 patients with cholangiocarcinoma, and in two (33%) of the six patients with metastatic biliary obstruction (primary tumours: colon and prostate) (table). Mutations were not detected in any of the controls or the patients with ampullary cancer.

Discussion

c-Ki-ras mutations were detected in patients with malignant disease (or presumed malignant disease) only. In relation to pancreatic cancer the sensitivity (50%) was disappointing, given the high prevalence (>90%) of c-Ki-ras mutations in pancreatic cancer. There are two possible explanations for the low sensitivity: (1) low cellularity in some bile samples, and (2) relative insensitivity of the PCR technique. It is likely that brushings of biliary strictures would be more likely to contain tumour cells than bile—biliary brush cytology has a much higher sensitivity compared with bile exfoliative cytology in the diagnosis of malignant bile duct strictures.7 The PCR technique we have used detects mutations at codon 12 only. It is likely that more sophisticated molecular techniques (for example, amplification refractory mutation system, single strand conformation polymorphism, DNA ligase PCR) or more sensitive detection methods (for example, gene scanning on a DNA sequencer) might increase the mutation positivity rate, and thus the value of the test.

Ras mutations were detected in patients with metastatic biliary obstruction, and in one patient with cholangiocarcinoma. The presence of the oncogene therefore does not establish a definitive diagnosis, but in the appropriate clinical setting is strong evidence of pancreatic cancer. There was, however, a small number (three) of patients with benign biliary strictures. The tissue diagnosis rate in our patients with pancreatic cancer was low at 20%, but a positive tissue diagnosis is achieved in only a minority of patients with pancreatic cancer in this country—for example, in the Yorkshire region, of patients registered with the Regional Cancer Registry in 1993 and 1994, a positive tissue diagnosis was achieved in only 35-6% and 36-4%, respectively (Yorkshire Regional Cancer Registry: personal communication). In summary, we have detected bile c-Ki-ras mutations in 10 of 20 patients with pancreatic cancer. The sensitivity (50%) is therefore too low to recommend the clinical application of this test, but this approach has potential as a means of establishing a diagnosis at the molecular level in patients with pancreatic cancer.
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