指導教授氏名 Instructing Professor Commissioned Professor	指導役割 Role
（自署）	指導全般の監督
（自署）	実験計画の立案、実験、評価の指導
（自署）	

学位論文要旨
Abstract of the Doctoral Dissertation

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論文題名 Title of Doctoral Dissertation: Stromal cells in the tumor microenvironment promote the progression of oral squamous cell carcinoma.
口腔扁平上皮癌間質による腫瘍進展促進への直接的関与

論文内容の要旨（2000字程度） Summary of Dissertation (approx. 800 words)

Oral squamous cell carcinoma (OSCC) is developing due to the interaction between the tumor parenchyma and the tumor stroma. At present, however, there are only reports of cancer parenchyma subordinately dominating the cancer stroma, and the influence of the cancer stroma on the biological properties of the cancer parenchyma is unknown. In this study, we investigated the possibility that the tumor stroma regulates the biological character of the tumor parenchyma.

Clinically, various subtypes of OSCC exist, including invasive carcinoma and verrucous carcinoma. Differences in the invasive ability of these subtypes results in marked differences in prognosis. The endophytic type (ED-type) OSCC can invade and occasionally metastasize. Conversely, the exophytic type (EX-type) OSCC, such as verrucous OSCC, presents an outward growth, does not invade the subepithelial connective tissue, does not metastasize and is therefore associated with a relatively good prognosis. To demonstrate that the cancer stroma directly regulates the biological character of cancer parenchyma, in the present study, the moderately differentiated human oral cancer cell line, HSC-3, was used as a cell model; human dermal fibroblasts (HDFs) were selected as the negative control of stromal cells, and verrucous squamous cell carcinoma-associated stromal cells (VSCC-SCs; derived from EX-type OSCC stroma) and squamous cell carcinoma-associated stromal cells (SCC-SCs; derived from ED-type OSCC stroma) were extracted from patients with OSCC to examine the effects of different stromal cells subtypes in the TME on the biological character of OSCC.

In vitro experiment, the morphology and proliferation of VSCC-SCs, SCC-SCs and HDFs were examined by the Giemsa staining, Immunofluorescence (IF) and MTS assay respectively. The results revealed that VSCC-SCs, SCC-SCs and HDFs had a spindle-shaped morphology. Moreover, the effects of VSCC-SCs, SCC-SCs and HDFs on the tumor nest formation, proliferation, invasion and migration of HSC-3 in vitro were examined by the Giemsa staining and IF staining, MTS assay, Transwell (Invasion), and Transwell (Migration) respectively. The results revealed that both VSCC-SCs and SCC-SCs inhibited the tumor nest formation and promoted the proliferation, invasion and migration of HSC-3 in vitro. SCC-SCs exerted a more prominent effect than VSCC-SCs while HDFs exerted a minimal effect. Furthermore, the VSCC-SCs, SCC-SCs and HDFs were mixed with HSC-3 to inject into the head of mice to construct the animal models and the whole head of mice contain the

tumor tissues were extracted after 4 weeks. The effects of VSCC-SCs, SCC-SCs and HDFs on the differentiation of HSC-3 in vivo were examined by the Hematoxylin and eosin (HE) staining. The results indicated that VSCC-SCs promoted the differentiation of HSC-3 and SCC-SCs inhibited the differentiation of HSC-3 in vivo. The effects of VSCC-SCs, SCC-SCs and HDFs on the bone invasion of HSC-3 in vivo were examined by the HE staining and tartrate-resistant acid phosphatase (TRAP) staining, which suggested that both VSCC-SCs and SCC-SCs promoted the bone invasion of HSC-3 in vivo and SCC-SCs exerted a more prominent promoting effect than VSCC-SCs while HDFs exerted an inhibitory effect. In addition, the effects of VSCC-SCs, SCC-SCs and HDFs on the proliferation, invasion and epithelial mesenchymal transformation (EMT) of HSC-3 in vivo were examined by the immunohistochemistry (IHC) and double-fluorescent immunohistochemical staining, respectively. The results demonstrated that both VSCC-SCs and SCC-SCs could promote the proliferation, invasion and EMT of HSC-3 in vivo and SCC-SCs exerted a more prominent promoting effect on the proliferation and EMT than VSCC-SCs while HDFs exerted a minimal effect on the proliferation, invasion and exerted an inhibitory effect on the EMT. Finally, the microarray data were used to predict genes in VSCC-SCs and SCC-SCs that may influence the progression of OSCC, and those with potential to influence the differential effects of VSCC-SCs and SCC-SCs on the differentiation of OSCC. It was found that C-X-C motif chemokine ligand 8 (CXCL8), mitogen-activated protein kinase 3 (MAPK3), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), C-X-C motif chemokine ligand 1 (CXCL1) and C-C motif chemokine ligand 2 (CCL2) may be involved in the crosstalk between VSCC-SCs, SCC-SCs and OSCC cells, which regulates the progression of OSCC. Intercellular adhesion molecule 1 (ICAM1), interleukin (IL)1B, Fos proto-oncogene, AP-1 transcription factor subunit (FOS), bone morphogenetic protein 4 (BMP4), insulin (INS) and nerve growth factor (NGF) may be responsible for the differential effects of VSCC-SCs and SCC-SCs on the differentiation of OSCC.

In conclusion, the present study demonstrated that VSCC-SCs promoted the differentiation, proliferation, invasion and migration of OSCC, while the SCC-SCs inhibited differentiation and promoted the proliferation, invasion and migration of OSCC. Compared with the VSCC-SCs, the SCC-SCs exerted an inhibitory effect on the differentiation and exerted a more potent promoting effect on the proliferation, invasion and migration of OSCC. Finally, CXCL8, CCL2, CXCL1, MAPK3 and PIK3CA in VSCC-SCs and SCC-SCs may regulate the progression of OSCC, and ICAM1, IL1B, FOS, BMP4, INS and NGF may underlie the differential effects of VSCC-SCs and SCC-SCs on the differentiation of OSCC. These findings may describe a potential regulatory mechanism in the progression of OSCC.