

## SHORT REPORT

## p73 gene mutations in gastric adenocarcinomas

E Pillozzi, C Talerico, A Platt, C Fidler, L Ruco

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**Background/Aims:** The p73 gene encodes a protein that shares structural and functional homology with the p53 gene product. The highest degree of homology is in the DNA binding domain, which is the region of p53 that is most frequently mutated in cancer. In contrast to p53 there is little evidence that p73 acts as a classic tumour suppressor gene. Because of the similarities between the p53 and p73 genes and the high frequency of mutation of p53, this study was designed to investigate the p73 gene in patients with gastric adenocarcinoma.

**Methods:** The mutational status of the p73 gene was investigated in a series of 13 cases of gastric adenocarcinoma from the antro-pyloric region and the gastro-oesophageal junction, using the polymerase chain reaction, single strand conformational polymorphism, and direct DNA sequencing.

**Results:** A glutamine to arginine mutation was detected in exon 5 of the p73 gene in a case of adenocarcinoma at the gastro-oesophageal junction.

**Conclusion:** Although limited to a small series of cases, these results suggest that p73 may have a potential pathogenetic role in this tumour.

The p73 gene maps to chromosome 1p36, a region frequently deleted in a variety of human cancers. p73 encodes a protein that shares structural and functional homology with the p53 gene product.<sup>1</sup> At the structural level, the highest degree of homology with p53 is in the DNA binding domain, which in p53 is the region most frequently mutated in cancer.<sup>2</sup> It is well known that p53 controls cell growth and apoptosis through binding to specific DNA sequences and the activation of target genes. p53 is activated in response to cellular stresses such as DNA damage, oncogene activation, and hypoxia.<sup>3</sup> However the p73 and p53 genes show some differences in their regulation. It has been suggested that a regulatory pathway involving MDM2 exists for p53, whereas in the case of p73, MDM2 upregulation reduces p73 transcription but does not induce its degradation.<sup>4</sup> The chromosomal location of p73 and its similarity to p53 gave rise to the idea that p73 was a tumour suppressor gene. Further studies have shown that p73 gene mutations are rare in cancer.<sup>5–7</sup> There are conflicting results on whether p73 is imprinted. In fact, in some normal tissues and tumours monoallelic expression has been demonstrated, whereas others show biallelic expression. In a subset of stomach, prostate, and lung tumours overexpression of wild-type p73 and biallelic expression compared with their normal tissue counterparts has been reported.<sup>6, 8</sup> All these findings seem to suggest that, despite the structural and functional similarities, p73 and p53 act very differently in the pathogenesis of cancer. At the moment, there is no evidence that p73 acts as a classic tumour suppressor gene. However, it has recently been reported that the p73 gene contains a second promoter that controls the expression of p73 variant ( $\Delta$ TA-p73), a species of p73 protein that lacks the N-terminal transactivation domain.

It has been speculated that  $\Delta$ TA-p73 is the p73 protein variant that acts as an oncogene because it is overexpressed in human cancer cells and its overexpression results in the malignant transformation of NIH3T3 fibroblasts and tumour growth in nude mice.<sup>9</sup>

“The chromosomal location of p73 and its similarity to p53 gave rise to the idea that p73 was a tumour suppressor gene”

Given the similarities between the p53 and the p73 genes and the high frequency of mutation of p53 in gastric adenocarcinomas, we chose to investigate a series of nine gastric adenocarcinomas from the antro-pyloric region and four adenocarcinomas at the gastro-oesophageal junction for mutations in the p73 gene.

## MATERIALS AND METHODS

## Samples

Nine cases of gastric adenocarcinoma and four cases of adenocarcinoma of the gastro-oesophageal junction and matched normal tissue obtained at surgery were collected. Frozen tissue was available from 10 patients, whereas only formalin fixed, paraffin wax embedded tissue was available from three patients (with gastro-oesophageal junction adenocarcinoma). The age of the patients ranged between 50 and 83 years. DNA was obtained by standard proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation.

