Detection of Mutant *K-ras* in a Kindred With Hereditary Pancreatic Cancer by Denaturing Gradient Gel Electrophoresis

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the US, and its frequency is rising. At the time of diagnosis, 96–99% of patients are incurable and will shortly die. Current methods to evaluate patients for pancreatic adenocarcinoma include endoscopic retrograde cholangiopancreatography (ERCP), abdominal CT, ultrasound, and serum markers. These methods of diagnosis are often insensitive or equivocal in early disease. Tumorigenesis is believed to involve the *K-ras* oncogene and the *DCC, p16, APC, bcl-2,* and *p53* tumor suppressor genes; screening for mutations or loss of heterozygosity in these genes may provide better diagnostic tests that are more sensitive and specific (Rozenblum et al. 1997). The study of families in which cancer is inherited in an autosomal dominant fashion has provided considerable insight into understanding the molecular basis for pancreatic cancer. We have previously reported an extensive kindred in which pancreatic cancer is inherited in an autosomal dominant fashion and is associated with development of pancreatic insufficiency prior to the diagnosis of cancer (Evans et al. 1995). We have utilized the DCode™ universal mutation detection system for denaturing gradient gel electrophoresis (DGGE) to identify patient samples with *K-ras* mutations.

Methods

Genomic DNA was isolated from tissue or from fluid obtained from patients at ERCP. *K-ras* exon 1 was amplified from genomic DNA using a thermal cycler and primers as described by Imai et al. (1991). Amplifications were carried out using 300 ng of DNA template in a buffer containing 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3, with 2 U of Taq DNA polymerase (Boehringer Mannheim), 200 M dNTPs, and 15 pmol of each primer in a total volume of 50 L. Reactions were denatured for 3 min at 95°C, which was followed by 37 cycles with the following profile: 95°C (20 sec), 55°C (60 sec), 72°C (40 sec). The 110 base pair (bp) product was run on a 3% agarose gel and visualized using ethidium bromide staining.

Successful samples were run on a 10% acrylamide 0–80% perpendicular DGGE gel at 150 V for 2 hr at 56°C, then stained with ethidium bromide to find the optimal conditions for parallel DGGE (Figure 1). This was determined to be 30–60%. Samples were then run on a parallel DGGE gel at 56°C for 4–5 hr at 150 V, and stained with ethidium bromide. Positive samples were sequenced using dye terminator chemistry and run on an ABI PRISM instrument (PerkinElmer, Inc.).

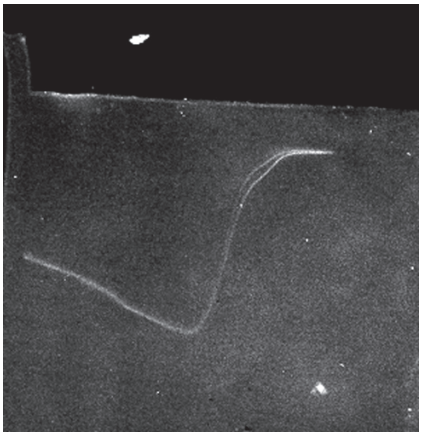

Fig. 1. 0–80% perpendicular DGGE with wild-type and mutant *K-ras* products. The fragment melts at a denaturant concentration of 48%.

Table 1. *K-ras* mutations in family X.

III.1 PreOp Juice*	III.2 PreOp Juice*	IV.19 Metaplasia	III.15 Dysplasia	III.16 Cancer In Situ	III.6 Cancer	III.17 Cancer	III.19 Metastasis
Codon 12 GGC→GAT	Codon 12 GGT→GAT	Codon 13 GGC→GAC	None	Codon 12 GGC→XXX	Codon 13 GGC→GAC	Codon 13 GGC→AGC	None

*For those patients who had not undergone pancreatectomy yet, pancreatic juice was obtained at ERCP and tested for *K-ras* mutation.

Results

Exon 1, containing codons 12 and 13, of the *K-ras* gene was examined for the presence of *K-ras* mutations by DGGE. Constitutional and pancreatic tissues or pancreatic juice in eight of the family members were evaluated. All samples that were positive by DGGE (Figure 2) were confirmed by DNA sequencing. Three of these individuals had *K-ras* mutations in codon 13 present in pancreatic cancer or precancerous tissue, and three had mutations in codon 12. There was no evidence of *K-ras* mutation in the metastatic pancreatic cancer tissue from individual III.19, even when DNA was subcloned, or from the ERCP fluid or dysplastic tissue from III.15 (Table 1).

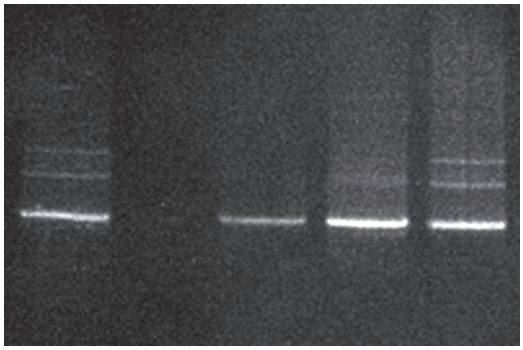

Fig. 2. Parallel DGGE of *K-ras* exon 1. Mutant and wild-type samples were run with a 30–60% denaturing gradient. Lane 1, positive control; lane 2, negative control; lane 3, ERCP fluid from patient III.1; lane 4, ERCP fluid from patient III.16.

Discussion

K-ras mutations are a common event in pancreatic adenocarcinoma. Screening for *K-ras* mutations along with a panel of other markers may prove useful in early diagnosis of this disease. DGGE is a practical tool in screening samples for the presence of such mutations.

References

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