EJGO European Journal of Gynaecological Oncology

Correlations of leukemia inhibitory factor and macrophage migration inhibitory factor with endometrial carcinoma

W. Xiao*, O. Jin*, S. Han, R. Nie, L. Zhu, X. Gao, L. Li

Department of Obstetrics and Gynecology, Forth Affiliated Hospital of Harbin Medical University, Harbin (China)

Summary

Objective: To investigate the correlations of leukemia inhibitory factor (LIF) and macrophage migration inhibitory factor (MIF) with endometrial carcinoma. *Materials and Methods:* The study included 113 endometrial specimens from the Fourth Affiliated Hospital of Harbin Medical University, collected from May 2006 to October 2008, classified into normal endometrium, simple hyperplasia, complex hyperplasia, atypical hyperplasia, and endometrial carcinoma. The LIF and MIF expression of all 113 specimens was detected with immunohistochemistrical (IHC) method. *Results:* The MIF expression in hyperplastic endometrium and endometrial carcinoma increased significantly as compared with that in normal endometrium ($p < 0.05$ and $p < 0.001$, respectively), and its expression in endometrial carcinoma was also remarkably higher than that in hyperplastic endometrium ($p < 0.001$). The expressions of LIF in atypical hyperplasia and endometrial carcinoma were also significantly higher than that in the normal endometrium ($p < 0.05$), but it is not obviously higher in simple hyperplasia and complex hyperplasia than in the normal endometrium ($p > 0.05$). Furthermore, the expression of LIF showed no statistical difference between hyperplastic endometrium and endometrial carcinoma. *Conclusion:* It could be speculated that MIF may be correlated with the occurrence of endometrial carcinoma. However, whether LIF also has a correlation with the occurrence of endometrial carcinoma still cannot be presumed.

Key words: Endometrial carcinoma; LIF; MIF.

Introduction

Endometrial carcinoma is one of the common malignant tumors in female genital tract, with increased morbidity and mortality in recent years. According to dependency on sex hormone, endometrial carcinoma is divided into type I and type II [1], and the former is estrogen-dependent tumor, accounting for 80%-85% of endometrial carcinoma. The carcinogenesis mechanism of endometrial carcinoma on molecular level is not clear. At present, it was considered to be associated with the abnormal expression of oncogene, tumor suppressor gene and DNA repair gene. Detection of tumor suppressor gene, oncogene or cytokine helps in analyzing the properties of endometrial carcinoma and understanding the relationship between tumor and tissue differentiation. This has great significance and value for further revealing the molecular biological mechanism of endometrial carcinoma and exploring new treatment strategies including combined gene therapy [2].

Oncogene C-erbB-2, also known as neu or HER-2, was discovered in 1981. It plays an important role in cell signal transduction, and is an important regulator in cell growth, differentiation, and survival. The overexpression of C-erbB-2 has important regulatory effect on tumor formation and growth process, and has a certain relationship with some biological behaviors of tumor. It exists in breast cancer, ovarian cancer, and renal cell carcinoma [3]. Macrophage migration inhibitory factor (MIF) is a type of multifunctional cytokine, and is an important regulatory factor in inflammatory and immune responses. MIF is related with growth factor-dependent cell proliferation, cell cycle change, angiogenesis, and tumor formation. At the same time, it directly influences the division of normal cells and oncogene-induced malignant transformation. MIF can also regulate immune response, inhibit function of tumor suppressor gene, and promote tumor angiogenesis, thus promoting the occurrence and development of tumor from multiple levels. In recent years, MIF has been a hotspot in tumor research [4]. At present, the correlations of C-erbB-2 and MIF with endometrial carcinoma have not been reported. Elucidation of these problems has important significance for early detection and treatment of endometrial carcinoma.

Endometrial hyperplasia is the most common cause of dysfunctional uterine bleeding in women of childbearing age. Part of endometrial hyperplasia lesions are precancerous lesions of endometrial carcinoma; thus a precise diagnosis and prompt treatment will be of great significance in preventing the progression of the disease [5]. Growth factors are a class of polypeptide substances, which widely exist in various tissues of the body. Endometrial hyperplasia is closely associated with the dysregulation of growth factors. Various kinds of growth factors and their related peptides, combining with various components of endometrial and the hormones secreted by

*Contributed equally to this work.

