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The endometrial stem cell markers notch-1 and numb are associated with endometriosis

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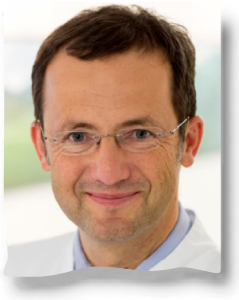

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KEY MESSAGE

Compared to controls, notch-1 expression in endometrial glands is significantly higher in patients with deep infiltrating endometriosis, whereas numb expression in luminal epithelium is significantly higher in eutopic endometrium of endometriosis patients. Further evaluation of stem cell markers notch and numb as biomarkers and therapeutic targets for endometriosis may be worthwhile.

ABSTRACT

Previous studies reported increased expression of the notch pathway-associated protein Musashi-1 in endometriosis. This case–control study investigates an association of the endometrial stem cell markers notch-1 and numb with endometriosis. Fifty-one endometriosis patients and 76 controls were recruited in the IVF unit and tertiary endometriosis referral centre of a university hospital. All subjects underwent transcervical endometrial biopsy and diagnostic laparoscopy. Expression of endometrial notch-1 and numb was assessed by immunostaining and correlated with clinical data. Association of stem-cell-marker expression with the presence of endometriosis was evaluated. Numb expression in the luminal epithelium was significantly higher in eutopic endometrium of endometriosis patients compared with controls (20.5% versus 16.5%, $P = 0.033$). Numb-positive single stromal cells were less frequent in endometrioma patients compared with other forms of endometriosis (0.3 versus 0.5 cells/visual field; $P = 0.028$). Notch-1 expression in endometrial glands was significantly higher in patients with deep infiltrating endometriosis compared with controls (39.1% versus 21.8%; $P = 0.045$). We conclude that stem cell markers notch-1 and numb of eutopic endometrium are associated with endometriosis and its clinical presentations, supporting the stem cell hypothesis of endometriosis. These findings could help develop promising research strategies applying endometrial stem cells as novel tools.

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Introduction

Endometriosis, the presence of endometrium outside the uterus, is a disorder with symptoms ranging from infertility and pain to a tumour-like proliferation affecting the integrity of pelvic organs (Giudice and Kao, 2004). Although the pathological mechanisms of endometriosis are still poorly understood, an altered eutopic endometrium has been postulated to constitute the source of the disorder (Sharpe-Timms, 2005). Stem cells have been candidates of interest because of their high proliferation potential and their ability to differentiate into multiple lineages, contributing to characteristic traits of endometriosis (Cervello et al., 2013; Sasson and Taylor, 2008). Adult stem cells have been functionally described in the endometrium obtained by routine transcervical biopsy, providing an easily accessible tool to study a possible contribution of endometrial stem cells to the pathogenesis of endometriosis (Schüring et al., 2011). We could demonstrate that expression of the stem cell marker Musashi-1, a modulator of the notch signalling pathway, is up-regulated in ectopic endometriotic lesions compared with healthy endometrium (Götte et al., 2008). In endometrial carcinoma cells, siRNA-depletion of Musashi-1 resulted in down-regulation of the stem cell marker notch-1 and increased apoptosis (Götte et al., 2011).

The adult stem cell markers notch and numb are evolutionary highly conserved proteins involved in the determination of cell fate. Proteins of the notch family are transmembrane receptors capable of interacting with Delta-like and Jagged ligands, resulting in the formation of an intracellular domain that regulates nuclear gene transcription (Talora et al., 2008). Notch signalling constitutes a central mechanism of multicellular development by controlling maintenance and fate of stem cells. The signal conveyed by the notch receptor is pleiotropic and context-specific, affecting numerous physiological processes of proliferation, differentiation and apoptosis (Weijzen et al., 2002). Notch malfunction has been associated with human disease and is evaluated as a potential target for cancer therapy (Ibrahim et al., 2017; Pannuti et al., 2010; Wu et al., 2010). Similar to notch, numb plays a critical role in the promotion of cell fate, contributing both to physiological cell development and proliferating diseases, e.g. neoplasms (Pece et al., 2004). Exerting a complex pattern of functions, numb can context-specifically suppress notch action and act as its functional antagonist. Numb is involved in the proteolytic degradation of notch via modulation of endocytosis, thus regulating notch-mediated signalling (McGill and McGlade, 2003; McGill et al., 2009). Given the emerging role of endometrial stem cells in the pathogenesis of endometriosis and their potential relevance as a future therapeutic target, we evaluated the possible role of notch-1 and numb in eutopic endometrium of patients with endometriosis compared with healthy controls.

