High mobility group box chromosomal protein 1 increases migration and α5β1 integrin expression through PI3K, Akt, c-Jun-dependent pathway in human chondrosarcoma cells

Keng, Y T; Liu, J F; Tang, C H
School of Pharmacy, China Medical University, Taichung, Taiwan. Central Laboratory, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan. Department of Pharmacology, School and Medicine, China Medical University and Hospital, Taichung Taiwan. Graduate Institute of Basic Medical Science, China Medical University, Taichung Taiwan
chtang@mail.cmu.edu.tw

ABSTRACT:
Chondrosarcoma is a type of highly malignant tumor with a potent capacity to invade locally and cause distant metastasis. And, invasion of tumor cells is the primary cause of therapeutic failure in the treatment of malignant chondrosarcomas. High mobility group box chromosomal protein 1 (HMGB)-1 is a widely studied, ubiquitous nuclear protein that is present in eukaryotic cells, and plays a crucial role in stabilizing nucleosomes, enabling nicking of DNA and inflammatory response. It also plays a crucial role in migration and metastasis of human cancer cells. Integrins are the major adhesive molecules in mammalian cells and have been associated with metastasis of cancer cells. However, the effects of HMGB-1 in migration and integrin expression in human chondrosarcoma cells are mostly unknown. Here, we found that HMGB-1 increased the migration and the expression of α5β1 integrin in human chondrosarcoma cells (JJ012 cells). Activations of phosphatidylinositol 3-kinase (PI3K), Akt and AP-1 pathways after HMGB-1 treatment were demonstrated. HMGB-1-induced expression of integrin and migration activity was inhibited by the specific inhibitor and mutant of PI3K, Akt and AP-1 cascades. Taken together, our results indicated that HMGB-1 enhances the migration of chondrosarcoma cells by increasing α5β1 integrin expression through the PI3K, Akt, and AP-1 signal transduction pathways.

METHODS:
Cell culture: The human chondrosarcoma cell line (JJ012) was kindly provided from the laboratory of Dr. Sean P Scully (University of Miami School of Medicine, Miami, FL, USA). The JJ012 cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum and maintained at 37°C in a humidified atmosphere of 5% CO2.

Migration assay: The migration assay was performed using Transwell (Costar, NY; pore size, 8-μm) in 24-well dishes. Before performing the migration assay, cells were pretreated for 30 min with different concentrations of inhibitors or vehicle control (0.1% DMSO). Approximately 1×104 cells in 100 μl of serum-free medium were placed in the upper chamber, and 300 μl of the same medium containing HMGB-1 was placed in the lower chamber. The plates were incubated for 24 hr at 37°C in 5% CO2; then cells were fixed in methanol for 15 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Each clone was plated in triplicate in each experiment, and each experiment was repeated at least three times. qPCR; Flow cytometric analysis; Western blot analysis; Transfection and reporter gene assay

RESULTS SECTION:
HMGB-1-triggered migration in chondrosarcoma cells was examined using the Transwell assay with correction of HMGB-1-induced proliferation effects on human chondrosarcoma cells. HMGB-1 directed human chondrosarcoma cell migration (Fig 1A-D). Previous studies have shown significant expression of integrin in human chondrosarcoma cells. We therefore, hypothesized that integrin may be involved in HMGB-1-directed chondrosarcoma cell migration. The expression of mRNA for the integrins in response to HMGB-1 was analyzed by qPCR analysis shown that HMGB-1 induced the expression of α5β1 integrin (Fig 1E). To confirm this finding, Flow cytometry analysis also showed that HMGB-1 induced the cell surface expression of α5β1 integrin dose-dependently (Fig 1F).

Interaction of HMGB-1 with its specific receptor RAGE on the surface of cancer cells has been reported to induce cancer invasion. Transfection of cells with RAGE siRNA suppressed the expression of RAGE (Fig 2A). HMGB-1-induced cell migration and integrin expression was also inhibited by RAGE siRNA but not by control siRNA (Fig 2A-C).