

Aberrant expression of epithelial leucine-rich repeat containing G protein-coupled receptor 5-positive cells in the eutopic endometrium in endometriosis and implications in deep-infiltrating endometriosis

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Objective: To characterize leucine-rich repeat containing G protein-coupled receptor 5-positive (LGR5⁺) cells from the endometrium of women with endometriosis.

Design: Prospective experimental study.

Setting: University hospital/fertility clinic.

Patient(s): Twenty-seven women with endometriosis who underwent surgery and 12 healthy egg donors, together comprising 39 endometrial samples.

Intervention(s): Obtaining of uterine aspirates by using a Cornier Pipelle.

Main Outcomes Measure(s): Immunofluorescence in formalin-fixed paraffin-embedded tissue from mice and healthy and pathologic human endometrium using antibodies against LGR5, E-cadherin, and cytokeratin, and epithelial and stromal LGR5⁺ cells isolated from healthy and pathologic human eutopic endometrium by fluorescence-activated cell sorting and transcriptomic characterization by RNA high sequencing.

Result(s): Immunofluorescence showed that LGR5⁺ cells colocalized with epithelial markers in the stroma of the endometrium only in endometriotic patients. The results from RNA high sequencing of LGR5⁺ cells from epithelium and stroma did not show any statistically significant differences between them. The LGR5⁺ versus LGR5⁻ cells in pathologic endometrium showed 394 differentially expressed genes. The LGR5⁺ cells in deep-infiltrating endometriosis expressed inflammatory markers not present in the other types of the disease.

Conclusion(s): Our results revealed the presence of aberrantly located LGR5⁺ cells coexpressing epithelial markers in the stromal compartment of women with endometriosis. These cells have a statistically significantly different expression profile in deep-infiltrating endometriosis in comparison with other types of endometriosis, independent of the menstrual cycle phase. Further studies are needed to elucidate their role and influence in reproductive outcomes. (Fertil Steril® 2017; ■:■-■. ©2017 by American Society for Reproductive Medicine.)

Key Words: Deep infiltrating endometriosis (DIE), epithelial mesenchymal transition (EMT), LGR5, macrophages, uterine aspirate

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Endometriosis, an estrogen-dependent benign disease characterized by the presence of endometrial tissue outside the uterine cavity, affects 10% to 15% of reproductive-age women and is a common cause of pelvic pain and/or infertility (1). Several theories have been proposed to explain its origin, but no consensus has been reached. For many years, the most accepted theory was Sampson's hypothesis, in which retrograde menstruation is the cause of endometriosis (2). However, there are limitations to Sampson's theory, and other investigators have pointed to coelomic metaplasia (3) or embryonic rests (4). These latter theories would explain the most frequently observed lesions, such as endometrioma (ovaries), pelvic lesions (pelvic cavity), and deep-infiltrating endometriosis (DIE) (other areas such as the intestines and urinary bladder); the presence of endometrial tissue in the myometrium is diagnosed as adenomyosis. However, none of these theories account for the cases of distant lesions outside the pelvis, such as in the brain or lungs. Thus, alternatively, more recent studies have suggested that bone marrow or endometrial stem cells may play a role in the origin of endometriosis (5–9).

The association between infertility and endometriosis and adenomyosis (10) has been widely studied (11). It is known that eutopic endometrium is altered in women with endometriosis (12), and that it diminishes endometrial receptivity and embryo implantation (13), but the mechanistic link remains unknown. There is a need to identify the molecules involved in the pathophysiology of the disease and to determine their impact on the eutopic endometrium and reproductive outcomes. Additionally, understanding these mechanisms in the eutopic endometrium may help to unravel the origin of endometriosis. Endometrial biopsies, also called uterine aspirates, may be a useful tool to assess these cytologic and molecular alterations and could contribute to the diagnosis of certain endometrial disorders (14).

Leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5), which has been described as a stem cell marker in the small intestine and in hair follicles (15–17), also has been found in endometrium (18). Recently, it was discovered that LGR5⁺ cells from healthy endometrium have a hematopoietic origin (19). Approximately half the population of LGR5⁺ cells coexpress CD45 (leukocyte marker) and CD163, suggesting its myeloid nature. It also is interesting that these cells appear to remain constant throughout the menstrual cycle (19, 20).

Bidirectional communication between primary and metastatic tumors has been demonstrated (21). Certain metastatic cancers shed cells into the blood circulation; these cells selectively migrate, engraft the original tumor, and contribute to the progression of the disease. In addition, one study observed a substantial reduction in liver metastasis when original colon tumors were depleted of LGR5⁺ cells (22). Further, selective destruction of LGR5⁺ cells led to temporary tumor regression in colorectal tumors, highlighting the important role that LGR5⁺ cells may have in the progression of certain tumors (23). Finally, a previous study (24) demonstrated in a rodent model of endometriosis that cells from endometriosis lesions migrated specifically to eutopic endometrium, modifying its normal gene expression profile. These migrating cells were mostly located close to blood vessels and aberrantly expressed the epithelial marker cytokeratin (CK) in the stromal compartment. Subsequent gene expression

analysis revealed that these cells expressed markers related to cell adhesion, stemness, and epithelial-mesenchymal transition process (EMT) as well as LGR5.

Therefore, we hypothesized that LGR5 could be involved in the pathophysiology of endometriosis. We determined whether there is an aberrant pattern of LGR5 and epithelial markers present in the uterine aspirates of women with endometriosis as compared with healthy endometrium, and we examined the nature and the possible role of these cells in endometriosis by characterizing their gene expression profile in different types of endometriosis.

MATERIALS AND METHODS

Sample Collection and Processing

A total of 39 uterine aspirates were collected between 2014 and 2016. Twenty-seven samples were obtained from women with endometriosis in treatment for diagnosis or for benign gynecologic disorders in collaboration with the Department of Gynecology of Vall d'Hebron University Hospital (Barcelona, Spain). Twelve additional specimens were obtained from healthy women donating eggs at IVI Barcelona S.L. All uterine biopsy samples were collected using a Cornier Pipelle under an approved protocol, and after written informed consent was obtained. The use of the uterine specimens was approved by the ethics committee of Vall d'Hebron Research Institute [Number PR (AMI) 298/2013].

All the participating women were premenopausal. The patients with endometriosis did not receive hormones for 6 months before surgery, and all the healthy women were egg donors stimulated with follicle-stimulating hormone (FSH). The biopsy samples were collected from the endometriosis patients at the time of laparoscopy, and from the donors at the time of egg retrieval. The clinical characteristics of the patients are detailed in [Supplemental Table 1](#) (available online).

A small fraction of the uterine aspirates was washed with phosphate-buffered saline solution (Gibco-Invitrogen) and placed into 4% formaldehyde for fixation and paraffin embedding. Formalin-fixed paraffin-embedded samples were sectioned for hematoxylin and eosin staining to determine the stage of the menstrual cycle and for immunofluorescence experiments. The stage of the cycle was determined by histologic examination by a pathologist according to the criteria of Noyes et al. (25). The remaining samples were carefully rinsed of blood and mucus and sliced into 1–2-mm³ fragments, and digestion was performed with 10 mg of collagenase type I (Sigma-Aldrich) in Dulbecco's modified Eagle's medium (with glucose and 10% of fetal bovine serum) (Gibco-Invitrogen). We incubated the samples overnight at 4°C before proceeding with immunocytochemistry and fluorescence activated cell sorting (FACS) of the LGR5^{+/-} cells.

The next day, epithelial and stromal cells were separated based on their size by means of gravity sedimentation and membrane filtration, as previously described elsewhere (19, 26). Subsequently they were treated with standard erythrocyte lysis solution.

In order to confirm the expression of the LGR5 in the migrating cells of rodent model of endometriosis, specimens

were generously provided by Dr. Hugh Taylor from Yale University (New Haven, CT).