Revised manuscript accepted for publication December 30, 2013

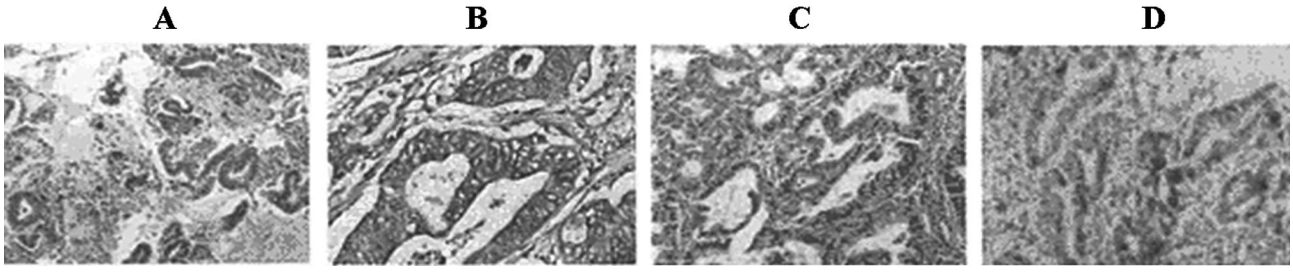

Figure 1. — Expressions of MIF and LIF in normal endometrium and endometrial carcinoma. A: Expression of MIF in normal endometrium; B: Expression of MIF in endometrial carcinoma; C: Expression of LIF in normal endometrium; D: Expression of LIF in endometrial carcinoma.

ovarian cells and body, constitute a complicated network to regulate the orderly cyclical changes of endometrium and maintain the stability of the endometrium [6]. Recently, the relationship between the growth factors and endometrium has drawn wide attentions. Thus, it is very important to carry out in-depth studies of the effects of growth factors on endometrial hyperplasia disorders and diseases. Leukemia inhibitory factor (LIF) and MIF have extensive biological effects including promoting cell proliferation and differentiation [7]. Recently, the relationship between growth factors and endometrial carcinoma has attracted more and more attentions. In this study, the correlations of LIF and MIF with endometrial carcinoma were investigated, in order to obtain new progress in studying the mechanism of endometrial hyperplasia.

Materials and Methods

Specimens

The study included 113 paraffin-embedded specimens from the department of Pathology, submitted from May 2006 to October 2008, collected for detection of LIF and MIF. All the 113 specimens were classified pathologically into normal endometrium (n=13), hyperplastic endometrium [(n=55, including simple hyperplasia (n=17), complex hyperplasia (n=18), atypical hyperplasia (n=20)] and endometrial carcinoma (n=45). All the subjects had not been treated with any hormone replacement therapy, chemotherapy or radiotherapy before they underwent curettage or surgery. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Harbin Medical University. Written informed consent was obtained from all participants. Specimens of endometrial carcinoma were further staged according to the FIGO staging criteria into Stage I (n=14), Stage II (n=1), and Stage III (n=13). They could also be graded into G1 (n=12), G2 (n=14), and G3 (n=19).

Immunohistochemistry

Immunohistochemistry analysis of 113 endometrium specimens was carried out with SP kits. Five μm-thick slices of paraffin-embedded specimens were dewaxed to hydrophile according to the routine procedure. Then the slides were incubated with 3% H_2O_2 in dark at room temperature for 15 minutes, followed by microwave heating antigen retrieval in 0.01 M citrate buffer (pH 6.0,) for 20 minutes. After cooling at room temperature, non-specific binding was blocked with normal goat serum for 30 minutes. Incubation with rabbit anti-human MIF or LIF was carried out at 4°C overnight before the slides were treated with SP kit according to the manufacturer's instruction, followed by sequential incubation with horseradish peroxidase-streptavidin and the peroxidase substrate 3'-diaminobenzidine. Nucleus was counterstained with hematoxylin.

Assessment of the results

Results were analyzed semi-quantitatively. The degree of the positive expression of LIF and MIF was the sum of the number and the intensity of the positive cells. No brown granules appearing in the endometrial tissue were regarded as negative (-); 1% to 5% of the endometrial cells appearing brown were regarded as weakly positive (+/-); 6% to 15% of the positive endometrial cells was regarded as moderate positive (+), and more than 16% of the positive endometrial cells were regarded as strong positive (++).

Statistical analysis

Results were analyzed using Chi-square test and $p < 0.05$ was considered as significant.

