学位論文内容の要旨

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学　位　論　文　題　名

Changes in High Endothelial Venules with Progression of Oral Squamous Cell Carcinoma

（　口腔扁平上皮癌進展に伴う高内皮細静脈の変化　）

キーワード（５つ）Oral squamous cell carcinoma, Tumor-associated high endothelial venules, Cancer microenvironment, prognosis predicting marker, T cell

Oral squamous cell carcinoma (OSCC) is a prevalent type of cancer originating in the oral mucosal epithelium, accounting for approximately 90% of oral malignancies. The progression of OSCC often involves potentially malignant oral disorders (OPMDs). Therefore, monitoring the abnormalities during OPMD development is crucial for early OSCC diagnosis and target therapy.

Angiogenesis, the process of new blood vessel formation, plays a critical role in tumor progression and metastasis, including in OSCC. High endothelial venules (HEVs) are specialized post-capillary venules composed of cuboidal blood endothelial cells. HEV endothelial cells express specialized lymphocyte ligands, such as peripheral node addressin (PNAd), which can be recognized by the HEV-specific antibody MECA-79. It is reported that the density of tumor-associated HEVs (TA-HEVs) was correlated with a favorable prognosis in many tumors, including OSCC, suggesting they may serve as independent prognostic markers. However, it was also reported that TA-HEVs in oral and pharyngeal

squamous cell carcinoma (OPSCC), characterized by thin walls and enlargement with numerous red blood cells, have been associated with lymph node metastasis.

These findings highlight the heterogeneity of TA-HEVs and suggest their function and prognostic value may differ. Thus, prognosis cannot be predicted solely by the number of TA-HEVs.

This study aims to investigate HEV changes during tumor progression using a mouse OSCC model.

We employed 4-nitroquinoline N-oxide (4NQO) to establish a dynamic OSCC model in C57BL/6 mice, observing changes in the oral mucosa weekly. The tongue tissues were histologically categorized as normal, dysplasia, and carcinoma based on the Hematoxylin and Eosin (H&E) staining results. The control mice fed regular water served as untreated group.

Immunohistochemistry (IHC) of CD31 revealed a significant increase in CD31-positive vessels with occluded lumens as the lesion progressed from normal to OSCC. Double staining with α-SMA and CD31 indicated a decrease in pericyte coverage in the dysplasia group, with a further reduction in the carcinoma group, suggesting that angiogenesis begins in the dysplastic stage but becomes increasingly immature in carcinoma.

We have previously shown that TA-HEVs present in human OSCC tumor tissue are positively correlated to a better prognosis. In this study, HEVs were observed in 4NQO-treated groups, including normal tissue, but not in the untreated group. The density of HEVs increased progressively from normal to dysplasia and carcinoma.

HEVs in dysplasia exhibited immature morphology, while carcinoma HEVs were more mature, with high and thick endothelial cells. These results suggest that TA-HEV density and maturation correlate with OSCC progression.

It was reported that HEVs not only function in recruiting T lymphocytes but also interact with T lymphocytes. Therefore, to determine the infiltrating timing of lymphocytes, IHC of CD3, CD8, and CD4 were performed. IHC for granzyme B indicated that CD8+ T cells became activated as lesions

progressed, correlating with HEV presence in dysplasia and carcinoma. Moreover, cytotoxic T-cell activation may promote HEV neogenesis in dysplasia. However, in the carcinoma group, CD3 T cells' density increased significantly compared with the untreated group; and the number of CD4 T cells increased slightly without significance; the density of CD8+ T cells was observed to be approximately 5 to 7 times higher in the carcinoma group than in the normal and dysplasia groups. These data suggested that the HEVs in carcinoma but not in dysplasia were correlated with the infiltration of CD8+ T cells. To further analyze the activation of CD8+ T cells, IHC of granzyme B was performed. The density of granzyme B and the ratio of activated CD8+ T cells were increased in dysplasia and carcinoma compared to normal. These findings indicated that the CD8+ T cells were activated as the lesion progressed. Moreover, HEVs observed in the dysplasia group have a positive correlation to activated cytotoxic T cells. Activated cytotoxic T cells may also promote HEVs neogenesis in dysplasia.

We have shown that the margin HEVs indicate a better prognosis in human OSCC. Consistent with the findings, in the 4NQO model, margin-TA-HEVs were more prevalent in exophytic tumors, whereas inside-TA-HEVs were more common in endophytic tumors. Although the number of HEVs did not significantly differ between tumor types, exophytic tumors exhibited higher CD8+ T-cell infiltration. Margin-TA-HEVs were more strongly associated with CD8+ T-cell accumulation, suggesting their role in improved prognosis.

In conclusion, our study demonstrated that the quantity, morphology, and distribution of HEVs change with OSCC progression. Additionally, margin-TA-HEVs were frequently located in exophytic tumors, and positively correlate with CD8+ T-cell infiltration, linking HEV location to OSCC prognosis. Identifying HEVs in OSCC may serve as a promising tool for the early diagnosis and prognosis prediction.