Review



# Expression Differences Between the Eutopic Endometrium With and Without Endometriosis and the Ectopic Endome-trium. A Re-Analysis of Arrays

Muhammad A Riaz , Ezekiel O Mecha , Charles O. A. Omwandho , Felix Zeppernick , Ivo Meinhold-Heerlein , Lutz Konrad *

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*Review*

# Expression Differences between the Eutopic Endometrium with and without Endometriosis and the Ectopic Endometrium. A Re-Analysis of Arrays

**Muhammad A. Riaz [1], Ezekiel Onyonka Mecha [2], Charles OA Omwandho [3], Felix Zeppernick [1], Ivo Meinhold-Heerlein [1] and Lutz Konrad [1]**

[1] Department of Gynecology and Obstetrics, University of Giessen, 35392 Giessen, Germany
[2] Department of Biochemistry, University of Nairobi, Nairobi, 00100 Nairobi, Kenya
[3] Department of Health Sciences, Kirinyaga University, Kirinyaga, 10300 Kirinyaga, Kenya
* Correspondence: to: Assoc Prof L. Konrad, Department of Gynecology and Obstetrics, Justus Liebig University, Feulgenstr. 10-12, 35392 Giessen, Germany. lutz.konrad@gyn.med.uni-giessen.de

**Abstract:** In the pathogenesis of endometriosis, the differences between the eutopic and ectopic endometrium as well as between the eutopic endometrium with and without endometriosis are repeatedly pointed out. Various mechanisms have been suggested to explain these changes among them epithelial-mesenchymal transition (EMT). Recently, we suggested based on immunohistochemical data that most of the changes occur after and not before implantation of endometrial cells into ectopic locations. Furthermore, the subtle changes between eutopic endometrium with and without endometriosis and maintenance of epithelial cell-to cell contacts only suggest a partial EMT. In this study, we have re-analyzed the mRNA expression array data of eutopic and ectopic endometrium with respect to expression changes and EMT. Especially, we found that the similarity between eutopic endometrium with and without endometriosis is extremely high (~99.1%). In contrast, eutopic endometrium compared to ectopic endometrium only shows an overall similarity of ~95.3%. Analysis of some EMT-associated genes revealed small differences in the mRNA expression levels of some members of the claudin family. The array data suggest that the changes in eutopic endometrium at the beginning of the disease are quite subtle and that the majority of differences occur after implantation into ectopic locations.

**Keywords:** endometrium; endometriosis; epithelial-mesenchymal transition; EMT; claudins; keratins

## Introduction

The histological appearance of endometrial glands and stroma sometimes with hemosiderin-laden macrophages outside the uterine cavity is still the definition of endometriosis used by pathologists worldwide [1]. In contrast to epithelial endometriosis which is quite rare, a large study of pelvic endometriosis showed a higher percentage (44.9%) of cases (123/274) as stromal endometriosis [2]. Additionally, we have recently provided strong indications that stromal endometriosis is also common (~53%) in catamenial pneumothorax if caused by ectopic endometrial lesions [3]. In many cases endometriosis causes pain and/or infertility [4].

Histological definition of endometriosis was recently described as outdated and that it no longer reflects the true scope and manifestations of the disease [5]. Furthermore, Taylor et al. [5] emphasized that the clinical presentation is varied, that the presence of pelvic lesions is heterogeneous, and that the manifestations of the disease outside the female reproductive tract remain poorly understood. They concluded that endometriosis is a systemic disease rather than a disease predominantly affecting the pelvis [5]. Although this criticism is justified in many points, we really lack an understanding of how the sometimes very small ectopic lesions might cause the systemic disease in many patients. We suggest including the uterus in this consideration, because suppression of menstrual bleeding with contraceptives and hysterectomies with or without laparoscopy cured endometrial pain in the majority of cases with low reoperations rates [6-9].

Although the Sampson hypothesis of retrograde menstruation [10] provides a reasonable model for ectopic endometrial tissue [11], it is still unclear why only 0.7-8.6% of women in the general

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population develop endometriosis [12]. Thus, several additional hypotheses such as inflammation, oxidative stress, disturbance of the peritoneal barrier and genetic/epigenetic changes have been put forward to explain this discrepancy [13-15].

