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ORIGINAL ARTICLE

Effects of MIF on proliferation, migration, and STAT1 pathway of colon cancer cells*

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Abstract

Objective This study aimed to investigate how macrophage migration inhibitory factor (MIF) regulates the interaction of signal transducer and activator of transcription 1 (STAT1) with CD74, and affects colon cancer proliferation and invasion.

Methods After transfecting MIF small interfering RNA into the SW480 cell line, the expression of STAT1 and CD74 mRNA was detected by qRT-PCR and western blotting. Transwell and MTT assays were performed to detect the colon cancer cell invasion and proliferation ability. Co-immunoprecipitation was used to detect the interaction between CD74 and STAT1 proteins in the treated and control groups.

Results The cellular biological assays (MTT and Transwell) showed that the proliferation and invasion ability of colon cancer cells decreased after MIF knockdown; the results showed significant statistical difference ($P < 0.05$). The results of the co-immunoprecipitation assay suggested that MIF knockdown in colon cancer cells could inhibit the binding of CD74 and STAT1 proteins; statistical difference was observed between the two groups ($P < 0.05$).

Conclusion MIF can increase the proliferation and invasion of colon cancer cells by promoting the combination of CD74 and STAT1.

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Key words: colon cancer; migration inhibitory factor; signal transducer and activator of transcription 1; cell proliferation; cell migration

Colon cancer is one of the most common malignant tumors; its morbidity ranks fourth among all the tumors in China [1]. As colon cancer typically exhibits locally infiltrating or distant metastasis, patients often need surgery. Prevention of rapid growth, metastasis, and recurrence of colon cancer are crucial to improve the prognosis of patients [2–3]. Therefore, curbing the proliferation and invasion ability of colon cancer cells has been a research focus in recent years. Macrophage migration inhibitory factor (MIF) can be rapidly secreted under the stimulation of extracellular microbial products or hypoxia environments, and binds to specific receptors to initiate downstream signal pathways that affect oncogenesis and tumor progression through interaction with tumor-related proteins [4–5]. Studies have shown that CD74 can act as a membrane receptor for MIF, which promotes the interaction of CD74 with transcription factors that regulate related downstream gene expression in cells [6–8]. Signal transducer and activator of transcription 1 (STAT1) was one of the first members of the STAT family to be discovered and studied. It is an important intracellular transcription factor that regulates tumor cell proliferation, apoptosis, and differentiation [9–10]. Our previous research has demonstrated the physical interaction of STAT1 and CD74 proteins in colon cancer cells. However, the role played by MIF in CD74/STAT1-mediated colon cancer invasion and metastasis needed further confirmation.

Materials and methods

Main experimental cells, materials, and reagents

Cell line

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The human colon cancer cell line SW480 was purchased from ATCC.

Reagents

DMEM medium and fetal bovine serum were purchased from GIBCO, USA. Small interfering RNAs (siRNAs) of MIF, STAT1, and CD74 were purchased from Wuhan Qingke Biotechnology Company. pCMV-Myc and pCMV-HA vectors were purchased from Shanghai Shuomeng Biological Company. pTalluc-DDR2 vector was purchased from Shanghai Bi Yuntian Biological Company. Polyclonal antibodies against HA-tag and anti-Myc were purchased from GIBCO, USA. Horseradish peroxidase (HRP) -conjugated goat anti-mouse IgG, HRP-conjugated goat anti-rabbit IgG, Anti-HA, and anti-Myc monoclonal antibodies were purchased from Wuhan Baoshide Company. A Lipofectamine 2000 transfection kit was purchased from Santa Cruz. Real-time quantitative PCR and reverse transcription were suitable for purchase from Japan's TAKARA Company. Immunochemoluminescence development reagent kits were purchased from Clontech, USA.

Instruments

A gel imaging system was purchased from Sigma, USA. Protein-A/G was purchased from Santa Cruz, USA. An LSLS-B50L vertical circular pressure steam sterilizer was purchased from Tuttnauer Company, USA. An all-around high-performance benchtop refrigerated centrifuge (Heaeus, Biofuge Stratos, Germany), a UV spectrophotometer (2540MK, Tuttnauer, USA), and a fluorescence analyzer (SCW-HS-840 type, OLYMPUS, Japan) were used in the experiments.

