

## Original Article

# Role of macrophage migration inhibitory factor in mesenchymal epithelial transition of cervical carcinoma cells

Fangfang Guo<sup>1,2</sup>, Xueqi Fu<sup>1</sup>, Jili Yang<sup>2</sup>, Xueying Zhang<sup>2</sup>, Dezhi Liu<sup>2</sup>, Wenjuan Feng<sup>2</sup>, Jinyu Liu<sup>2</sup>, Jinhong Qi<sup>2</sup>, Huifang Cao<sup>2</sup>, Li Qi<sup>3</sup>, Jingyuan Ren<sup>2</sup>

<sup>1</sup>Edmond H. Fischer Signal Transduction Laboratory, School of Life Sciences, Jilin University, Changchun, Jilin, PR China; <sup>2</sup>Jilin Province Tumor Hospital, Changchun, Jilin, PR China; <sup>3</sup>Chaoyang District People's Hospital of Jilin Province, Changchun, Jilin, PR China

Received December 9, 2016; Accepted February 20, 2017; Epub September 1, 2017; Published September 15, 2017

**Abstract:** Cervical cancer is a kind of female malignant tumor with increasing incidence recently. Macrophage migration inhibitory factor (MIF) is a major tumor facilitating factor. The previous study suggests that there was a correlation between MIF and migration or invasion of tumors. Epithelial mesenchymal transition (EMT) is the basis for tumor invasion and migration. Therefore, this study utilized MFI to treat cervical carcinoma Hela cells, and the mechanism of EMT was also further analyzed. Cervical carcinoma Hela cells were transfected with pFenesil MIF siRNA plasmids, following by real-time fluorescent quantitative PCR to detect MIF levels. MTT assay was then utilized for evaluate the proliferative activity of Hela cells after transfection. The cell invasion and migration were examined. The expression of E-cadherin and Vimentin were also detected. The results indicated that the MIF was positively expressed in Hela cells, whose MIF mRNA level was increased after the transfection ( $P < 0.05$ ). Compared to the control or blank group, the transfected group had elevated proliferative activity with elongated incubation time ( $P < 0.05$ ). Both invasion and migration functions of transfected cells were significantly potentiated ( $P < 0.05$ ) compared to the control or blank group. E-cadherin expression level was also decreased in experimental group. MIF was also expressed in cervical carcinoma Hela cells. Elevated MIF level could facilitate the cell invasion and migration, and elevate the Vimentin and decrease E-cadherin expression, thus facilitating EMT.

**Keywords:** Macrophage migration inhibitory factor, cervical carcinoma, Hela cells, epithelial-mesenchymal transition

## Introduction

Cervical carcinoma is one malignant tumor that severely affects the female's health. It has become the second popular malignant tumor in women [1], and only next to breast cancer, plus younger age of onset [2]. Invasion and metastasis are standards for malignancy of cancer, and are triggered by various factors and steps, as it usually takes long time period from precancerous lesion to invasive cancer [3]. Epithelial mesenchymal transition (EMT) existed in epithelial derived malignant tumor, and mainly in those tumor cells originated from endothelial and transformed into mesenchymal-like features. Certain specific markers, such as epithelial marker E-cadherin, have rapidly decreased expression level, while the mesenchymal mark-

er, Vimentin, had significantly increased expression level. These factors significantly potentiated motility, invasiveness and metastatic potency of malignant tumor cells [4, 5]. Previous study [5] has suggested aggression and metastasis features of malignant tumors. At the early phase, tumor cells have already showed several signs of EMT. The distal metastasis could be effectively inhibited if one can reverse the occurrence or progression of EMT during tumor treatment [6]. Macrophage migration inhibitory factor (MIF) is a kind of cytokine with pluripotent biological functions, and plays an important role in tumor occurrence and progression [7]. This study selected cervical carcinoma Hela cells as *in vitro* model, on which MIF was added for intervention, following by Transwell assay to detect cell migration/invasion ability, and

