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Endometrial and decidual stromal precursors show a different decidualization capacity

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Abstract

Endometrial stromal cells (EnSCs) and decidual stromal cells (DSCs) originate from fibroblastic precursors located around the vessels of the human nonpregnant endometrium and the pregnant endometrium (decidua), respectively. Under the effect of ovarian or pregnancy hormones, these precursors differentiate (decidualize), changing their morphology and secreting factors that appear to be essential for the normal development of pregnancy. However, the different physiological context – that is, non-pregnancy vs pregnancy – of those precursors (preEnSCs, preDSCs) might affect their phenotype and functions. In the present study, we established preEnSC and preDSC lines and compared the antigen phenotype and responses to decidualization factors in these two types of stromal cell line. Analyses with flow cytometry showed that preEnSCs and preDSCs exhibited a similar antigen phenotype compatible with that of bone marrow mesenchymal stem/stromal cells. The response to decidualization in cultures with progesterone and cAMP was evaluated by analyzing changes in cell morphology by microscopy, prolactin and IL-15 secretion by enzyme immunoassay and the induction of apoptosis by flow cytometry. In all four analyses, preDSCs showed a significantly higher response than preEnSCs. The expression of progesterone receptor (PR), protein kinase A (PKA) and FOXO1 was studied with Western blotting. Both types of cells showed similar levels of PR and PKA, but the increase in PKA RI subunit expression in response to decidualization was again significantly greater in preDSCs. We conclude that preEnSCs and preDSCs are equivalent cells but differ in their ability to decidualize. Functional differences between them probably derive from factors in their different milieus.

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Introduction

Endometrial stromal cells (EnSCs) and decidual stromal cells (DSCs) are the main cellular components of the human nonpregnant endometrium and the pregnant endometrium (decidua), respectively. These stromal cells originate from fibroblastic precursors located around the vessels (Wynn 1974, Ferenczy & Guralnick 1983) and are related to pericytes and mesenchymal stem/stromal cells (MSCs) (Dimitrov *et al.* 2010, Munoz-Fernandez *et al.* 2018, 2019). The latter two are considered analogous or identical cell types (da Silva Meirelles *et al.* 2008, 2016, Caplan & Correa 2011). From their perivascular location, fibroblastic precursors secrete chemokines that may determine the different types of leukocytes in the endometrium and decidua (Munoz-Fernandez *et al.* 2018). A predecidual reaction, which begins close to the vessels and extends through the endometrium, occurs under the effect of ovarian hormones during the luteal phase of the menstrual cycle. In this reaction, fibroblastic precursors differentiate into decidualized EnSCs, changing their morphology to a rounder shape and secreting several distinctive factors such as prolactin (PRL) (Telgmann & Gellersen 1998, Bergeron 2000). These cells are discarded during menstruation, but if pregnancy occurs, the process of precursor decidualization continues in the presence of pregnancy hormones (Wynn 1974, Ferenczy & Guralnick 1983). This is essential for DSCs to acquire properties that allow embryo implantation, the control of trophoblast invasion and appropriate maternal–fetal immune relationships.

We and other authors have used the term 'predecidual stromal cells' (preDSCs) to refer to the perivascular stromal precursors in the decidua that differentiate into decidualized DSCs (Olivares *et al.* 1997, Kyurkchiev

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et al. 2010, Munoz-Fernandez et al. 2018). Likewise, stromal precursors in the nongestational endometrium can be referred to as preEnSCs. Therefore, preEnSCs and preDSCs can be hypothesized to correspond to the same type of cell in two different physiological situations: nonpregnancy and pregnancy. Nevertheless, a relevant point that needs to be ascertained is whether the gestational milieu, conditioned by the effect of local hormones, cytokines and growth factors, is associated with any phenotypic or functional changes in preDSCs compared to preEnSCs.

PreEnSCs, obtained from the endometrium or menstrual blood, and preDSCs, obtained from decidua, can be cultured *in vitro* to establish cell lines. This has made it possible to analyze the antigen phenotype and functions of these cells (Dimitrov *et al.* 2008, Munoz-Fernandez *et al.* 2012, 2018). Furthermore, under the effects of progesterone (P4) and cAMP, stromal precursors decidualize *in vitro* as they do *in vivo*: the cells become rounder and secrete PRL (Huang *et al.* 1987, Olivares *et al.* 1997). Menstrual blood, although a wasting tissue, is an appropriate source of stromal cells that have been used in many basic and clinical studies (Hida *et al.* 2008, Zhong *et al.* 2009, Ulrich *et al.* 2013, Sugawara *et al.* 2014). In the work reported here, we established preEnSC lines from menstrual blood and preDSC lines from first-trimester decidua and compared the antigen phenotype and functions of these two types of cell line. As expected, they had similar phenotypic markers; however, they showed differences in the process of decidualization.

