From pancreatic intraepithelial neoplasia
to cancer: a dramatic progression with K-ras status analysis

Carla FERNANDEZ (1), Bruno CHETAILLE (1), Anne-Marie TASEI (1), Bernard SASTRE (2), José SAHEL (3), Marie-José PAYAN-DEFAIS (1)

SUMMARY
Pancreatic ductal carcinomas are thought to arise from precursor ductal lesions called pancreatic intra-epithelial neoplasias or PanINs. We report the case of a woman suffering from idiopathic chronic pancreatitis associated with PanINs lesions who developed six years later an invasive ductal carcinoma. Immunohistochemistry for p53, HER-2/neu and genetic analysis of K-ras oncogene were performed at different stages of disease and revealed that the PanINs and the carcinoma did not express p53 and HER-2/neu gene products whereas a K-ras mutation was present at the carcinoma stage. The relationship between cancer and chronic pancreatitis and the main difficulties concerning the early diagnostic of pancreatic cancer are discussed.

RÉSUMÉ
Les adénocarcinomes canalaux du pancréas ont probablement pour origine des néoplasies intra-épithéliales des canaux pancréatiques appelées PanINs. Nous rapportons le cas d’une patiente souffrant de pancréatite chronique idiopathique associée à des lésions de PanINs, qui développait un carcinome invasif du pancréas après six ans d’évolution. Une étude immunohistochimique de p53 et HER-2/neu et une analyse moléculaire de l’oncogène K-ras ont été réalisées à différents stades de la maladie. Les résultats montrent que les PanINs et le carcinome n’exprimaient ni p53 ni HER-2/neu tandis qu’une mutation de K-ras était présente au stade de carcinome invasif. Les liens entre cancer et pancréatite chronique ainsi que les principales difficultés concernant le diagnostic précoces du cancer pancréatique seront discutées.

Case report
A 49-years old woman presented with recurrent attacks of acute pancreatitis. She had no history of alcohol or tobacco consumption. She had undergone a cholecystectomy without result. At endoscopic retrograde cholangiopancreatography (ERCP), a localized stenosis of the main pancreatic duct in the pancreatic body was observed (figure 1). A tumor was suspected and the patient underwent a corporeal pancreatectomy. The resected pancreas was processed in its entirety for histological examination. No stenosis or tumor was found but numerous foci of chronic pancreatitis were present. They were characterized by an advanced fibrosis, peri-ductular inflammatory infiltrates and protein plugs (figure 2a) and were intermixed with areas of normal parenchyme. The lesions of chronic pancreatitis were particularly prominent downstream the presumed stenosis zone. Moreover, multiple foci of PanIN-1 and PanIN-2 were present in almost the entire resected pancreas. They were extended to the proximal margin of resection (figure 2a). Lastly, a limited focus of PanIN-3 interesting medium and large pancreatic ducts was observed upstream the presumed stenosis. All usual causes of chronic pancreatitis were excluded and the diagnosis of idiopathic pancreatitis was done. The patient was followed-up yearly by cholangiopancreatography with pancreatic duct brushing. Atypical cells were regularly observed (figure 2c) and the patient underwent, at her request, a pancreatico-gastric anastomosis. The patient rapidly developed insulin-dependent diabetes. One year later, she was found to have a mass in the remaining tail of the pancreas on imaging studies and elevation of the CA 19-9 tumor marker. The patient underwent a corporeal pancreatectomy and a proximal pancreatico-gastric anastomosis. The histological examination of the resected pancreas showed a chronic pancreatitis with severe lesions of chronic pancreatitis and extensive lesions of PanIN-2 and PanIN-3 involving the main pancreatic duct and collateral branches. PanIN-3 lesions were far more numerous than within the first resection. The pancreas tail fragment was normal. The patient rapidly developed insulin-dependent diabetes. One year later, she was found to have a mass in the remaining tail of the pancreas on imaging studies and elevation of the CA 19-9 tumor marker.
marker in the serum. A distal pancreatectomy was done and an infiltrative ductal carcinoma interesting almost all the pancreas tail was diagnosed (figure 2g). The pancreas adjacent to the tumor showed extensive foci of PanIN-3 (figure 2a). PanIN-1 and PanIN-2 were rare. The patient died 8 months after this last resection.