Immunofluorescence

We performed immunofluorescence in eight eutopic endometrium samples to confirm the presence of LGR5 in mice endometrial GFP⁺ cells. Moreover, we analyzed the colocalization of LGR5 with the epithelial marker E-cadherin (ECAD). Once the colocalization was confirmed, we transferred the results to humans. We studied LGR5 colocalization with ECAD and CK in eutopic endometrium biopsy samples from 12 healthy women and 20 patients with endometriosis on 3- μ m tissue sections. The slides were incubated at 55°C overnight and treated in a xylene and ethanol circuit. Then they were treated with ammonium chloride (NH₄Cl) for 15 minutes and 20 minutes with citrate (pH 6.0) (Abgent) at 95°C in a water bath followed by blocking by using 5% normal goat serum (NGS) (Invitrogen) and 5% bovine serum albumin (Sigma-Aldrich) for 1 hour at room temperature.

The primary antibodies used were monoclonal rabbit anti-LGR5 antibody (Abgent) in a 1:30 dilution, monoclonal rat anti-GFP antibody (B-Bridge) in a 1:50 dilution, monoclonal mouse anti-E-cadherin antibody (Santa-Cruz) in a 1:150 dilution, and monoclonal mouse anti-pan-cytokeratin antibody (Santa-Cruz) in a 1:50 dilution. They were incubated overnight at 4°C. The secondary antibodies used were goat Alexa647 anti-rabbit (Invitrogen) and goat Alexa488 anti-mouse (Invitrogen), all in 1:500 dilutions, which were incubated for 45 minutes at room temperature. We used ProLong Gold antifade reagent with 6-diamino-2-phenylindole (DAPI; Invitrogen) to visualize the nuclear DNA. Immunoreaction without primary antibodies and without antibodies were performed as controls. Visualizations and pictures were obtained with an OlympusBX61 microscope.

FACS of LGR5^{+/-} cells

Uterine aspirates (eutopic endometrium) from 5 healthy women and 13 women with endometriosis were stained and sorted using a BD FACS ARIA I instrument. After the collagenase treatment, all samples were separated by gravity sedimentation and then filtered using 50- μ m mesh to obtain epithelial and stromal fractions. Samples were treated with erythrocyte lysis buffer then were blocked with 5% bovine serum albumin (Sigma-Aldrich) for 1 hour at room temperature. The primary antibody was monoclonal rabbit anti-LGR5 antibody (1 μ L per million of cells; BioNova Scientific), and the secondary was goat Alexa647 anti-rabbit (Invitrogen) in a 1:500 dilution. To discard the dead cells, the samples were stained with the DAPI probe (5 μ g/mL; Invitrogen). The LGR5^{+/-} cells were collected separately in TRIzol (Invitrogen) and stored at -80°C. Thirty-eight paired samples were obtained, which comprised LGR5⁺ cells from each uterine aspirate. The percentage of LGR5⁺ cells was analyzed by FCS Express5.0. To confirm that LGR5⁺ cells had been specifically sorted, we subjected them to cytopspin with 5,000 cells on a slide, and we stained them with the same antibody that was used for immunofluorescence.

RNA Extraction

Total RNA from sorted cells was isolated in two steps. First, the cells were lysed using a 1-mL syringe. After treatment with chloroform, the aqueous phase was precipitated with 70% ethanol in a volume proportion 1:1. Second, the samples were passed through columns (step 2 of the RNeasy micro kit; Qiagen). We then followed the protocol according to the manufacturer's suggested conditions. The quality of the RNA was determined using a Pico Chip with the Agilent 2100 Bioanalyzer. All 36 samples used for RNA high sequencing had RIN (RNA integrity number) \geq 7.

RNA High Sequencing

Preliminary study. To prove the feasibility of the sample to be sequenced, we performed a preliminary study using four samples (two from patients with endometriosis and two from healthy donors). After the separation of epithelium and stroma and cell sorting (LGR5^{+/-} cells), we obtained 16 samples. The library was constructed using TruSeq Stranded Total RNA LT with the Ribo-Zero Gold, Set A kit (Illumina). Due to the small concentration of RNA, the fragmentation step was eliminated to prevent RNA degradation.

According to the standard protocols, purified amplicons were pooled in equimolar and paired-end sequenced flow cell 2 \times 50nt on an Illumina Hi-Seq2000 platform in the Genomics Unit of the Centre of Genomic Regulation (Barcelona). A total of eight paired-end (2 \times 50) pairs of FASTQ files were obtained. Basic quality controls were performed with FASTQC (27), FastX-Toolkit (28), and PRINSEQ (29). Paired-end (forward-reverse) sample merging and the remaining steps of the bioinformatics analysis were performed with CLC Bio Genomics Workbench software, version 8.0.2 (Qiagen). Alignment and mapping were against the current human genome (30). Counts were normalized with the standard reads per kilobase per million (RPKM) method (31).

Complete study. After testing the feasibility of the sample, a total of 36 eutopic endometrium samples—5 healthy donor, 3 endometriomas, 4 DIE, 3 pelvic endometriosis, and 3 adenomyosis, each with their respective LGR5^{+/-} cells—were sequenced as explained earlier. We studied different comparisons (Table 1), and we performed a biological significance analysis for comparisons 2, 4, 7, 9, and 10. In comparisons 2 and 4, the biological significance analysis was performed searching for gene set enrichment analysis against the KEGG (32) and GO databases (33) with the GAGE (34) and Pathview (35) bioconductor packages. The common differentiated expressed genes (DEG) between comparisons 7, 9, and 10 were determined and their biological significance analysis was conducted using Ingenuity Pathway Analysis (IPA) (Ingenuity Systems). The ideal set size for IPA core analysis from gene expression data is typically 200–3,000. Therefore, in comparisons 7, 9, and 10 we analyzed a total of 3,000 codifying DEG with the increased log₂ fold change (FC) and false discovery rate (FDR) < .01 in each comparison.

Subsequently, to minimize the effect of the differences among stimulated and nonstimulated cycles, we subtracted the DEG found to be significantly expressed throughout

TABLE 1

Statistically significant differences between LGR5^{+/-} cells in healthy women and women with endometriosis.

Comparison	DEG	Codifying DEG	Significance
LGR5 ⁺ healthy vs. LGR5 ⁺ endometriosis	0	0	FDR<0.01
LGR5 ⁻ healthy vs. LGR5 ⁻ endometriosis	502	299	FDR<0.01 ^a
LGR5 ⁺ healthy vs. LGR5 ⁻ healthy controls	0	0	FDR<0.01
LGR5 ⁺ endometriosis vs. LGR5 ⁻ endometriosis	394	306	FDR<0.01 ^a
LGR5 ⁺ endometrioma vs. LGR5 ⁺ pelvic	0	0	FDR<0.01
LGR5 ⁺ endometrioma vs. LGR5 ⁺ adenomyosis	0	0	FDR<0.01
LGR5 ⁺ DIE vs. LGR5 ⁺ endometrioma	14023	5374	FDR<0.001 ^a
LGR5 ⁺ adenomyosis vs. LGR5 ⁺ pelvic	0	0	FDR<0.01
LGR5 ⁺ DIE vs. LGR5 ⁺ pelvic	14567	5753	FDR<0.001 ^a
LGR5 ⁺ DIE vs. LGR5 ⁺ adenomyosis	17200	6852	FDR<0.001 ^a

Note: DEG in different comparisons. DEG = differentiated expressed genes; FDR = false discovery rate; LGR5⁺ = leucine-rich repeat containing G protein-coupled receptor 5-positive. LogFold change = ±2.

^a Comparison with statistically significant difference (FDR<.01/FDR<.001).