Previous studies have suggested that the eutopic endometrium with and without endometriosis is different [16,17], assuming that initiation of endometriosis might start in the endometrium. One of the mechanisms which have been suggested to play a role is epithelial-mesenchymal transition (EMT). EMT is involved in wound healing, fibrosis, tissue regeneration, inflammation and cancer metastasis [18-20] and was classified into: (1) type I EMT during embryonic development, (2) type II EMT during wound healing and tissue regeneration, and (3) type III EMT associated with cancer [19]. The gradual remodelling of the epithelial cell architecture is a multi-stage process, characterized by the first EMT hallmark: the loss of epithelial markers resulting in disruption of cell-cell contacts, remodelling of the cytoskeleton and loss of apical-basal polarity. This is then followed by the second hallmark of EMT namely the acquisition of mesenchymal markers [18-22]. The cellular changes often result in a mesenchymal phenotype with spindle-like cell shape, increased cell migration, invasion and cell survival (resistance to anoikis) [22,23]. Despite these significant changes only a small set of transcription factors (TFs) or master regulators of EMT are involved. These include the Snail family proteins Snail1 (Snail), Snail2 (Slug), Zinc finger E-box binding (Zeb) homeobox family proteins Zeb1 and Zeb2, and TWIST family proteins Twist1 and Twist2 [24].

The first evidence of EMT in endometriosis in vivo was described for pelvic endometriosis by immunohistochemistry with EMT markers such as cytokeratin, E/N-Cadherin, Vimentin, and S100A4 [25]. After attachment to the peritoneum the reverse process called mesenchymal-epithelial transition (MET) was postulated to occur for peritoneal and deep infiltrating endometriosis [25]. Similarly, a decreased expression of cytokeratin in ectopic compared to eutopic endometrium was found [26,27], however, we demonstrated a stable expression of keratin 18, 19 and mucin-1 in eutopic and ectopic epithelial cells without any loss of the epithelial phenotype [28]. It needs to be emphasized again that ectopic endometriotic lesions still consist of epithelial glands surrounded by stromal cells without an apparent mesenchymal phenotype of the epithelial cells [1,28].

In this study we re-analyzed mRNA/cDNA arrays to take a closer look at the key differences between endometrium with and without endometriosis and between the eutopic and ectopic endometrium. In this review, particular attention was paid to possible mRNA expression changes of EMT-associated genes.

## 2. Materials and methods

For this systematic review we followed the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines [29].

### *2.1. Search strategy and eligibility criteria*

We performed a systematic search using PubMed from 1990 up to 1. April 2023. PubMed was searched for "endometriosis" in conjunction with "array", "mRNA expression", and "cDNA library". All human studies reporting original data concerning mRNA expression/cDNA/array and eutopic/ectopic endometrium/endometriosis as well as related studies in all languages were included in this review. Studies were excluded if they were not published in established peer reviewed journals.

### *2.2. Study Selection*

Results from the initial searches were collated, and duplicates deleted. Title and abstract screening were completed independently by two authors (MAR and LK). Full texts were retrieved and reviewed independently by MAR and LK and each study evaluated for inclusion using the pre-determined eligibility criteria. Any disagreements were resolved via discussion among the authors, and consensus achieved. Additional studies were identified through forward and backward chaining of the included studies.

### *2.3. Data Extraction and Synthesis*

Data was extracted independently by MAR and LK. We looked for the keywords: E/N-cadherin, Snail1, Slug (also known as Snail2), Twist, claudin(s), occludin, integrin(s), keratin(s), and

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transforming growth factor-betas (TGF-βs). Data included, but were not limited to title, author, journal, year of publication, population studied, phase of the menstrual cycle, results and outcomes. Results were synthesized in a thematic manner. The authors independently identified recurring genes in the final list of included studies. This final list of genes was discussed until consensus reached by the authors. A meta-analysis was not possible in this review due to the heterogeneity of methodology and results obtained in the papers included in the study.

**Results**

A total of 14 studies were included in the analysis (Fig. 1), 5 comparing eutopic endometrium with and without endometriosis (Table 1). The comparison resulted in a total of 1,195 of 129,937 (0.92%) genes or samples with altered mRNA expression (Table 1). A slight regulation of claudin-3, claudin-6, claudin-10 and claudin-14 ranging from 0.59 up to 2.3 was described in only 2 of 5 manuscripts (Table 2) [30,32]. Additionally, TGF-β3 expression was also found to be increased in eutopic endometrium of endometriosis patients compared to eutopic endometrium without endometriosis (Table 2) [30,32]. Not any of the other EMT-associated genes was found in more than one study to be regulated.

