ORIGINAL ARTICLE: ENDOMETRIOSIS

Possible involvement of signal transducer and activator of transcription-3 in cell–cell interactions of peritoneal macrophages and endometrial stromal cells in human endometriosis

Fumiko Itoh, M.D.,[a,b] Yoshihiro Komohara, M.D., Ph.D.,[b] Kiyomi Takaishi, M.D., Ph.D.,[a] Rituo Honda, M.D., Ph.D.,[a] Hironori Tashiro, M.D., Ph.D.,[a] Satoru Kyo, M.D., Ph.D.,[c] Hidetaka Katabuchi, M.D., Ph.D.,[a] and Motohiro Takeya, M.D., Ph.D.[b]

[a] Department of Obstetrics and Gynecology and [b] Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan; and [c] Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

Objective: To investigate interactions between peritoneal macrophages and endometrial stromal cells (ESCs) involved in the development of endometriosis.
Design: Clinicopathologic and in vitro studies.
Setting: Department of Obstetrics and Gynecology and Department of Pathology, Kumamoto University Hospital.
Patient(s): Women undergoing laparoscopy or laparotomy to treat endometriosis or other benign gynecologic conditions.
Intervention(s): We collected samples of peritoneal fluid (ascites), endometrium, and endometriotic tissues. We cocultured ESCs in vitro with or without human macrophages.
Main Outcome Measure(s): Macrophage phenotypes in peritoneal fluid were determined via immunostaining. Proliferation of ESCs and activation of signal transducer and activator of transcription-3 (Stat3) in cocultures were evaluated.
Result(s): The endometriosis group had a significantly higher total number of macrophages in ascites compared with the control group, but the ratios of CD163+ alternatively activated macrophages (M2) in the two groups did not differ significantly. Coculture with M2 macrophages significantly up-regulated ESC proliferation and Stat3 activation in ESCs in vitro. Proliferation of ESCs was suppressed after Stat3 was down-regulated by small interfering RNA. Stat3 was activated in epithelial cells and ESCs in human endometriotic lesions.
Conclusion(s): Interactions between M2 macrophages and ESCs via Stat3 activation may play an important role in the development of endometriosis. (Fertil Steril® 2013;■:■–■. ©2013 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, macrophages, endometrial stromal cells, Stat3

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/itohf-stat3-macrophages-endometriosis/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received September 3, 2012; revised December 8, 2012; accepted January 21, 2013.
F.I. has nothing to disclose. Y.K. has nothing to disclose. K.T. has nothing to disclose. R.H. has nothing to disclose. H.T. has nothing to disclose. S.K. has nothing to disclose. H.K. has nothing to disclose. M.T. has nothing to disclose.
This study was supported in part by Grants-in-Aid for Scientific Research (C21592137 to H.K., 21790388 to M.T.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
F.I. and Y.K. contributed equally to this work.
Reprint requests: Yoshihiro Komohara, M.D., Ph.D., Kumamoto University, Graduate School of Medical Sciences, Department of Cell Pathology, Honjo 1-1-1, Chuou-ku, Kumamoto 860-8556, Japan (E-mail: ycomo@kumamoto-u.ac.jp).

Fertility and Sterility® Vol. ■, No. ■, ■ 2013 0015-0282/$36.00
Copyright ©2013 American Society for Reproductive Medicine, Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.fertnstert.2013.01.133

Endometriosis is a common gynecologic disorder in which growth of endometrial-like tissue occurs outside the uterine cavity (1). This disorder affects at least 5%–10% of all women of reproductive age and up to 30% of infertile women (1–3). Surgical removal of lesions and hormonal suppression are the current gold standards of therapy, but both

approaches are associated with various side effects and a high incidence of relapse (4, 5).

The importance of peritoneal macrophages in the development of endometriosis has been known for the past 3 decades (6–12). Peritoneal fluid volume is increased and the total number of macrophages is elevated in ascites from patients with peritoneal endometriosis (6–8). Several macrophage-derived cytokines, such as interleukin (IL)-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α, and transforming growth factor-β, also have elevated levels in ascites from patients with pelvic endometriosis (13–17). These cytokines stimulate endometrial stromal cells (ESCs) and are believed to be associated with endometriosis development (18–20). In addition, after macrophages are stimulated with soluble factors derived from ESCs, they secrete many factors, including IL-8, that activate ESCs (21–23). These findings indicate that interactions between macrophages and ESCs may be associated with the development of endometriosis.