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menstrual phases and stimulated and natural cycles according to previous reports in the literature (36, 37). Although both studies were based on the same array platform (Affymetrix Human Genome U133Plus2.0 Array), several studies have shown that the platforms used in this study (Hi-Sec and arrays) are comparable (38–45).

Statistical Analysis

Significance testing for DEG was implemented with EDGE testing (46) with a statistical significance criteria of alpha <.01, adjusting for multiple testing with the FDR method (47). We considered genes statistically significant if their expression was FDR<.01 with FC ± 2.

RESULTS

Immunofluorescence

Previously we reported that GFP⁺ cells colocalize with CK in the endometrium of endometriotic mice (24). We confirmed the colocalization of these GFP⁺ cells with the LGR5 marker. In five of eight cases we identified colocalization with the epithelial marker ECAD in this compartment (data not shown).

These findings translated to human endometrium: the presence of LGR5 was observed in both epithelium and stroma in 12 healthy and 20 diseased endometrial samples. It is interesting that LGR5⁺ cells colocalized aberrantly with CK and ECAD in the stroma from 13 and 16 of the 20 endometriotic patients, respectively, whereas there was no colocalization in any of 12 healthy donors (Supplemental Table 2, available online). An example of this colocalization is shown in Figure 1A and 1B.

FACS of LGR5^{+/-} cells

After FACS, we obtained around 5% of LGR5⁺ cells of the total uterine aspirate. Although not statistically significant, we found that the LGR5⁺ cell percentage in DIE trended higher than in the other types of the disease. We also confirmed that all the sorted LGR5⁺ cells were positive in the cytospin (Supplemental Fig. 1, available online).

RNA high Sequencing of LGR5^{+/-} cells

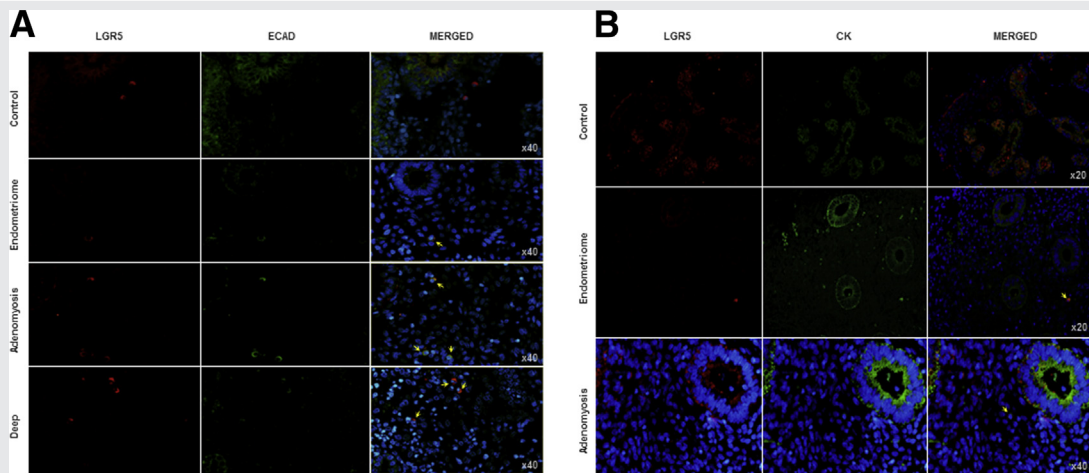
Preliminary study results. No statistically significant differences were observed in the gene expression profile of LGR5⁺ cells obtained from the epithelial versus the stromal compartment (FC ± 2 and FDR<.01). Therefore, we decided not to separate the compartments in subsequent samples.

Complete study results. No statistically significant differences (FC ± 2 and FDR<.01) were observed after comparing healthy versus endometriotic LGR5⁺ cells in 36 samples. We excluded from the study 6,348 DEG in total: 5,315 genes that are significantly expressed throughout the menstrual cycle and 1,033 DEG between the natural and stimulated cycles. Furthermore, we did not observe differences between the LGR5^{+/-} cells from the group of healthy women (FC ± 2 and FDR<.01). Nevertheless, we did find statistically significant differences (FC ± 2 and FDR<.01) in comparison 2 (502 DEG) and comparison 4 (394 DEG) (Table 1).

In the comparison 4, specific myeloid cell markers were overexpressed, including CD33, CD300E, CD300LF, CD300LB, and CD200R1. We also found overexpression of monocyte and macrophage markers, such as CD11b (ITGAM), CD163, CD86, CD209, CD14, CD180, CD68, CD84, CD1C, CD1A, CD45, CD53, CD300C, CD1B, CD300A, CD80, CD36, CD74, and CD93. When analyzing the biological significance, we obtained 16 up-regulated pathways (Fig. 2A). Some of them were related to the immune system, such as chemokine signaling pathways (hsa04062) which was the top enrichment score, followed by Toll-like receptor signaling pathway (hsa04620), Fc epsilon RI signaling pathway (hsa04664), phagosome (hsa04145), natural killer cell-mediated cytotoxicity (hsa04650), cell adhesion molecules (CAMs, hsa04514), and antigen processing and presentation (hsa04612).

Furthermore, hematopoietic cell lineage (hsa04640) was also present. As shown in Figure 2B and Supplemental Figure 2 (available online), LGR5⁺ cells overexpressed hematopoietic cell lineage markers. Among the myeloid lineage, CFU-GM (colony forming unit granulocyte, monocyte) node showed the majority of overexpressed markers represented. All these findings strongly suggest that LGR5⁺ cells may be

FIGURE 1



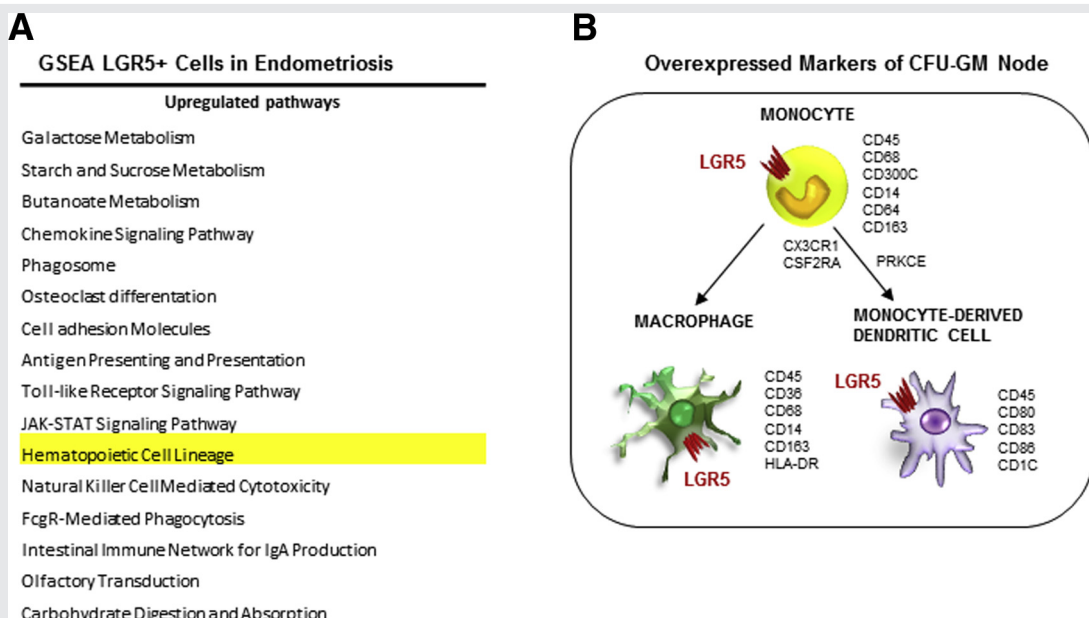
(A) Colocalization of leucine-rich repeat containing G protein-coupled receptor (LGR5) with E-cadherin (ECAD) in human eutopic endometrium. (B) Colocalization of LGR5 with cytokeratin (CK) in human eutopic endometrium. In the left panels, LGR5 is dyed in red; in the central panel, the epithelial markers are dyed in green; in the right panel, both markers are merged. The first rows show the control group, where there is no coexpression of the markers in the stroma. The bottom row represents the colocalization of both markers in each type of endometriosis (except pelvic endometriosis); in B, we did not find colocalization in deep-infiltrating endometriosis. Cells coexpressing both markers are shown with yellow arrows. Magnification is shown on the right for each row.