In 9 studies comparison of the eutopic endometrium with the ectopic endometrium showed a high percentage (4.74%, 15,234/321,149) of genes/samples with an altered mRNA expression (Table 3). In total, the altered expression of genes/samples in the ectopic endometrium compared to the eutopic endometrium (4.74%) is ~5x higher compared to the eutopic endometrium with and without endometriosis (0.92%, Tables 1,3).

The comparison of eutopic with ectopic endometrium revealed increased expression of claudin-1, -5, -6, -9, -11, -15, -17, and -22 (Table 4). Remarkably, claudin-11 showed the highest scores of increased ectopic gene expression ranging from 54.5 up to 100 (Table 4). Claudin-2, -3, -4, -7, -10, and -22 demonstrated a slight to modest decreased expression in ectopic endometrium compared to eutopic endometrium (Table 4). Furthermore, TGF-β3 expression was also increased in ectopic endometrium compared to eutopic endometrium (Table 4).

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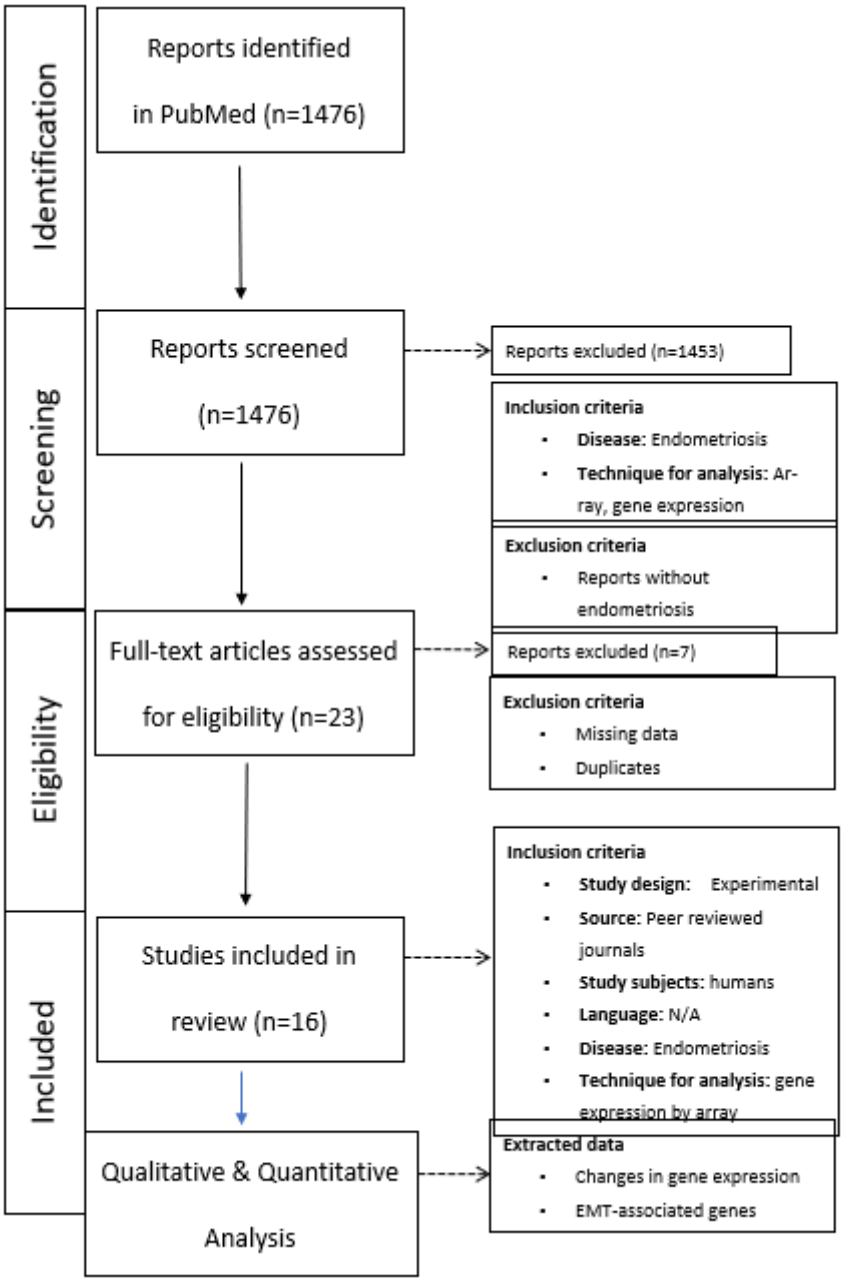


**Figure 1. PRISMA flowchart of literature search and data selection.** This systematic retrospective review is based upon literature research conducted in PubMed. The main focus was on mRNA/cDNA array analysis, EMT and endometriosis in the eutopic and ectopic endometrium. These reports were carefully read and data extracted.

