European Journal of Obstetrics & Gynecology and Reproductive Biology 204 (2016) 88–98

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Comprehensive study of angiogenic factors in women with endometriosis compared to women without endometriosis

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ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form 12 June 2016
Accepted 25 July 2016

Keywords:
Endometriosis
Angiogenic factors
Gene expression
Peritoneum

ABSTRACT

Objective: Endometriosis is a benign gynaecological disease, affecting women during their reproductive years. Angiogenesis represents a crucial step in the pathogenesis of endometriosis, because endometriotic lesions require neovascularization. In this study several angiogenesis-related genes have been studied in the context of endometriosis. Some of the analyzed angiogenic factors as well as their interactions were studied the first time regarding a possible association with endometriosis.
Study design: This case-control study consisted of 205 biopsies of 114 patients comprising 61 endometriosis patients and 53 control patients. Among them in 29 cases paired samples were obtained. *VEGFA, VEGFR2, HIF1A, HGF, NRP1, PDGFB, FGF18, TNFα, TGFB2, EPHB4, EPO* and *ANG* mRNA expression was analyzed by qRT-PCR in ectopic tissue samples, in eutopic endometrium of women with and without endometriosis, and in unaffected peritoneum of women with and without endometriosis.
Results: VEGFR2, HIF1A, HGF, PDGFB, NRP1 and *EPHB4* are overexpressed in ectopic lesions compared to eutopic tissues. *VEGFR2, HGF, PDGFB, NRP1*, and *EPHB4* showed highest mRNA levels in peritoneal implants, in contrast *HIF1A* showed the highest expression in ovarian endometriomas. Correlation analyses of angiogenic factors in ectopic lesions revealed the strongest associations between *VEGFR2, PDGFB*, and *EPHB4*. We further showed a significant upregulation of *VEGFR2, HIF1A* and *EPHB4* in eutopic endometrium of women with endometriosis compared to that of controls and a trend towards upregulation of *HGF*. Additionally, a significant downregulation for *HIF1A, HGF* and *EPHB4* was observed in unaffected peritoneal tissues of women with endometriosis compared to controls.
Conclusion: We identified new genes (*EPHB4* and *NRP1*) that may contribute to angiogenesis in endometriosis beside known factors (*VEGFA, VEGFR2, HIF1A, HGF*, and *PDGFB*). Correlation studies revealed the putative importance of *EBHB4* in association with endometriosis. Our analyses support preliminary reports that angiogenic factors seem to be differently expressed in peritoneal implants, ovarian endometriomas and deep infiltrating endometriosis. Our observation that angiogenic factors are differently expressed in the unaffected peritoneum of women with endometriosis compared to women without endometriosis underlines the importance of the peritoneum in the establishment of endometriosis.

Introduction

Endometriosis is a benign gynaecological disease characterized by the presence of functional endometrial glands and stroma outside the uterine cavity. Although it is one of the most common gynaecological diseases, the precise pathogenic mechanisms of this condition remain unsolved. Endometriosis is a multifactorial disease in which angiogenesis plays a major role, because similar to tumor and metastatic spread, endometriotic lesions require neovascularization [1,2]. Establishment, proliferation and survival

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http://dx.doi.org/10.1016/j.ejogrb.2016.07.500
0301-2115/

of endometriotic lesions are dependent on the establishment of an adequate blood supply [1–4].

Angiogenesis is regulated by growth factors and therefore several angiogenesis-related genes have been studied in the context of endometriosis, of which *VEGF* and its receptor are the most intensely investigated [1,4–14]. Recently it was shown that VEGFA is regulated by TGF-β1 through the ID1 pathway in women with endometriosis [15]. This pathway is similar to pathophysiology of tumor angiogenesis. Hypoxia inducible factor-1α (HIF1A) is another part of the biological system that plays a key role during tumor angiogenesis and in the pathophysiology of endometriosis. Levels of HIF1A and phospho-STAT3, its upstream signalling molecule, are significantly higher in eutopic endometrium from women with endometriosis when compared with women without the disease [16]. Besides the intensively studied *VEGF*, it has been shown that the growth of endometriosis can be regulated by many other cytokines such as *HGF* [17], Transforming Growth Factor Beta-2 (*TGFB2*) [18], Tumor necrosis factor-alpha (*TNF-α*) [19], Erythropoietin (*EPO*) [20] or Angiogenin (*ANG*) [21].

