Aberrant expression of the pluripotency marker SOX-2 in endometriosis

Expression of the pluripotency factors SOX-2, OCT-4, KLF-4, and NANOG was analyzed by quantitative real-time polymerase chain reaction, immunohistochemistry, and immunofluorescence microscopy in the endometrium, myometrium, and endometriotic tissue of 36 patients. Aberrant expression of SOX-2 may indicate a stem cell origin of endometriosis, whereas the presence of all progenitor markers in endometrial tissue marks the endometrium as a potential source for induced pluripotent stem cell generation. (Fertil Steril® 2011:95:338–41. ©2011 by American Society for Reproductive Medicine.)

Key Words: Stem cells, SOX-2, KLF-4, OCT-4, NANOG, OCT-4, endometriosis, endometrium, telomerase, induced pluripotent stem cells, adenomyosis

Stem cells persist in a quiescent, slowly proliferating state in an environment called the stem cell niche. Transit amplifying cells derived from multipotent stem cells are characterized by their high proliferative potential, progressively acquiring differentiation markers and ultimately producing terminally differentiated cells. Aberrant stem cell function may contribute to the pathogenesis of endometriosis, the growth of endometrioid tissue outside the uterine cavity, causing dysmenorrhea, dyspareunia, subfertility, or noncyclic pelvic pain. Stem cells that are inappropriately shed during retrograde menstruation or ectopically subserved during lymphovascular metastasis may contribute to the pathogenetic process, because their high proliferative potential promotes rapid clonal expansion. The monoclonal origin of some endometriotic lesions, the long-term culturing properties of cell clones established from endometriotic lesions, and the isolation of progenitor cells with high differentiation potential from menstrual blood support the stem cell hypothesis of endometriosis. Adenomyosis is caused by extensive endometrial invagination of the basal endometrium. The stem cell niche may be altered in adenomyosis, because variations in the niche may promote smooth muscle differentiation, causing myometrial hyperplasia.

Numerous putative stem cell markers have been described. Differentiated cells can be reprogrammed by the ectopic expression of specific transcription factors, including SOX-2, OCT-4, KLF-4, NANOG, and c-MYC, thus forming induced pluripotent stem (iPS) cells. SOX-2 regulates both embryonic and adult stem cell determination, differentiation, and proliferation. In this study, we investigated whether SOX-2 and associated pluripotency markers are expressed in human endometrium, possibly marking early progenitor cells, and whether endometriosis and adenomyosis are associated with altered number and location of SOX-2–expressing stromal and epithelial progenitor cells.

This study received institutional review board approval and written consent was provided by all patients. Tissue specimens (endometrial biopsies [n = 12] and adenomyotic lesions [n = 6] from hysterectomized uteri and endometriotic lesions [n = 18]) for immunohistochemical investigations were acquired between 2002 and 2006 at Münster University Hospital from 36 women aged 28–56 years (mean, 36 years) undergoing operation. Eighteen patients had endometriosis (9 ovarian, 6 rectovaginal, 3 peritoneal), 6 patients had hysterectomy because of adenomyosis, and 2 patients (6 proliferative/6 secretory phase) undergoing hysterectomy because of myoma. After surgery, hematoxylin-stained endometrial sections were assessed by experienced histopathologists referring to established histologic criteria and investigated by immunohistochemistry. Adenomyosis was diagnosed when endometrial glands and stroma were identified within the myometrium, with glands being present approximately 2.5 mm below the endometrium. Specimens of eight additional patients with hysterectomies (aged 42–62 years; mean, 47 years) diagnosed with leiomyoma (n = 5), endometriosis (n = 1), or adenomyosis (n = 2) were analyzed by quantitative real-time PCR (qPCR). Three proliferative, three secretory, and two samples of unknown cycle phase were included. Fresh hysterectomy specimens were brought to the pathology laboratory on ice and opened by an
An experienced pathologist. One quarter of the endometrial surface was scraped with a scalpel to obtain a pure endometrial sample. The uterine wall was serially sectioned, and macroscopically normal myometrium from the outer half of the uterine wall was cut from the slices to serve as a pure myometrial sample. All samples were immediately snap-frozen, stored in liquid nitrogen, and studied by qPCR.