**Table 1.** Genome-wide analysis of eutopic endometrium with and without endometriosis.

| Endometrium healthy | Endometrium with endometriosis | Endometriotic lesions, all | OMA | PE | DIE | References |
|---|---|---|---|---|---|---|
| N=7 | N=8, 206/12.686 (1.6%) | n.d. | n.d. | n.d. | n.d. | 30 |
| N=41 | N=43, 95/12.651 (0.8%) | N=19 lesions, not spec. | Not spec. | Not spec. | Not spec. | 31 |
| N=16 | N=21, 885/54.600 (1.62%) | n.d. | n.d. | n.d. | n.d. | 32 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| N=6 | N=10, 9/22.000 (0.04%) | n.d. | n.d. | n.d. | n.d. | 33 |
| N=18 | N=31 0/28.000 (0%) | n.d. | n.d. | n.d. | n.d. | 34 |
| **Sum** | **1.195/129.937 (0.92%)** | | | | | |

Altered mRNA expression is shown as total values and as % in brackets. OMA, endometrioma; PE, peritoneal endometriosis; DIE, deep infiltrating endometriosis; n.d., not done; not spec., not specified.

**Table 2.** Up- or down-regulation of EMT-associated genes in eutopic endometrium without vs with endometriosis.

| Genes | Up-regulation | Down-regulation | References |
|---|---|---|---|
| Claudin-3 | - | 0.59 | 32 |
| Claudin-6 | 1.54 | - | 32 |
| Claudin-10 | 2.3 | - | 30 |
| Claudin-14 | - | 0.65 | 32 |
| TGF-β3 | 100 | - | 30 |
| | 3.14 | - | 32 |

**Table 3.** Genome-wide analysis of eutopic and ectopic endometrium.

| Endometrium healthy | Endometrium with endometriosis | Endometriotic lesions, all | OMA | PE | DIE | References |
|---|---|---|---|---|---|---|
| n.d. | N=3 | N=3 (paired) 8/4,133 (0.2%) | N=3 | n.d. | n.d. | 35 |
| n.d. | N=23 | N=23 (paired) 1,413/23,040 (6.1%) | N=23 | n.d. | n.d. | 36 |
| n.d. | N=12 | N=12 (paired) 0/1,176 (0%) | n.d. | n.d | N=12 | 37 |
| n.d. | N=12 | N=25 (paired) 904/9,600 (4684*) (9.4%/19.3%*) | N=6 | N=5 | N=1 | 38 |
| N=5 (not used for the array) | N=5 | N=5 (paired) 13/1,176 (940*) (1.1%/1.38*) | N=5 | n.d. | n.d. | 39 |
| n.d. | N=10 | N=10 (paired) 1,146/53,000 (2.16%) | yes | yes | Not. spec | 40 |
| n.d. | N=4 | N=4 (paired) 36/44,928 (0.08%) | N=4 | n.d. | n.d. | 41 |
| n.d. | N=6 | N=6 (paired) 5,600/53,000 (10.6%) | N=6 | n.d. | n.d. | 42 |
| n.d. | N=18 | N=18 (paired) 847/29,421 (2.88%) | N=18 | n.d. | n.d. | 43 |
| n.d. | N=6 | N=6 (paired) 1,366/47,000 (2.9%) | N=6 | n.d. | n.d. | 44 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| n.d. | N=17 | N=18 (paired) 3,901/54,675 (7.1%) | n.d. | N=18 | n.d. | 45 |
| **Sum** | **15,234/321,149 (314,821*) (4.74%/4.84%*)** | | | | | |

*In two studies numbers of mRNA changes per gene were included. Calculation change per gene only slightly increases the rates of change by 0.1% (4.74% vs. 4.84%). OMA, endometrioma; PE, peritoneal endometriosis; DIE, deep infiltrating endometriosis; n.d., not done; not spec., not specified.

