Mutations of the DPC4/Smad4 Gene in Biliary Tract Carcinoma


Abstract

A candidate tumor suppressor gene, DPC4, located at 18q21.1, has recently been shown to be inactivated in half of pancreatic adenocarcinomas. The close developmental relationship of the pancreas and biliary tract prompted us to determine the role of DPC4 in the multistep carcinogenesis of biliary tract carcinoma. A search for mutations in the genomic sequence of the highly conserved COOH-terminal domain of DPC4 (exons 8–11) was performed by single-strand conformational polymorphism analysis. Five of 32 (16%) primary biliary tract carcinomas had point mutations in the DPC4 sequence. Interestingly, inactivation of DPC4 was especially common in carcinomas originating from the common bile duct (four of eight specimens analyzed), suggesting an important role for DPC4 in the development of this subtype of biliary tract tumor.

Introduction

The biliary tract and the ventral pancreas anlagen both originate from the same bud along the distal foregut of the developing embryo. This close developmental relationship may be one explanation for the observation that the p16 and K-ras genes are commonly mutated in both biliary tract tumors and pancreatic carcinomas (1–4), whereas this mutation pattern is unusual for other cancer types. We recently identified the tumor suppressor gene DPC4 (deleted in pancreatic carcinoma, locus 4) at human chromosome 18q21.1 (5). DPC4 is a member of the highly conserved family of Smad proteins that are involved in the transduction of signals from the transforming growth factor β family of cytokines; DPC4 has subsequently been designated as Smad4 (6). DPC4 is inactivated, either by homozygous deletion or by point mutation together with a chromosomal deletion, in approximately half of pancreatic carcinomas (5, 7). In the initial report, one xenograft tumor that had been derived from a primary biliary tract carcinoma was found to have a homozygous deletion of the DPC4 region (5). This finding, together with the other known similarities of the pancreas and the biliary tract system, prompted us to study the DPC4 gene sequence in biliary tract carcinomas.

Here we report the mutational analysis of 32 primary biliary tract tumors in exons 8–11 from the DPC4 gene by SSCP analysis. Our results demonstrate that DPC4/Smad4 is indeed involved in the tumor formation of biliary tract cancers. Furthermore, our results suggest that DPC4 mutations are frequent in common bile duct cancer, although they are rare in biliary tract cancers of other locations.
Results and Discussion

Exons 8–11 of the DPC4 gene were successfully amplified by PCR in 32 primary biliary tract carcinoma specimens (21 intrahepatic bile duct carcinomas, 3 Klatskin's tumors, and 8 common bile duct carcinomas). SSCP analysis revealed a reproducible abnormal banding pattern in 5 of 32 (16%) cases (see examples in Fig. 1). Sequence analysis of the aberrant SSCP products identified point mutations causing changes in the predicted DPC4 protein sequence in all five cases (Table 1). Case BC40 carried a nonsense mutation at codon 343, exon 8, predicting a truncation of the DPC4 protein. Case 15533T had an Ala-to-Thr amino acid replacement at codon 433, exon 9. Cases 3aT, 6280T, and 12aT had nonconservative amino acid replacements in exon 11 (Arg to His at codon 494, Arg to Gly at codon 502, and Cys to Trp at codon 523, respectively). The latter two mutations were located at highly conserved amino acid residues in the Smad homology region 2b (MH2b) (Fig. 2, a–c). In three cases (3aT, 6280T, and 12aT) in which constitutional normal DNA from the patient was available, the somatic nature of the mutation could be demonstrated (Fig. 2c). Furthermore, case 3aT was the only mutation-positive case with adequate neoplastic cellularity (>60%) in which fresh-frozen tissue from both the tumor and corresponding normal tissue was available, allowing determination of allelic loss in the DPC4 region. Microsatellite marker D18S46 showed a homozygous allelic pattern, whereas marker D18S363 showed loss of one allele (results not shown). Because marker D18S363 is located within less than one megabase of the DPC4 region, and the majority of chromosomal deletions identified in human tumors span several megabases, this result is highly suggestive of deletion of the second allele of the DPC4 gene in this tumor (7, 9). In an attempt to establish LOH frequency for the remaining tumor specimens, we could not generate conclusive LOH data for the following reasons: (a) the majority of the tumor specimens had a neoplastic cellularity of only 30–50% on dissection, and for all but three cases, no constitutional normal tissues were available; both of these factors severely impair the interpretation of the LOH data; (b) the LOH analysis revealed the upper allele to be far more frequently the target of the signal imbalances than the lower allele. This strongly suggests technical problems in the analysis, which may be related to poor quality of the DNA, as is often observed with DNA isolated from formalin-fixed tissue; and (c) the template may have been insufficient in the PCR reactions, an artifact that has been described (10).

It is not unlikely that the 16% DPC4 mutation frequency we observed in biliary tract carcinomas is an underestimate of the prevalence of DPC4 mutations in this tumor type. Most importantly, the presence of nonneoplastic cells in primary tumor samples precludes PCR-based detection of homozygous deletions (11), a mechanism of gene inactivation known to be of importance for the DPC4 gene (5, 8, 12). In addition, the present mutation analysis was restricted to exons 8–11 of DPC4, which together span the entire conserved COOH-
terminal Smad homology region (Fig. 2a). However, because 84% of the DPC4 mutations reported thus far are confined to this region, the number of unidentified mutations is expected to be low (5, 8, 12–16).

Mutational analyses have suggested that inactivation of DPC4 is restricted to particular tumor types. Inactivation of DPC4 was found in 54% of pancreatic carcinomas (1, 5, 8) and in 17% of colorectal carcinomas (13), but only in a minority (<7%) of cancers from other anatomical sites (5, 8, 12–20). Our finding of DPC4 mutations in 16% of biliary tract tumors adds a third tumor type to the DPC4 target spectrum. It may be of interest that four of five DPC4 mutations identified here were in cancers from the common bile duct. Of note, a previously reported biliary tract tumor harboring a homozygous deletion of DPC4 was also derived from the common bile duct (5). It is tempting to speculate that the tumor suppression pathway in which DPC4 participates is only operative in particular gastrointestinal organs. Elucidation of the functional properties of DPC4 in transforming growth factor ß-like signaling pathways may not only aid our understanding of the function of the protein but also uncover the mechanism underlying the tissue specificity of the involvement of DPC4 in human tumorigenesis.

References


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