**Table 1.** Primer for MIF

Target gene	Primer sequence	Temp (°C)
MIF	5'-CACCATGAAGCCTACTGTGTTCC-3' 5'-TTAAACCATTCCGGCAGCAGCGG-3'	60
GAPDH	5'-GCCAAGGTCATCCATGACAA CTTTGG-3' 5'-GCCTGCTCACCCACCTTCTTG ATGTC-3'	60

**Table 2.** MIF mRNA expression in cervical carcinoma Hela cells

Group	MIF mRNA
Transfection	1.045±0.064*#
Control	0.137±0.032
Empty plasmid	0.106±0.023

Note: \*, P<0.05 compared to control group; #, P<0.05 compared to empty plasmid group.

western blotting for cell expression level of E-cadherin and Vimentin.

## Materials and methods

### Cell culture

Hela cells were purchased from Baili Biotech (China) and cultured in RPMI1640 medium, by using an incubation chamber at 37°C with 5% CO<sub>2</sub>.

### MIF transfection on Hela cells

Hela cells at log-phase were transfected with pGenesil MIF siRNA or pGenesil empty plasmids, following with the manual instruction of Lipofectamine™ 2000.

### Real-time fluorescent quantitative PCR for MIF mRNA expression in Hela cells

Total of 200 ng RNA was extracted 48 h after following TRIzol instruction. cDNA was then synthesized based on polyA tails of mRNA. PCR amplification was then performed by using cDNA as the template with specific primers (**Table 1**) under the following conditions: 95°C for 30 s, followed by 40 cycles each containing 95°C for 5 s and 60°C for 30 s.

### Proliferative activity of Hela cells by MTT assay

After 24 h, 48 h or 72 h of transfection, cell proliferative activity was measured by adding 5 mg/mL MTT solution for 4 h incubation. After quenching, DMSO was added into each well

(150 µL per well). Absorbance values of each well at 570 nm were measured 10 min later.

### Transwell assay for invasion and migration of Hela cells

Matrigel was added into Transwell chamber, which was hydrated by serum-free medium under 37°C for 1 h. Transfected cells were seeded into the upper chamber of Transwell apparatus, while RPMI1640 medium was added into the lower chamber. Giemsa dye was used for cell stain.

### Western blotting for E-cadherin and vimentin protein expression

Cells at post-log phase were collected for extracting total proteins, which were separated by 8% SDS-PAGE. After electrophoresis, the membrane was blocked, incubated with primary antibody dilutions for 30 min (1:200 for E-cadherin and Vimentin; 1:500 for β-actin; Santa Cruz, USA), and secondary antibody dilution (1:2000) for 1 h. The membrane was then developed in substrate A and B, followed by the analysis of optical density values.

### Statistical analysis

SPSS17.0 software was used for data processing, and expressed as mean ± standard deviation (SD). Enumeration data were compared by chi-square test, while analysis of variance (ANOVA) was employed for compare means across groups. Student t-test was used for comparing means among groups. A statistical significance was defined when P<0.05.

## Results

### MIF mRNA expression of Hela cells after transfection

The measurement of MIF mRNA showed positive expression in Hela cells. The transfection of MIF plasmid significantly increased the mRNA levels compared to control or empty plasmid transfection group (P<0.05). No significant difference was discovered between control and empty group (P>0.05, **Table 2**).

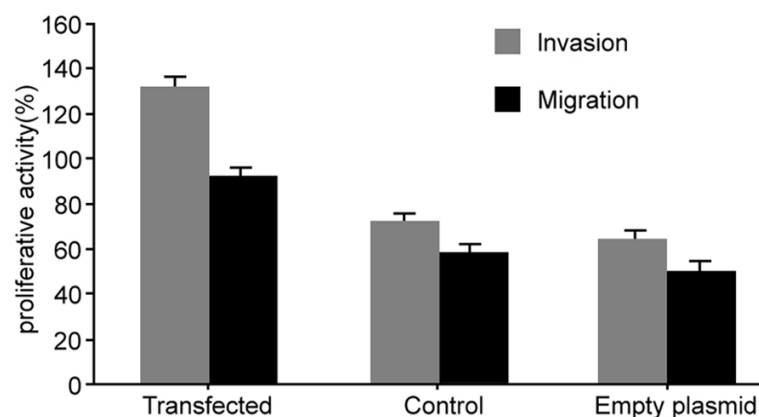
### Proliferative activity of Hela cells

