GYNECOLOGY

Aberrant expression of erythropoietin in uterine leiomyoma: implications in tumor growth

Ryoko Asano, MD; Mikiko Asai-Sato, MD, PhD; Yohei Miyagi, MD, PhD; Taichi Mizushima, MD; Makiko Koyama-Sato, MD, PhD; Yoji Nagashima, MD, PhD; Masataka Taguri, PhD; Hideya Sakakibara, MD, PhD; Fumiki Hirahara, MD, PhD; Etsuko Miyagi, MD, PhD

OBJECTIVE: Myomatous erythrocytosis syndrome is a rare complication of uterine leiomyoma caused by erythropoietin (EPO) that is produced by tumor cells. We assessed the EPO expression in leiomyomas and investigated the effects of EPO on the tumor growth.

STUDY DESIGN: Tissue samples were collected from 114 patients with uterine leiomyomas who underwent myomectomy or hysterectomy in Yokohama City University Hospital. From 17 patients, the corresponding normal myometrium was also collected. All samples were analyzed for EPO messenger RNA (mRNA) expression by real-time reverse transcription-polymerase chain reaction. EPO protein expression was determined by an enzyme-linked immunosorbent assay. The relationships between EPO expression and clinicopathological features were retrospectively analyzed using the patients' charts. Blood vessel density and maturity were assessed using hematoxylin-eosin staining and CD34 immunohistochemistry.

RESULTS: EPO mRNA expression was detected in 108 of 114, or 95%, of the leiomyomas. The mean EPO mRNA expression in the leiomyoma was higher than the corresponding normal myometrium (3836 ± 4122 vs 1455 ± 2141; $P = .025$ by Wilcoxon rank test). The EPO mRNA expression in the leiomyomas varied extensively among samples, ranging from undetectable levels to 18-fold above the mean EPO mRNA of normal myometrium. EPO protein production was observed concomitant with mRNA expression. A positive correlation of leiomyoma size and EPO mRNA expression was shown by Spearman rank correlation coefficient ($\rho = 0.294$; $P = .001$), suggesting the involvement of EPO in leiomyoma growth. The blood vessel maturity was also significantly increased in EPO-producing leiomyomas (high vessel maturity in high vs low EPO group: 67% vs 20%; $P = .013$ by Fisher exact test).

CONCLUSION: This report demonstrates that EPO is produced in most of conventional leiomyomas and supports a model in which EPO accelerates tumor growth, possibly by inducing vessel maturity. Our study suggests one possible mechanism by which some uterine leiomyomas reach a large size, and the understanding of EPO expression patterns in these tumors may be useful for management of the patients with leiomyomas.

Key words: erythropoietin, myomatous erythrocytosis syndrome, uterine leiomyoma, vessel maturity

Cite this article as: Asano R, Asai-Sato M, Miyagi Y, et al. Aberrant expression of erythropoietin in uterine leiomyoma: implications in tumor growth. Am J Obstet Gynecol 2015;213:199.e1-8.

Uterine leiomyoma is a benign tumor, frequently affecting women of reproductive age. The estimated cumulative incidence of uterine leiomyoma by the age of 50 years is greater than 70%.[1] The progression of uterine leiomyomas can follow a variety of courses. Some tumors stay small for years, whereas others may enlarge tremendously and rapidly. Estrogen and progesterone are known to support the growth of uterine leiomyoma[2]; however, the mechanism by which some leiomyomas grow to a great size remains unclear.

Erythropoietin (EPO) is a glycoprotein hormone essential to the regulation of erythrocyte production.[3] The possible association between EPO and leiomyoma was first observed in the case of myomatous erythrocytosis syndrome (MES) by Thomson and Marson in 1953.[4] This rare clinical condition was defined as erythrocytosis with uterine leiomyoma and diagnosed by restoration of normal hematological values following the removal of the uterine leiomyoma.[5]

The recent studies of MES have reported the expression of EPO protein or

From the Departments of Obstetrics, Gynecology, and Molecular Reproductive Science (Drs Asano, Asai-Sato, Mizushima, Koyama-Sato, Sakakibara, Hirahara, and E. Miyagi) and Biostatistics and Epidemiology (Dr Taguri), Graduate School of Medicine, Yokohama City University, and Division of Molecular Pathology and Genetics, Kanagawa Cancer Center Research Institute (Dr Y. Miyagi), Yokohama, and Department of Surgical Pathology, Tokyo Women's Medical University Hospital, Tokyo (Dr Nagashima), Japan.

Received Sept. 8, 2014; revised Feb. 11, 2015; accepted Feb. 14, 2015.

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (no. 24592524) (M.A.-S.).

The authors report no conflict of interest.

Corresponding author: Mikiko Asai-Sato, MD, PhD. mikikos@yokohama-cu.ac.jp

0002-9378/$36.00 • • http://dx.doi.org/10.1016/j.ajog.2015.02.016

messenger RNA (mRNA) in the uterine leiomyomas of MES patients, which suggests that EPO produced by leiomyoma cells may play a role in the development of this syndrome.[6-8] According to the largest clinicopathological review of MES cases by LevGur and Levie,[9] the anatomical sizes of the leiomyoma were often very large, and half the MES cases were postmenopausal. Therefore, we hypothesize that EPO may act as a factor that stimulates the enlargement of leiomyomas in both premenopausal and postmenopausal women.