The vast majority of publications covering this topic investigated mainly single factors whereas comprehensive studies are missing. Nevertheless, studies of endometriosis patients are available in which gene expression profiling of virtually all genes were performed, thus including genes that are involved in angiogenesis. The identification of genes and biological pathways that contribute to angiogenesis and therefore to endometriosis are of significant benefit by providing insight into the biology of endometriosis and eventually to improve treatment. To address this question, we re-analyzed the MIAME data reported by Khan et al. [22]. We selected angiogenesis-related factors which showed a significant or at least distinct expression difference between eutopic and ectopic endometrium of women with endometriosis and included *VEGFA*, *VEGFR2*, *HIF1A*, *HGF*, *PDGF*, *TGFB2*, *TNFα* in our study. Additionally, we investigated *NRP1*, *EPHB4*, *EPO*, *ANG*, and *FGF18* which previously have only been analyzed in the context of angiogenesis other than endometriosis [23–27].

The majority of publications adressing angiogenesis in endometriosis focus on the analysis of ectopic lesions [28,29]. Only few studies investigated other biological components such as the peritoneal fluid [30] or the healthy, unaffected peritoneum [31]. Nevertheless, endometriosis is considered a multifactorial disease in which the angiogenic potential of both the endometrium and the peritoneal environment could promote lesion establishment and survival [32]. Therefore, a major aim of the current study is to investigate the gene expression profile of several angiogenesis-related genes not only in the eutopic and ectopic endometrium of women with and without endometriosis, but also in the unaffected peritoneum. Furthermore, correlation analysis of these genes is performed in order to get a better understanding of their association to each other and to identify putative pathways which are involved.

Material and methods

In the current study we investigated the following angiogenic factors: *VEGFA* and its receptor *VEGFR2*, hypoxia-inducible factor (*HIF1A*), hepatocyte growth factor (*HGF*), platelet-derived growth factor (*PDGF*), Neuropilin 1 (*NRP1*), Ephrin Receptor (*EPHB4*), Transforming Growth Factor Beta-2 (*TGFB2*), Tumor Necrosis Factor Alpha (*TNFa*), Erythropoietin (*EPO*), Angiogenin (*ANG*) and Fibroblast Growth Factor 18 (*FGF18*). To evaluate the impact of these factors on endometriosis, we investigated their expression (i) in ectopic and eutopic endometrium of women with endometriosis including paired samples, (ii) in eutopic endometrium of women with and without endometriosis and (iii) in unaffected peritoneum of women with and without endometriosis. Samples were collected between 2010 and 2015 and were analyzed under a protocol approved by the institutional review board of the Medical University of Vienna (reference number 545/2010). Signed informed consent was obtained from all patients. Tissue samples were collected during laparoscopic surgery due to the suspicion of endometriosis with or without infertility. Eutopic endometrium was collected via dilatation and curettage. The tissue samples have been collected in the context of a prospective cohort study called EMMA Study (EndoMetriosisMarkerAustria). The diagnosis of endometriosis was made based upon histologic or visual inspection of possible endometriosis lesions. Staging was performed according to the revised American Fertility Society (rAFS) classification guidelines. Exclusion criteria were pregnancy or breastfeeding less than 6 months prior to the beginning of the

Table 1
Patient characteristics. Numbers of patients in each of the indicated subgroups are shown. Numbers in parentheses indicate the fraction of patients (%) in each column in the proliferative and secretory cycle phases or with low and high stages [55] (na, status not available).

		Total ($n = 114$)	Controls ($n = 53$)	Endometriosis ($n = 61$)
Age (years)	Mean ± STD	114	34.7 ± 6.3	32.1 ± 6.4
	na	0	0	0
BMI	Mean ± STD	109	23.6 ± 5.3	22.5 ± 4.6
	na	5	4 (80.0%)	1 (20.0%)
Cycle phase	Proliferative	41	17 (41.46%)	24 (58.54%)
	Secretory	57	31 (54.39%)	26 (45.61%)
	na	16	5 (31.25%)	11 (68.75%)
Staging	I or II	23	–	23 (100.0%)
	III or IV	38	–	38 (100.0%)
	na	53	53 (100.0%)	0 (0.0%)
Ethnicity	Caucasian/white	98	38 (38.78%)	60 (62.22%)
	Hispanic	4	3 (75.0%)	1 (25.0%)
	Asian	2	2 (100.0%)	0 (0.0%)
	na	10	10 (100.0%)	0 (0.0%)
Smoking	Yes	34	19 (55.88%)	15 (44.12%)
	No	73	28 (38.36%)	45 (61.64%)
	na	7	6 (85.71%)	1 (14.29%)