Ribonucleic acid was isolated (RNeasy Mini kit; Qiagen, Hilden, Germany) including a DNAse digestion step, and reverse transcribed (First-Strand cDNA Synthesis System; Fermentas, St.Leon-Rot, Germany). Quantitative real-time PCR was performed on triplicates as previously described (14), utilizing exon-spanning TaqMan probes for human SOX-2 (Hs00415716_m1), NANOG (Hs02387400_g1), OCT-4 (Hs00742896_s1), KLF-4 (Hs00358836_m1), and 18S rRNA (Hs99999901_s1) (Applied Biosystems, Darmstadt, Germany). Relative quantification was performed using the 2^{-DD_{Ct}} method (22), normalizing to 18S rRNA expression.

Immunohistochemistry was performed as previously described (14): antigen retrieval was achieved by incubating slides in citrate buffer (pH 6.0; Dako, Carpenteria, CA) for 30 minutes in a steamer. Blocked paraffin sections were incubated with mouse anti-human SOX-2 monoclonal antibody (1:100; R&D Systems, Minneapolis, MN) for 16 hours at 4°C. In negative controls, primary antibodies were omitted. Primary antibodies were detected using anti-mouse EnVision (Dako) and 3-amino-9-ethylcarbazole (AEC) substrate, with hemalum counterstaining (Merck, Darmstadt, Germany). SOX-2–positive cells were quantified in a blinded manner on 168–1,033 visual fields (320 μm × 430 μm) per numerically coded slide at 320× magnification using a Zeiss Axioskop100.
The proliferative compared with the secretory phase (stromal cell numbers were significantly increased by 68% compared with secretory- but not proliferative-phase endometrium of healthy donors (Fig. 1H). Glandular SOX-2 expression was heterogeneous and did not significantly differ between all investigated entities (Fig. 1I).

Our study demonstrates expression of the pluripotency factors SOX-2, OCT-4, KLF-4, and NANOG in human endometrium, myometrium, and endometriotic tissues. SOX-2 colocalized with telomerase, whose activity is associated with the immortality of embryonic stem cells and (endometrial) carcinoma cells (8, 14, 23), emphasizing the stem cell character of SOX-2-positive cells. A potential caveat is associated with the lack of nuclear immunofluorescence staining of SOX-2 and telomerase reverse transcriptase, which may indicate absence of regulatory activity in the nucleus or may represent nucleocytoplasmic shuttling of the telomerase holoenzyme during the assembly process (23).

SOX-2-positive cells were frequently found in a perivascular location, albeit in lower quantities compared with the adult stem cell markers CD146, STRO-1, and CD90, which show a more widespread perivascular staining pattern (24, 25). These findings are in agreement with the identification of bone marrow–derived mesenchymal stem cells populating the endometrium, and with the concept of lymphovascular metastasis as a pathogenetic route for endometriosis (6, 24).

Reprogramming of differentiated cells to an embryonic stem cell–like state by forced expression of OCT-4, SOX-2, KLF-4, and c-MYC (15) can be more easily induced in cells already expressing some of these factors (16, 17). The novel finding of an expression of SOX-2, OCT-4, KLF-4, and NANOG marks the endometrium as an attractive potential source for the generation of iPS cells. Further research needs to establish whether all four factors are expressed by the same putative endometrial stem cell as a prerequisite for applying this technology. Because human endometrium can be obtained by low-invasive techniques, the generation of autologous iPS cells from endometrium may represent a promising future perspective.

SOX-2-positive stroma cell numbers were significantly increased in the proliferative phase. Reflecting SOX-2 functions in cell cycle progression and mitogenic signaling (19), this supports the stem cell concept of cyclic endometrial regeneration (2, 3, 6, 7). Future investigations of endometrial samples according to menstrual cycle phase may be worthwhile. Significantly increased numbers of SOX-2-positive stroma cells in endometriotic lesions compared with secretory endometrium support the stem cell concept of endometriosis. Future studies need to address the full differentiation potential of endometrial SOX-2–positive cells, to provide formal proof of their stem cell properties. Furthermore, histopathologic investigations of the predictive value of stromal SOX-2 expression in expanded patient collectives, which need to be complemented by functional studies, are warranted to firmly establish its clinical relevance for the pathogenesis of endometriosis.

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REFERENCES