

## Telomerase gene expression in Intraductal Papillary-Mucinous Tumors (IPMT): preliminary findings

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**Abstract.** *Background and aim.* The surgical management of IPMT is based upon a preoperative suspicion of malignancy, that is difficult to obtain from the available diagnostic tools. *Methods:* Telomerase gene expression was investigated by means of hTERT/RT-PCR on total RNA from peripheral blood, tumour and non-tumour pancreatic samples of 2 patients with IPMT. *Results:* Histological diagnosis was mild-grade dysplasia in the first case and invasive carcinoma in the second. Telomerase expression was undetectable in all the samples derived from the first case. Blood and tumour samples from the second patient were positive for telomerase mRNA expression, while the pancreatic non-tumour specimen was not. *Conclusions:* The following suggestions are made: 1) the telomerase gene expression seems to be implicated in the malignant evolution of IPMT; 2) the malignant transformation may be limited to a single area of the gland; 3) the presence of invasive carcinoma may be preoperatively suspected by peripheral venous blood sample collection. A possible clinical employment of telomerase gene expression determination in the management of IPMT is thus hypothesized.

**Key words:** Intraductal papillary mucinous tumour, pancreas, telomerase, telomerase reverse transcriptase (hTERT), cancer therapy

### Introduction

Intraductal papillary mucinous tumours (IPMT) are rare, recently characterized neoplasms belonging to the group of pancreatic cystic tumours and presenting clinicopathological features distinct from those of common pancreatic ductal cell carcinoma. They were firstly described in 1982 (1), and further classified in 1997 as adenoma, borderline or carcinoma (in situ or invasive) tumours (2, 3). In the last years, from a number of reports by the most important surgical Institutions, IPMT emerged as a very interesting disease (4-10). The neoplasm is clinically evident in more than 80% of the cases, recurrent acute pancreatitis without

recognised biliary or alcoholic etiology being the most frequent expression. The diagnosis is based upon CT and MNR imaging, showing a pancreatic cystic mass, while pancreatography and pancreatoscopy usually evidence irregular Wirsung dilatation and abundant mucinous production. Retrospective radiological and histopathological findings suggested the existence of 2 different variants of the tumour, the main duct tumour (MDT) being prognostically worse than the branch duct tumour (BDT), owing to the more frequent extension to the whole pancreatic ductal epithelium and the more likely presence of carcinoma (11). Nevertheless, the natural history of IPMT remains partially unclear. At the moment of diagnosis,

30-40% of the cases harbour invasive carcinoma, 20-30% in situ carcinoma and the remaining 30-50% intraductal papillomatosis with simple dysplasia. In the reported series, resectability is 90-100%, while mortality and morbidity are similar to those related to pancreatic surgery for ductal adenocarcinoma; 5-year survival is 100% for adenomas and border-line tumours, 80-90% for in situ carcinomas and 50-70% for invasive carcinomas.

From these suggestive clinical results, there are no doubts that IPMT represents a good surgical indication; however, a number of different pathological features may be found into pancreatic lesions presenting similar clinical and radiological aspects. Thus, at present, it cannot be stated that the diagnosis of IPMT absolutely indicate the need for surgical intervention, nor, owing to the eventual occult multifocality, that surgical resection should be limited to the site of the visible lesion, or, on the other hand, that a total pancreatectomy has to be preferred as a rule (12-16).

The surgical management of IPMT should be mainly improved by pre or intra-operative diagnostic tools allowing us to demonstrate or estimate the risk of malignancy, and suggesting the presence of multiple neoplastic foci or diffuse pre-neoplastic modifications. In the present work, we prospectively evaluated the expression of telomerase (hTERT: human telomerase reverse-transcriptase, a specialized enzyme able to indefinitely maintain the replication process of the tumour cells by the synthesis and extension of DNA at the chromosome ends) in 2 recently observed cases, as a marker of malignancy in IPMT, with potential clinical applications.

## Material, methods and results

Telomerase mRNA expression was determined by RT-PCR on total RNA preparations derived from peripheral blood, tumour and non-tumour pancreatic samples of 2 patients. For each patient 3 samples were collected and examined for TERT RT-PCR expression: a preoperative peripheral venous blood collection (PB: peripheral blood), a tumour specimen from the resected bioptic fragments (T: tumour) and a non-tumourous one, named as peritumoral (PT: peritumoral).