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similar to monocytes or their derivatives. This fact is reinforced by the presence of the overexpressed markers CD45, CD68, CD300C, CD14, and CD163 (Fig. 2B).

Finally, statistically significant differences ($FC \pm 2$ and $FDR < .001$) between pelvic endometriosis, endometrioma, and adenomyosis against DIE were observed (Table 1). After

FIGURE 2



(A) Leucine-rich repeat containing G protein-coupled receptor 5-positive (LGR5⁺) cells from patients with endometriosis from enriched KEGG up-regulated pathways. The LGR5⁺ cells are related to immune system pathways as well as hematopoietic cell lineage (highlighted in yellow). (B) Overexpressed markers in CFU-GM node. Putative model where LGR5⁺ monocytes transform to their derivatives: macrophages and monocyte-derived dendritic cells. Cytokines CX3CR1 and CDF2RA participate in monocyte differentiation.

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biological analysis of comparisons 7, 9, and 10 we found an overexpression of inflammatory response, immune cell trafficking, and hematologic system development and function pathways in LGR5⁺ cells from DIE compared with other subtypes. Molecular and cellular functions such as activation of leukocytes and myeloid cells or inflammatory response were observed; also the genes CCL1, CCL11, DEFB4A, DEFB103A, CRH, PPM1D and PRKCE were present in LGR5⁺ cells that were overexpressed in DIE. All molecular and cellular functions and the implicated genes are listed in [Supplemental Table 3](#) (available online).

DISCUSSION

Our previous study (24) showed an abnormal epithelial phenotype in the stromal compartment of the eutopic endometrium of mice with induced endometriosis. In the present study we assessed this abnormal epithelial cell type that expresses the stem cell marker LGR5. Further, we postulated that this marker could have a role in the disease. For that reason we studied LGR5⁺ cells in the human eutopic endometrium of endometriotic women. We have demonstrated that 70% and 80% of the patients with endometriosis presented an abnormal colocalization of LGR5 with CK and ECAD, respectively, in the stromal compartment of the eutopic endometrium. Strikingly, this coexpression was not found in healthy women, showing that LGR5⁺ cells seem to behave differently in women with endometriosis. To our knowledge, this is the first time that this process has been reported in the eutopic endometrium of endometriotic women. In our opinion, this characteristic phenotype could potentially be used as a diagnostic maker of endometriosis.

Moreover, previous studies reported the presence of 1% to 2% of LGR5⁺ cells in healthy endometrium and we observed 2% to 7% of LGR5⁺ cells in the eutopic endometrium of women with endometriosis. It is interesting that, in accordance with other studies, our results showed no differences between the percentage of LGR5⁺ cells present in epithelium and stroma in gene expression after RNA high sequencing analysis (19). Although LGR5 has been well described in some tissues as a stemness marker (48), recently it was demonstrated that it does not seem to play this role in the human endometrium (19).

The endometrium is a highly dynamic tissue that changes through the menstrual cycle. In our study, endometrial samples were obtained randomly throughout the menstrual cycle. For this reason, to normalize and compare the results, we excluded the genes that significantly changed along the menstrual cycle and in stimulated cycles. This has the advantage that our results can be interpreted independently of the phase of the menstrual cycle, but it is also limiting in that some relevant genes involved in pathways that change through the menstrual cycle can be inadvertently excluded.

We are aware that we obtained the differentiated expressed genes through the menstrual cycle and under FSH stimulation from two arrays studies. For this reason, we used the IPA software, where we only take into account the DEG that codify for proteins. Several studies have been done using available data sets from different platforms (49–55), and it has been

demonstrated in other studies that DEG from arrays and RNA high sequencing were similar (38–45).

The gene expression profile of LGR5⁺ human endometrial cells in women with endometriosis showed that members of the Wnt pathway were down-regulated, in contrast to the migrating cells of the model where Wnt7a was overexpressed. These results also support the evidence that LGR5⁺ cells may not behave as stem cells in human eutopic endometrium, as previous works have demonstrated (19).

We also observed overexpression of certain hematopoietic markers in LGR5⁺ cells such as CD33, CD300E, CD300LF, CD300LB, and CD200R1, supporting the fact that LGR5⁺ cells from the human endometrium of women with endometriosis seem to have myeloid lineage, as has been previously demonstrated to occur in healthy endometrium (19). In our work, the majority of overexpressed markers such as CD45, CD68, CD300C, CD14, and CD163 belong to the colony forming unit–macrophages/dendritic cells (CFU-M/DC). These findings indicate that LGR5⁺ cells of endometriotic eutopic endometrium are monocytes and their derivatives. It is known that monocytes and macrophages are also directly related to innate immune response, so it seems plausible that LGR5⁺ cells are monocytes capable of transdifferentiating into macrophages and dendritic cells, which explains the activation of the mononuclear phagocyte and immune-regulatory effector (M-PIRE) system (56) (see Fig. 2B).

Other genes related to this cell lineage are overexpressed in LGR5⁺ cells. For instance, CX3CR1 is a chemokine involved in the adhesion and migration of leukocytes, and CSF2RA is a cytokine that controls the production, differentiation, and function of granulocytes and macrophages. Several dendritic cell markers such as CD1C, CD1E, CD83, CD207, and HLA-DR.

Previous works in a mice endometriosis model (24) suggested that a selective migration of cells to the eutopic endometrium could be regulated by the EMT process, as they expressed aberrant epithelial markers in the stroma. Our findings suggest that EMT may explain the fact that LGR5⁺ cells display a monocyte gene expression profile as well as cytokeratin in the stroma of the eutopic endometrium. This is also reinforced by the overexpression in LGR5⁺ cells of MMP12, which is a matrix metalloproteinase involved in the degradation of the extracellular matrix and involved in the EMT process (57).

A recent published article (19) demonstrated the presence in the endometrium of CD45⁺ and CD45⁻ LGR5⁺ cells, and it is interesting that there were no statistically significant gene expression profile differences among these groups. That study also discussed the existence of two different origins of LGR5⁺ cells: LGR5⁺ cells from bone marrow and LGR5⁺ eutopic endometrium resident cells. That is, there appears to be a population of LGR5⁺ macrophage-like cells derived from bone marrow that are CD45⁺, and another perivascular population of LGR5⁺ macrophage-like cells that are CD45⁻. In our study, we mostly observed overexpression of the myeloid markers in our LGR5⁺ population. Taking into account that a significant rate of LGR5⁺ coexpress CD45⁺, we tend to conclude that the LGR5⁺ cells come from the bone marrow. However, we cannot fully exclude the hypothesis that LGR5⁺ may also come from a transdifferentiation of stromal fibroblasts into an epithelial phenotype by an EMT process, which could

potentially lead to the migration of these cells to produce endometrial lesions outside of the uterus.

Macrophages are also involved in tissue repair and remodeling (58–60). In some tumors, macrophages produce factors that foster tumor progression through the production of soluble mediators that support the proliferation, angiogenesis, survival, and invasion of malignant cells (61). It is known that DIE usually represents an aggressive type of disease with increased invasion, proliferation, and angiogenesis in comparison with the other types of endometriosis (1). It is interesting that when we compared LGR5⁺ cells from different types of endometriosis, we found seven overexpressed genes in DIE: DEFB103A, DEFB4B, CCL1, CCL11, CRH, PPM1D, and PRKCE. All these genes are related to inflammatory processes and may have an impact in reproductive outcomes (Fig. 3).