Results

Expression of MIF

MIF primarily expresses in the cytoplasm of glandular epithelial cells and some mesenchymal cells. Sometimes the nucleus of glandular epithelial cells may be stained. Thus, the cytoplasm of glandular epithelial cells or mesenchymal cells appearing brown were considered to be positive (Figure 1A, B). According to the pathological classification, 113 specimens were classified into normal endometrium, simple hyperplasia, complex hyperplasia, atypical hyperplasia, and endometrial carcinoma. As can be seen in Table 1, among the 45 cases of endometrial carcinoma, there were 42 MIF-positive cases. Its positive rate (91.11%) was obviously higher than that in the normal endometrium group and hyperplastic endometrium group (23.07% and 65.45%, $p < 0.001$ and $p < 0.05$, respectively). As compared with the positive rate in the normal endometrium group (23.07%), the MIF-positive rates in both the complex hyperplasia group (66.67%) and the atypical hyperplasia group (75.00%) increased remarkably ($p < 0.05$), but there was no statistical difference in the simple hyperplasia group ($p > 0.05$). As far as the hyperplastic endometrium was concerned, no significant difference existed in the MIF-positive rates of the three groups ($p > 0.05$) (Table 1). If grading endometrial carcinoma into G1, G2, and G3, the MIF-positive rate was highest in the G3, but there was no statistical significance between G1, G2, and G3 (Table 2).

Table 1. — *Expression of MIF in the endometrium of the different groups.*

Group	Total cases	Intensity of the positive expression				Positive rate (%)
		-	+/-	+	++	
Normal endometrium	13	10	2	1	0	23.07
Simple hyperplasia	17	8	6	3	0	52.94
Complex hyperplasia	18	6	5	5	2	66.67
Atypical hyperplasia	20	5	4	5	6	75.00
Endometrial carcinoma	45	3	9	15	18	91.11

Table 2. — *Expression of MIF in endometrial carcinoma.*

Grades	Total cases	Intensity of the positive expression				Positive rate (%)
		-	+/-	+	++	
G1	12	1	3	4	4	91.66
G2	14	1	3	6	4	92.86
G3	19	1	2	8	8	94.74

Table 3. — *Expression of LIF in the endometrium of the different groups.*

Group	Total cases	Intensity of the positive expression				Positive rate (%)
		-	+/-	+	++	
Normal endometrium	13	9	2	2	0	30.76
Simple hyperplasia	17	8	7	2	0	52.94
Complex hyperplasia	18	7	5	4	2	61.10
Atypical hyperplasia	20	5	6	6	3	70.00
Endometrial carcinoma	45	14	15	13	3	68.88

Table 4. — *Expression of LIF in endometrial carcinoma.*

Grades	Total cases	Intensity of the positive expression				Positive rate (%)
		-	+/-	+	++	
G1	12	5	2	2	3	58.33
G2	14	4	3	4	6	71.43
G3	19	5	3	7	8	73.68

Expression of LIF

LIF also expresses mainly in the cytoplasm of glandular epithelial cells and some mesenchymal cells. Thus, the cytoplasm of glandular epithelial cells or mesenchymal cells appearing brown was considered to be positive (Figure 1C, D). There were 31/45 cases of endometrial carcinoma, 30/55 cases of hyperplastic endometrium and 4/13 cases of normal endometrium expressing LIF (Table 3). The positive rate of LIF in the normal endometrium group (30.76%) was remarkably lower than that in the atypical hyperplasia group (70.00%) and the endometrial carcinoma group (68.88%) ($p < 0.05$), but it showed no statistical difference from that in the simple hyperplasia group (52.94%) or the complex hyperplasia group (61.10%). The positive rates of LIF in the three hyperplasia groups showed no significant difference between each other and were not different significantly from that in the endometrial carcinoma group ($p > 0.05$) (Table 3). In the different grades of endometrial carcinoma, the LIF positive rate increased with the grade, but showed no statistical significance between the three grades (Table 4).

Discussion

The main purpose of this study was to investigate the correlations of LIF and MIF with endometrial carcinoma. Results of immunohistochemical experiments show that the positive rate and intensity of MIF and LIF expression increase with the elevation of histological grade of endometrial carcinoma, but the differences are not statistically significant ($p > 0.05$). This conforms to the authors' original experimental expectation and assumption, and is consistent with existing viewpoints and results of other researchers. LIF is a cytokine with multiple biological functions. Currently, its biological effects in reproductive medicine have been a hot point in China [6-8]. LIF expresses in the endometrial glandular epithelial cells and increases in the secretory phase. In the reproductive cycle, LIF is associated with the uterine function and the regulation of endometrial growth. Endometrial glandular epithelial cells are the main production of LIF, which changes cyclically with the menstrual cycle and reaches a peak in the secretory phase, especially in the mid-secretory phase, suggesting that the expression of LIF may be directly or indirectly controlled by ovarian hormones [9, 10]. Some studies showed that adding exogenous LIF to the cultured sheep endometrial cells can stimulate cell proliferation in a dose-dependent manner, indicating that LIF may relate to the regulation of endometrial growth, self-renewal, and uterine functions [11]. In this study, the positive rate of LIF in endometrial cancer was only 70%, which may be explained by several reasons. Firstly, in the past, despite that blood LIF changes cyclically with the menstrual cycle has usually been described, no detection of LIF in tissues has virtually been reported. LIF may express weakly in normal tissue and thereby the positive rate of LIF in endometrial cancer cannot be higher than that in the normal endometrium [12]. Secondly, in the hyperplastic conditions, LIF is regulated abnormally by hormones, and thus it may be reduced in the endometrial tissues. It was reported that the expression of LIF shows no significant difference in various types of endometrial carcinoma [13]. Thus, the roles of LIF in the endometrial carcinoma cannot be verified by the results obtained in this study.