A novel concept of macrophage phenotypes was recently proposed. Interferon-γ induces the classic activation of macrophages, whereas anti-inflammatory cytokines, such as IL-10, IL-4, and IL-13, stimulate alternative activation (24–26). These macrophage phenotypes are called M1 and M2, respectively. CD163 is a marker of M2 macrophages that is believed to be polarized to the alternatively activated or anti-inflammatory phenotype (24–27). M2 peritoneal macrophages were closely related to the development of endometriosis in a murine endometriosis model in that they induced angiogenesis in lesions (28).

We previously demonstrated that activation of signal transducer and activator of transcription-3 (Stat3) is involved in interactions between macrophages and ovarian cancer cells (29). Stat3 is a key signal transducer and regulator of macrophage activation associated with several oncogenic signaling pathways, such as proliferation, survival, angiogenesis, and immunosuppression (30). On the basis of all these data, we hypothesized that interactions between macrophages and ESCs may be important in the development of endometriosis and that Stat3 activation may be associated with interactions between macrophages and ESCs.

MATERIALS AND METHODS

Human Samples

Samples of ascites were collected from 25 female patients with endometriosis and a control group of 15 female patients without endometriosis (but with uterine fibroma or ovarian dermoid cyst) who were scheduled to undergo laparoscopy or laparotomy at Kumamoto University Hospital. Women who had received steroid hormone therapy within 3 months of surgery were excluded from the study. Peritoneal fluid samples were transported to the laboratory under sterile conditions and were used immediately. Endometrial samples were cut from uteri collected by laparotomy from women without endometriosis. Endometriotic lesions were obtained after surgical removal by laparotomy. All samples manifested superficial peritoneal endometriosis (red and black lesions). The women with endometriosis all had peritoneal lesions with endometriotic tissue. The presence or absence of endometriosis was confirmed visually at surgery and by histologic analysis. The extent of endometriosis was scored according to the American Society for Reproductive Medicine classification (31). The phase of the menstrual cycle was determined according to the most recent menstrual period and was confirmed by visualization of a corpus luteum or dominant follicle, or by histologic dating of the endometrium. All patients provided written informed consent for participation in the study. The Kumamoto University Hospital Review Board approved the study protocol (Project 1370).

Immunostaining of Macrophages in Peritoneal Fluid

Cytospin (Shandon) specimens of ascites on glass slides were fixed in 4% paraformaldehyde for 10 minutes and immediately kept at -80°C. Immunostaining was started at the same time. Slides were incubated with mouse anti-CD68 (PG-M1; Dako) or anti-CD163 (AM-3K; TransGenic) antibodies. After use of the secondary antibody horseradish peroxidase–labeled goat anti-mouse immunoglobulin (Nichirei), macrophages were visualized with diaminobenzidine. CD163 expression was scored semiquantitatively according to staining intensity (1, weak; 2, intermediate; 3, strong). Normal mouse IgG and rabbit IgG (Dako) were used as negative controls, and these control antibodies detected no positive signals. Although M1 macrophages also express CD163, CD163 protein expression by M1 macrophages is too weak to be detected by routine immunohistochemical methods (32). We therefore used CD163 as the M2 marker in this study.

ESCs and Cell Lines

Primary ESCs (pESCs) were isolated by digestion of endometrial tissue fragments with 0.5% collagenase as previously described (33). Samples of endometrium at the proliferative phase were obtained from premenopausal patients who had undergone hysterectomy for uterine fibromas and had no evidence of endometriosis. To establish immortalized ESCs (iESCs), stromal cells isolated from noncancerous endometrium were purified and transduced with the human telomerase reverse transcriptase gene by using retroviral infections as described previously (34). We confirmed that these cells expressed human telomerase reverse transcriptase, maintained the structural characteristics of ESCs, and continued to divide at least over PD50. Primary ESCs and iESCs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Primary ESCs in monolayer culture after the third passage were >99% pure according to immunocytochemical staining with antibodies (all from Dako) to vimentin (V9), CD10 (SS2/36), cytokeratin factor, and leukocyte common antigen (2B11 + PD7/26).

Coculture Assays

Peripheral blood mononuclear cells were obtained from three healthy adult female volunteer donors without endometriosis. CD14+ monocytes were purified from peripheral blood mononuclear cells by positive selection, via magnetic

Download English Version:

https://daneshyari.com/en/article/6182427

Download Persian Version:

https://daneshyari.com/article/6182427

Daneshyari.com