Each specimen was obtained with the patient's informed consent under standard conditions of sampling.

Ex-situ evaluation of the cystic areas was performed, and samples of the main cystic wall and of normal pancreatic tissue far from the interested area were harvested. PB (10 ml) was collected in a tube containing 0.1% EDTA as anticoagulant and after Histopaque-1077 (Sigma Aldrich) treatment the cellular fraction was submitted to total RNA extraction by Trizol method (17) (Life Technologies). The pancreatic bioptic fragments (T and PT), collected in culture medium, were washed twice with PBS buffer within 30 min, cut into smaller fragments and immediately processed for RNA extraction or rapidly frozen in liquid nitrogen. One microgram of total RNA was reverse transcribed as already described (18). Integrity of RNA and adequate cDNA synthesis were confirmed using the ribosomal protein L7 specific primers (17, 19); the telomerase RT-PCR expression was evaluated with the human TERT specific primers (20) and the specificity of its PCR cDNA product (of 281 bp) was further assessed by restriction analysis with *ApaI* enzyme (data not shown). The PCR conditions were as described (19-20) and the PCR products were analysed on 2% agarose gel electrophoresis.

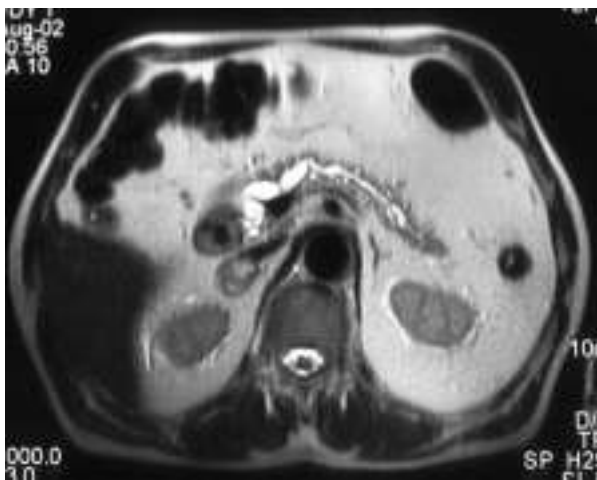
In 2002 a 57-year-old woman, after the second severe episode of acute pancreatitis (serum lipase >20.000) occurring in 4 years, the first of which (1997) having been treated by cholecistectomy, was found by cholangio-MNR to have a cephalo-pancreatic cystic mass, 2 cm in size (fig. 1), communicating with the Wirsung duct, that appeared focally dilated (5 mm in size); pancreatoscopy showed abundant mucinous secretion, and ERCP showed a normal distal Wirsung duct. Endoscopic brushing did not find malignant cells. CA19.9 was 27 U/ml. She was treated by cephalic pancreaticoduodenectomy, as neither intraductal papillary mucinous hyperplasia nor dysplasia were identified at the intra-operative frozen sections examination from the resected edge. Telomerase RT-PCR expression was undetectable in all the samples, even if they expressed at comparable levels the house-keeping gene ribosomal protein L7 (fig. 3). Histological diagnosis was mild-grade dysplasia.

Abdominal CT and cholangio-MNR were further employed to investigate multiple cystic pan-

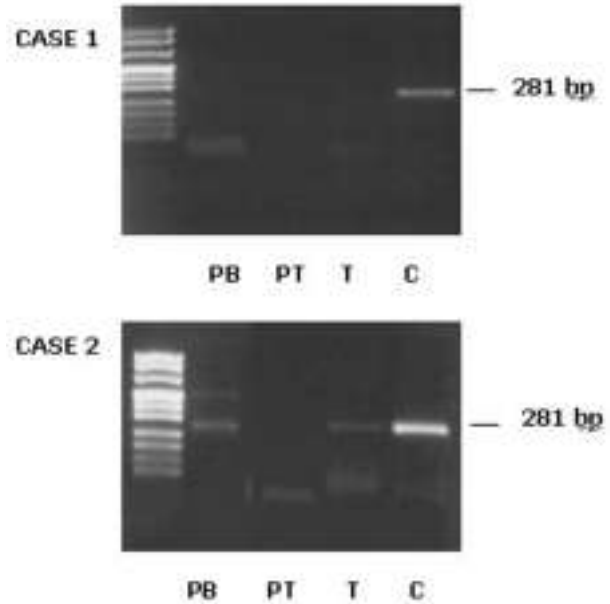


**Figure 1.** Case 1. The cholangio-MR shows the presence of a cystic dilatation of the Wirsung duct in its cephalic part, 20 mm in size. The remaining pancreatic duct appears to be normal.