Research methods

Cell culture

Colon cancer cells were cultured with DMEM medium containing 10% fetal bovine serum and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin), in a 37 °C 5% CO_2 incubator. The cells were passaged when the cell density reached 70% or more. After washing with the medium, the cells were digested with trypsin containing 0.02% EDTA for 2 to 3 min. The digested cells were mixed with the same volume of serum-containing medium to terminate the digestion. The cells were then observed under a microscope. The adherent cells were pipetted, and centrifuged at 10,000 r/min for 5 min to remove the supernatant. Thereafter, the cell suspension was counted and inoculated into a new culture flask to continue the cultivation.

Transfection

When the colon cancer cells reached 70% density, they were transfected with siRNAs or overexpression plasmids of STAT1 and CD74. A 10 μg DNA sample was diluted and mixed with 240 μL serum-free DMEM medium, and incubated for 5 min. Then, 10 μL Lipofectamine 2000 transfection reagent was diluted with 250 μL DMEM without antibiotics and serum. After incubation for 5 min, the two solutions were mixed and incubated at room temperature for 20 min. The medium in a six-well plate was replaced with DMEM containing the transfection mixture, and the plate was placed into an incubator. After 6 h of culturing, the medium was discarded and the culturing was continued using DMEM containing 10% fetal bovine serum. Solutions A and B were configured according to the ratios 2 μL Lipo2000 + 100 μL Opti-MEM and 2.5 μL siRNA/NC + 100 μL Opti-MEM, respectively, and incubated at 37 °C for 5 min. Solutions A and B were gently mixed and incubated at 37 °C for 15 min. Then, 200 μL of the mixed liquid was added to each well, shaken, and incubated at 37 °C for 4–6 h.

Quantitative real-time PCR

Total RNA was isolated from cells using Trizol (Invitrogen). Reverse transcriptions were performed using a PrimeScript™ RT reagent kit (Takara, Dalian, China) according to the manufacturer's instructions. RT-PCR assays were performed under the following conditions: 37 °C for 15 min, 85 °C for 5 s, and 4 °C for 10 min. qRT-PCR was performed with SYBR®Premix Ex Taq™ (TaKaRa), according to the manufacturer's protocol, on an Applied Biosystems StepOne-Plus Real-Time PCR System. GAPDH was used as the loading control. Each reaction was done in triplicate.

Invasion assay

A 500 μL sample of serum-free DMEM medium was added to a 24-well plate, a Transwell chamber was placed into the well, and the cell density was adjusted to 1×10^6 cells/well. The cells were transfected with either STAT1/CD74 over-expressing plasmids or MIF-transfected siRNA and cultured at 37 °C for 48 h. The liquid in the culture was aspirated and the cells were washed gently with PBS and fixed with methanol for 20 min. Then, they were stained with 0.1% crystal violet at room temperature for 30 min and washed gently with PBS thrice. The cells were carefully wiped onto the upper layer of a microporous membrane with a cotton swab, and counted at 200× magnification under an inverted microscope. The cells moved to the lower layer of the microporous membrane, upon which each field was randomly counted five times and the average was taken.

Cell proliferation assay

Cells were seeded into 96-well plates at a density of 2000 cells/well and incubated at 37 °C for 1 to 5 days. Then, the cells were incubated with MTT solution (0.5 mg/mL) at 37 °C for 4 h and the medium was replaced with 150 μL DMSO. A microplate reader was used to detect the absorbance at 570 nm. All the experiments were repeated thrice independently and there were 5 samples per group.

Co-immunoprecipitation

Cell extracts were incubated with the primary antibody or normal IgG overnight at 4 °C and then incubated with Protein A/G PLUS-Agarose for 2 h at 4 °C. Thereafter, the agarose was collected and washed with lysis buffer.

Statistical methods

All the data obtained in this study were analyzed using SPSS 19.0 software. The results are presented as mean ± standard deviation; the statistical differences were determined using the *t*-test. Differences were considered significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$ (NS: no sense).

Results

MIF has no significant effect on the expression of STAT1 and CD74 in colon cancer cells

First, we transfected siRNA into colon cancer cells to knockdown MIF. Then, PCR and western blot assays were performed to detect the mRNA and protein expressions of STAT1 and CD74. The results showed that MIF knockdown did not significantly change the expression of either STAT1 or CD74 compared to that in the control group ($P > 0.05$), indicating that MIF did not affect the expression of STAT1 or CD74 mRNA and protein (Fig. 1a, 1b).