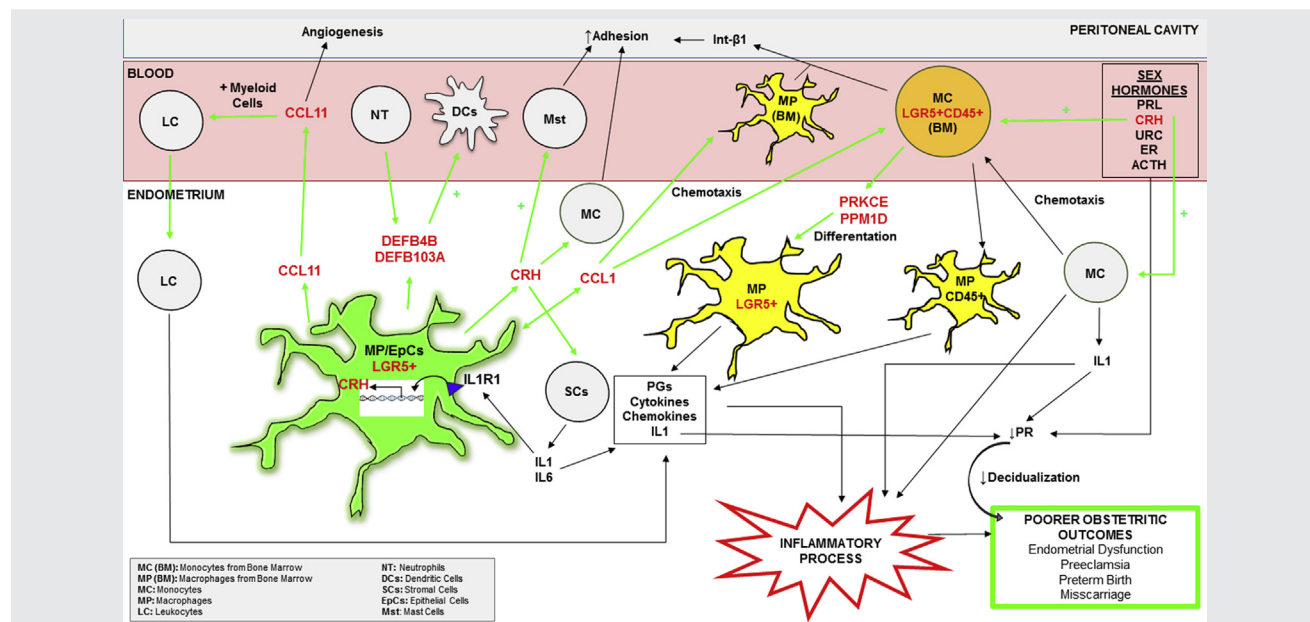
The innate defenses of the human endometrium play a critical role in the maintenance of an environment hospitable to fertilization, fetal implantation, and successful pregnancy. Circulating monocytes migrate into tissues where they differentiate into macrophages, which plays an important role in the initiation, maintenance, and resolution of inflammatory responses. These functions are mediated through the production of innate effectors such as proinflammatory interleukin-1 (62). Interleukin-1 activates uterine epithelial cells to induce DEFB4B, an innate defensin. Chemokine (C-C motif) ligand 1 (CCL1) is a chemokine released by monocytes and macrophages that acts as a chemoattractant for neutrophils and monocytes into different tissues and promotes the expression

of integrin- β , which is involved in embryo-adhesion processes (63). Therefore, it seems plausible that LGR5⁺ cells in DIE could be responsible for recruiting more immune cells in the endometrium by overexpressing CCL1 and potentially affecting embryo implantation.

On the other hand, CCL11, is a chemokine initially identified as a specific chemoattractant protein for eosinophils. However, recent studies have indicated that it has a role in mediating the activity of myeloid cells during development and pathological states (64) and that it may have a function in the endometrium other than as an eosinophil chemoattractant (65). Further studies have shown that the concentration of CCL11 is elevated in the peritoneal fluid of women with severe endometriosis (64, 66) and that CCL11 has angiogenic activity (66), in directly mediating angiogenic responses (67), both processes present in DIE. Additionally, Hornung et al. (64) explained that CCL11 interacts with other cytokines and immune cells to contribute to an inflammatory reproductive tract environment, leading to endometrial or blastocyst dysfunction and potentially impairing implantation.

Corticotropin-releasing hormone (CRH) is found in both epithelial and stromal endometrial compartments (68), although it is mainly produced by epithelial cells (69). It is secreted at inflammatory sites and serves as an autocrine and paracrine modulator (70), with proinflammatory properties that influence both innate and acquired immune responses (71). These properties have been reported to be involved in endometriosis (72). Moreover, CRH has been found to participate in an immune-

FIGURE 3



Leucine-rich repeat containing G protein-coupled receptor 5–positive (LGR5⁺) cells from deep-infiltrating endometriosis. The figure shows LGR5⁺ cells (in green) and the seven overexpressed genes in deep-infiltrating endometriosis: DEFB103A, DEFB4B, CCL1, CCL11, CRH, PPM1D, and PRKCE (in red). We can observe the effects of the genes in other cell types and the consequences of their expression; they are all related to inflammatory processes, which have an impact on reproductive outcomes.

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regulatory manner in ovulation, luteolysis, decidualization, embryo implantation, and maintenance of human pregnancy (70–74). An up-regulation of CRH has been observed in abortions (70, 75). Thus, it seems plausible that CRH may have a negative reproductive effect on endometriotic endometrium.

Protein phosphatase 1D magnesium-dependent, delta isoform (PPM1D), controls a number of critical cellular functions such as proliferation, cell cycle arrest, and programmed cell death. It also is implicated in the differentiation and regulation of the activity of hematopoietic stem cells (76) and appears to be overexpressed in ovarian clear cell carcinoma (77), one of the ovarian cancers often associated with endometriosis. Although the evidence is not conclusive, these initial findings may help us to understand the link between these two pathological entities.

Finally, protein kinase C epsilon (PRKCE) plays a major role as a critical mediator of several signaling cascades in activated macrophages (78) and is involved in monocyte-derived dendritic cells differentiation (79). These data support our proposed theory that LGR5⁺ cells are monocytes and their derivatives (Fig. 2B), and they are involved in the innate immune response. It is interesting that PRKCE seems to be overexpressed exclusively in monocytes (80).

Although our results are promising, we are aware that the study has several limitations. First, the number of samples we assessed was not very large, so we have been restrictive in our statistical analyses. Additionally, the egg donors did not undergo a laparoscopy before the biopsy. Previous reports have observed endometriosis in 4% of asymptomatic women undergoing laparoscopic tubal ligation (81). Therefore, to minimize the possible misdiagnosis of endometriosis we carefully selected fertility-proven donors without dysmenorrhea or cysts in the ovaries.

Taken together, our results show for the first time aberrant LGR5⁺ cells coexpressing epithelial markers in the stromal compartment of women with endometriosis that have a significantly different expression profile in DIE. All these findings suggest eutopic endometrium may have different gene signatures that depend on the type or aggressiveness of the disease, which has implications for reproductive outcomes. Further research is required to elucidate these issues.