Materials and methods

Samples

For preDSC lines, samples from elective vaginal terminations of first-trimester pregnancies (6–11 weeks) were obtained from 20 healthy women. None of the abortions were pharmacologically induced. The specimens were obtained by suction curettage at the Clínica El Sur in Malaga or the Clínica Ginegranada in Granada. For the preEnSC lines, 21 healthy women donated menstrual blood samples. All women were aged 20 to 35 years, and those who were using any medication or with infectious, autoimmune or other systemic or local disease were excluded.

For MSC lines, leftover samples of bone marrow aspirates from seven hematologically normal donors were obtained at the Hospital de Baza (Baza, Granada).

All donors provided a written informed consent. The Research and Ethics Committee of the University of Granada approved the study.

Isolation, culture and decidualization of cell lines

To establish preDSC lines, we used the method described by Kimatrai *et al.* (2003). For preEnSC and MSC lines, the procedure started with the dilution in phosphate buffered saline (PBS) of menstrual blood and bone marrow aspirate samples, respectively, followed by centrifugation on a Ficoll-Paque (Sigma-Aldrich) density gradient. Only preparations with >95% viable cells, as determined by trypan blue exclusion, were used. Purified cells, confirmed by the co-expression of CD10 and CD29 (95–100% positive cells) and the absence of CD45 and cytokeratin, were maintained and expanded in Opti-MEM medium (Invitrogen) with 3% fetal calf serum (FCS). In this low serum-containing medium, cell lines showed a stable antigen phenotype and functional activities for 8–12 weeks (up to five passages) (Oliver *et al.* 1999, Garcia-Pacheco *et al.* 2001, Kimatrai *et al.* 2003, 2005). The maternal origin of each preDSC line was confirmed by comparison with its corresponding trophoblast obtained from the same sample, using short tandem repeat markers and quantitative-fluorescent PCR (Devyser AB, Hägersten, Sweden).

For decidualization, preDSC, preEnSC and MSC lines were cultured to 70% confluence and incubated in complete medium (OptiMEM plus 3% FCS) with 300 nM P4 and 500 mM 8-bromo-cAMP (Sigma-Aldrich) for up to 28 days. Every 3–4 days the culture medium was changed and the factors were added again (Leno-Duran *et al.* 2014).

Antibodies

The monoclonal antibodies (mAbs) used in this study for flow cytometry analysis were CD9-fluorescein isothiocyanate (FITC), CD10-phycoerythrin (PE), CD15-allophycocyanin (APC), CD19-PE, CD31-FITC, CD34-FITC, CD34-brilliant violet 421, CD44-FITC, CD45-FITC, CD62P-PE, CD73-PE, CD80-PE, CD86-APC, CD140b-PE, CD146, HLA-DR-PE, ICAM-1(CD54)-FITC, podoplanin-Alexa Fluor® 647, VCAM-1 (CD106)-PE, W5C5-APC, cytokeratin (Biolegend, San Diego, CA, USA), CD13 (OKM13, Ortho Diagnostic Systems, Beerse, Belgium), CD29-APC (Caltag Laboratories, Burlingame, CA, USA), CD90-FITC (eBioscience, San Diego, CA, USA), CD140b, nestin, BAFF-FITC, CXCL13-APC (R&D Systems), anti-alpha smooth muscle (α-SM) actin-FITC or cyanine 3 (Cy3) (Sigma-Aldrich) and HLA-G-APC (Abcam). The isotype controls were immunoglobulin IgM, IgG1-FITC, IgG1-PE, IgG1-APC or IgG2-PE (Biolegend). The secondary antibodies were FITC-labeled goat anti-mouse IgM and Alexa Fluor® 488-labeled goat anti-mouse IgG (Invitrogen). For Western blot analysis, we used anti-protein Kinase A (PKA) Riα/β (which recognizes both RIα and Riβ; 1:100), anti-PKA Cα/β/γ (which does not distinguish among Cα, Cβ and Cγ; 1:100) anti-progesterone receptor (PR; 1:100) (Santa Cruz Biotechnology), anti-human Foxo1 antibody (1 µg/mL; R&D Systems), anti-mouse IgG-horseradish peroxidase (HRP) (1:5000; Santa Cruz Biotechnology), anti-sheep IgG HRP-conjugated (1:1000; R&D Systems), anti-β actin (1:5000) and anti-α-tubulin antibody (1:1000) (Sigma-Aldrich).