The proliferative activity of cells after transfection was gradually increased with elongated incubation time (24 h, 48 h and 72 h), as signifi-

**Table 3.** Proliferative activity of Hela cells after transfection

Group	12 h	24 h	48 h
Transfected	59.92±1.12*.#	78.79±2.39*.#	88.63±2.71*.#
Control	25.23±8.34	39.72±5.74	45.95±4.21
Empty plasmid	22.38±7.24	36.29±4.35	43.75±2.58

Note: \*, P<0.05 compared to control group; #, P<0.05 compared to empty plasmid group.



**Figure 1.** Invasion and migration of Hela cells after transfection. \*, P<0.05 compared to control group; #, P<0.05 compared to empty plasmid group.

cantly higher compared to the control or empty plasmid groups (P<0.05, **Table 3**).

#### *Migration and invasion of Hela cells after transfection*

The detection of cell invasion and migration found significantly enhanced potency in experimental cells as compared to control group (P<0.05, **Figures 1 and 2**).

#### *Expression of E-cadherin and vimentin proteins in Hela cells*

Western blotting was employed to test expressions of E-cadherin and Vimentin proteins in Hela cells after transfection. The results showed significantly decreased E-cadherin expression and elevated Vimentin expression (P<0.05, **Table 4; Figure 3**).

#### **Discussion**

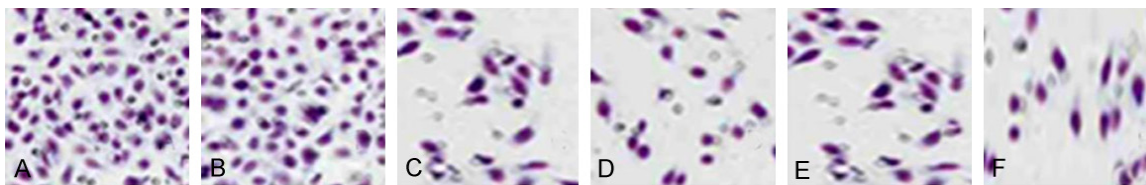
Cervical cancer is one common malignant tumor in women, with the younger onset age and increasing incidence. It is the second popular malignant tumor in females, only next to breast carcinoma, and thus severely affects

women health [8]. The treatment for cervical cancer includes surgery, chemo- and radio-therapy [9]. There are various factors affecting the prognosis of cervical cancer, including clinical stage, vascular invasion/mesenchymal infiltration and histology grade [10]. Recently, with the development of modern molecular biology, further research has been performed searching for tumor related factors at molecular level. Current opinions agree that the pathogenesis of cervical cancer is under the direction of multiple genes inside our body [11]. Bloom and Bennett identified MIF as one cytokine during the research for late onset hypersensitive reaction. MIF can inhibit the motility of macrophage, and is one cytokine with T lymphocyte origin that possibly partici-

pates in tumor related immune mechanism. Initial study about MIF revealed its pro-inflammatory factor nature, as it can activate various acute and chronic inflammation by cascade signal transductions. Ohkawara *et al.* reported high expression level of MIF in tissues with chronic inflammation, such as hepatitis, gastritis and pancreatitis [12]. Later researches, however, reported that the MIF is one barrier between inflammation and malignant tumors. MIF is produced by activated T cells, and can affect cell mitosis, induce malignant transformation, thus accelerating tumor occurrence and progression [13]. Specifically, MIF could inhibit p53 gene expression, induce cell apoptosis, and facilitate tumor growth. The inhibitory role of p53 gene expression by MIF presents as chronic inflammation, such as gastritis or hepatitis, which may eventually develop into malignant tumors [14]. Therefore, this study analyzed the correlation between MIF and EMT in cervical carcinoma Hela cells, and related mechanisms.

