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Induction and Down-regulation of *Sox17* and Its Possible Roles During the Course of Gastrointestinal Tumorigenesis

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BACKGROUND & AIMS: The activation of Wnt/β-catenin signaling causes the development of gastric and colon cancers. Sox17 represses Wnt/β-catenin signaling and is down-regulated in colon cancer. This study was designed to elucidate the role of Sox17 during the course of gastrointestinal tumorigenesis. **METHODS:** *Sox17* expression was examined in gastrointestinal tumors of mouse models and humans. The roles of Sox17 in gastric tumorigenesis were examined by cell culture experiments and by construction of *Sox17* transgenic mice. **RESULTS:** *Sox17* was induced in *K19-Wnt1/C2mE* mouse gastric tumors and *K19-Wnt1* preneoplastic lesions, where Wnt/β-catenin signaling was activated. Consistently, Wnt activation induced *Sox17* expression in gastric cancer cells. In contrast, Sox17 was rarely detected by immunohistochemistry in gastric and colon cancers, whereas strong nuclear staining of Sox17 was found in >70% of benign gastric and intestinal tumors. Treatment with a demethylating agent induced *Sox17* expression in gastric cancer cells, thus indicating the down-regulation of *Sox17* by methylation. Moreover, transfection of *Sox17* in gastric cancer cells suppressed both the Wnt activity and colony formation efficiency. Finally, transgenic expression of *Sox17* suppressed dysplastic tumor development in *K19-Wnt1/C2mE* mouse stomach. **CONCLUSIONS: Sox17 plays a tumor suppressor role through suppression of Wnt signaling. However, *Sox17* is induced by Wnt activation in the early stage of gastrointestinal tumorigenesis, and *Sox17* is down-regulated by methylation during malignant progression. It is therefore conceivable that Sox17 protects benign tumors from malignant progression at an early stage of tumorigenesis, and down-regulation of *Sox17* contributes to malignant progression through promotion of Wnt activity.**

BASIC–ALIMENTARY TRACT

The binding of the Wnt ligand to a Frizzled receptor destabilizes the β-catenin degradation complex containing adenomatous polyposis coli (APC), AXIN, and glycogen synthase kinase 3β (GSK3β), allowing the nuclear translocation of β-catenin, followed by the transcriptional activation of the Wnt target genes.[1] This canonical Wnt signaling (Wnt/β-catenin signaling) plays a key role in the maintenance of intestinal stem cells and progenitor cells.[2,3] Moreover, the constitutive activation of Wnt/β-catenin signaling causes gastrointestinal tumorigenesis in both human beings[4,5] and mice.[6,7] It has also been shown that β-catenin nuclear accumulation, a hallmark of Wnt activation, is particularly enhanced in the invasion front and metastasized colon cancer cells, suggesting that promotion of Wnt/β-catenin signaling is important for malignant progression.[8] Platelet-derived growth factor and hepatocyte growth factor, as well as tumor necrosis factor-α, have been shown to promote Wnt/β-catenin signaling activity in tumor cells.[9–11] On the other hand, down-regulation of Wnt antagonists such as secreted frizzled-related proteins contributes to gastric and intestinal tumorigenesis by boosting Wnt/β-catenin signaling activity.[12–14] These results suggest that the enhancement of Wnt activity by the induction of Wnt promoters or down-regulation of Wnt antagonists is important for gastrointestinal carcinogenesis.

Sox17 and other Sox family members, Sox3, Sox7, and Sox9, have been shown to inhibit Wnt/β-catenin signaling.[15–18] The *Sox* gene family was first identified by homology to the high mobility group box of the sex-determining gene *SRY*.[19] *Sox17*-null mouse embryos exhibit a deficiency of definitive endoderm,[20] and overexpression of *Sox17* in embryonic stem cells results in the establishment of stable endoderm progenitors.[21] These results indicate that Sox17 plays a key role in definitive endoderm development. On the other hand, *Sox17* expression is down-regulated in colon cancer cells by promoter methylation,[22] and the expression of *Sox17*

Abbreviations used in this paper: APC, adenomatous polyposis coli; DAC, 5-aza-2′-deoxycytidine; GSK3β, glycogen synthase kinase 3β; HMG, high mobility group; PGE_2, prostaglandin E_2; RT-PCR, reverse-transcription polymerase chain reaction; TCF, T-cell factor.

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in colon cancer cells reduces the efficiency of the colony formation.[22,23] These results suggest that Sox17 is a tumor suppressor for colorectal cancer development.

Activation of Wnt/β-catenin signaling has been shown to cause the development of gastric tumors as well as intestinal tumors.[6,7,24,25] However, *Sox17* expression during the course of gastrointestinal tumorigenesis has not been fully investigated yet. We herein show the *Sox17* expression to be unexpectedly induced at the early stage of gastric and intestinal tumors both in human and mouse models. Sox17 and β-catenin have been shown to cooperate to function as a transcription factor in definitive endoderm development.[26,27] Interestingly, we found that Sox17 target genes were induced in mouse gastric tumors, thus suggesting that Sox17 plays a role in early tumorigenesis in cooperation with β-catenin. Moreover, the transgenic expression of *Sox17* in mouse stomach suppressed dysplastic tumor development in *K19-Wnt1/C2mE* mice.[7] These results suggest that Sox17 prevents malignant progression as a tumor suppressor at an early stage of tumorigenesis, and down-regulation of *Sox17* causes tumor progression.