In this study, we assessed the expression of EPO mRNA and protein in a set of 114 uterine leiomyomas. We determined the correlation between EPO expression and tumor size to explore a potential role of EPO in the growth of uterine leiomyomas. Moreover, the patients' clinicopathological features, including intratumor blood vessels, were assessed in the context of tumor EPO production to determine a possible mechanism by which EPO functions to induce growth in leiomyomas.

Materials and Methods

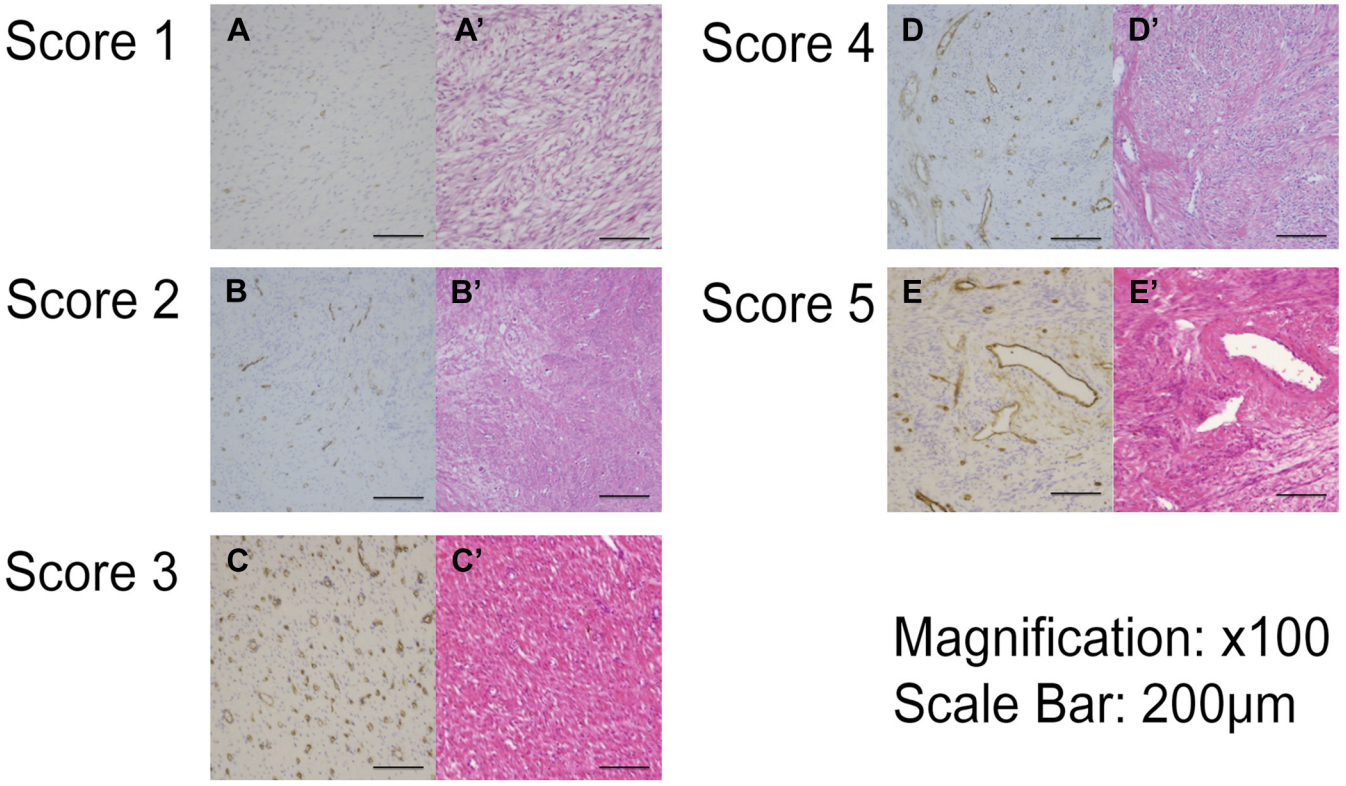
Patients and tissue samples

One hundred fourteen uterine leiomyoma tissue samples were collected from women who underwent hysterectomy or myomectomy between 2005 and 2012 at Yokohama City University Hospital. The reasons for the surgery included leiomyoma, uterine prolapse, ovarian cancer, and cervical cancer. In the cases of samples complicated with malignant disease, the absence of myometrial invasion was confirmed microscopically. Among the leiomyoma nodules, the largest leiomyoma nodule of the patient was chosen, and the center part of the nodule with minimum degeneration was sampled for analysis.

Corresponding normal myometrium tissue samples were also collected from 17 patients selected randomly. The patients' clinicopathological features were assessed retrospectively using medical records. Menopause was defined as the absence of menstrual period for over 12 months.

To determine the largest leiomyoma nodule, diameters were measured on magnetic resonance images taken within 1 year prior to surgery. The leiomyoma was measured by ultrasonography at least 1 month prior to the surgery and reexamined by magnetic resonance imaging when a change in tumor size was detected. The samples were divided and (1) snap frozen in liquid nitrogen and stored at −80°C or (2) fixed in formalin and embedded in paraffin, for subsequent analysis.

FIGURE 1
Scoring maturity of the blood vessels



A—**E** shows immunohistochemical staining of CD34, and **A′**—**E′** shows hematoxylin-eosin staining of the corresponding sections. Magnification, ×100. The sample with a score of 5 is from the case of MES.

MES, myomatous erythrocytosis syndrome.

Asano. Erythropoietin expression in uterine leiomyoma. Am J Obstet Gynecol 2015.