Prolactin and IL-15 analysis

The concentration of PRL and IL-15 in supernatants, collected from decidualized cells at the indicated times, was determined by enzyme immunoassay (Roche and R&D Systems) according to the manufacturer's instructions.

Flow cytometry analysis

Surface and intracellular antigens, as well as hypodiploid apoptotic cells, were studied in an FACScan cytometer as previously described (Blanco *et al.* 2009).

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells with the TRIzol isolation method, and cDNA was synthesized with Oligo-dT primers and Moloney murine leukemia virus H minus ribonuclease reverse transcriptase (Invitrogen) according to the manufacturer's protocol. For RT-PCR, we used a 2720 Thermal Cycler (Applied Biosystems). The reaction mixture (total volume 20 µL) contained cDNA (the equivalent of 100 ng RNA), 200 nM deoxy-NTPs, 0.5 U GoTaq polymerase (Biotools, Madrid, Spain) and 800 nm of each primer. After initial incubation for 3 min at 95°C, each cycle consisted of 95°C for 30 s, 55°C for 45 s and 72°C for 45 s, for a total of 35 cycles. The RT-PCR products were size-separated on ethidium bromide-stained 2% agarose gels (PanReact AppliChem, Barcelona, Spain) and a 100-bp DNA ladder was included in each run.

The following primers, obtained from the Instituto de Parasitología y Biomedicina, Granada, Spain, were used:

β2-microglobulin (β2MG): 5′-CTCGCGCTACTCTCTCTTTCTGG-3′ (forward) and 5′-TCTACATGTCTCGATCCCACTTAA-3′ (reverse).

Prolactin: 5′-TTCAGGATGAACCTGGCTGAC-3′ (forward) and 5′-GGGTTCATTACCAAGGCCATC-3′ (reverse).

Western blot

To detect proteins, cells were lysed in ice-cold lysis buffer (140 mM NaCl, 10 mM Tris–HCl, 2 M EDTA, 1% NP-40, 50 mM NaF, 1 mM PMSF, 10 mM iodoacetamide, 5 mM sodium pyrophosphate, 50 mM phenylarsine oxide and protease inhibitors) for 30 min. Proteins in the supernatants were resolved on 10% SDS-PAGE gels and detected as reported previously (Leno-Duran *et al.* 2014). Band intensity was quantified with Adobe Photoshop software; intensity values were normalized against the respective loading controls.

Statistical analysis

All experiments were repeated at least three times with different cell lines. Statistical analysis was carried out using GraphPad Prism 7 software. The data were analyzed with Welch's test for unequal variances. Values of $P<0.05$ were considered significant (*$P<0.05$, **$P<0.01$ and ***$P<0.001$).

Results

Antigen phenotype compatible with bone marrow MSC lines in both preEnSC and preDSC lines

The phenotypic profile of preEnSC lines obtained from menstrual blood showed the characteristic markers previously detected in preDSC lines (Munoz-Fernandez *et al.* 2018). An extensive, comparative flow cytometric analysis of preEnSC and preDSC lines with a panel of 28 antigens confirmed that both types of cell exhibited an equivalent phenotype, with no statistically significant differences for all the antigens studied. More than 95% of cells in the preEnSC and preDSC lines expressed CD9, CD10 (an EnSC marker (Sumathi & McCluggage 2002)), CD13, CD29, CD44, CD73, CD90, α-SM actin, nestin and podoplanin and lacked CD15, CD19, CD31, CD34, CD45, CD62P, cytokeratin and HLA-DR expression (Fig. 1 and Table 1). The expression profile for these antigens was equivalent to that observed in bone marrow MSC lines. The exceptions were BAFF and CXCL13, which were expressed by preEnSCs and preDSCs, but not by MSCs (Table 1) (Munoz-Fernandez *et al.* 2012, Gargett *et al.* 2016). PreDSC and preEnSC lines also expressed the endometrial MSC (eMSC) markers CD140b, CD146 and SUSD2 (Gargett *et al.* 2016) (Fig. 1 and Table 1).

