No Mutations of the Smad2 Gene in Human Sporadic Gastric Carcinomas

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Background: The majority of cancer cells escape from TGF-β-mediated growth control. However, the mechanism of resistance to the growth inhibitory effects by TGF-β is not clear. TGF-β signaling is initiated when the type I receptor phosphorylates the SMAD proteins, Smad2 and Smad3. Recently, mutations of Smad2 have been detected in human colon and lung cancers. Mutation of coding sequences of Smad2 in gastric carcinomas has not yet been elucidated adequately.

Methods: PCR-SSCP analysis of the entire coding region of Smad2 in 35 human sporadic gastric cancers and eight gastric cancer cell lines was performed using 11 sets of intron-based primers.

Results: No mutations of Smad2 were detected in any tumor or cell line.

Conclusions: The results suggest that mutation of Smad2 does not play a key role in human stomach carcinogenesis.

Key words: gastric carcinoma – Smad2 – mutation

INTRODUCTION

The loss of sensitivity to negative growth regulators is an important step in the development of malignant tumors. Transforming growth factor-β (TGF-β) is a potent antiproliferative polypeptide and has been proposed to play a pivotal role in suppressing tumorigenicity. The signal transduction by TGF-β involves the formation of a heteromeric type I and type II receptor complex (1,2). TGF-β binds directly to TGF-β type II receptor (TGF-βRII), which is a constitutively active transmembrane serine/threonine kinase that recruits TGF-β type I receptor (TGF-βRI) into the receptor complex. TGF-βRII phosphorylates TGF-βRI in the GS domain, a region rich in glycine and serine residues. Phosphorylation of TGF-βRI propagates the signal to Smad2 and Smad3, downstream substrates of the type I kinase.

Smad2 maps on chromosome 18q21. Two candidate tumor suppressor genes, DCC and DPC4/Smad4, have been cloned and identified from this chromosome region (14–16). Loss of heterozygosity (LOH) on this chromosome region is frequently found in human pancreatic cancers and alterations of DCC and DPC4/Smad4 are involved in many human tumors (15–19). Moreover, mutations of Smad2 have been reported in colorectal and lung cancers (14,20,21). However, mutation of Smad2
Mutation of Smad2 in gastric carcinomas

gastric cancer has not yet been elucidated adequately. Recently, the genomic structure of Smad2 was determined (22). The Smad2 protein is encoded by 467 codons distributed in 12 exons. Exon 1 of Smad2 has two different splicing variants in the promoter region, named exons 1a and 1b. Translation start codon is located in exon2. MH-1 and MH-2 domains are encoded within exons 2–5 and 6–11, respectively. In this study, we performed polymerase chain reaction–single strand conformational polymorphism (PCR–SSCP) analysis of the entire coding region of Smad2 in human sporadic gastric carcinomas using 11 intron-based primers.

MATERIALS AND METHODS

TUMORS AND CELL LINES

A total of 35 sporadic gastric cancers obtained by surgery and eight gastric cancer cell lines (TMK-1, KATO-III, HSC-39, MKN-1, MKN-7, MKN-28, MKN-45 and MKN-74) were examined. A representative tumor specimen and non-neoplastic mucosa from the surgical margin were frozen in liquid nitrogen immediately after surgical resection and stored at −80°C until isolation of DNA samples. TMK-1 cell line (poorly differentiated gastric adenocarcinoma) was established in our laboratory (23). KATO-III and HSC-39 cell lines (signet ring cell carcinoma) were kindly provided by Dr Sekiguchi (University of Tokyo) and Dr Yagihara (Hiroshima University), respectively (24). The other five human gastric carcinoma cell lines of the MKN series (MKN-1, adenosquamous carcinoma; MKN-7, MKN-28 and MKN-74, well differentiated adenocarcinoma; MKN-45, poorly differentiated adenocarcinoma) were kindly provided by Dr Suzuki (Fukushima Medical College). They were routinely grown in RPMI-1640 (Nissui Pharmaceutical, Tokyo) containing 10% fetal bovine serum (FBS) (Whittaker M.A.Bioproducts, Walkersville, MD) under conditions of 5% CO₂ in air at 37°C.

DNA EXTRACTION

High molecular weight DNA samples from fresh-frozen tissues and cell lines were prepared using the phenol–chloroform method after treatment with sodium dodecyl sulfate and proteinase K (25).