creatic masses in a 72-year-old man, who had a single acute pancreatitis without evident etiology. He had irregular areas of cystic dilatation, mostly localized in the head of the gland, the greatest of which measured 22 mm in size, while almost three others were 6-7 mm in size; the Vater papilla had a typical bulging aspect into the duodenum; pancreatoscopy diagnosed the presence of abundant mucinous secretion, and ERCP showed the Wirsung duct markedly dilated and tor-



**Figure 2.** Case 2. The cholangio-MR shows in the head of the pancreas the presence of multiple cystic lesions; the remaining Wirsung duct is irregular and partially dilated all along the gland.



**Figure 3.** RT-PCR detection of TERT (281 bp) in peripheral blood (PB), in tumour and non-tumourous (peritumoral) pancreatic biopsic specimens (T, PT) derived from IPMT patients. C refers to HT1080 human fibrosarcoma cells examined as positive control for TERT mRNA expression.

tuos all along the pancreas corpus and tail (fig. 2). The brushing cytology was negative. CA19.9 was 1 U/ml. During the operation, the resection was enlarged owing to the presence, at microscopic examination, of papillary hyperplasia in two subsequent section edges; a total pancreatectomy was finally performed. Multifocal high-grade dysplasia-in situ carcinoma, associated with aspects of invasive carcinoma into the main cystic areas, were present in this patient; diffuse intraductal papillary hyperplasia involved the main and the secondary pancreatic ducts all along the gland. Telomerase RT-PCR expression was positive in blood and tumour samples (PB, T), while the pancreatic non-tumour specimen (PT) was not (fig. 3).

The 2 patients are well and alive at 13 and 12 months follow-up.

## Discussion

Telomerase is a ribonucleoprotein complex that synthesizes telomeric repeats onto chromosomal ends,

compensating for progressive telomere shortening during cellular division, thus allowing the cell to undergo indefinite proliferation (21). Starting in the first '80thies, from a wide amount of experimental and clinical data, a growing interest was addressed to the expression of this gene, that was suspected to be involved in cancer progression as a critical step (22). Whereas somatic cells do not have telomerase activity, this has been found in up to 85% of the most common cancers, suggesting that telomerase detection may represent a useful marker in the diagnosis of cancer, a good prognostic factor and also a possible target for cancer therapy (23). Several possible methods are available to investigate telomerase. First of all, the protein activity may be searched by the telomerase repeat amplification protocol (TRAP) assay, using a PCR-based test that may detect telomerase activity in as few as 10 immortal cells. The test is highly sensitive and allows a quantitative evaluation, but some false positive results have been reported in activated lymphocytes and in benign tumours (24); furthermore, some tissues has been shown to contain inhibitors for TRAP assay. The second method consists in the evaluation of telomerase gene expression, that can be done by investigation of the expression of 2 main components: human telomerase RNA (hTR) and human telomerase reverse transcriptase mRNA (hTERT). Northern blot analysis and RT-PCR analysis, respectively, are the methods of choice for hTR and hTERT identification; and in situ hybridisation and immunohistochemistry, respectively, are further in situ diagnostic tools allowing to detect telomerase expression on a wide group of samples, such as archive paraffin embedded specimens. The strategy targeting telomerase by hTERT mRNA RT-PCR detection appears to be the one that deserves the most important advantages, being the only fast enough to be employed in the clinical practice (25).