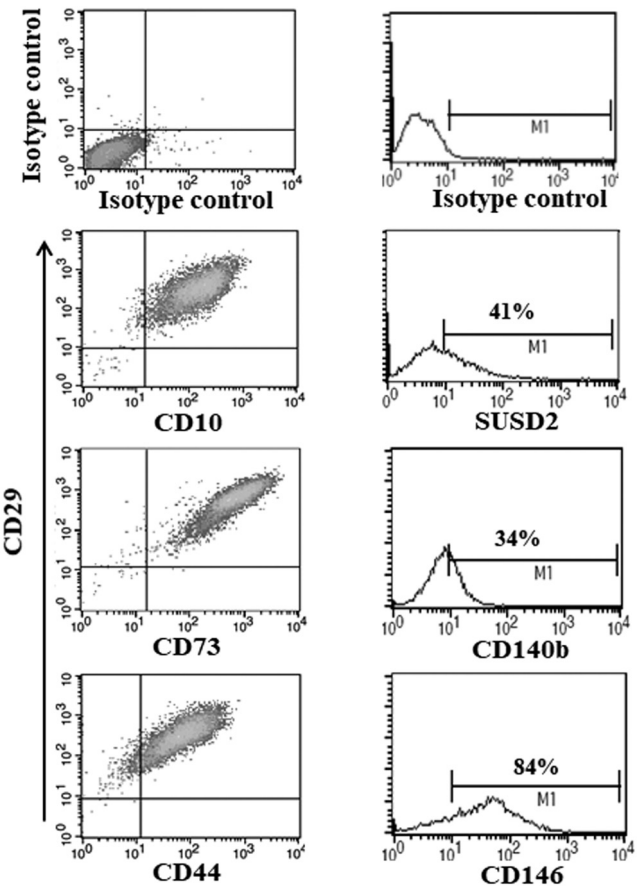

Figure 1 Antigen phenotype of preEnSCs obtained from menstrual blood. Flow cytometry analysis of preEnSC cells stained with antibodies for different cell surface antigens. The figures show the results for a single representative established cell line.

Table 1 Antigen expression by preEnSC, preDSC and bone marrow MSC lines as determined by flow cytometry.

% Positive cells			
Antigen	preEnSC ($n=21$)	preDSC ($n=20$)	MSC ($n=7$)
CD9	>95	>95	ND
CD10	>95	>95	>95
CD13	>95	>95	>95
CD15	<1	<1	<1
CD19	<1	<1	<1
CD29	>95	>95	>95
CD31	<1	<1	<1
CD34	<1	<1	<1
CD44	>95	>95	>95
CD45	<1	<1	<1
CD54	75–87	72–91	45–69
CD62P	<1	<1	<1
CD73	>95	>95	>95
CD80	5–33	0–73	ND
CD86	10–22	0–79	ND
CD90	>95	>95	>95
CD106	0–22	0–17	0–10
CD140b	30–92	72–84	ND
CD146	42–85	67–82	68–75
α-SM actin	>95	>95	>95
BAFF	72–84	52–66	<1
CXCL13	18–33	20–32	<1
Cytokeratin	<1	<1	ND
HLA-DR	<1	<1	<1
HLA-G	0–18	7–22	<1
Nestin	>95	>95	>95
Podoplanin	>95	>95	>95
SUSD2	34–79	33–55	21–35

ND, Not determined.

Stronger evidence of markers and functions associated with decidualization in preDSC lines than in preEnSC lines

PreDSC and preEnSC lines can differentiate *in vitro*, as they do *in vivo*. Under the effect of the decidualizing agents P4 and cAMP, these cells became rounder, secreted PRL and IL-15 and underwent apoptosis (Dunn *et al.* 2003, Leno-Duran *et al.* 2014, Sharma *et al.* 2016).