**Table 4.** Up- or down-regulation of EMT-associated genes in eutopic endometrium compared to ectopic endometrium.

| Genes | Up-regulation | Down-regulation | References |
|---|---|---|---|
| Claudin-1 | 6.64 | - | 45 |
| | 0.87-2.85 | - | 43 |
| Claudin-2 | - | 0.45-0.55 | 43 |
| Claudin-3 | - | 0.14 | 45 |
| | - | 0.06 | 43 |
| | - | 0.58 | 35 |
| Claudin-4 | - | 0.11 | 45 |
| | - | 0.1 | 43 |
| Claudin-5 | 4,31 | - | 45 |
| | 7.46 | - | 43 |
| Claudin-6 | 1.05 | - | 43 |
| Claudin-7 | - | 0.19 | 45 |
| | - | 0.12 | 43 |
| Claudin-8 | - | 0.28 | 43 |
| Claudin-9 | 2.16 | - | 43 |
| Claudin-10 | - | 0.17 | 43 |
| Claudin-11 | 54.05 | - | 45 |
| | 69.3 | - | 43 |
| | 100 | - | 40 |
| Claudin-15 | 1.31-2.07 | - | 43 |
| Claudin-17 | 1.25 | - | 43 |
| Claudin-22 | - | 0.17 | 43 |
| TGF-β3 | 4.86 | - | 43 |
| | 0.9-1.7 | - | 39 |

## Discussion

In this study, the comparison of the eutopic endometrium with and without endometriosis revealed only a slight difference (0.92%) in mRNA expression. Accordingly, there were very few changes in the EMT-associated genes. Only some claudins (N=3) and TGF-β3 were abnormally expressed, whereas expression of the other EMT-associated genes were not mentioned in more than one study. In contrast, comparison of eutopic endometrium with ectopic endometrium showed a clearly higher number (4.74%) of impaired gene expression. Similarly, more claudins (N=15) and TGF-β3 were abnormally expressed, while EMT-regulating transcription factors such ZEB1, and

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Snail1/2 (Slug), were not described in more than one study. Of note, mRNA expression of claudin-11 was often strongly increased in ectopic endometrium compared to eutopic endometrium. Overall, it was noticeable that ovarian endometriosis was examined very frequently, but peritoneal and deep infiltrating endometriosis only very rarely. No study has up to date examined the proportion of stroma and epithelium in the eutopic and ectopic endometrium, which is possible using markers such as CD10 and keratins. Only one study using cell picking, revealed no differences in gene expression between eutopic and ectopic endometrium [38].

Our observation that there are only very few differences in eutopic endometrium with and without endometriosis is supported by a recently published meta-analysis, which did not show a single differently expressed gene in the mid-secretory phase [46]. Remarkably two other studies about miRNAs [47] and methylation patterns [48] also reported similar results. Methylation pattern revealed only 1 of 42.990 probes (0.002%) to be different between eutopic endometrium with and without endometriosis, while 12378 methylation patterns (28.8%) were dissimilar between eutopic and ectopic endometrium [47]. Similarly, the microRNA differences between eutopic endometrium with and without endometriosis was comparatively low with 15/1105 (=1.36%) but noticeably more frequent between eutopic and ectopic endometrium 156/1105 (=14.1%) [48]. These studies are consistent with our results that changes in eutopic endometrium with and without endometriosis are minimal, and that the differences between eutopic and ectopic endometrium are clearly larger.

Although a recent bioinformatic analysis of three microarray datasets emphasized the importance of EMT in the development of endometriosis due to down-regulation of E-cadherin (CDH1) [49], another study ruled out EMT in the endometrium, because only the endometrial epithelial cells but not the stromal cells showed DNA alterations/mutations [50]. As demonstrated in our previous review on EMT, the main problem with many studies on EMT is that mostly only acquisition of mesenchymal markers and not the loss of epithelial markers, in particular the cell-to-cell contacts, have been examined [51]. Thus, loss of cell-to-cell contacts, the first hallmark of EMT, does not occur in eutopic endometrium with and without endometriosis as well as in ectopic endometrium, and the epithelial phenotype of the cells is still clearly preserved [1,28]. The small increase in mesenchymal gene expression of endometrial epithelial cells does not allow the assumption of a cell transition to mesenchymal cells, but at most the conclusion of a partial EMT without the loss of the epithelial cell characteristics [51].

Gene expression between eutopic and ectopic endometrium showed about ~5 times more differences (4.74%) compared to that of eutopic endometrium with and without endometriosis (0.92%). We can thus conclude with a high degree of certainty that most of the differences in gene expression did not occur before but after implantation of the endometrial cells. This conclusion supports our hypothesis of a partial EMT without loss of the epithelial phenotype, which we had put forward from immunohistochemical analysis of EMT-associated proteins of the eutopic and ectopic endometrium [51]. Although we cannot completely rule out that circulating endometrial cells (CECs) may have been altered by EMT prior to implantation, there has been no study to date showing alterations of CECs by EMT. Remarkably, endometrial tissue fragments from endometriosis and control patients did not differ in their implantation potential on chorionic allantois membranes (CAMs) in vitro. The authors suggested that implantation is possibly determined by external factors regulating influences on the endometrial implants [52]. Similarly, Nap et al. [53] showed that the integrity of endometrial tissue architecture determined the success of implantation of human endometrium into CAM ectopic locations in vitro.