MIF can promote the binding of STAT1 and CD74 proteins

In co-immunoprecipitation assays, the lysates of colon cancer cells co-transfected with MIF siRNAs and pCMV-HA-STAT1, pCMV-Myc-CD74 plasmids and those only transfected with pCMV-HA-STAT1, pCMV-Myc-CD74 plasmids were extracted. The lysates were precipitated with polyclonal antibodies of anti-Myc or pre-immune and detected with HA-monoclonal antibodies. Bands were detected for both the direct output lysate (input) and lysate precipitated by anti-Myc polyclonal antibody; however, the MIF knockdown group (siMIF) showed a significantly lower band intensity than the control group (Fig. 2). These results suggested that MIF knockdown inhibited the binding of STAT1 to CD74 protein.

MIF promotes colon cancer cell proliferation and invasion via STAT1 and CD74

The results of the Transwell assay showed that the number of cells passing through the Transwell membrane increased more significantly in the group overexpressing STAT1/CD74 and MIF knockdown than in the group that only overexpressed STAT1/CD74 (Fig. 3a, 3b). This result indicated that MIF promoted the proliferation and invasion of colon cancer cells through the STAT1/CD74 axis.

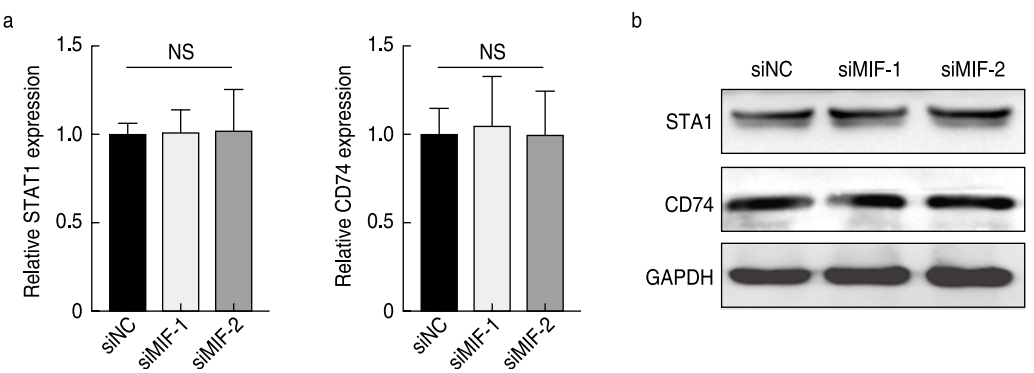

Fig. 1 MIF has no significant effect on STAT1 and CD74 mRNA (a) and protein (b) expression

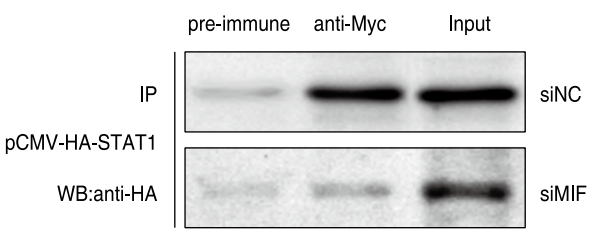

Fig. 2 Co-immunoprecipitation assay indicated that MIF knockdown inhibited CD74 and STAT1 protein combination

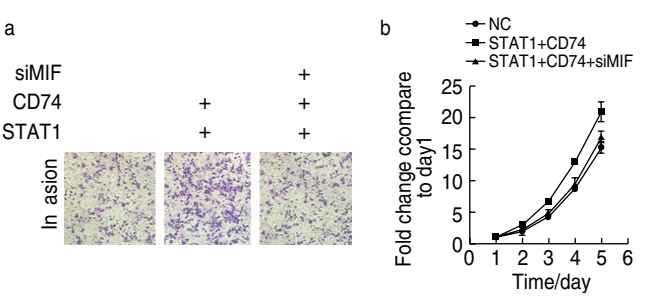

Fig. 3 MIF affects colon cancer cell invasion (a) and proliferation (b) through CD74 and STAT1

Discussion

Recurrence and metastasis are the key factors that affect the prognosis and quality of life of colon cancer patients, and have become the focus of current clinical and basic research. Although some progress has been made in the clinical efficacy of radiotherapy, chemotherapy, and molecular targeted therapy for colon cancer, the overall prognosis of patients still has great room for improvement. Malignant biological behaviors such as unlimited proliferation and invasion of tumor cells are important factors that promote rapid progress of colon cancer. Therefore, research focusing on the regulation of abnormally expressed genes involved in tumor cell proliferation and invasion can provide potential molecular targets and new ideas for clinical diagnosis and treatment.