Methods

Surgical specimens were fixed in Bouin’s fixative (first resection) or 10% buffered formalin (second and third resections) and paraffin-embedded. All available sections were reviewed and the PanINs lesions were classified using the criteria defined by Hruban et al [6]. HER-2/neu and p53 expression was evaluated immunohistochemically using the following antibodies: Herceptest kit (Dako) and monoclonal mouse anti-human p53 antibody (Dako).

Genomic DNA was extracted from three adjacent 10 μm-thick sections and the K-ras gene analysis was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described [1]. The principle of this method is to introduce by PCR a BstNI restriction site in the K-ras sequence. Thus, the enzyme cleaves the wild type sequence in two fragments, one of which is 106 bp in length, whereas a mutation at codon 12 leads to a BstNI-resistant 135 bp fragment [1]. The mutant-enriched PCR-RFLP method increases the amount of mutant allele by cleavage of the wild-type one before the second amplification step and detects one mutant allele in up to 10² wild type allele [12].

Results

Sections from the two first resections containing the main pancreatic duct and the largest amount of PanIN-2 and PanIN-3 and sections from the distal pancreatectomy displaying both PanIN-3 and carcinoma were chosen for immunohistochemical and genetic studies. PanIN-2 and PanIN-3 lesions of the three surgical specimens as well as the infiltrating ductal carcinoma did not show nuclear accumulation of p53 or HER-2/neu overexpression. HER-2/neu was observed only in Langerhans islets (figure 2f). The DNA from all selected sections was successfully amplified. Samples from the corporeal pancreatectomy and the pancreatica-duodenectomy only harbored the wild type K-ras sequence. In theory, the wild-type ras allele present in normal pancreatic tissue is unlikely to mask the presence of a mutant allele because of the high sensitivity of the technique [12]. On the contrary, both the wild type and mutant forms were detected in DNA from the distal pancreatectomy (figure 3). It is possible that PanIN-3 adjacent to the tumor harbored the mutant sequence too but they were too close to the tumor to be reliably analyzed alone without microdissection.

Discussion

It has been recently suggested that pancreatic intraepithelial neoplasias (PanIN) are non invasive neoplastic precursor lesions [2-4] but only four patients with histologically proven PanINs leading to infiltrating carcinoma have been reported [3, 4]. So, our report gives an additional evidence for a direct link between PanIN and carcinoma, and is the first case with a p53, HER-2 and K-ras evaluation. Currently, the major evidence for the PanIN-carcinoma progression comes from molecular analysis as PanIN share most of the genetic alterations observed in invasive carcinoma [2, 4, 5]. An important step should be made between PanIN-1 and PanIN-2, the former being very frequent even in normal pancreas [13] and the later having a high malignant potential for developing cancer [2]. K-ras activation by point mutation in codon 12 of the K-ras oncogene is the most common molecular abnormality seen in pancreatic carcinoma (80 to 100% of cases) [1, 2, 5, 8, 14]. It has been observed in PanIN of all grades [2, 5, 13, 14] and seems to be an early event of pancreatic carcinogenesis [2]. Three patients with a K-ras mutation in pancreatic juice identified 18 to 40 months before the development of an invasive carcinoma have been reported [7, 15]. The overexpression of HER-2/neu (c-erbB2), a proto-oncogene member of the EGF-receptor family, appears also from the PanIN-1 stage and is present in 50 to 80% of carcinomas [2, 5]. On the contrary, the inactivation of the p53 tumor suppressor gene seems to be a late event in the progression to cancer. It does not arise until the PanIN-3 stage but occurs in only 40 to 80% of infiltrative carcinoma [2, 5]. In our case, p53 and HER-2/neu expression was normal in both PanINs and ductal carcinoma, and K-ras mutation at codon 12 was detected simultaneously with the cancer and several years after PanINs were diagnosed. These data suggest that other genes, yet identified or not, may be involved in the PanIN to ductal carcinoma progression. Recently, several other phenomenon’s, such as telomere shortening and inactivation of the tumor suppressor genes p16 at an intermediate stage and less frequently DPC4 and BRCA2 at a late stage have been demonstrated in pancreas carcinogenesis [2].

