CXCL12/CXCR4 Promotes Laryngeal and Hypopharyngeal Squamous Cell Carcinoma Metastasis by MMP-13-Dependent Invasion via ERK1,2/AP-1 Pathway

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Introduction

Cancer of the larynx and hypopharynx is one of the most common head and neck malignancy. Squamous cell carcinoma (SCC) is the most common histopathologic type of the laryngeal and hypopharyngeal malignancies, and lymph node metastasis is frequently seen in laryngeal and hypopharyngeal SCC and it directly contributes to the prognosis of patients with laryngeal and hypopharyngeal SCC. In spite of many advances in surgery and cancer therapy in recent years, there was no significant improvement in survival rates of head and neck squamous cell carcinoma patients. Therefore, understanding the detailed mechanism by which LHSCC metastasize to local lymph node is of importance and urgently needed.

In this report, we demonstrated that LHSCC express high levels of functional CXCR4 receptor, which is a native receptor for the chemokine stromal-cell-derived factor-1 (SDF-1/CXCL12). Immunohistochemical analysis of primary tumor samples from LHSCC patients revealed significant expression of both CXCR4 and CXCL12. The higher expression of CXCR4 is correlated with the patients who have lymph node metastasis and distant metastasis. Reverse transcriptase-polymerase chain reaction and Western blot examination demonstrated that the CXCR4 mRNA and protein were expressed in LHSCC cell lines as well. However, the expression of CXCL12 mRNA was not observed in LHSCC cell lines. We further showed that CXCL12 treatment enhanced the activation of ERK pathway and the motility/invasion abilities of LHSCC cell lines and that were blocked by treatment with CXCR4 antagonist (AMD3100) and specific MEK inhibitor (U0126). Since MMPs are critically involved in cell invasiveness, we thus examined which MMP is possibly involved in the CXCL12-induced chemoinvasion of LHSCC cells. In this study, we investigated the possible role of CXCL12/CXCR4 axis and its related signaling in LHSCC. Our results show that LHSCC not only highly express CXCR4 but also its ligand, CXCL12. Treatment with CXCL12 enhanced LHSCC cell migration and chemoinvasion. RT-PCR and Western blot analysis showed that MMP-13 was a downstream effector gene of CXCL12/CXCR4 signaling. In addition, the up-regulation of MMP-13 was mediated by Erk1/2 pathway and subsequent transcriptional factor AP-1.

Methods

1. RNA extraction and RT-PCR assay
2. Migration and Invasion assay
3. Western blotting and Immunohistochemistry staining
4. Nuclear and cytosol protein separation

Results

Conclusions

CXCL12 enhances LHSCC cell invasion through paracrinically activates CXCR4 and in turn triggering an ERK/c-Jun-dependent MMP-13 up-regulation. It provide new information regarding signaling pathways that may regulate CXCL12-induced metastasis in LHSCC.