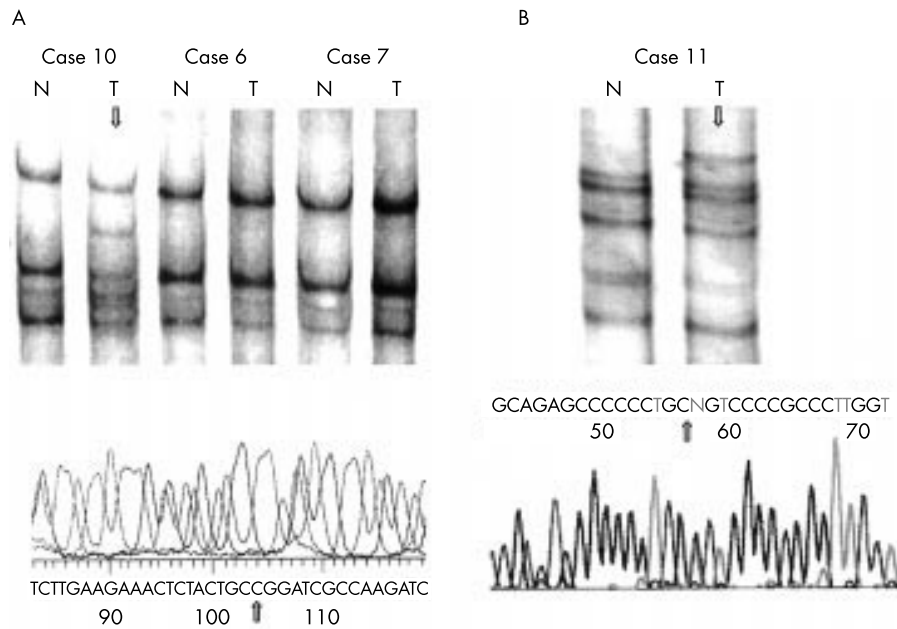
## Polymerase chain reaction

DNA was used as template in the polymerase chain reaction (PCR) to amplify the 13 coding exons (2 to 14) of the p73 gene. The primer sequences have been published previously.<sup>5</sup> PCR amplification consisted of 32 cycles of 95°C for one minute, 58–62°C for one minute, and 72°C for one minute, after the initial Taq Gold activation (10 minutes at 95°C). The MgCl<sub>2</sub> concentration varied between 1.5 and 2.5 mM. Amplification products were then run on a 1% agarose gel stained with ethidium bromide to check the reaction.

## Single strand conformational polymorphism

Mutational analysis of the p73 gene was performed using single strand conformational polymorphism as a screening technique to spot base substitutions in the amplification products. After heat denaturation in a formamide containing buffer, 10  $\mu$ l aliquots of each PCR amplification product were loaded on to a 0.5 $\times$  mutation detection enhancement gel (FMC Bioproducts Rockland, Maine, USA) in 0.6 $\times$  Tris borate EDTA buffer and run at 25 mA at 4°C for four hours. The gel was then stained and visualised using the silver staining kit (Biorad,

**Abbreviations:** PCR, polymerase chain reaction; SCCP, single strand conformational polymorphism



**Figure 1** (A) Single strand conformational polymorphism (SSCP) analysis of exon 5 of the p73 gene shows a band shift in the tumour tissue (T) compared with the normal tissue (N) in case number 10. DNA sequencing detected a base substitution (arrow) of A → G (nucleotide 571; accession number, NM005427) causing an amino acid change (CAG → CGG; Gln → Arg). (B) SSCP analysis shows a band shift in the the tumour tissue from case 11. Sequencing showed that it was a silent mutation of GCC → GCT (Ala → Ala; nucleotide 1118; accession number, NM005427).

Hercules, California, USA). Cases in which a band shift was observed were sequenced directly.

### Sequencing

The direct sequencing reaction was performed using the FS ready reaction dye terminator sequencing kit (Applied Biosystems, Foster City, California, USA), according to the manufacturer's instructions.