MIF is stored in immune cells such as monocyte macrophages, B cells, T cells, and dendritic cells. It is also present in epithelial cells, the epithelial cytoplasm, and endocrine cells, which can promote the initiation and development of inflammatory diseases [11]. Studies have also shown that MIF is highly expressed in gliomas, prostate cancer, nasopharyngeal cancer, and colon cancer, and is involved in the regulation of tumorigenesis and cancer development. Its functional mechanism includes the inhibition of the tumor suppressive effects of p53, CD74/CD44, MAPK, or other signaling pathways that regulate tumor cell growth, apoptosis, spread, and angiogenesis [12–16].

CD74 is a membrane antigen related to MHC-II restricted antigen presentation. It exists both in the cell membrane and cytoplasm. Generally, CD74 is expressed in lymphocytes, monocytes/macrophages, Langham cells, dendritic cells, activated T lymphocytes, and some thymic epithelial cells. Studies on CD74 have mostly focused on the field of cellular immunology. Recently, however, the role and functional mechanism of CD74 in tumors has attracted the attention of many scientists. Some have reported that the expression intensity of CD74 has a negative correlation with the prognosis of patients with gastric cancer. Other studies have found that CD74 can be used as a biological molecular marker to predict the prognosis of patients with pancreatic and lung cancers. Furthermore, high expression of CD74 in colon cancer can also promote cancer development [17–21]. With regard to the functional mechanism, it has been proved that MIF interacts with the cell membrane receptor CD74; CD74 in the cytoplasmic segment can activate the nuclear transcription factor NF-κB to regulate the transcription of downstream targeted genes. MIF can also function through the interaction of CD7/CD44 to activate the Src family protein tyrosine kinase, causing ERK phosphorylation; or by regulating cell proliferation by activating Syk and the PI3K/AKT pathway [22–25]. It has been widely reported that MIF binds to the cell membrane receptor CD74 to exercise its functions. Therefore, the intracellular molecular mechanism and signaling pathway are the focus of research in this field.

STAT1 is a DNA-binding protein that can work as a nuclear transcription factor to regulate the transcription and expression of genes related to cell proliferation, invasion, and immune response, and plays an important role in the initiation and development of malignant tumors. Our previous research demonstrated that STAT1 can bind to CD74 protein in colon cancer cells. In this study, we demonstrated that MIF inhibition reduces the binding of CD74 and STAT1. Furthermore, the results of cell biology experiments showed that the proliferation and invasion ability of colon cancer cells were significantly decreases upon MIF knockdown. Therefore, we conclude that MIF regulates the malignant biological behavior of colon cancer by affecting the binding of CD74 and STAT1. Moreover, we noticed that, with regard to the tumor extracellular environment, i.e., tumor microenvironment formation and positive feedback effects, immune evasion is also an important factor for tumor recurrence and metastasis. A large number of studies have reported that MIF, CD74, and STAT1 play important roles in local or systemic inflammation-related diseases, and that their molecular interaction mechanisms are our next research directions.

In summary, MIF silencing may inhibit the proliferation, migration, and invasion of colon cancer cells. The underlying mechanism probably regulates the binding of CD74 and STAT1 proteins, thereby affecting the expression of the STAT1 pathway. This study provides new ideas for targeted treatment of colon cancer by silencing the effect of MIF expression on colon cancer cells.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin, 2020, 70: 7–30.
2. Feng RM, Zong YN, Cao SM, *et al*. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? Cancer Commun (Lond), 2019, 39: 22.
3. Yokota J. Tumor progression and metastasis. Carcinogenesis, 2000, 21: 497–503.
4. Bernhagen J, Calandra T, Mitchell RA, *et al*. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature, 1993, 365: 756–759.
5. Roger T, Froidevaux C, Martin C, *et al*. Macrophage migration inhibitory factor (MIF) regulates host responses to endotoxin through modulation of Toll-like receptor 4 (TLR4). J Endotoxin Res, 2003, 9: 119–123.

