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Macrophage migration inhibitory factor interacts with thioredoxin-interacting protein and induces NF-κB activity

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ABSTRACT

The nuclear factor kappa B (NF-κB) pathway is pivotal in controlling survival and apoptosis of cancer cells. Macrophage migration inhibitory factor (MIF), a cytokine that regulates the immune response and tumor-igenesis under inflammatory conditions, is upregulated in various tumors. However, the intracellular functions of MIF are unclear. In this study, we found that MIF directly interacted with thioredoxin-interacting protein (TXNIP), a tumor suppressor and known inhibitor of NF-κB activity, and MIF significantly induced NF-κB activation. MIF competed with TXNIP for NF-κB activation, and the intracellular MIF induced NF-κB target genes, including c-IAP2, Bcl-xL, ICAM-1, MMP2 and uPA, by inhibiting the interactions between TXNIP and HDACs or p65. Furthermore, we identified the interaction motifs between MIF and TXNIP via site-directed mutagenesis of their cysteine (Cys) residues. Cys^{57} and Cys^{81} of MIF and Cys^{36} and Cys^{120} of TXNIP were responsible for the interaction. MIF reversed the TXNIP-induced suppression of cell proliferation and migration. Overall, we suggest that MIF induces NF-κB activity by counter acting the inhibitory effect of TXNIP on the NF-κB pathway via direct interaction with TXNIP. These findings reveal a novel intracellular function of MIF in the progression of cancer.

1. Introduction

Nuclear factor kappa B (NF-κB) is a key transcription factor that plays an important role in the regulation of diverse biological responses, including immune responses, inflammation, proliferation, survival and angiogenesis, and pathological responses, including tumor promotion and metastasis [1]. In mammals, the NF-κB family comprises five proteins: NF-κB1 (p50/105), NF-κB2 (p52/100), Rel A (p65), Rel B and c-Rel. NF-κB activation is induced by the phosphorylation of inhibitor of NF-κB (IκB). In unstimulated conditions, NF-κB is predominantly present in the cytoplasm, where it is bound to IκB proteins [2]. NF-κB is activated by various stimuli, such as growth factors, cytokines, interleukin-1 (IL-1), tumor-necrosis factor (TNF) and hormones [3,4]. Many studies have reported that NF-κB regulates cancer development and progression and is constitutively activated in lung carcinoma, breast cancer, lymphoma, and leukemia cell lines [5–8]. Moreover, NF-κB has been found to be present at a significantly increased level, this level has been shown to correlate with poor prognostic outcome in ovarian cancer and glioblastoma [9,10]. Inactivation of NF-κB signaling or gene knockout of NF-κB has been shown to promote anti-tumor responses [11,12]. In a recent report, targeting NF-κB signaling showed therapeutic efficacy in ovarian and other cancers.

Macrophage migration inhibitory factor (MIF) is a proinflammatory mediator that plays an important role in the innate immune response. MIF was originally identified as a soluble molecule or cytokine induced

by activated T cells in a delayed-type hypersensitivity response [13]. This protein is expressed in various cell types, such as monocytes/macrophages, T lymphocytes, and eosinophils, epithelial cells, and endothelial cells after stimulation with inflammatory molecules or exposure to stress [14]. MIF has been intensively studied in several inflammatory and autoimmune diseases, including sepsis, rheumatoid arthritis, atherosclerosis, asthma, and acute respiratory distress syndrome (ARDS) [15–20]. MIF expression has been found to be significantly increased in various tumor types, including prostate tumors, breast cancer, melanomas, colon carcinomas, and lung cancer [21–25]. Furthermore, various studies have found that MIF also induces tumor growth by controlling immune responses and promoting tumor-associated angiogenesis [26,27]. Recombinant MIF (rMIF) accelerates cell growth and migration. Decreased cell growth and migration and increased apoptosis have been observed following removal of MIF by gene deletion, inactivation of MIF by mutation or blockade of MIF by antibodies, small interfering RNA, a MIF anti-sense plasmid or a MIF inhibitor [23,28–38]. These results showed that the blockade of MIF decreased the activity of a Rho GTPase family member, Rac1, increased phosphorylation of p53 and decreased Akt phosphorylation. MIF knockdown was found to lead to a decrease in tumor necrosis factor-α, interleukin-6 (IL-6), and interleukin-10 (IL-10) expression. MIF can bind to cell surface receptors, such as CD74, CD44, and CXCRs, or enter cells via endocytosis [39–41]. It can directly bind to JAB1, a co-activator of the AP-1 transcriptional complex and tumor suppressor p53, and induce a tumorigenic signaling pathway associated with tumor progression [42,43].