PCR–SSCP ANALYSIS

All samples were examined for mutations in exons 1-11 of the Smad2 by PCR–SSCP analysis. Each exon was amplified by the 11 sets of PCR primers which were designed to amplify the entire coding region, including each splicing site, of Smad2. All samples were examined for mutations in exons 1-11 of the Smad2 and cell lines were prepared using the phenol-chloroform method after treatment with sodium dodecyl sulfate and proteinase K (25).

RESULTS

To examine alterations of Smad2, we performed PCR–SSCP analysis in gastric carcinoma cell lines. We could not find any SSCP variants in PCR products for exons 1-11 (Fig. 1). We also analyzed alterations of the Smad2 in gastric carcinoma tissues and their corresponding normal mucosae. In cases 208 and 308, we detected an SSCP variant in the DNA fragments including exon 4 (Fig. 1). This variant was not detected in the corresponding normal tissue. DNA fragments which showed different mobilities were reamplified by PCR and sequenced. There were 6 bp deletions in the polythymidine tract (T₁₈) of intron 3 in these two cases (Fig. 2).

DISCUSSION

Mutations of Smad2 have been detected in colon and lung cancers (14, 20, 21). In colon cancer, Eppert et al. (14) focused on the MH-1 and MH-2 domains and identified four missense mutations (6%) in 66 sporadic cases. They found that three of them were functionally mutated. Riggsin et al. (20) detected a 42 bp deletion...
and a homozygous deletion (11%) among 18 colorectal cancers. In lung cancer, a missense mutation and a 9 bp in-frame deletion (3.5%) among 57 cases were detected (21). Most of them were identified within the MH-2 domain (exons 9 and 11). Interestingly, most of the mutations in DPC4/Smad4 were detected within the MH-2 domain (16). The remaining case was identified within the MH-1 domain (exon 4).

However, there were no mutations of Smad2 in 101 axillary negative breast carcinomas, 76 sarcomas, 30 primary esophageal squamous cell carcinomas, seven esophageal squamous cell carcinoma cell lines and 25 neuroblastoma cell lines (14,26,27). In gastric cancer, two cases showed 6 bp deletions in polythymidine tract of the gene. These two cases revealed replication error positive phenotype and mutations in the polyadenine tract of TGF-βRII were also found (28). Polythymidine tract was located at position -39 to -56 at the splicing site of the intron 3–exon 4 junction. This deletion in polythymidine tract was also detected in two sporadic colon cancers and five colon cancer cell lines and there were no splicing abnormalities in Smad2 cDNAs (29). Therefore, it may not cause splicing abnormalities in cases 208 and 308.

We could not identify any mutations in the PCR products for other exons in eight cell lines and 35 cancers, even though the conditions for PCR–SSCP were varied. The length of PCR products for exons 1a, 1b, 3, 5 and 6 ranged from 172 to 330 bp. On the other hand, the PCR products for exons 2, 4 and 7–11 were over 400 bp. There may be some false negative cases. Further examination by sequencing analysis is needed to confirm our results. We performed sequencing analysis in these exons in eight cell lines and four cancers. However, we could not detect any mutation.
The mechanism of resistance to the growth inhibitory effects by TGF-β is still unclear. TGF-β RII and RI, Smad2, Smad3 and Smad4 are needed for TGF-β signaling. Mutations of TGF-β RII have been reported in several human cancers (30–34). However, in our previous study, we demonstrated that mutations of the gene were rare events in human sporadic gastric cancer (28). Mutations of TGF-β RII are more infrequent than that of TGF-β RII in human cancers (35). Somatic mutations in Smad4 are frequently observed in pancreatic cancers (16), but less frequently in other types of cancers. Powell et al. (36) reported one case (3%) of nonsense mutation of Smad4 in a study of 35 gastric carcinomas. No mutations of Smad3 in human colorectal cancer were detected and no report was found of mutation of Smad3 in gastric cancer (37). Taken together, the mutations of TGF-β receptors, Smad2, Smad3 and Smad4 are infrequent and may not play a pivotal role in the escape from growth regulation by TGF-β in gastric cancer.

Except for mutational inactivation of TGF-β receptors and downstream substrates of these receptors, new mechanisms by which inhibitory Smads block the activation of the pathway-restricted Smads are provided. Smad6 and Smad7 proteins which were recently identified appear to block TGF-β signaling (38–40). They bind to TGF-β RI and interfere with the phosphorylation of Smad2 and Smad3. These inhibitory Smads may confer the escape from growth regulation by TGF-β in cancer cells.

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References