To further determine whether preDSCs and preEnSCs are equivalent cells, we compared their ability to differentiate in response to P4 and cAMP. Interestingly, while both types of cells changed their fibroblastic-like appearance to a rounder morphology, the percentage of rounded cells was significantly higher in decidualized preDSCs. In addition, decidualized preDSCs secrected significantly more PRL, a widely used marker of decidualization, than decidualized preEnSCs. Other activities associated with the process of decidualization, such as IL-15 production and apoptosis (Dunn *et al.* 2003, Leno-Duran *et al.* 2014, Sharma *et al.* 2016), were also significantly greater in preDSC than in preEnSC lines (Fig. 2). Even after prolonged incubation with P4 and cAMP, preEnSCs did not reach the level of PRL production and apoptosis observed in preDSCs (Fig. 2B and D). Due to the close relationship of bone marrow MSCs with preEnSCs and preDSCs (Table 1), we also analyzed MSC lines cultured under decidualizing conditions for comparison. Intriguingly, although MSCs changed their shape to a rounder morphology and expressed PRL mRNA, secretion of this factor was not detected (Supplementary Fig. 1, see section on supplementary materials given at the end of this article).

Expression of progesterone receptor, protein kinase A and FOXO1 in preEnSCs and preDSCs

The nuclear receptors for progesterone (PR) and protein kinase A (PKA)-mediated cAMP signaling are crucial for decidualization (Brar *et al.* 1997, Telgmann *et al.* 1997,

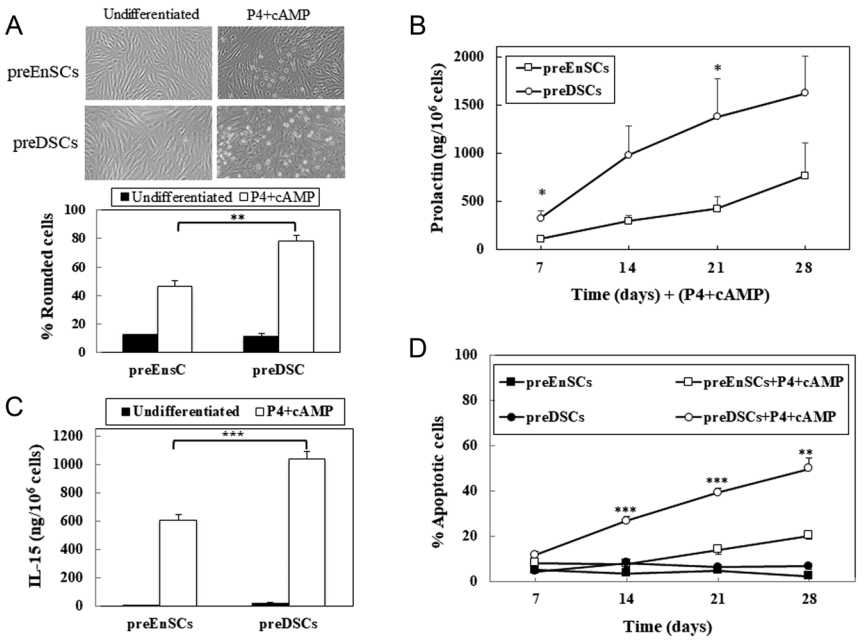

Figure 2 Comparative analysis of decidualized EnSCs and DSCs. (A) Morphological changes in undifferentiated preEnSCs and preDSCs after 7 days of culture with P4 and cAMP. Bars show the percentage of rounded cells after quantification of representative areas ($n=3$ per sample) with ImageJ. (B) Time course of PRL secretion by preEnSCs and preDSCs after incubation with P4 and cAMP. PRL production by nondecidualized (undifferentiated) cells was zero at all time points (not shown). (C) Secretion of IL-15 after 7 days of culture without (undifferentiated) or with P4 and cAMP. (D) Induction of apoptosis upon incubation without (undifferentiated) or with P4 and cAMP for up to 28 days. Sub-G1 apoptotic cells were analyzed every 7 days. Error bars show the S.E.M. of five (B and D) and three (A and C) independent experiments with different cell lines. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ (preENSC + P4 + cAMP vs preDSC + P4 + cAMP).