Our analysis of EMT-associated genes showed altered expression patterns only for claudin-3, -6, -10, and -14 and TGF-β3 in eutopic endometrium with and without endometriosis while comparison between eutopic and ectopic endometrium revealed aberrant gene expression of 11 claudins (claudin-1 up to claudin-11), and claudin-15, -17, and -22. The highest increase in expression was reported for claudin-11 [40,42,45]. In contrast to two reports that described an impaired expression of claudin-3 in endometriosis [54,55], we recently found an unchanged protein localization in the eutopic endometrium with and without endometriosis and also in the ectopic endometrium [56] which might be due to different fixation protocols used. Similarly, we found a high abundance (~98%) of claudin-10 in nearly all endometrial and endometriotic glands, but no differences in the claudin-10 positive endometrial glands between cases with and without endometriosis [57]. A significantly higher expression of claudin-10 was detected in the ectopic endometrium of deep

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infiltrating and ovarian endometriosis [57]. Interestingly, a shift in claudin-10 from a predominant apical localization in the eutopic endometrium to a more pronounced basal/cytoplasmic localization in the ectopic endometria of all three endometriotic entities (ovarian, peritoneal, deep infiltrating) was observed. Of note, despite the impaired endometriotic localization of claudin-10, the epithelial phenotype was retained in all glands [57].

A decreased expression of Claudin-7 was observed in ectopic compared to eutopic endometrium in the array studies [42,45] as well as with immunohistochemistry [54]. Claudin-7 was identified primarily at the basolateral junctions of the glandular epithelial cells in eutopic endometrium as well as in the ectopic lesions in nearly all glands and cysts [58]. Quantification of claudin-7 localization showed a slight increase in peritoneal and deep infiltrating endometriosis compared to eutopic endometrium [58].

In three array studies a strong increase in claudin-11 mRNA expression in ectopic compared to eutopic endometrium was described [40,42,45], however, without any supporting protein data. In contrast, we observed only a moderately decreased abundance of claudin-11 in ovarian endometriosis compared to eutopic endometrium [58]. Claudin-11 was localized mainly in the apicolateral junctions in nearly all glandular epithelial cells of the eutopic endometrium. Interestingly, deregulation of claudin-11 localization to basal or basolateral localization in ovarian, peritoneal, and deep infiltrating endometriosis was observed. Silencing of claudin-11 decreased invasiveness of endometriotic epithelial 12Z cells significantly in endometriotic epithelial 49Z cells [58]. None of the other claudins have been analyzed in more depth to date.

Beyond the three isoforms of the TGF-βs, TGF-β1-3, expression of TGF-β3 mRNA was increased in eutopic endometrium with endometriosis compared to those without endometriosis [30,32]. Similarly, TGF-β3 gene expression was also higher in ectopic compared to eutopic endometrium [39,42]. TGF-β1 showed the highest expression compared to TGF-β2 and TGF-β3 in the human endometrium [59]. TGF-β3 was increased at menstruation remaining high during the proliferative phase and was preferentially expressed in the stroma. In contrast, TGF-β1 was increased in the peritoneal fluid (PF) of women with endometriosis compared to those without the disease, while TGF-β3 was not altered [60]. However, higher PF and serum levels of TGF-β1, -β2, and –β3 were observed in women with endometriosis compared to controls [61].

### Strength and limitations

This is the first study evaluating the similarities and differences in gene expression between eutopic endometria with and without endometriosis with special emphasis on EMT-associated genes. One limitation lies in the fact that the total number of samples is higher than the real number of genes. However, the analysis was less about absolutes and more about relative values.

## Conclusions

Results of this study clearly show very little differences in gene expression between eutopic endometrium with and without endometriosis. In contrast, the differences between eutopic and ectopic endometrium are much larger. We suggest that the main changes happen after and not before implantation. Remarkably, there were also few differences in expression of EMT-associated genes with complete absence of the master genes. It can therefore be assumed that there is at most only a partial EMT, with no loss of the epithelial phenotype, and that EMT plays only a minor if not negligible role in the initiation of endometriosis.

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