To our knowledge, this is the first report evaluating telomerase mRNA expression in patients with IPMT. A few studies previously reported the usefulness of telomerase activity measurement in pancreatic juices endoscopically harvested (24, 26) and in fine-needle aspiration (27) from patients with pancreatic ductal adenocarcinoma. In a single Japanese work telomerase activity was evaluated by means of the

TRAP assay in 28 patients with IPMT (28): for the distinction of intraductal carcinoma and adenoma, reported sensitivity, specificity, predictive value for positive and negative results were 85%, 100%, 100% and 88%, respectively; this allowed a significant improvement of the diagnostic accuracy, compared to the cytological assessment that was made in the same patients. Although these interesting results, the evaluation of telomerase activity may have been partially hampered by the inactivation of the enzyme by proteases in the pancreatic juice. Therefore, RT-PCR assessment of telomerase gene expression may better define the effective status of the ongoing malignant process involving the ductal epithelium. Unpublished results from the same Author confirm that resected specimens from patients with intraductal carcinoma showed telomerase gene expression.

From our preliminary data, three aspects appear worth of consideration. First of all, although insufficient data exist to prove the point, it seems likely that telomerase gene expression is related to the malignant evolution of IPMT. This finding is not surprising, considering the very large amount of published observations about the implication of telomerase in the cancer process. However, a clear consensus still doesn't exist about the exact nature of IPMTs, sometimes considered as a simple cystic and benign neoplasia. The biological behaviour of the neoplasm remains partially unclear, and very different patterns of clinical outcome have been reported, that cannot be explained by available clinical, radiological and histological findings (29). Similarities in telomerase expression between IPMT with carcinoma and the classical variant of ductal adenocarcinoma rise the suspicion of a common carcinogenic pathway (30). From this point of view, IPMT natural history, that has been reported to be very slowly in some cases (31), provide a great model to study the pancreatic carcinogenic process (6).

Second, the evaluation we made on the neoplastic and non-neoplastic tissue focus on a very important question: is the disease multifocal? A field (multicentric) cancerization has been reported for IPMTs, originating from multiple molecularly distinct precursor lesions, polyclonal in origin and metachronous in clinical expression (32), so that one should be aware for

the potential for multifocal tumours. In the second patient, having an invasive carcinoma in the head of the gland, telomerase wasn't expressed in pancreatic tissue far from the neoplastic area, even if preoperative radiological imaging evidenced a diffuse irregular dilatation of the main pancreatic duct, and histological analysis confirmed the intraductal papillary mucinous hyperplasia to involve the whole Wirsung duct. Telomerase gene expression determination in pancreatic tissue represents a specific tool to identify areas of malignant evolution inside the cysts, but it does not recognize the presence of pre-neoplastic foci, thus it may not be employed during surgery when examining the resection edge. When deciding the extension of the resection, a cautious approach should be applied, because the disease may in fact involve only a part of the gland, being the remaining pancreas normal: the quality of life must in such cases be taken into account, and organ function preserving procedures are appropriate if the intra-operative sections study provides that distal pancreatic duct harbour a normal epithelium. On the contrary, we do not agree with Authors proposing a complete resection only for intra-operative finding of intraductal dysplasia in the pancreatic edge, considering the persistence of simple papillary mucinous hyperplasia to represent a really incomplete treatment of the disease (4).

Third, peripheral venous blood sample collection showed in the described patients a good reliability in the distinction of the benign and malignant form of IPMT. Telomerase resulted expressed in the case of carcinoma, and it was not in the mild-grade dysplasia. These results may be explained by the fact that some cells from the tumour may circulate into the blood, where a polymerase chain reaction-based assay may reveal them also when present at a very few number, even though a contamination from telomerase-expressing lymphocytes may not be excluded. The value of this observation must be confirmed to prove the sensibility and specificity of telomerase gene expression in the peripheral blood evaluations to be advantageous when compared with the invasive and technically demanding percutaneous and endoscopic techniques of sampling. Very few reports focused until now on such a possibility, warranting additional studies (28).

## Conclusion

RT-PCR detection of telomerase gene expression may give suspicion of the presence of invasive carcinoma in IPMT, influencing the surgical decision. Further clinical studies are needed to confirm the usefulness of such observation. At the present, we follow our patients with IPMT by telomerase expression evaluation in peripheral venous blood sample; a larger study has been started, taking into consideration patients affected by pancreatic ductal "classical" adenocarcinoma too. Preliminary findings are very promising.

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