Ras Activation Mediates Wisp-1-induced Increases In Cell Motility And Matrix Metalloproteinase Expression In Human Osteosarcoma

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Introduction: WISP-1 is a cysteine-rich protein that belongs to the CCN (Cyr61, CTGF, Nov) family of matrix cellular proteins. Osteosarcoma is a type of highly malignant tumor with a potent capacity to invade locally and cause distant metastasis. However, the effect of WISP-1 on migration activity in human osteosarcoma cells is mostly unknown. In this study, we examined the role of WISP-1 in metastasis in human osteosarcoma.

Methods: Osteosarcoma migration was examined using Transwell, wound healing, and invasion assay. The Ras, Raf-1, MEK, ERK, and NF-kB phosphorylations were examined by using western blotting. The qPCR was used to examine the mRNA expression of matrix metalloproteinase (MMP). A transient transfection protocol was used to examine NF-kB activity.

Results: WISP-1 has been reported to stimulate the directional migration and invasion of human cancer cells [1,2]. However, the expression of WISP-1 in human osteosarcoma cells is largely unknown. First, we examined tissues obtained from human osteosarcoma patients for the expression of WISP-1 by using immunohistochemistry. Expression of WISP-1 in the tissue from osteosarcoma patients was significantly higher than that in normal bone (Fig. 1A&B). Quantitative data for protein expression of WISP-1 are shown in Fig. 1B. To elucidate a link between WISP-1 expression and osteosarcoma migration, we next examined the migratory activity of human osteosarcoma by using the Transwell assay. Stimulation of osteosarcoma cells (HOS and U2OS cells) with WISP-1 promoted cell migration (Fig. 1C). In addition, WISP-1 dose-dependently increased chemokinesis activity (Fig. 1D&E). Furthermore, WISP-1 enhanced the invasive activity of U2OS cells through a Matrigel basement membrane matrix (Fig. 1F). Thus, the expression of WISP-1 was associated with a metastatic phenotype of osteosarcoma cells.

A previous study showed significant expression of MMP-1, -2, -3, -8, -9, and -13 in human osteosarcoma cells [3]. We therefore hypothesized that one of these MMPs is involved in WISP-1-directed migration of osteosarcoma cells. Incubation of cells with WISP-1 increased the expression of MMP-2 and MMP-9, but not that of the other MMPs, as assessed by qPCR analysis (Fig. 2A). WISP-1 also increased MMP-2 and MMP-9 enzyme activity in the supernatant, as measured by the zymography assay (the total protein loading was used for loading control; Fig. 2B). Furthermore, incubation of U2OS cells with WISP-1 enhanced MMP-2 and MMP-9 protein expression (Fig. 2C). To examine whether MMP-2 and MMP-9 are involved in WISP-1-induced cell migration, MMP-2 and MMP-9 inhibitors were used. Pretreatment of cells with the MMP-2 or MMP-9 inhibitor blocked WISP-1-induced cell migration and chemokinesis activity (Fig. 2D&E). Furthermore, transfection of cells with MMP-2 or MMP-9 siRNA abolished WISP-1-mediated cell motility (Fig. 2D). Therefore, WISP-1 increased cell migration through up-regulation of MMP-2 and MMP-9 in human osteosarcoma cells.

In addition, the Ras and Raf-1 inhibitor or siRNA abolished WISP-1-induced cell migration and MMPs expression. On the other hand, activation of the Ras, Raf-1, MEK, ERK, and NF-kB signaling pathway after WISP-1 treatment was demonstrated, and WISP-1-induced expression of MMPs and migration activity were inhibited by the specific inhibitor, and mutant of MEK, ERK, and NF-kB cascades. Taken together, our results indicated that WISP-1 enhances the migration of osteosarcoma cells by increasing MMP-2 and MMP-9 expression through the αvβ3 integrin receptor, Ras, Raf-1, MEK, ERK, and NF-kB signal transduction pathway.

Discussion: The prognosis of osteosarcoma patients with distant metastasis is generally considered very poor. Thus, preventing human osteosarcoma metastasis is an important issue. Our study showed that the interaction between WISP-1 and alphavbeta3 integrin increases the expression of MMP-2 and MMP-9 via a pathway involving Ras, Raf-1, MEK, ERK, and NF-kB, as well as increases the migration of human osteosarcoma cells. The discovery of a WISP-1-mediated signaling pathway helps us understand the mechanism of human osteosarcoma metastasis and may lead to the development of an effective therapy.

Significance: Osteosarcoma is a debilitating, although not always fatal, high-grade malignant bone neoplasm common in children and adolescents. Osteosarcoma shows a predilection for metastasis to the lungs. Therefore, it is important to develop an effective adjuvant therapy to prevent osteosarcoma metastasis. Here we found that WISP-1 is play critical role in osteosarcoma metastasis.

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