MIF is a protein comprising 115 amino acids. It is a multifunctional cytokine controlled by the hypothalamic-pituitary. Besides the activated T lymphocytes, LIF also can be produced by numerous tissue cells. It can promote the occurrence of various inflammatory diseases. Some scholars believe that MIF inhibits the cytotoxicity of NK cell against tumor cells via the inactivation of p53 and induces neovascularization for tumor formation, and thus plays an important role in the occurrence and development of tumor. With the effects of MIF, tumor cells and macrophages are involved in tumor angiogenesis, and thereby promoting tumor growth and metastasis [14]. It was also reported that MIF can stimulate macrophages to produce

several kinds of inflammatory mediators, which could kill tumor cells [15]. Thus, MIF may play a dual role in the occurrence and development of tumor. In the researches about the relationship between MIF and endometriosis, it has been suggested that MIF limits macrophages in the ectopic foci and enhances the secretory function of macrophages to produce the growth factor and angiogenesis factor. Furthermore, MIF can directly stimulate the proliferation of vascular endothelial cell, promoting the formation of blood vessel, and thus being conducive to the maintenance and growth of ectopic endometrium [16]. Apoptosis plays an important role in maintaining the normal cyclical change of endometrial cells and the apoptosis of endometrium is regulated by apoptosis-related genes and associated with the function of sex hormone and its receptor, cytokines, and enzymes. Apoptosis disorders may be one of the causes of endometrial carcinoma [17]. MIF can inhibit apoptosis through some ways, such as inhibiting the p53 gene [18]. In this study, the positive rate of MIF reached more than 90%, indicating that there is a close relationship between MIF and endometrial cancer. However, it acts by which means still requires further study. The positive rates of MIF were high in atypical hyperplasia and endometrial carcinoma, indicating that MIF not only is related to the abnormal endometrial hyperplasia, but also plays an important role in the process of cellular malignant change [19]. A study by Levy *et al.* showed that MIF also expresses highly in ovarian cancer cells [20]. In recent years, with the development of genomics and molecular biological technology, it has been realized that the formation and development of malignant tumors is a process with multi-step and multiple genes involved. More and more tumor-associated factors have been discovered, and conducted to many in-depth researches. Therefore, to identify a key factor in all aspects of tumor progression has a very important practical significance. It can facilitate the detection of a certain type of tumor and quantify the indicators, and thus provides a meaningful reference to the diagnosis of cancer, the differential diagnosis, prognostic assessment, and the selection of an individualized treatment. In-depth study of LIF and MIF is expected to get some new developments in the research field of the mechanism of endometrial carcinoma.

Acknowledgements

This work was supported by the Specialized Research Fund for the Natural Science Foundation of Heilongjiang Province, People's Republic of China (nr. H201429).

References

[1] Hagemann T., Robinson S.C., Thompson R.G., Charles K., Kulbe H., Balkwill F.R.: "Ovarian cancer cell-derived migration inhibitory factor enhances tumor growth progression and angiogenesis". *Mol. Cancer Ther.*, 2007, *6*, 1993.

[2] Mutter G.L.: "Endometrial intraepithelial neoplasia (EIN): will it bring order to chaos? The Endometrial Collaborative Group". *Gynecol. Oncol.*, 2000, *76*, 287.

[3] Tsudo T., Harada T., Iwabe T., Tanikawa M., Nagano Y., Ito M., *et al.*: "Altered gene expression and secretion of interleukin-6 in stromal cells derived from endometriotic tissues". *Fertil. Steril.*, 2000, *73*, 205.

[4] Piccinni M.P.: "T cells in normal pregnancy and recurrent pregnancy loss". *Reprod. Biomed. Online*, 2006, *13*, 840.

[5] Cameron S.T., Critchley H.O., Buckley C.H., Kelly R.W., Baird D.T.: "Effect of two antiprogestins (mifepristone and on apristone) on endometrial factors of potential importance for implantation". *Fertil. Steril.*, 2007, *67*, 1046.