6. Cournia Z, Leng L, Gandavadi S, *et al*. Discovery of human macrophage migration inhibitory factor (MIF)-CD74 antagonists via virtual screening. J Med Chem, 2009, 52: 416–424.
7. Fan C, Rajasekaran D, Syed MA, *et al*. MIF intersubunit disulfide mutant antagonist supports activation of CD74 by endogenous MIF trimer at physiologic concentrations. Proc Natl Acad Sci U S A, 2013, 110: 10994–10999.
8. Leng L, Metz CN, Fang Y, *et al*. MIF signal transduction initiated by binding to CD74. J Exp Med, 2003, 197: 1467–1476.
9. Kaler P, Owusu BY, Augenlicht L, *et al*. The role of STAT1 for crosstalk between fibroblasts and colon cancer cells. Front Oncol, 2014, 4: 88.
10. Ramana CV, Gil MP, Schreiber RD, *et al*. STAT1-dependent and independent pathways in IFN-γ-dependent signaling. Trends Immunol, 2002, 23: 96–101.
11. Roger T, David J, Glauser MP, *et al*. MIF regulates innate immune responses through modulation of Toll-like receptor 4. Nature, 2001, 414: 920–924.
12. Camlica H, Duranyildiz D, Oguz H, *et al*. The diagnostic value of macrophage migration inhibitory factor (MIF) in gastric cancer. Pathol Oncol Res, 2008, 14: 79–83.
13. Lechien JR, Kindt N, Costa Pde A, *et al*. MIF in head and neck cancer: a new therapeutic target? Rev Laryngol Otol Rhinol (Bord), 2013, 134: 67–74.
14. Lin S, Wang M, Liu X, *et al*. Association of genetic polymorphisms in MIF with breast cancer risk in Chinese women. Clin Exp Med, 2017, 17: 395–401.
15. Richard V, Kindt N, Saussez S, *et al*. Macrophage migration inhibitory factor involvement in breast cancer (Review). Int J Oncol, 2015, 47: 1627–1633.
16. Rosengren E, Bucala R, Aman P, *et al*. The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction. Mol Med, 1996, 2: 143–149.
17. Bozzi F, Mogavero A, Varinelli L, *et al*. MIF/CD74 axis is a target for novel therapies in colon carcinomatosis.J Exp Clin Cancer Res, 2017, 36: 16.
18. Cheng SP, Liu CL, Chen MJ, *et al*. CD74 expression and its therapeutic potential in thyroid carcinoma. Endocr Relat Cancer, 2015, 22: 179–190.
19. McClelland M, Zhao L, Carskadon S, *et al*. Expression of CD74, the receptor for macrophage migration inhibitory factor, in non-small cell lung cancer. Am J Pathol, 2009, 174: 638–646.
20. Metodieva G, Nogueira-de-Souza NC, Greenwood C, *et al*. CD74-dependent deregulation of the tumor suppressor scribble in human epithelial and breast cancer cells. Neoplasia, 2013, 15: 660–668.
21. Nagata S, Jin YF, Yoshizato K, *et al*. CD74 is a novel prognostic factor for patients with pancreatic cancer receiving multimodal therapy. Ann Surg Oncol, 2009, 16: 2531–2538.
22. Baron N, Deuster O, Noelker C, *et al*. Role of macrophage migration inhibitory factor in primary glioblastoma multiforme cells. J Neurosci Res, 2011, 89: 711–717.
23. Becker-Herman S, Arie G, Medvedovsky H, *et al*. CD74 is a member of the regulated intramembrane proteolysis-processed protein family. Mol Biol Cell, 2005, 16: 5061–5069.
24. Shi X, Leng L, Wang T, *et al*. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. Immunity, 2006, 25: 595–606.
25. Leng L, Bucala R. Insight into the biology of macrophage migration inhibitory factor (MIF) revealed by the cloning of its cell surface receptor. Cell Res, 2006, 16: 162–168.

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