Materials and methods

Patients

Written informed consent was obtained from all patients included in the study, which was carried out according to the principles of the Helsinki Convention and approved by the local ethics committee (1 IX Greb, from 2001-09-19). In a university-based IVF unit, female patients undergoing standardized diagnostics prior to assisted reproductive techniques were subject to transcervical endometrial biopsy as published previously (Schüring et al., 2011). Briefly, endometrial biopsies were obtained between cycle days 22 and 24 with a Probet catheter (Gynemed, Lensahn, Germany). Exclusively patients undergoing diagnostic laparoscopy in the tertiary endometriosis referral centre of the university were included in the study. Operative procedures were performed by specialized fertility surgeons, who classified endometriosis according to the ASRM criteria (American Society for Reproductive Medicine, 1997). Clinical diagnosis was histopathologically confirmed by an experienced pathologist. To exclude a confounding influence by different cycle phases in patients with ovulatory disorders, endometrium was staged according to established criteria (Noyes et al., 1950). Anthropometric and clinical data were obtained from the patient records.

Immunohistochemistry

Sample collection followed the BRISQ Tier 1 requirements (Simeon-Dubach et al., 2012). Endometrial tissue was fixed in 10% formalin and embedded in paraffin using standard procedures, and documented at the Department of Pathology of Münster University Hospital. Paraffin blocks were stored at ambient temperature for less than 7 years. Consecutive 3 µm sections were cut from paraffin blocks and placed on poly-L-lysine-coated coverslips. Dried coverslips were deparaffinized, rehydrated and treated with target retrieval solution (pH 6.1, Dako, Glostrup, Denmark) for 35 min in a steamer, followed by three washes in phosphate-buffered saline (PBS). Sections were blocked with peroxidase (Dako), followed by a second block with 10% Aurion BSA (Dako) for 30 min. Sections were then washed in PBS and incubated with numb antibodies (1:70, rabbit polyclonal IgG, Santa Cruz Biotechnology) or with notch-1 antibodies (1:50, rabbit polyclonal IgG, Santa Cruz Biotechnology) diluted with Dako Real Antibody diluent, for 16 h at 4°C. Normal rabbit immunoglobulin fraction (# X0903, Dako) was diluted to the same protein concentration as the respective notch/numb rabbit polyclonal IgGs and served as a negative control. After three washes, primary antibodies were detected using anti-rabbit EnVision systems and AEC-substrate chromogen (7 min) according to the manufacturer (Dako). Sections were counterstained with Mayers haemalum (Merck, Darmstadt, Germany) and embedded in Kaiser's glycerol gelatin (Merck).

Microscopic evaluation

Microscopic evaluation was performed by three independent investigators (BD, MG, AK) blinded for the patient's diagnosis using an Axiophot 100 microscope, equipped with a CCD camera and Axiovision Software (Zeiss, Göttingen, Germany). Staining intensity was evaluated at 20 × magnification for five visual fields (700 µm × 525 µm) per stained section. The stromal staining, a diffuse cytoplasmic staining of the glands and a diffuse cytoplasmic staining of the luminal epithelium were assessed according to the following score: 0: weak staining, 1: medium staining, 2: intense staining. Distinct membranous staining of the glands and the luminal epithelium were assessed as a percentage, ranging from 0% (lowest intensity) to 100% (highest intensity). In addition, marker-positive stromal cell nests and single stromal cells were counted and expressed as mean cell number per visual field. Only samples with unambiguous staining results were included in the final analysis. Thirteen samples were excluded due to the presence of an ambiguous gradient-like staining pattern.

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