### RESULTS

The p73 gene is composed of 14 exons. Because exon 1 is a non-coding exon, we performed the mutational analysis from exon 2 to 14. We studied four gastro-oesophageal junction adenocarcinomas and nine antral gastric adenocarcinomas. Histologically, two of the tumours from the gastro-oesophageal junction were adenocarcinomas with a signet ring component, one was an adenocarcinoma, and the other was predominantly a signet ring cell carcinoma. Of the nine gastric antral adenocarcinomas, histologically two were adenocarcinomas of the mucinous type, five were intestinal type, and two were signet ring type. In one patient with gastro-oesophageal adenocarcinoma, two base changes were found in exon 5 (AF079085). The first base change was an A → G change at position 571 of the cDNA (accession number, NM005427), which caused an amino acid change (arginine instead of glutamine); this mutation was not found in the corresponding normal tissue, indicating that it was a true mutation (fig 1A). The second base change was a T → C change at position 629 (accession number, NM005427), which was also found in the normal tissue and was interpreted as a polymorphism. The presence of the mutation was confirmed in a repeated PCR. Histologically, this case was an adenocarcinoma of the gastro-oesophageal junction, found in an 83 year old man. A silent base substitution C → T (nucleotide 1118; accession number, NM005427) was seen in another case of gastro-oesophageal adenocarcinoma (fig 1B).

### DISCUSSION

Gastric cancer is one of the most common cancers worldwide. Most (approximately 65%) gastric cancers develop in the

antro-pyloric region, whereas 15% develop in the cardia, with the remaining cases developing in the corpus. Histologically, adenocarcinomas arising in the gastric cardia are almost identical in terms of growth pattern, degree of differentiation, and spread to adenocarcinomas arising in the setting of Barrett's oesophagus. The p53 gene has been found to be mutated in almost 50% of gastric cancers and is thought to play a key role in the adenocarcinomas that develop in Barrett's oesophagus.<sup>10</sup>

"The finding of a single mutation in the total of 13 cases is consistent with previous reports showing that p73 mutations are rare in solid tumours"

In our study, we report a mutational analysis of the p73 gene in a series of gastric adenocarcinomas. In one of the 13 cases studied a p73 mutation was found. This was a glutamine to arginine mutation in exon 5. It was a case of gastro-oesophageal junction adenocarcinoma. It should be noted that exon 5 encodes part of the DNA binding site of p73, encompassing exon 5 through to exon 8, which is the functional domain with the greatest degree of homology with p53.<sup>2</sup> Most of the p53 mutations found in cancers occur in the DNA binding domain of the p53 gene. It has been suggested that the N-terminally truncated p73 isoform ( $\Delta$ TA-p73) may act as oncogene.<sup>9</sup> Thus, we can speculate that the mutation in exon 5 may play a pathogenic role in tumorigenesis, causing conformational changes that might be relevant in neoplastic transformation, and may increase the oncogenicity of the  $\Delta$ TA-p73 variant. The finding of a single mutation in the total of 13 cases is consistent with previous reports showing that p73 mutations are rare in solid tumours.<sup>3-7</sup> Previous reports on the analysis of p73 in large series of gastric adenocarcinomas have failed to find p73 gene mutations. Nevertheless, it would seem that further studies of the mutational status of p73 gene in gastric cancer are warranted in patients from diverse populations. The other base substitutions (T629C and C1118T) were found to be polymorphic variants of the gene that have already been described, and which did not result in amino acid changes.<sup>5,6</sup>

**Take home messages**

- The p73 gene encodes a protein that shares structural and functional homology with the p53 tumour suppressor gene product
- A glutamine to arginine mutation was detected in exon 5 of the p73 gene in a case of adenocarcinoma at the gastro-oesophageal junction
- Although limited to a small series of cases, our results suggest that p73 may have a potential pathogenetic role in adenocarcinoma at the gastro-oesophageal junction, and further studies of p73 gene mutation in gastric adenocarcinoma are warranted

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