論 文 の 内 容 の 要 旨

論文題目　Transcription Factor SOX9 in Gastric Cancer

（胃癌における転写因子 SOX9 の意義）

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SOX9 is a member of the SOX [Sry-related high-mobility group (HMG) box] family, is required for the development and differentiation of multiple cell lineages, and has been implicated in several types of cancer; however, there are few reports of SOX9 expression in gastrointestinal cancers. To clarify the significance of SOX9 in gastric carcinoma (GC), immunohistochemical staining of SOX9 and the CpG-island methylation status of SOX9 were evaluated and compared with clinicopathologicalfactors. It was concluded that upregulation of SOX9 is related to GC development. On the other hand, downregulation of SOX9 is associated with GC progression.

The increase of SOX9 occurred from the normal-metaplasia-adenoma transition and SOX9 expression was correlated with CDX2 expression, which is known to play a role in intestinal metaplasia. SOX9 was strongly expressed in 83% (20/24) of gastric adenoma, in which Ki67 was also expressed. Furthermore, MTT assay revealed that SOX9 promoted normal gastric epithelial cell (GES-1) proliferation. Moreover, PI3K/Akt activation was induced by overexpression of SOX9 in GES-1 cells. SOX9 was frequently expressed in the early stage gastric cancer. Correlation between the expression of SOX9 and clinicopathological factors was more significant in the

intestinal type than in the diffuse type. The findings underline a novel oncogenic function of SOX9 in the early stage of intestinal type gastric carcinogenesis through regulating PI3K/Akt.

SOX9 function in GC cell lines was then studied. *In vitro*, MKN7 gastric cancer cells transfected with SOX9 siRNA proliferate more rapidly than control cells by MTT assay. It was also examined whether SOX9 could inhibit Epithelial-Mesenchymal Transition (EMT) in gastric cancer. Knockdown of SOX9 in MKN74 cells induced conversion of circular-shaped cells with cell-cell contact to spindle-shaped cells and improved cell migration, shown by the wound healing assay. MKN7 and NUGC3 cells treated with SOX9 siRNA also induced cell migration. Next, the effect of SOX9 on the expression of EMT marker mRNA and proteins was examined by real-time PCR and Western blotting. Knockdown of SOX9 in MKN74 cells decreased the expression level of E-cadherin, and increased SLUG, ZEB1, and Vimentin expression, which are known as EMT. Comparable results were observed in MKN7 and NUGC3 cells transfected with SOX9 siRNA.

To further study the role of SOX9 in EMT, SOX9 was transfected into GES-1 cells transiently and stably. SLUG levels were significantly reduced by overexpression of SOX9, concomitant with re-expression of E-cadherin. The relationship between E-cadherin and SOX9 in human gastric cancer tissues was further investigated, and a positive correlation was elucidated between SOX9 and E-cadherin. SOX9 may inhibit EMT in the progression of gastric cancer by downregulating SLUG and thereby upregulating E-cadherin. Taken together, these results suggest that SOX9 may

contribute to the development of gastric cancer by promoting cell proliferation; on the other hand, SOX9 downregulation induces EMT during progression of GC.

The expression of SOX9 was decreased in 43% of GC tissues. SOX9 methylation was detected in 48% of GCs and correlated with the low expression of SOX9 protein. The decrease of SOX9 expression in advanced GC was related to the methylation of SOX9 promotor during GC progression. An interesting finding in this study was that the expression of SOX9 protein was frequently lost or down regulated in the EBV-positive GC cases. SOX9 expression was significantly downregulated in 4 GC cell lines when infected with EBV (MKN1-EBV, MKN7-EBV, TMK1-EBV and NUGC-3-EBV), and one original EBV-positive GC cell lines, KT. Downregulation of SOX9 expression may be associated with the development of EBV-associated GC. More SOX9 promotor methylation in gastric cancer samples from patients with EBV infection (86%, 6/7) was detected than in those without EBV infection (46%, 52/114, P = 0.041). Furthermore, 68% (5/8) of EBV-infected GC cell lines (MKN1-EBV, MKN7-EBV, TMK1-EBV, NUGC3-EBV and KT) that show relatively low expression were detected SOX9 methylation. These findings might support that SOX9 methylation was accelerated by EBV infection and the subsequent silencing of SOX9 expression may correlate with the progression of gastric cancer.

In summary, SOX9 expression was increased in the development of GC and decreased throughout its progression. The increase might be related to intestinal-type gastric carcinogenesis and influence the proliferation of GC cells via PI3K/Akt. Downregulation of SOX9 seems to contribute to the progression of GC by regulating

EMT. Methylation of SOX9 promoter increased during progression and could be the cause of SOX9 suppression in advanced cancer. SOX9 promotor methylation was also related to EBV-associated GC, which shows low expression of SOX9. SOX9 is closely related to gastric cancer.