学 位 論 文 の 要 旨

氏 名　殿元 康仁

学 位 論 文 名	Differential Expression of *RUNX* Genes in Human Esophageal Squamous Cell Carcinoma: Downregulation of *RUNX3* Worsens Patient Prognosis
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著 者 名	Yasuhito Tonomoto, Mitsuo Tachibana, Dipok Kumar Dhar, Toshinao Onoda, Kohkichi Hata, Hideyuki Ohnuma, Tsuneo Tanaka, Naofumi Nagasue

INTRODUCTION

In general, alterations in genetic and epigenetic pathways that play important roles in diverse cellular functions, such as cellular proliferation, differentiation, signal transduction, DNA repair, and methylation status of gene promoters, have been identified in several carcinomas, although much less is known about the genetic pathways responsible for the malignant phenotype of esophageal squamous cell carcinoma(SCC). The *RUNX* proteins are a family of transcriptional factors that have essential functions during embryogenesis and development whereas deregulation in expression of *RUNX*s is often linked to tumor formation. There has as yet been no study describing precise expression, prognostic impact and methylation status of *RUNX*s in esophageal SCC.

We evaluated the differential expression pattern of *RUNX* genes in human esophageal SCC samples by a highly sensitive real time RT-PCR method. In addition we examined not only the expression but also the methylation status of *RUNX3* by methylation specific PCR. Also a correlation between *RUNX*3 and Smad4 expression was sought.

MATERIALS AND METHODS

Resected specimens from 61 patients who underwent curative resection for esophageal SCC were used to identify the expression of *RUNX*1, *RUNX*2 and *RUNX*3 by real time RT-PCR. The mRNA level of each type of *RUNX* gene was expressed as a ratio between its own expression and *GAPDH* (a housekeeping gene) expression and, was referred as normalized expression. Localization of *RUNX*3 mRNA was done by in situ hybridization. *RUNX*3 expression was evaluated separately in the normal mucosa and cancer tissue according to a semiquantitative scoring system. Among 61 cases, paraffin sections were available in 56 cases and were used for immunohistochemical analysis. Expression of Smad4 was evaluated by immunohistochemistry. Methylation status of *RUNX3* was analyzed by methylation specific PCR. The modified DNA was used as template for MSP using primers specific for either the methylated or unmethylated *RUNX*3 promoter sequences.

RESULTS AND DISCUSSION

*RUNX*3 had significant impact on patient prognosis with worse survival in *RUNX*3-negative group. In early tumors (T1/T2), the prevalence of lymph vessel invasion and number of metastatic lymph nodes were significantly higher in *RUNX*3-negative tumors. The downregulation of *RUNX3* gene is associated with a worse prognosis in esophageal SCC and, therefore, *RUNX*3 may serve as a useful prognostic marker for this carcinoma. Also significantly decreased expression of *RUNX*3 was noted in large tumors indicating that *RUNX*3 may have a tumor suppressor role in esophageal SCC. Furthermore, *RUNX*3 became a strong prognostic factor only in Smad4-positive tumors. This may indicate a strong association between the Smad4, *RUNX*3 and apoptosis. Also the methylation status of *RUNX3* promoter had significant correlation with loss of *RUNX3*

expression. Aberrant hypermethylation of the *RUNX3* gene promoter region may be one of the crucial factors responsible for downregulation of *RUNX3* transcripts in esophageal SCC.

CONCLUSION

Downregulation of *RUNX*3 may play a role in disease progression of esophageal SCC. A significant survival advantage of *RUNX*3 in only Smad4 positive cases may suggest a synergism between the *RUNX* and TGF-β family. Also aberrant hypermethylation of the promoter region of the *RUNX3* might be one of the crucial mechanisms responsible for loss of expression during malignant progression of the esophageal SCC and thus may help to identify new targets for therapeutic intervention.