In this study, we utilized pGenesil MIF siRNA plasmid to transfect Hela cells, whose MIF mRNA level was found to be up-regulated, sug-

## MIF and tumor invasion



**Figure 2.** Invasion and migration of HeLa cells after transfection. A. Invasion of transfected cells; B. Migration of transfected cells; C. Invasion of control cells; D. Migration of control cells; E. Invasion of empty cells; F. Migration of empty cells.

**Table 4.** Expression of E-cadherin and vimentin proteins in HeLa cells after transfection

Group	E-cadherin	Vimentin
Experiment	0.46±0.32*.#	1.08±0.87*.#
Control	1.23±1.01	0.61±0.17
Empty plasmid	1.31±0.09	0.58±0.19

Note: \*, P<0.05 compared to control group; #, P<0.05 compared to empty plasmid group.



**Figure 3.** Western blotting bands of E-cadherin and Vimentin proteins.

gesting that the MIF exists in the cervical carcinoma cells. Previous study [15] revealed abundant expression of liver carcinoma, esophageal cancer, pulmonary carcinoma, and head-neck squamous carcinoma, plus the close correlation with lymphatic metastasis of various tumors. Clinical study has been performed to find the correlation between MIF expression in cervical carcinoma with clinical stage, pathology differentiation, lymph node metastasis and tumor size. The expression of MIF can reflect prognosis of cancer, as it is significantly different in endothelial carcinoma tissues compared to normal cervical tissues. These findings suggest that MIF could inhibit apoptosis of cervical cells, and elevate proliferative activity, therefore, endowing malignant transformation potency of cervical cells [16].

By detecting proliferative activity, this study discovered the significantly elevated proliferation of transfected HeLa cells at 24 h, 48 h and 72

h, accompanying with the remarkably enhanced invasion and migration abilities. These results suggested that abundant expression of MIF could facilitate invasion and migration potency of HeLa cells. Previous study has suggested the involvement of MIF in invasion, metastasis and prognosis of malignant tumors, indicating it as one potential tumor facilitating cytokine via inducing EMT, and may enhance invasiveness of tumors, such as pancreatic carcinoma [17]. Other reports also suggested the abundant production of MIF by malignant tumor cells via cascade reaction to activate ERK1/2 protein kinase and oncogenic factor Ras, therefore, modulating neo-angiogenesis and tumor invasion [18]. Moreover, MIF can initiate continuous proliferation of neural stem/progenitor cells, modulate their cell cycle, and facilitate the growth, invasion and metastasis [19]. In another study, pEGFP-N1-MIF was transfected into SiHa cells, whose *in vitro* proliferation and motility functions were significantly increased [20], as consistent with this study.

A complete EMT process mainly consists of three stages: Firstly malignant tumor cells must acquire the morphological feature of fibroblast. Then those epithelial markers had decreased expression level with signal transduction pathway, accompanied with up-regulation of mesenchymal markers. Eventually, extra-cellular matrix was gradually degraded to export from cytoplasm toward focal tissues or even distal metastasis [21-23]. E-cadherin is one Ca<sup>2+</sup>-dependent transmembrane glycoprotein molecule in body epithelial tissues, and exerts important roles in intra-cellular connection, therefore, which is considered as a specific epithelial marker. Vimentin is one mesenchymal-derived protein marker. Its abnormal expression could induce alternation of cytoskeletal proteins, and facilitate cell migration [24, 25]. In this study, we observed significantly lower

E-cadherin and higher Vimentin proteins in transfected Hela cells, suggesting that MIF over-expression could facilitate Vimentin expression. When suppressing the E-cadherin expression in Hela cells, and decreasing intracellular adhesion potency of tumors cells, the EMT was occurred.