Materials and Methods

Mouse Models

Construction of $Apc^{\Delta 716}$, *cis-*$Apc^{\Delta 716}$ *Smad4* (+/−) knockout (*cis-*$Apc^{\Delta 716}$ *Smad4*), *K19-Wnt1*, *K19-C2mE*, and *K19-Wnt1/C2mE* (*Gan* for Gastric neoplasia) mouse models has been described previously.[6,7,28,29] Briefly, $Apc^{\Delta 716}$ mice carry a heterozygous mutation in the *Apc* gene, which develop intestinal polyps. *cis-*$Apc^{\Delta 716}$ *Smad4* mice are compound heterozygotes of *Apc* and *Smad4* which develop invasive intestinal adenocarcinoma. *K19-Wnt1* and *K19-C2mE* mice express *Wnt1* and a combination of *Ptgs2* and *Ptges*, respectively, in gastric epithelial cells. *K19-Wnt1* mice develop gastric preneoplastic lesions, whereas *K19-C2mE* mice show inflammation-associated metaplastic hyperplasia. *Gan* mice expressing *Wnt1*, *Ptgs2*, and *Ptges* are compound transgenic mice of *K19-Wnt1* and *K19-C2mE*, which develop dysplastic gastric tumors. To construct *K19-Sox17* mice, mouse *Sox17* cDNA fragment was subcloned into pBluescript (Stratagene, La Jolla, CA) with cytokeratin 19 (K19) gene promoter and SV40 poly(A) cassette. The expression vector was microinjected into the fertilized eggs of F1 (C3H and C57BL/6) mice to obtain *K19-Sox17* mice. Two *K19-Sox17* lines, no. 5 and

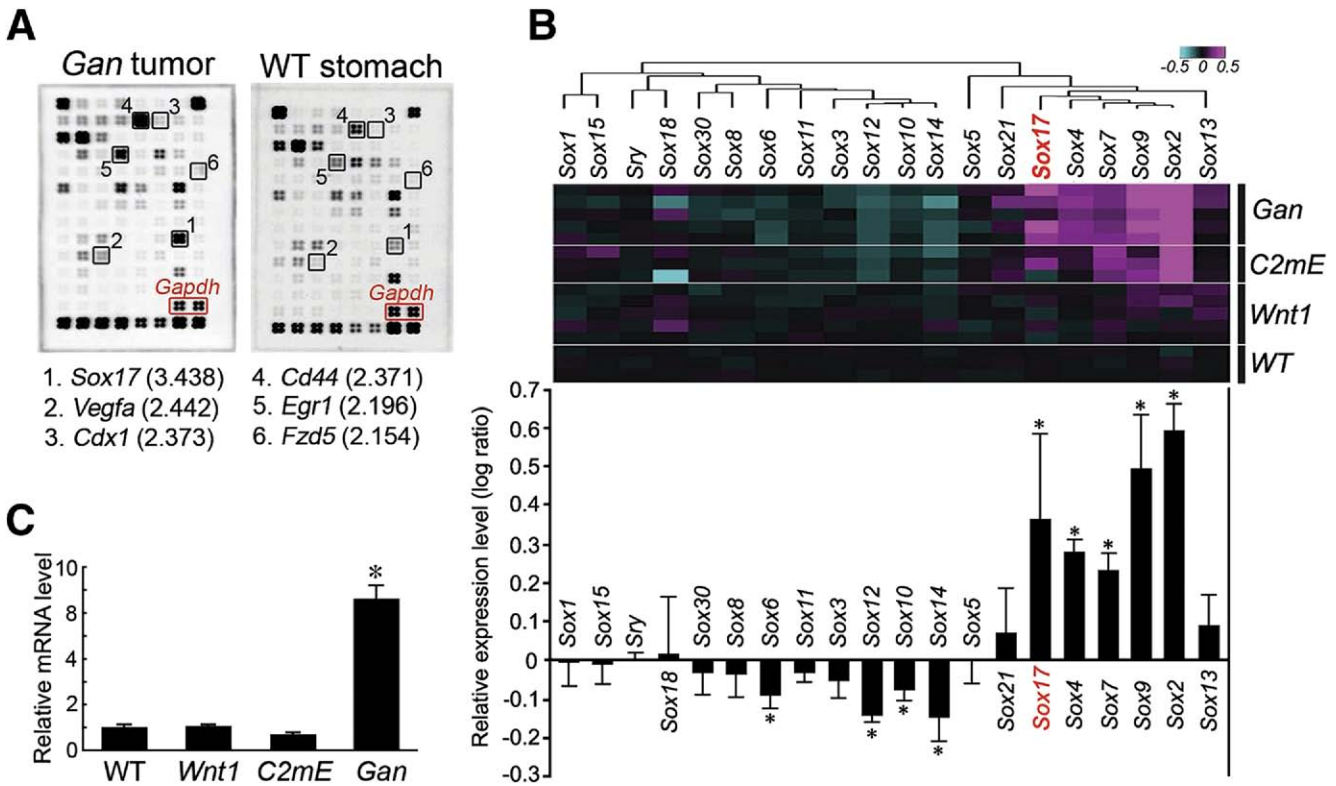

Figure 1. Induction of *Sox17* in *Gan* mouse gastric tumors. (*A*) Results of filter array analysis of Wnt pathway in *Gan* mouse tumors and wild-type mouse (WT) stomach. *Squares* indicate up-regulated genes in *Gan* tumors, and fold increases are indicated in *parentheses*. (*B*) Expression profiles of *Sox* family genes in *Gan*, *K19-C2mE* (*C2mE*), *K19-Wnt1* (*Wnt1*), and WT mice. The gene expression levels are shown in log10 ratios to wild-type as shown in the *cyan-magenta color bars* (*top*) and the mean log10 ratios in *Gan* tumors to the wild-type are shown in bar graph (mean ± SD) (*bottom*). (*C*) The relative *Sox17* mRNA levels of gastric tissues of indicated genotypes to the wild-type mouse stomach (mean ± SD). (*B* and *C*) *$P < .05$ versus wild-type mouse stomach.