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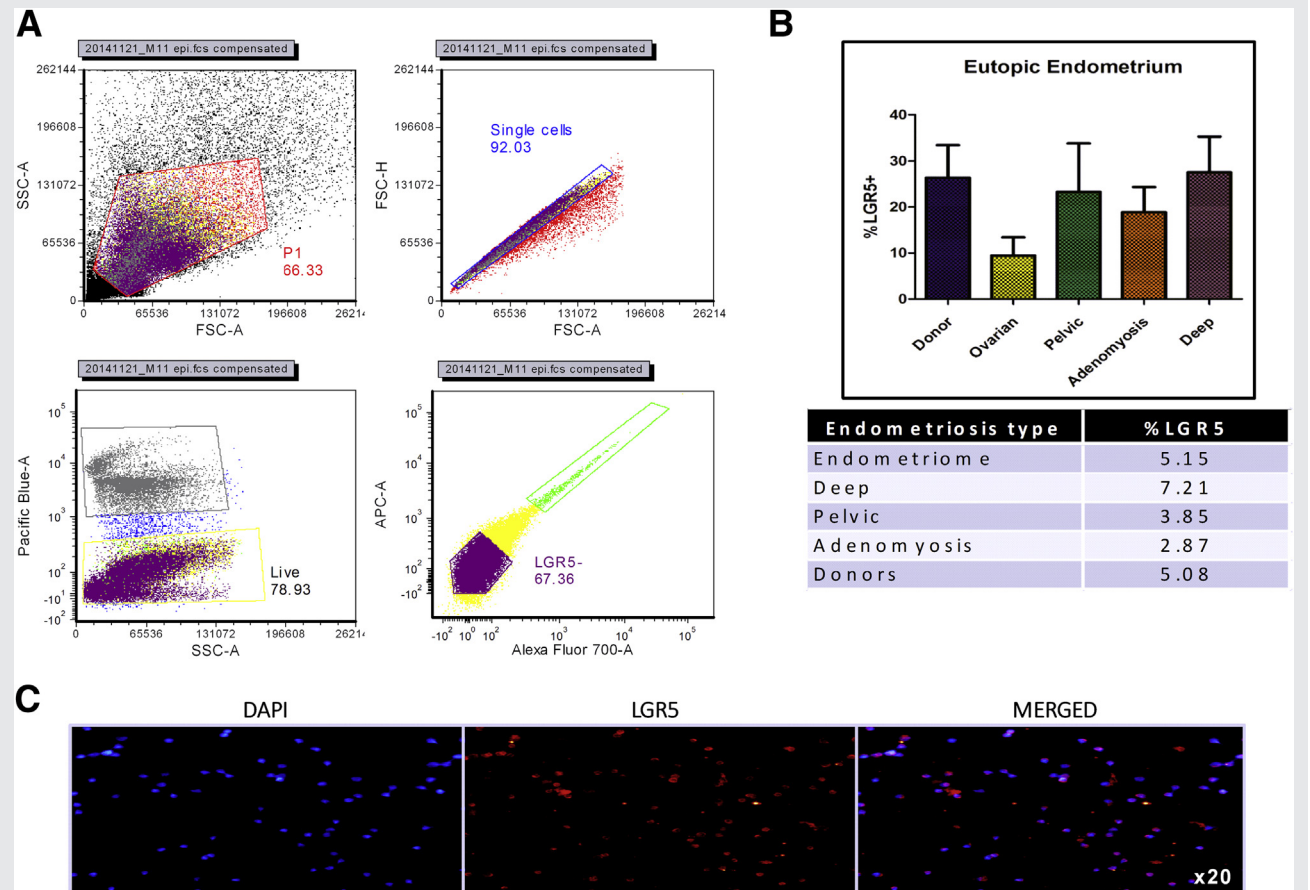
REFERENCES

- Giudice LC. Clinical practice: endometriosis. *N Engl J Med* 2010;362:2389–98.
- Sourial S, Tempest N, Hapangama DK. Theories on pathogenesis of endometriosis. *Int J Reprod Med* 2014;2014:179515.
- Ferguson BR, Bennington JL, Haber SL. Histochemistry of mucosubstances and histology of mixed müllerian pelvic lymph node glandular inclusions. Evidence for histogenesis by müllerian metaplasia of coelomic epithelium. *Obstet Gynecol* 1969;33:617–25.
- Sasson I, Taylor H. Stem cells and the pathogenesis of endometriosis. *Ann NY Acad Sci* 2008;1127:106–15.
- Pluchino N, Taylor HS. Endometriosis and stem cell trafficking. *Reprod Sci* 2016;23:1616–9.
- Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells* 2007;25:2082–6.
- Li T, He H, Liu R, Wang S-X, Pu D-M. Isolation and identification of epithelial and stromal stem cells from eutopic endometrium of women with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2014;178:89–94.
- Djokovic D, Calhaz-Jorge C. Somatic stem cells and their dysfunction in endometriosis. *Front Surg* 2015;1:1–8.
- Figueira PGM, Abrão MS, Krikun G, Taylor H. Stem cells in endometrium and their role in the pathogenesis of endometriosis. *Ann NY Acad Sci* 2011;1221:10–7.
- Vila-Vives JM, Martínez-Conejero JA, Pellicer A. Effect of adenomyosis on implantation [letter]. *Reprod Biomed Online* 2012;24:584.
- Petraglia F, Serour GI, Chapron C. The changing prevalence of infertility. *Int J Gynaecol Obstet* 2013;123(Suppl 2):S4–8.
- Tamareis JS, Irwin JC, Goldfien GA, Rabban JT, Burney RO, Nezhat C, et al. Molecular classification of endometriosis and disease stage using high-dimensional genomic data. *Endocrinology* 2014;155:4986–99.
- Kwon HE, Taylor HS. The role of HOX genes in human implantation. *Ann NY Acad Sci* 2004;6:75–9.
- Campoy I, Lanau L, Altadill T, Sequeiros T, Cabrera S, Cubo-Abert M, et al. Exosome-like vesicles in uterine aspirates: a comparison of ultracentrifugation-based isolation protocols. *J Transl Med* 2016;14:180.
- Barker N, van Es JH, Jaks V. Very long-term self-renewal of small intestine, colon, and hair follicles from cycling Lgr5⁺ stem cells. *Cold Spring Harb Symp Quant Biol* 2008;73:351–6.
- Haegebarth A, Clevers H. Wnt signaling, Lgr5, and stem cells in the intestine and skin. *Am J Pathol* 2009;174:715–21.
- Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007;449:1003–7.
- Gil-Sanchis C, Cervelló I, Mas A, Faus A, Pellicer A, Simón C. Leucine-rich repeat-containing G-protein-coupled receptor 5 (Lgr5) as a putative human endometrial stem cell marker. *Mol Hum Reprod* 2013;19:407–14.
- Cervelló I, Gil-Sanchis C, Santamaría X, Faus A, Vallvé-Juanico J, Díaz-Gimeno P, et al. Leucine-rich repeat-containing G-protein-coupled receptor 5-positive cells in the endometrial stem cell niche. *Fertil Steril* 2017;107:510–9.e3.
- Krusche CA, Kroll T, Beier HM, Classen-Linke I. Expression of leucine-rich repeat-containing G-protein-coupled receptors in the human cyclic endometrium. *Fertil Steril* 2007;87:1428–37.
- Kim MY, Oskarsson T, Acharyya S, Nguyen DX, Zhang XHF, Norton L, et al. Tumor self-seeding by circulating cancer cells. *Cell* 2009;139:1315–26.
- de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoecck JD, Hung J, et al. A distinct role for Lgr5⁺ stem cells in primary and metastatic colon cancer. *Nature* 2017;543:676–80.
- Shimokawa M, Ohta Y, Nishikori S, Matano M, Takano A, Fujii M, et al. Visualization and targeting of LGR5⁺ human colon cancer stem cells. *Nature* 2017;545:187–92.
- Santamaría X, Massasa EE, Taylor HS. Migration of cells from experimental endometriosis to the uterine endometrium. *Endocrinology* 2012;153:5566–74.
- Noyes RW, Hertig AIRJ. Dating the endometrial biopsy. *Fertil Steril* 1950;1:3–25.
- Simón C, Piquette GN, Frances A, Polan ML. Localization of interleukin-1 type I receptor and interleukin-1 beta in human endometrium throughout the menstrual cycle. *J Clin Endocrinol Metab* 1993;77:549–55.
- Andrews S. FastQC: a quality control tool for high throughput sequence data. Version 0.11.5. Babraham Bioinformatics. Available at: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. Accessed: August 3, 2016.
- FASTX-Toolkit, version 0.0.13, Hannon Laboratory, Cold Spring Harbor Laboratory. Available at: http://hannonlab.cshl.edu/fastx_toolkit/index.html. Accessed: February 2, 2010.
- Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. *Bioinformatics* 2011;27:863–4.
- Ensembl GRCh37 Release 75, Homo sapiens. EMBL-EBI. Available at: <http://grch37.ensembl.org/index.html>. Accessed: February 7, 2014.
- Aanes H, Winata C, Moen LF, Østrup O, Mathavan S, Collas P, et al. Normalization of RNA-sequencing data from samples with varying mRNA levels. *PLoS One* 2014;9:e89158.
- Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res* 2012;40(Suppl D):D109–14.

33. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. *Nat Genet* 2000;25:25–9.
34. Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics* 2009;10:161.
35. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics* 2013;29:1830–1.
36. Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, et al. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 2006;147:1097–121.
37. Van Vaerenbergh I, Fatemi HM, Blockeel C, Van Lommel L, In'T Veld P, Schuit F, et al. Progesterone rise on HCG day in GnRH antagonist/rFSH stimulated cycles affects endometrial gene expression. *Reprod Biomed Online* 2011;22:263–71.
38. Black MB, Parks BB, Pluta L, Chu T, Allen BC, Wolfinger RD, et al. Comparison of microarrays and RNA-Seq for gene expression analyses of dose-response experiments. *Toxicol Sci* 2014;137:385–403.
39. Nookaew I, Papini M, Pornputtpong N, Scalcinati G, Nielsen J, Fagerberg L, et al. A comprehensive comparison of RNA-Seq-based transcriptome analysis from reads to differential gene expression and cross-comparison with microarrays: a case study in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2012;40:10084–97.
40. Izadi F, Zarrini HN, Kiani G, Jelodar NB. A comparative analytical assay of gene regulatory networks inferred using microarray and RNA-seq datasets. *Bioinformatics* 2016;12:340–6.
41. Zhang W, Yu Y, Hertwig F, Thierry-mieg J, Zhang W, Thierry-Mieg D, et al. Comparison of RNA-seq and microarray-based models for clinical endpoint prediction. *Genome Biol* 2015;16:133.
42. Fumagalli D, Blanchet-cohen A, Brown D, Desmedt C, Gacquer D, Michiels S, et al. Transfer of clinically relevant gene expression signatures in breast cancer: from Affymetrix microarray to Illumina RNA-sequencing technology. *BMC Genomics* 2014;15:1008.
43. Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y. RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res* 2008;18:1509–17.
44. Bradford JR, Hey Y, Yates T, Li Y, Pepper SD, Miller CJ. A comparison of massively parallel nucleotide sequencing with oligonucleotide microarrays for global transcription profiling. *BMC Genomics* 2010;11:282.
45. Sultan M, Schulz MH, Richard H, Magen A, Klingenhoff A, Scherf M, et al. A Global view of gene activity and alternative splicing by deep sequencing of the human transcriptome. *Science* 2008;321:956–60.
46. Storey JD, Leek JT, Bass AJ. EDGE: extraction of differential gene expression, version 2.5.2. *Bioconductor.org*. Available at: <http://bioconductor.org/packages/release/bioc/html/edge.html>. Accessed: March 2015.
47. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol* 1995;57:289–300.
48. Barker N, Clevers H. Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells. *Gastroenterology* 2010;138:1681–96.
49. Ben-porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet* 2008;40:499–507.
50. Venet D, Dumont JE, Detours V. Most random gene expression signatures are significantly associated with breast cancer outcome. *PLoS Comput Biol* 2011;7:e1002240.
51. Segal E, Friedman N, Koller D, Regev A. A module map showing conditional activity of expression modules in cancer. *Nat Genet* 2004;36:1090–8.
52. Luo H, Ye H, Ng HW, Shi L, Tong W, Mattes W, et al. Understanding and predicting binding between human leukocyte antigens (HLAs) and peptides by network analysis. *BMC Bioinformatics* 2015;16(Suppl 13):S9.
53. Vang YS, Xie X. HLA class I binding prediction via convolutional neural networks. *Bioinformatics* April 21, 2017, Published online April 21, 2017.
54. Wirapati P, Sotiriou C, Kunkel S, Farmer P, Pradervand S, Haibe-kains B, et al. Meta-analysis of gene expression profiles in breast cancer: toward a unified understanding of breast cancer subtyping and prognosis signatures. *Breast Cancer Res* 2008;10:R65.
55. Varn FS, Ung MH, Lou SK, Cheng C. Integrative analysis of survival-associated gene sets in breast cancer. *BMC Med Genomics* 2015;8:11, 2015;1–16.
56. Ben-Ezra JM, Koo CH. Langerhans' cell histiocytosis and malignancies of the M-PIRE system. *Am J Clin Pathol* 1993;99:464–71.
57. Madala SK, Pesce JT, Ramalingam TR, Wilson MS, Minnicozzi S, Cheever AW, et al. Matrix metalloproteinase 12-deficiency augments extracellular matrix degrading metalloproteinases and attenuates IL-13-dependent fibrosis. *J Immunol* 2010;184:3955–63.
58. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity* 2016;44:450–62.
59. Lech M, Anders HJ. Macrophages and fibrosis: How resident and infiltrating mononuclear phagocytes orchestrate all phases of tissue injury and repair. *Biochim Biophys Acta* 2012;7:989–97.
60. Wattanani S, Tornero D, Graubardt N, Memanishvili T, Monni E, Tatarishvili J, et al. Monocyte-derived macrophages contribute to spontaneous long-term functional recovery after stroke in mice. *J Neurosci* 2016;36:4182–95.
61. Low HB, Png CW, Li C, Wang DY, Justin SB. Monocyte-derived factors including PLA2G7 induced by macrophage-nasopharyngeal carcinoma cell interaction promote tumor cell invasiveness. *Oncotarget* 2016;7:55473–90.
62. Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM. Lipopolysaccharide-induced IL-1 β production by human uterine macrophages up-regulates uterine epithelial cell expression of human β -defensin 2. *J Immunol* 2006;176:6647–55.
63. Shi Y-L, Luo X-Z, Zhu X-Y, Li D-J. Combination of 17 β -estradiol with the environmental pollutant TCDD is involved in pathogenesis of endometriosis via up-regulating the chemokine I-309–CCR8. *Fertil Steril* 2007;88:317–25.
64. Hornung D, Dohrn K, Sotlar K, Greb RR, Wallwiener D, Kiesel L, et al. Localization in tissues and secretion of eotaxin by cells from normal endometrium and endometriosis. *J Clin Endocrinol Metab* 2000;85:2604–8.
65. Zhang J, Lathbury LJ, Salamonsen LA. Expression of the chemokine eotaxin and its receptor, CCR3, in human endometrium. *Biol Reprod* 2000;62:404–11.
66. OuYang Z, Osuga Y, Hirota Y, Hirata T, Yoshino O, Koga K, et al. Interleukin-4 induces expression of eotaxin in endometriotic stromal cells. *Fertil Steril* 2010;94:58–62.
67. Salcedo R, Young HA, Ponce ML, Ward JM, Kleinman HK, Murphy WJ, et al. Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3⁺ endothelial cells. *J Immunol* 2001;166:7571–8.
68. Gravanis A, Makrigiannakis A, Zoumakis E, Margioris AN. Endometrial and myometrial corticotropin-releasing hormone (CRH): its regulation and possible roles. *Peptides* 2001;22:785–93.
69. Zoumakis E, Chatzaki E, Charalampopoulos I, Margioris AN, Angelakis E, Koumantakis E, et al. Cycle and age-related changes in corticotropin-releasing hormone levels in human endometrium and ovaries. *Gynecol Endocrinol* 2001;15:98–102.
70. Kalantaridou SN, Zoumakis E, Makrigiannakis A, Lavasidis LG, Vrekoussis T, Chrousos GP. Corticotropin-releasing hormone, stress and human reproduction: an update. *J Reprod Immunol* 2010;85:33–9.
71. Makrigiannakis A, Zoumakis E, Kalantaridou S, Mitsiades N, Margioris A, Chrousos GP, et al. Corticotropin-releasing hormone (CRH) and immunotolerance of the fetus. *Biochem Pharmacol* 2003;65:917–21.
72. Vergetaki A, Jeschke U, Vrekoussis T, Taliouri E, Sabatini L, Papakonstanti EA, et al. Differential expression of CRH, UCN, CRHR1 and CRHR2 in eutopic and ectopic endometrium of women with endometriosis. *PLoS One* 2013;8:e62313.
73. Mastorakos G, Karoutsou EI, Mizamtsidi M. Corticotropin releasing hormone and the immune/inflammatory response. *Eur J Endocrinol* 2006;155(Suppl 1):S77–84.
74. Kalantaridou SN, Makrigiannakis A, Zoumakis E, Chrousos GP. Stress and the female reproductive system. *J Reprod Immunol* 2004;62:61–8.
75. Minas V, Jeschke U, Kalantaridou SN, Richter DU, Reimer T, Mylonas I, et al. Abortion is associated with increased expression of FasL in decidual leukocytes and apoptosis of extravillous trophoblasts: a role for CRH and urocortin. *Mol Hum Reprod* 2007;13:663–73.