### Conclusion

MIF was positively expressed in cervical carcinoma Hela cells. The elevation of MIF level could facilitate its invasion, migration, and EMT occurrence. With the advancement of molecular and genetic study, increasing number of tumor related cytokines have been identified for potential diagnosis and treatment of cancers. In clinical practice, effective inhibition of MIF thus may provide new strategy for inhibiting cervical cancer invasion/metastasis. Therefore, improving treatment efficacy, although detailed mechanism still requires further studies for elaboration.

### Acknowledgements

This work was supported by National Natural Science Foundation of China (NO. 81402257).

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Xueqi Fu, Edmond H. Fischer Signal Transduction Laboratory, School of Life Sciences, Jilin University, Changchun 130012, Jilin, PR China. Tel: +86-431-81969061; Fax: +86-431-81969061; E-mail: xueqifue3e@163.com

### References

- [1] Lao G, Liu P, Wu Q, Zhang W, Liu Y, Yang L, Ma C. Mir-155 promotes cervical cancer cell proliferation through suppression of its target gene LKB1. *Tumour Biol* 2014; 35: 11933-8.
- [2] Yoshinaga K, Ito K, Moriya T, Nagase S, Takano T, Niikura H, Sasano H, Yaegashi N, Sato Y. Roles of intrinsic angiogenesis inhibitor, vasohibin, in cervical carcinomas. *Cancer Sci* 2011; 102: 446-51.
- [3] Bifulco C, McDaniel K, Leng L, Bucala R. Tumor growth-promoting properties of macrophage migration inhibitory factor. *Curr Pharm Des* 2008; 14: 3790-801.
- [4] Copple BL. Hypoxia stimulates hepatocyte epithelial to mesenchymal transition by hypoxia-inducible factor and transforming growth factor-beta-dependent mechanisms. *Liver Int* 2010; 30: 669-82.
- [5] Fernebro E, Bendahl PO, Dictor M, Persson A, Fernö M, Nilbert M. Immunohistochemical patterns in rectal cancer: application of tissue microarray with prognostic correlations. *Int J Cancer* 2004; 111: 921-8.
- [6] Cannito S, Novo E, Compagnone A, Valfrè di Bonzo L, Busletta C, Zamara E, Paternostro C, Povero D, Bandino A, Bozzo F, Cravanzola C, Bravoco V, Colombatto S, Parola M. Redox mechanisms switch on hypoxia-dependent epithelial-mesenchymal transition in cancer cells. *Carcinogenesis* 2008; 29: 2267-78.
- [7] Rendon BE, Willer SS, Zundel W, Mitchell RA. Mechanisms of macrophage migration inhibitory factor (MIF)-dependent tumor microenvironmental adaptation. *Exp Mol Pathol* 2009; 86: 180-5.
- [8] Conroy H, Mawhinney L, Donnelly SC. Inflammation and cancer: macrophage migration inhibitory factor (MIF)--the potential missing link. *QJM* 2010; 103: 831-6.
- [9] Bansal N, Herzog TJ, Shaw RE, Burke WM, Deutsch I, Wright JD. Primary therapy for early-stage cervical cancer: radical hysterectomy vs radiation. *Am J Obstet Gynecol* 2009; 201: 485, e1-9.
- [10] Kosmas C, Mylonakis N, Tsakonas G, Vorgias G, Karvounis N, Tsavaris N, Daladimos T, Kalinoglou N, Malamos N, Akrivos T, Karabelis A. Evaluation of the paclitaxel-ifosfamide-cisplatin (TIP) combination in relapsed and/or metastatic cervical cancer. *Br J Cancer* 2009; 101: 1059-65.
- [11] Guo Y, Hou J, Luo Y, Wang D. Functional disruption of macrophage migration inhibitory factor (MIF) suppresses proliferation of human H460 lung cancer cells by caspase-dependent apoptosis. *Cancer Cell Int* 2013; 13: 28.
- [12] Ohkawara T, Nishihira J, Takeda H, Asaka M, Sugiyama T. Pathophysiological roles of macrophage migration inhibitory factor in gastrointestinal, hepatic, and pancreatic disorders. *J Gastroenterol* 2005; 40: 117-22.
- [13] Roger T, David J, Glauser MP, Calandra T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* 2001; 414: 920-4.
- [14] Bach JP, Deuster O, Balzer-Geldsetzer M, Meyer B, Dodel R, Bacher M. The role of macrophage inhibitory factor in tumorigenesis and central nervous system tumors. *Cancer* 2009; 115: 2031-40.
- [15] Mawhinney L, Armstrong ME, O'Reilly C, Bucala R, Leng L, Fingerle-Rowson G, Fayne D, Keane MP, Tynan A, Maher L, Cooke G, Lloyd D, Conroy H, Donnelly SC. Macrophage migration inhibi-