[6] Dabbous M.K., North S.M., Haney L., Tipton D.A., Nicolson G.L.: "Effects of mast cell-macrophage interactions on the production of collagenolytic enzymes by metastatic tumor cells and tumor-derived and stromal fibroblasts". *Clin. Exp. Metastasis*, 1995, *13*, 33.

[7] Patel A.V., Feiqelson H.S., Talbot J.T., McCullough M.L., Rodriguez C., Patel R.C., *et al.*: "The role of body weight in the relationship between physical activity and endometrial cancer: results from a large cohort of US women". *Int. J. Cancer*, 2008, *123*, 1877.

[8] Menon U., Skates S.J., Lewis S., Rosenthal A.N., Rufford B., Sibley K., *et al.*: "Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer". *J. Clin. Oncol.*, 2005, *23*, 7919.

[9] Agarnal R., Whang D.H., Alvero A.B., Visintin I., Lai Y., Segal E.A., *et al.*: "Macrophage migration inhibitory factor expression in ovarian cancer". *Am. J. Obstet. Gynecol.*, 2007, *196*, 348.e1.

[10] Danforth K.N., Tworoger S.S., Hecht J.L., Rosner B.A., Colditz G.A., Hankinson S.E.: "Breastfeeding and risk of ovarian cancer in two prospective cohorts". *Cancer Causes Control*, 2007, *18*, 517. Epub 2007 Apr 21.

[11] Wright T.C. Jr., Massad L.S., Dunton C.J., Spitzer M., Wilkinson E.J., Solomon D.: "2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ". *Am. J. Obstet. Gynecol.*, 2007, *197*, 340.

[12] Mahutte N.G., Matalliotakis L.M., Goumenou A.G., Koumantakis G.E., Vassiliadis S., Arici A.: "Elevations in peritoneal fluid macrophage migration inhibitory factor are independent of the depth of invasion or stage of endometriosis". *Fertil. Steril.*, 2004, *82*, 97.

[13] Ohkawara T., Takeda H., Miyashita K., Nishiwaki M., Nakayama T., Taniguchi M., *et al.*: "Regulation of Toll-like receptor 4 expression in mouse colon by macrophage migration inhibitory factor". *Histochem. Cell. Biol.*, 2006, *125*, 575.

[14] Xia X.A., He C.Z., Li L.H., Lu P., Pu H., Ren D.J., *et al.*: "Expression of macrophage migration inhibitory factor is associated with gynecologic malignant tumor". *Obstet. Gynecol.*, 2006, *36*, 9.

[15] Beaulieu E., Green L., Elsby L., Alourfi Z., Morand E.F., Ray D.W., *et al.*: "Identification of a novel cell type - specific intronic enhancer of macrophage migration inhibitory factor (MIF) and its regulation by mithramycin". *Clin. Exp. Immunol.*, 2010, *163*, 178.

[16] Sakamoto W., Nishihira J., Fujie K., Iizuka T., Handa H., Ozaki M., *et al.*: "Effect of vitamin E on production of macrophage migration inhibitory factor (MIF) by macrophages". *Biofactors*, 1999, *10*, 139.

[17] Lobanok E.S., Mezhevikina L.M., Belianovich L.M., Petrova R.R., Vasilevich I.B., Volotkovski- I.D., et al.: "The influence of LIF (Leukemia Inhibitory Factor) on the functional status of R1 mouse embryonic stem cells". *Biomed. Khim.*, 2009, *54*, 570.

[18] Albrecht S., Schols H.A., van den Heuvel E.G., Voragen A.G., Gruppen H.: "CE - LIF - MSn profiling of oligosaccharides in human milk and feces of breast - fed babies". *Electrophoresis*, 2010, *31*, 1264.

[19] Rodat A., Couderc B., Feurer B., Couderc F.: "A new evaluation technique for the detection of impurities in purified proteins via CE with native UV - LIF". *Electrophoresis*, 2010, *31*, 396.

[20] Levy C.S., Slomiansky V., Gattelli A., Nahmod K., Pelisch F., Blaustein M., *et al.*: "Tumor necrosis factor alpha induces LIF expression through ERK1/2 activation in mammary epithelial cells". *J. Cell. Biochem.*, 2010, *110*, 857.

Address reprint requests to:
W. XIAO, M.D.
Department of Obstetrics and Gynecology,
Forth Affiliated Hospital of
Harbin Medical University,
31 Yinhang Street
Harbin 150001 (China)
e-mail: weixiaovcn@yeah.net