Kaya *et al.* 2015). The progesterone receptor has two isoforms, PR-A and PR-B, whereas in the case of PKA, an enzymatic complex formed by two regulatory (R) and two catalytic (C) subunits, three distinct C subunits (Cα/β/γ) and four R subunits (RIα/β and RIIα/β) have been identified (Skalhegg & Tasken 2000). It has been reported that EnSCs express the Cα and Cβ isoforms as well as all four regulatory subunits of PKA, although expression of the RIα isoform is higher than the rest (Telgmann *et al.* 1997, Kim *et al.* 1998). We compared the expression of PR and PKA between preEnSC and preDSC. As shown in Fig. 3A, both types of cells showed similar basal levels of the two isoforms of PR and the catalytic (Cα/β/γ) and Iα/β regulatory (RIα/β) subunits of PKA. In addition, expression of the regulatory subunit of PKA and the PR-A isoform increased after decidualization in both cell types. Interestingly, upregulation of PKA RIα/β was significantly higher in decidualized preDSCs than in decidualized preEnSCs (Fig. 3A, right panel). These results were confirmed by a time course expression analysis of PKA RIα/β in response to decidualization (Fig. 3B). We further explored differences in the signaling pathways in response to decidualization by analyzing the expression of the transcription factor FOXO1, a downstream mediator of decidualizing factors (Buzzio *et al* 2006, Labied *et al* 2006). As expected, incubation with P4 and cAMP induced the expression of FOXO1 in preEnSC and preDSC but without significant differences between both cell types (Fig. 3A).

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Discussion

There is considerable confusion in the terminology regarding EnSCs and DSCs. Because the endometrium differentiates into decidua when gestation occurs, some authors consider EnSCs as precursor cells and DSCs as differentiated cells. The fact that decidualization has been observed *in vivo* and *in vitro*, in both the endometrium and decidua (Wynn 1974, Ferenczy & Guralnick 1983, Huang *et al.* 1987, Olivares *et al.* 1997), indicates that there must be precursor cells in both tissues, which we have called preEnSCs and preDSCs, respectively (Olivares *et al.* 1997). These two types of precursors may be considered the same type of cell in two different physiological situations, that is, nongestation and gestation. In fact, our analysis of the expression of 35 antigens showed an equivalent antigen phenotype for both precursors (Fig. 1 and Table 1). However, we observed that processes associated with decidualization, for example, change in the cellular morphology to a rounder shape, secretion of PRL and IL-15 and apoptosis (Dunn *et al.* 2003, Leno-Duran *et al.* 2014, Sharma *et al.* 2016), were significantly more evident in preDSCs than in preEnSCs (Fig. 2). To our knowledge, this is the first study designed to compare markers of decidualization processes in preEnSCs and preDSCs.

Our results could be attributed to the fact that we compared stromal cells from a wasting tissue (menstrual blood) to stromal cells from a developing tissue (decidua). However, although normal menstrual blood and decidua harbor apoptotic cells (Tabibzadeh 1996, von Rango *et al.* 1998), the cell cultures developed to establish cell lines select only healthy, proliferative cells to survive, which correspond to precursor cells in both tissues (Meng *et al.* 2007, Hida *et al.* 2008, Patel *et al.* 2008, Munoz-Fernandez *et al.* 2019). In this connection, stromal cells from menstrual blood and decidua have been used in numerous basic and clinical studies (Hida *et al.* 2008, Zhong *et al.* 2009, Ulrich *et al.* 2013, Sugawara *et al.* 2014, Alshabibi *et al.* 2018, Sadeghi *et al.* 2019). Furthermore, a similar