## MIF and tumor invasion

- tory factor (MIF) enzymatic activity and lung cancer. *Mol Med* 2014; 20: 729-35.
- [16] Liu N, Jiang N, Guo R, Jiang W, He QM, Xu YF, Li YQ, Tang LL, Mao YP, Sun Y, Ma J. MiR-451 inhibits cell growth and invasion by targeting MIF and is associated with survival in nasopharyngeal carcinoma. *Mol Cancer* 2013; 12: 123.
- [17] Funamizu N, Hu C, Lacy C, Schetter A, Zhang G, He P, Gaedcke J, Ghadimi MB, Ried T, Yfantis HG, Lee DH, Subleski J, Chan T, Weiss JM, Back TC, Yanaga K, Hanna N, Alexander HR, Maitra A, Hussain SP. Macrophage migration inhibitory factor induces epithelial to mesenchymal transition, enhances tumor aggressiveness and predicts clinical outcome in resected pancreatic ductal adenocarcinoma. *Int J Cancer* 2013; 132: 785-94.
- [18] Grieb G, Merk M, Bernhagen J, Bucala R. Macrophage migration inhibitory factor (MIF): a promising biomarker. *Drug News Perspect* 2010; 23: 257-64.
- [19] He XX, Yang J, Ding YW, Liu W, Shen QY, Xia HH. Increased epithelial and serum expression of macrophage migration inhibitory factor (MIF) in gastric cancer: potential role of MIF in gastric carcinogenesis. *Gut* 2006; 55: 797-802.
- [20] Huang XH, Jian WH, Wu ZF, Zhao J, Wang H, Li W, Xia JT. Small interfering RNA (siRNA)-mediated knockdown of macrophage migration inhibitory factor (MIF) suppressed cyclin D1 expression and hepatocellular carcinoma cell proliferation. *Oncotarget* 2014; 5: 5570-80.
- [21] Grünert S, Jechlinger M, Beug H. Diverse cellular and molecular mechanisms contribute to epithelial plasticity and metastasis. *Nat Rev Mol Cell Biol* 2003; 4: 657-65.
- [22] Qureshi R, Arora H, Rizvi MA. EMT in cervical cancer: its role in tumour progression and response to therapy. *Cancer Lett* 2015; 356: 321-31.
- [23] Lee MY, Shen MR. Epithelial-mesenchymal transition in cervical carcinoma. *Am J Transl Res* 2012; 4: 1-13.
- [24] Liew PL, Hsu CS, Liu WM, Lee YC, Lee YC, Chen CL. Prognostic and predictive values of Nrf2, Keap1, p16 and E-cadherin expression in ovarian epithelial carcinoma. *Int J Clin Exp Pathol* 2015; 8: 5642-9.
- [25] Mrozik KM, Cheong CM, Hewett D, Chow AW, Blaschuk OW, Zannettino AC, Vandyke K. Therapeutic targeting of N-cadherin is an effective treatment for multiple myeloma. *Br J Haematol* 2015; 171: 387-99.