76. Uyanik B, Grigorash BB, Goloudina AR, Demidov ON. DNA damage-induced phosphatase Wip1 in regulation of hematopoiesis, immune system and inflammation. *Cell Death Discov* 2017;3:17018.
77. Tan DSP, Lambros MBK, Rayter S, Natrajan R, Vatcheva R, Gao Q, et al. PPM1D is a potential therapeutic target in ovarian clear cell carcinomas. *Clin Cancer Res* 2009;15:2269–81.
78. Castrillo A, Pennington DJ, Otto F, Parker PJ, Owen MJ, Boscá L. Protein kinase C is required for macrophage activation and defense against bacterial infection. *J Exp Med* 2001;194:1231–42.
79. Aksoy E, Amraoui Z, Goriely S, Goldman M. Critical role of protein kinase C δ for lipopolysaccharide-induced IL-12 synthesis in monocyte-derived dendritic cells. *Eur J Immunol* 2002;32:3040–9.
80. Oshevski S, Bousse-kerdile MC, Clay D, Levashova Z, Debili N, Vitral N, et al. Differential expression of protein kinase C isoform transcripts in human hematopoietic progenitors undergoing differentiation. *Biochem Biophys Res Commun* 1999;263:603–9.
81. Bloski T, Pierson R. Endometriosis and chronic pelvic pain: unraveling the mystery behind this complex condition. *Nurs Womens Health* 2008;12:382–95.

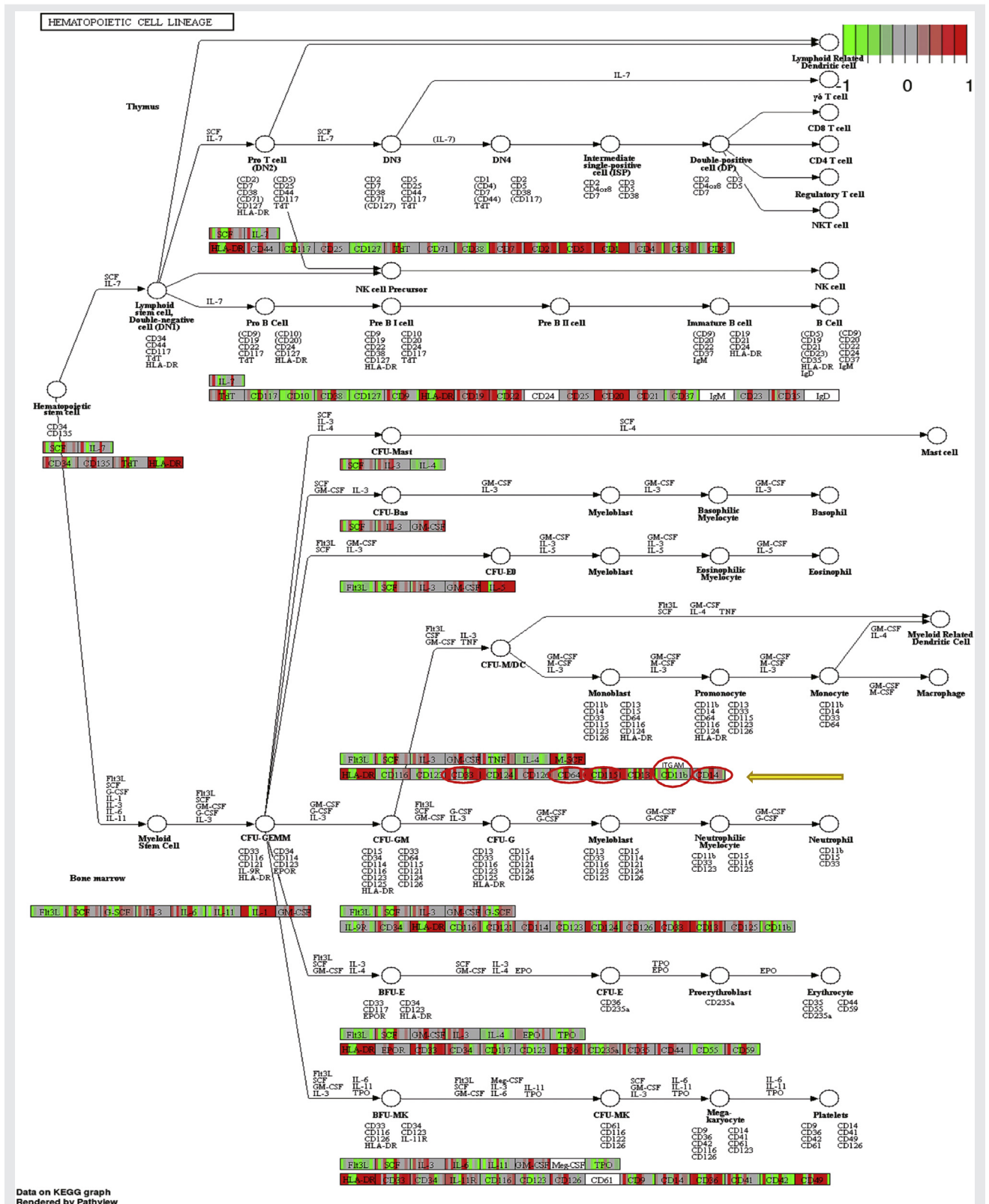
SUPPLEMENTAL FIGURE 1



(A) FACS plots of leucine-rich repeat containing G protein-coupled receptor 5-positive (LGR5⁺) sorted cells. (B) Percentage of LGR5⁺ sorted cells by FACS. (C). Immunofluorescence of LGR5⁺ cells sorted by FACS.

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SUPPLEMENTAL FIGURE 2



Hematopoietic cell lineage (hsa04640). Overexpressed differentiated expressed genes (DEG) in the hematopoietic cell lineage. The overexpressed genes are shown in red and the down-regulated in green. Every box contains the information for the 14 studied patients with endometriosis. Overexpressed (red encircled) markers match with monocyte/macrophages markers (CD11b (ITGAM), CD14, CD33, and CD64).

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