Our case report highlights another critical point in pancreatic pathology, the relationship between chronic pancreatitis and pancreatic cancer. Two of the patients with PanINs identified several years before the onset of pancreatic cancer reported by Brat suffered from chronic pancreatitis too [3]. Prospective and retrospective studies have shown a 3 to 19-fold increased risk for pancreatic cancer in patients with chronic pancreatitis regardless of its etiology [9-11]. Chronic inflammation, glandular destruction and presumably increased cell turn over should be implicated [9]. A high frequency of PanINs and K-ras mutations (18 to 62% of cases) [7, 8, 12] have been reported in chronic pancreatitis and could account for the increased risk for developing cancer. As this risk seems maximal in the first ten years [10] and PanINs and K-ras mutation frequency does not increase with the pancreatitis duration [7], it is suggesting that a subgroup of patients may be particularly exposed [12]. However, if PanIN-1A, PanIN-1B and PanIN-2 are seen in respectively 100%, 69% and
33% of chronic pancreatitis, high-grade dysplasia lesions (PanIN-3) are nearly never observed and p53 immunostaining is always negative [7], that is why identifying PanIN-3 in specimen with chronic pancreatitis as well as in disease-free pancreas must incite on a close follow-up. However, the pancreas is a hardly accessible organ and there is actually no reliable screening test for pancreatic cancer [2]. Indeed, it is likely that ductal epithelium changes and K-ras mutations that can be evaluated on pancreatic juice do not inevitably lead to ductal carcinoma [7]. So, the development and the evaluation of new screening tests allowing identifying patients with a high risk for developing cancer at the pre-carcinoma stage should be a priority for the near future. Lastly, the case reported here as well as spatial distribution studies show that PanINs are evenly distributed throughout the pancreas [13] and probably represent, at least in our case, a diffuse disease. It is likely that if our patient had undergone an earlier complete pancreatectomy, she would be still alive. So, the only pre-cancerous status of PanINs and their uncertain prognosis associated to the gravity of pancreatic surgery make difficult the establishment of standardized management and require additional studies.

In conclusion, this case report illustrates both the malignant potential and the diffuse nature of pancreatic intra-epithelial neoplasias. It highlights that major challenges for the next years lie in a better understanding of tumorigenesis mechanism leading to the development of early screening test for pancreatic cancer and management procedures for the pre-carcinoma lesions.

ACKNOWLEDGMENTS: We are grateful to N. Dahmane and G. Monges for helpful counseling and to Bernard Chazeau for technical assistance.
REFERENCES


12. Wakabayashi T, Sawabu N, Watanabe H, Morimoto H, Sugioka G. Detection of K-ras point mutations at codon 12 by PCR-RFLP. Lanes 1-4: absence of enzymatic digestion before the second PCR. Lanes 1'-4': mutant-enriched PCR by enzymatic digestion before the second PCR. Lanes 1/1' and 2/2': corporeal pancreatectomy, lanes 3/3': pancreaticoduodenectomy, lanes 4/4': distal pancreatectomy with infiltrating ductal carcinoma. 135 bp fragments, that are diagnostic of mutation, appear only at the carcinoma stage. Note that the carcinoma DNA contains both the mutant (135 bp) and the wild type (106 bp) fragments. Products are shown on a 3% agarose gel with ethidium bromide.


REFERENCES


