Macrophage migration inhibitory factor up-regulates $\alpha V \beta 3$ integrin expression and cell migration via PI3K, Akt and NF-κB-dependent pathway in human chondrosarcoma cells

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INTRODUCTION:

Chondrosarcoma is a malignant cancer that results in abnormal bone and cartilage growth. It is a skeletal tumor with various grades of malignancy, rapidly evolving, and with a strong tendency to metastasize that may migrate to lung, with low responsiveness to chemotherapy. Chondrosarcoma is the second most frequent primary malignant tumor of bone, representing approximately 25% of all primary osseous neoplasms. Chondrosarcoma is a tumor with highly diverse features and behavior patterns, ranging from slow-growing non-metastasizing lesions to highly aggressive metastasizing sarcomas.Macrophage migration inhibitory factor (MIF) is expressed and secreted in response to mitogens and integrin-dependent cell adhesion, which is an upstream activator of the innate response that mediates the recruitment and retention of monocytes via CD74 and associated chemokine receptors, and it plays an essential role in tumor progression and metastasis. However, the effects of MIF in chondrosarcoma are mostly unknown. Integrins are the major adhesive molecules in mammalian cells and have been associated with metastasis of cancer cells. We found that MIF increased cell surface expression of αvβ3 integrin and the migration through αvβ3 over expression via PI3K, Akt and NF-κB pathway in human chondrosarcoma cells.

METHODS:

Cell culture: The human chondrosarcoma cell lines (SW1353 and JJ012) were purchased from American Type Culture Collection Cells were cultured in DMEM and αMEM supplemented with 10% FBS and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) Serum and maintained at 37°C in a humidified atmosphere of 5% CO2.

Migration assay; Western blot analysis; Transfection and reporter gene assay; Quantitative real-time PCR; flow cytometry analysis; Immunofluorescence assay.

RESULTS:

MIF has been reported to stimulate directional migration and invasion of human cancer cells. To examine the effects of NOV on chondrosarcoma cell migration, the Transwell assay was used. JJ012 was treated with various concentrations of MIF and significantly directed cells migration (Fig1.A). It has been reported that MIF was concerned with some specific integrins. We hypothesized that integrin-signaling pathway may be involved in MIF-directed chondrosarcoma cell migration. Pretreatment of cells for 30 min with anti-αvβ3 monoclonal antibody (mAb) markedly inhibited the MIF-induced cancer migration (Fig. 1B). Chondrosarcoma cells were incubated with various concentrations of MIF, and the cell expression of $\alpha v \beta 3$ integrin was determined using flow cytometry (Fig. 1C). Chondrosarcoma cells were pretreated with cyclicRGD, or cyclicRAD for 30min followed by stimulation with MIF, Migration analysis indicated that the tetrameric RAFT-RGD-Cy5 had a tenfold higher affinity for its soluble receptor integrin αvβ3. It can be targeted by specific arginine-glycine-aspartic acid (RGD)-containing peptides binding integrin αvβ3. The nonspecific RAFT-RAD-Cy5 did not interact with integrin αvβ3 (Fig. 1D). Therefore, αvβ3 integrin is mediated MIF-induced cell migration.

Phosphoinositide 3-kinase (PI3K) respond to a wide variety of signaling molecules controlled primarily by their adaptor subunit. It plays a crucial role in effecting alterations in a broad range of cellular functions in response to extracellular signals. To explore whether PI3K is involved in MIF-induced αvβ3 integrin expression, PI3K inhibitor Ly294002, wortmannin and PI3K siRNA were used. As shown in Fig. 2A, pretreatment of cell with Ly294002, wortmannin and PI3K siRNA inhibited MIF-induced αvβ3 integrin expression. It has been reported that PI3K is an upstream regulator of the phosphorylation of Akt, which is a serine/threonine protein kinase that plays a key role in multiple cellular processes. Akt is in response to PI3K activation, phosphorylates and regulates the activity of a number of targets including kinases, transcription factors and other regulatory molecules, we then examined whether MIF stimulation also enhances the association of PI3K with Akt. Pretreatment of cells for 30 min with Akt inhibitor inhibited the MIF-induced αvβ3 integrin expression. Transfection of cells with Akt mutant reduced MIF-mediated αvβ3 integrin expression (Fig. 2B). NF-κB activation is necessary for the migration and invasion of human cancer cells. We further examined activation of the NF-κB after MIF stimulation. Stimulation of cells with MIF increased NF-κB-luciferase activity (Fig. 3A and Fig. 3B). In addition, PI3K and Akt inhibitors or siRNA reduced MIF-increased NF-κB leuciferase activity (Fig. 3C). Therefore, PI3K、Akt and NF-κB signaling pathways are involved in MIF-mediated cell migration and αvβ3 integrin expression.

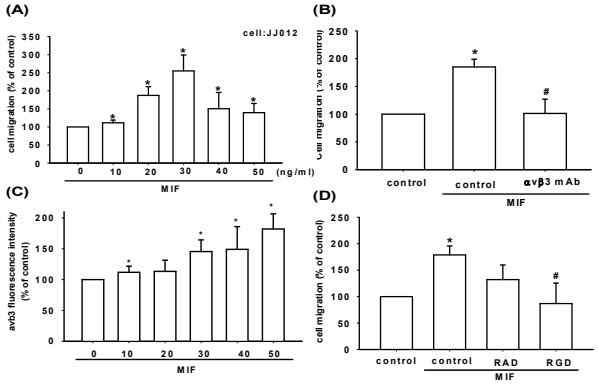

Fig 1 MIF-directed chondrosarcoma cells migration through αvβ3 integrin.

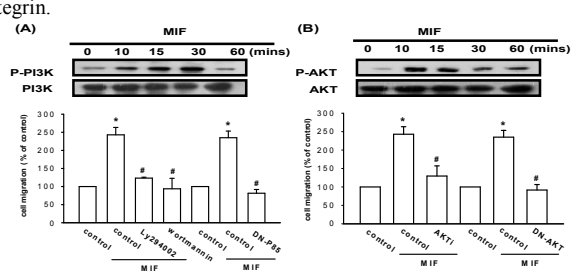

Fig 2 PI3K, Akt are involved in MIF-induced αvβ3 integrin expression.

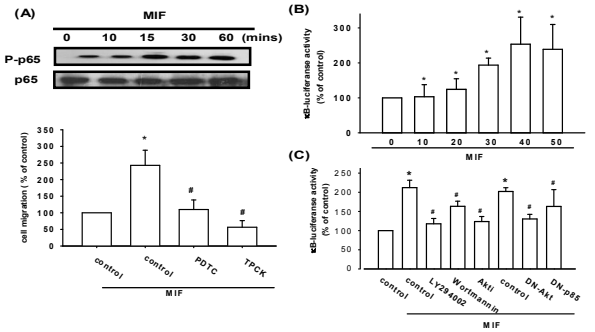

Fig 3 ILK, Akt are involved in MIF-induced αvβ3 integrin activity.

DISCUSSION:

We present a novel mechanism of MIF-directed migration of human chondrosarcoma cells by upregulation αvβ3 integrin. MIF increases αvβ3 integrin expression and activity by binding to the CD74 receptor and activation of PI3K/Akt, which in turn activates NF-κB, resulting in the activations of αvβ3 integrin and contributing the migration of chondrosarcoma cells.