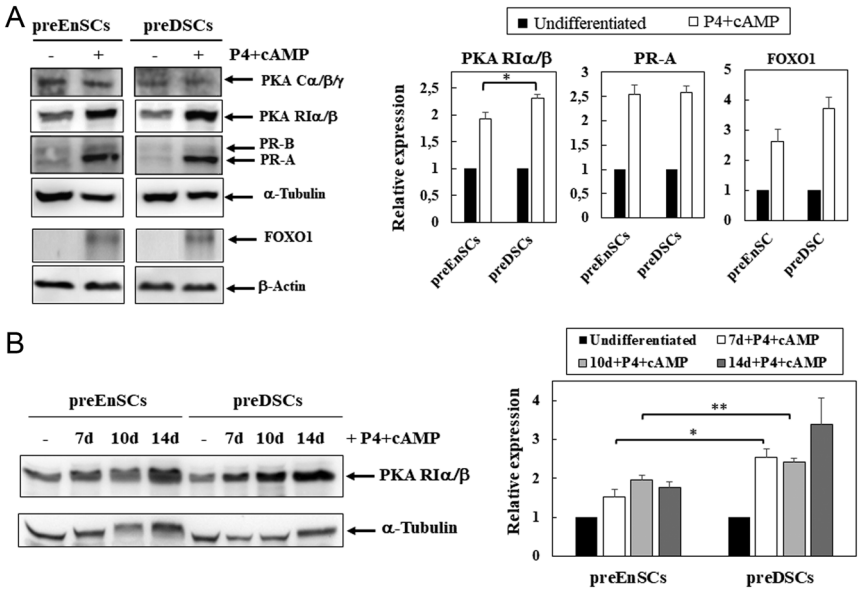

Figure 3 Expression of PR, PKA and FOXO1 in response to decidualization. (A) preEnSCs and preDSCs were incubated with P4 and cAMP for 10 days, and the expression of PR isoforms, PKA catalytic (Cα/β/γ) and regulatory (RIα/β) subunits and FOXO1 was determined by Western blot. (B) Expression of PKA RIα/β was analyzed by Western blot in preEnSCs and preDSCs after incubation for 7, 10 and 14 days with P4 + cAMP. α-tubulin and β-actin were used as controls for loaded protein. A representative experiment is shown (left panels). Bar charts show the relative expression of PR-A (A), PKA RIα/β (A, B) and FOXO1 (A) after band intensity analysis from three different Western blots (right panels).

antigen phenotype and capacity to decidualize were observed in preEnSC lines obtained from endometrial biopsies rather than menstrual blood (Supplementary Fig. 2). Because menstrual blood is easily obtained by noninvasive methods, it was used as the source of endometrial stromal cells in our study.

Decidualized cells depend on the constant activation of cAMP-dependent PKA for the efficient expression of their characteristic phenotype and markers, including PRL. Earlier work found that, after decidualization with 17β-estradiol, MPA (medroxyprogesterone acetate) and relaxin, there were no differences in the expression of the PKA subunits except for the RIα isoform, which was downregulated at the protein level (Telgmann *et al.* 1997, Kim *et al.* 1998). In the present work, we compared the expression of the C and RI subunits in preEnSCs and preDSCs and found that both types of cells showed similar levels of these proteins. This finding confirmed that decidualization did not regulate the expression of PKA C. Interestingly, we found that PKA RI was upregulated after treatment with P4 and cAMP. Both decidualized EnSCs and DSCs maintained high levels of PKA RI expression throughout the time course studied here, although the increase was significantly greater in DSCs. The discrepancies between our data and those of Telgmann et al. may be due to the different experimental conditions used to establish, maintain and decidualize cell lines (Telgmann *et al.* 1997). Because PKA formed by RI subunits was reportedly a more efficient activator of cAMP-responsive elements than PKA that contained RII (Stakkestad *et al.* 2011), the larger increase in RI in preDSCs may explain their greater ability to decidualize. In addition to PKA, we also analyzed the expression of PR as the other key mediator of decidualization signaling. Our results show that there was no difference between preDSCs and preEnSC in either the basal expression of PR or the increase in the PR-A isoform in response to decidualization, suggesting that the different behaviors in the two cell types may not be due to differences at this stage of progesterone signaling. Moreover, the downstream mediator FOXO1, which has been reported to play a role in the differentiation of endometrial cells (Buzzio *et al.* 2006, Labied *et al.* 2006, Kajihara *et al.* 2013), did not seem to be involved in the different response of preEnSC and DSC to decidualization.

Gestation is an immunological challenge for the mother, and several lines of experimental evidence support the key role of DSCs in maintaining immunological tolerance to avoid rejection of the fetus (Dunn *et al.* 2003, Blanco *et al.* 2008, 2009, Munoz-Fernandez *et al.* 2012, Nancy *et al.* 2012). The production of IL-15 by decidualized cells appears to be related to the maintenance of decidual NK cells (Dunn *et al.* 2003). Concerning apoptosis, this was previously reported to occur in parallel with decidualization, as a physiological phenomenon necessary to limit the lifespan of DSCs and allow placental development to proceed (Leno-Duran *et al.* 2014). Conversely, in the absence of gestation, these activities do not necessarily need to be carried out by EnSCs, although these cells are 'standing by' in case pregnancy takes place. Although the findings available to date regarding antigen phenotype, perivascular location, relation to MSCs and secreted factors support that preEnSCs and preDSCs are the same type of cell, they are nevertheless located in two different physiological milieus: nongestational and gestational, respectively. Furthermore, preDSCs remain *in vivo* for a longer period (up to 11 weeks of gestation before the voluntary termination of pregnancy) than preEnSCs (up to 4 weeks before menstruation) before they can be isolated to establish cell lines. These distinct milieus, which involve a different environment in terms of cytokines, hormones and growth factors, together with differences in their natural history, are the probable causes of the differences observed between preEnSCs and preDSCs, as suggested by others (Kyurkchiev *et al.* 2010). Because decidualization is a complex phenomenon, further research is necessary to understand the molecules involved in this process as well as the genetic or epigenetic factors that, under the influence of the milieu, may regulate the likelihood of decidualization.