Thioredoxin-interacting protein (TXNIP) is a multifunctional protein involved in a variety of cellular processes, including the regulation of differentiation, the cell cycle, cancer, cell aging, metabolism and inflammation [44]. The TXNIP level has been shown to increase in HL-60 cells treated with 1,25-(OH)2D3 [45]. TXNIP expression is upregulated by various stresses, such as H_2O_2 exposure, UV irradiation, heat shock, serum deprivation, and transforming growth factor-β stimulation [46,47]. In addition, anti-cancer and anti-proliferative reagents increase the expression of TXNIP in cancer cells [48,49]. TXNIP is also involved in immune cell regulation. Dendritic cells derived from TXNIP-deficient mice have been shown to exhibit defects in regulating T cell activation and proliferation [50]. TXNIP plays an important role in the development and function of natural killer cells [51]. Additionally, TXNIP inhibits the expression and the antioxidant function of thioredoxin (TRX) through direct interaction [52]. Recent studies have reported that the loss of TXNIP in hematopoietic stem cells caused severe damage under stress conditions [53,54]. TXNIP plays a key role in IL-1 secretion via interaction with NLRP3. In addition, endotoxin shock was found to be exacerbated by LPS in TXNIP-deficient mice [55,56]. TXNIP is a tumor suppressor protein that is significantly downregulated in a variety of tumors, including breast, renal, and gastrointestinal cancers [57–59]. It also inhibits cell proliferation and regulates p27 stability through direct interaction with Jab1 [60]. In addition, the overexpression of TXNIP has been shown to suppress tumor growth and metastasis in a mouse model of melanoma. Recently, TXNIP was shown to inhibit hepatocarcinogenesis by suppressing the TNF-dependent NF-κB signaling pathway [61].

Based on the evidence that NF-κB, MIF and TXNIP were involved in tumor progression, we hypothesized that MIF might regulate the link between NF-κB signaling and TXNIP. We investigate whether MIF can directly participate in TXNIP-mediated NF-κB signaling pathways and demonstrate that MIF positively regulates NF-κB activity via direct interaction with TXNIP and increases cell proliferation and migration. Here, we propose a novel intracellular function of MIF in cancer progression by controlling the interaction between NF-κB and TXNIP.

2. Materials and methods

2.1. Cell culture

HeLa (human epithelial carcinoma) and 293T (human embryonic kidney fibroblast cell line) were obtained from the American Type Culture Collection (Manassas, VA). HeLa cell was maintained in MEM (WELGENE Inc., Gyeongsan, Korea) and 293T cell was cultured in DMEM (WELGENE Inc., Gyeongsan, Korea) at 37 °C and 5% CO_2. All culture mediums were supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin.

2.2. Transient transfections and stable cell lines

Transient transfections of 293T cells were performed using Lipofectamine and Plus Reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The cells were transfected with GST vector, GST tagged MIF, GST tagged MIF C57S, GST tagged MIF C60S, GST tagged MIF C81S, GST tagged MIF C57S/C81S, FLAG vector, FLAG tagged TXNIP, FLAG tagged MIF and HA tagged HDAC1, HA tagged p65, FLAG tagged TXNIP N-constructs, FLAG tagged TXNIP C-constructs, FLAG tagged TXNIP-C36S, FLAG tagged TXNIP-C49S, FLAG tagged TXNIP-C63S, FLAG tagged TXNIP-C120S. HeLa cells were transiently transfected with GST vector, GST tagged MIF, FLAG vector, FLAG tagged TXNIP, FLAG tagged MIF, FLAG tagged MIF C57S/C81S and HA tagged HDAC1, HA tagged p65 using Lipofectamine and Plus Reagent. To establish stable cell lines, the shRNA TXNIP or shRNA MIF plasmid was transfected into HeLa cells. Forty-eight hours later, the cells were selected with 1000 μg/ml neomycin or 0.5 μg/ml puromycin until drug-resistant colonies became visible. TXNIP shRNA plasmid was obtained from Qiagen (Valencia, CA). MIF shRNA plasmid was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.3. NF-κB luciferase assay

HeLa cells were transiently cotransfected with expression vectors, pNF-κB–Luc plasmid (Stratagene, La Jolla, CA) and cytomegalovirus-Renilla luciferase construct (pRL–CMV) (Promega, Madison, WI), a Renilla-derived luciferase reporter plasmid for transfection control, using Lipofectamine and Plus reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. Luciferase assays were performed according to instructions supplied with the Luciferase Assay Kit (Promega, Madison, WI). Firefly luciferase and Renilla luciferase activities were measured by the Dual Luciferase Reporter Assay System (Promega, Madison, WI) on a luminometer (Turner Designs, Sunnyvale, CA).

2.4. RNA isolation and quantitative real-time PCR

Total RNA was extracted from HeLa cells using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's-instructions. Total RNAs were reverse-transcribed to first strand cDNAs by using ReverTra Ace® qPCR RT Kit (Toyobo) and analyzed by real time PCR (Takara Bio) using SYBR Green PCR Master Mix (Takara Bio) with specific primers. The mRNA expression level was calculated using GAPDH as a control. The primer sequences were as follows: TXNIP, forward 5′-CTC GTG TCA AAG CCG TTA GGA-3′ and reverse 5′-GTC AAG AAA AGC CTT CAC CCA-3′; MIF, forward 5′-CCG GAC AGG GTC TAC ATC AAC TAT TAC-3′ and reverse 5′-TAG GCG AAG GTG GAG TTG TTC C-3′; c-IAP2, forward 5′-TGG AAG CTA CCT CTC AGC CTA C-3′ and reverse 5′-GGA ACT TCT CAT CAA GGC AGA-3′; Bcl-xL, forward 5′-GAA CGG CGG CTG GGA T-3′ and reverse 5′-AGC GGT TGA AGC GTT CCT G-3′; TRAF1, forward 5′-GCC CTT CCG GAA CAA GGT C-3′ and reverse 5′-CGT CAA TGG CGT GCT CAC-3′; TNF-α, forward 5′-TGC TTG TTC CTC AGC CTC TT-3′ and reverse 5′-