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

学位論文要旨
Abstract of the Doctoral Dissertation

岡山大学大学院医歯薬学総合研究科

教育研究分野 Department	Department of Oral Pathology And Medicine	身分	大学院生	氏名 Name	May Wathone Oo

論文題名
Title of Doctoral Dissertation

Resident stroma-secreted chemokine CCL2 governs myeloid-derived suppressor cells in the tumor microenvironment
腫瘍間質由来 CCL2 は骨髄由来免疫抑制細胞の腫瘍微小環境への動員に関与する

Oral squamous cell carcinoma (OSCC) is one of the most common solid tumors of the head and neck. OSCC consists of two major components: malignant epithelium and stroma, which make up the tumor microenvironment (TME). The stromal components play an active role in supporting tumor cells for progression, invasion, and metastasis. The stromal components contain a heterogeneous population of cells that derive from resident stromal cells and recruiting cells, namely cancer-associated fibroblasts (CAFs), tumor-associated endothelial cells (TECs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs). MDSCs are bone marrow-derived immature myeloid cells possessing immune suppressive functions. CCL2 (C-C motif chemokine ligand 2), also known as monocytic chemotactic protein (MCP-1), and CCR2 (the CCL2 receptor) play a crucial role in MDSC migration. CCL2 is secreted by tumors such as prostate cancer, breast cancer, malignant glioma, colorectal cancer, and lung carcinoma. However, the role of stroma-secreted CCL2 in MDSC recruitment in OSCC remains unclear.

In the present study, we sought new insights into the interconnection between resident stroma and MDSCs in the TME. To this end, we established a novel patient-derived stroma xenograft (PDSX) model. We performed sequential transplantation in BALB/c nude mice, including (i) bone marrow transplantation (BMT) of GFP-expressing cells to trace the BMDCs and (ii) co-xenografting of patient-derived stroma (two cases, designated PDS1 and PDS2, isolated from two OSCC patients) with oral cancer cells (HSC-2) after 28 days of BMT. The PDSX model permits an *in vivo* recreation of the TME to reproduce the interaction between the resident stroma and MDSCs, generating a system resembling the human cancer-TME interaction under natural growth conditions. As a control, we established a co-xenograft model of a normal human dermal fibroblast cell line (HDF) with HSC-2 cells. As another control for this model, we xenografted recipient mice with HSC-2 alone. Using these animal models, we investigated (i) whether the resident stroma alters the infiltration of BMDCs into the TME; (ii) whether the resident stroma alters MDSCs recruitment; and (iii) the gene expression signature of stromal cells, a profile that is expected to be essential for the migration of MDSCs.

First, we evaluated whether the tumor-stroma interaction alters BMDCs migration *in vitro* and *in vivo*. We performed a Transwell migration assay and wound closure assay for *in vitro* assessment and GFP IHC detection for *in vivo* assessment. The data provided that PDS1, PDS2, and HDF all promoted BMDCs migration *in vitro* and recruitment *in vivo*. Further, we evaluated whether the resident stroma alters MDSCs recruitment. Murine MDSCs are known to express CD11b and GR1 and to provide immunosuppression by producing arginase 1 (Arg1). Therefore, we identified MDSCs by performing multicolor fluorescent detection on Arg1-positive cells that exhibited co-staining with GFP, CD11b, and GR1. Notably, multicolor immunofluorescence revealed that the PDS co-xenografts recruited Arginase-1/CD11b/GR1/GFP quadruple-positive cells, which are myeloid-derived suppressor cells (MDSCs), to the TME, whereas the

HDF co-xenograft did not. These findings suggested that patient-derived stroma promotes MDSCs infiltration into the TME. To clarify the underlying molecular mechanism by which co-xenografted stromal cells (PDS1 and PDS2) promoted MDSCs recruitment, we next performed microarray analysis to identify the differentially expressed genes (DEGs) in PDS1 and PDS2 compared to HDF. We observed that PDS1 and PDS2 expressed CCL2 mRNA (encoding C-C motif chemokine ligand 2) at higher levels than HDF.
Indeed, when we confirmed the CCL2 protein expression in co-xenografts tissues, PDS co-xenografts contained significantly higher proportions of CCL2-positive stromal cells than the HDF co-xenograft. CCR2 has been reported to serve as the primary functional receptor for CCL2 in the TME, and the CCL2/CCR2 axis is known to play a crucial role in tumor progression. Therefore, we next examined whether the recruitment of CCR2-positive MDSCs to the TME in the co-xenograft models is different. CCR2/Arginase-1/CD11b/GR1 quadruple-positive MDSCs (receiver cells) were significantly recruited in PDSX models more than in the HDF co-xenograft. Consistent with these results, either treatment of tumor/stroma co-cultures with an inhibitor of CCL2 synthesis, bindarit, or treatment of BM cells with CCR2 antagonist, RS 102895, significantly inhibited the PDS-driven migration of BM cells *in vitro*. Further, to investigate whether blocking CCR2 in xenograft models inhibits the recruitment of MDSCs in OSCC TME, we injected CCR2 antagonist intraperitoneally (3mg/kg, the other day) into the tumor/stromal co-xenografts. Then, we evaluated the MDSCs by multicolor immunofluorescent staining. Interestingly, the CCR2 antagonist significantly reduced the infiltration of CCR2/Arginase-1/CD11b/GR1 quadruple-positive MDSC in PDSX co-xenografts than HDF co-xenograft.

Taken together, the CCL2/CCR2 axis mediates MDSCs recruitment to the TME in OSCC, and stroma-secreted CCL2 is important in the MDSCs recruitment. Therefore, targeting key stromal factors likely will serve as a crucial strategy for treating OSCC. The interaction of stroma-secreted CCL2 with CCR2-positive MDSCs is essential for three complex factorial crosstalk between cancer cells, stromal cells, and MDSCs, and thus promises to constitute an attractive target for OSCC treatment.