In previous publications, we and others demonstrated the close relationships between preEnSCs, preDSCs and MSCs (Dimitrov *et al.* 2008, 2010, Munoz-Fernandez *et al.* 2012, 2018, 2019). Our present results, which show that both types of precursor – one from the endometrium, and one from the decidua – have an antigen phenotype in common with bone marrow MSCs (Table 1), confirm these relationships and support the view proposed by some authors (Taylor 2004, Du & Taylor 2007) that bone marrow-derived MSCs may be the source of EnSCs and hence of DSCs. Despite the close relationships among preEnSCs, preDSCs and bone marrow MSCs, we detected differences in the expression of BAFF and CXCL13, which was positive in preEnSCs and preDSCs but negative in MSCs. These differences may be due to the immunological interactions of bone marrow MSCs, when they arrive in the endometrium or decidua, with local immune cells (Vacca *et al.* 2015). Furthermore, bone marrow MSCs demonstrated a low capacity to decidualize, given that they did not secrete PRL in response to P4 and cAMP (Supplementary Fig. 1). Our results suggest that through a gradual process of progression from MSCs (bone marrow) to preEnSCs (endometrium) and then to preDSCs (decidua), stromal cells acquire the decidualization capacity essential for the normal development of pregnancy (Dunn *et al.* 2003). Thus, further elucidation of the initiation and progression of decidualization capacity may help to better understand diseases such as endometriosis, in which cells are unable to decidualize (Patel *et al.* 2017).

PreDSCs and preEnSCs also appear to correspond to endometrial MSCs (eMSCs), that is, clonogenic, self-

renewing, multipotent cells that can differentiate into adipogenic, osteogenic, chondrogenic and myogenic lineages. Like preEnSCs and preDSCs, eMSCs are CD146+, CD140b+ and SUSD2+, decidualize, are found in perivascular sites, and have also been associated with pericytes (Gargett & Masuda 2010, Kyurkchiev *et al.* 2010, Spitzer *et al.* 2012, Munoz-Fernandez *et al.* 2018). However, to date, eMSCs have been isolated only by cell sorting, whereas the preEnSC and preDSC lines obtained in the present work from endometrium and decidua were enriched by cell culture.

Like MSCs (Uccelli *et al.* 2008), preDSCs and preEnSCs exhibit immunoregulatory activity *in vivo* and *in vitro* (Vacca *et al.* 2015). Human DSCs were found to have beneficial effects in steroid-refractory graft-vs-host disease in humans (Ringden *et al.* 2013, 2018). Likewise, we recently demonstrated the therapeutic effect of human preDSCs in an immune-based mouse model of recurrent spontaneous abortion (Munoz-Fernandez *et al.* 2019). These findings identify DSCs, and probably EnSCs, as potentially important components of cell therapies for immune-mediated diseases. The availability of endometrial and decidual stromal cells, and the straightforward techniques needed to purify and expand them, may help to further research on their clinical applications in the treatment of autoimmune and inflammatory diseases. Our findings thus raise the possibility of testing DSCs and EnSCs in therapeutic trials to determine which cell type would be more suitable (Simoni & Taylor 2018, Queckborner *et al.* 2019).

In conclusion, our results suggest that although stromal precursors from endometrium and decidua have a similar phenotype, they differ in their response to decidualization factors, with preDSCs being significantly more responsive. The similarity of these precursors with MSCs suggests an important therapeutic potential for inflammatory diseases.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/REP-19-0465.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M J R M performed conception and design, administrative support, collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. J M P performed administrative support, collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. R M A performed administrative support, collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. T L performed collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. O B performed collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. R M F performed collection and/or assembly of data, experimental procedures, data analysis and interpretation and final approval of manuscript. E G O performed conception and design, financial support, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing and final approval of manuscript. C R-R performed conception and design, financial support, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing and final approval of manuscript. E G O and C R-R jointly supervised this work.

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