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Decidualization modulates the mesenchymal stromal/stem cell and pericyte characteristics of human decidual stromal cells. Effects on antigen expression, chemotactic activity on monocytes and antitumoral activity

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ABSTRACT

Decidual stromal cells (DSCs) are the most abundant cellular component of human decidua and play a central role in maternal–fetal immune tolerance. Antigen phenotyping and functional studies recently confirmed the relationship of DSCs with mesenchymal stem/stromal cells (MSCs) and pericytes, the latter two cell types being closely related or identical. The present study investigated the effect of decidualization, a process of cell differentiation driven by progesterone (P4) and other pregnancy hormones, on the MSC/pericyte characteristics of DSCs. To this end we isolated undifferentiated DSC (preDSC) lines that were decidualized in vitro (dDSC) by the effect of P4 and cAMP. Using flow cytometry, we found significant downmodulation of the expression of the MSC/pericyte markers α-smooth muscle actin, nestin, CD140b, CD146 and SUSD2 in dDSCs. The dDSCs did not differ, compared to preDSCs, in the expression of angiogenic factors (characteristic of pericytes) HGF, FGF2, ANGPT1 or VEGF according to RT-PCR results, but had significantly increased PGF expression. In migration assays, preDSC-conditioned media had a chemotactic effect on the THP-1 monocytic line (characteristic of pericytes), and this effect was significantly greater in dDSC-conditioned media. Media conditioned with dDSC, but not with preDSC, induced apoptosis in 4 out of 6 different tumor cell lines (characteristic of MSCs) according to propidium iodide staining and flow cytometry results. Our findings show that decidualization induces phenotypic and functional changes in the MSC/pericyte properties of DSCs that may have a role in the normal development of pregnancy.

1. Introduction

Decidual stromal cells (DSCs) are the most abundant cells in the decidua, the maternal part of the human placenta in close contact with fetal trophoblast. In the decidua, DSCs play a key role in the normal development of pregnancy by controlling trophoblast invasion, and by exerting immunoregulatory activities (Nancy et al., 2012; Macklon and Brosens, 2014; Sharma et al., 2016). DSCs originate from fibroblastic perivascular precursors (preDSCs) also detected in nongestational endometrium (Wynn, 1974; Ferenczy and Guralnick, 1983); under the effect of progesterone (P4) and other pregnancy hormones, these precursors differentiate into decidualized DSCs (dDSCs). During the decid-

Abbreviations: α-SM actin, α-smooth muscle actin; ANGPT1, angiopoietin 1; APC, allophycocyanin; CD, cluster of differentiation; CM, conditioned media; CXCL12, C-X-C motif chemokine 12; dDSC, decidualized DSC; DSC, decidual stromal cell; eMSC, endometrial MSC; FGF2, fibroblast growth factor 2; HGF, hepatic growth factor; MSC, mesenchymal stem/stromal cell; NFC, negative fold change; P4, progesterone; PFA, paraformaldehyde; PGF, placental growth factor; PreDSC, undifferentiated DSC; PRL, prolactin; SUSD2, sushi domain-containing 2; VEGF, vascular endothelial growth factor; WJ-MSC, Wharton's jelly mesenchymal stem cell

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ualization process, dDSCs leave the perivascular space toward the extravascular zone, become rounder, and secrete prolactin (PRL) (Supplementary Fig. 1) and other proteins such as IL-15 (Wynn, 1974; Braverman et al., 1984; Richards et al., 1995; Bergeron, 2000). It is therefore likely that DSCs exert functions different from those of non-differentiated preDSC in promoting differentiation to the dDSC state (Blanco et al., 2008).

The isolation and maintenance of highly purified human preDSC lines in culture allowed us to study the antigen expression and functions of these cells. These lines exhibited characteristics equivalent to those of their corresponding fresh cells (Munoz-Fernandez et al. 2012, Vento-Tormo et al., 2018). Because of their antigenic phenotype, expression of stem cell markers, capacity to differentiate into the mesenchymal lineage, and immunoregulatory activity in vivo and in vitro, preDSCs have been identified as mesenchymal stem/stromal cells (MSCs). These properties, together with the expression of endometrial MSC (eMSC) markers (CD140b, CD146 and SUSD2 [sushi domain-containing 2]) (Gargett et al., 2016), strongly suggest that preDSCs correspond to eMSCs (Muñoz-Fernandez et al., 2018). Furthermore, like MSCs, DSCs were shown to exert therapeutic activities in immune-based diseases in humans and experimental animal models (Ringden et al., 2018; Muñoz-Fernández et al., 2019). Additionally, due to the perivascular location of preDSCs, their contractile and chemotactic activity, expression of pericyte markers and angiogenic factors, and immunoregulatory activity, it is plausible that preDSCs are also a form of pericytes (Munoz-Fernandez et al. 2018). Interestingly, several lines of experimental evidence support that MSCs and pericytes are also closely related, or may even be the same type of cell (Caplan and Correa, 2011; da Silva Meirelles et al., 2016). In this connection, we previously showed in isolated preDSC clones that MSC, pericyte and decidual characteristics are all displayed by the same cells (Muñoz-Fernandez et al., 2018; Muñoz-Fernández et al., 2019).

Because decidualization induces morphological, locational and functional changes in DSCs, the present study was designed to investigate the effects of decidualization on MSC/pericyte characteristics in these cells. A controversial characteristic of MSCs is their antitumor activity (Galland and Stamenkovic, 2020); however, several reports confirmed that Wharton's jelly mesenchymal stem cells (WJ-MSCs), which have MSC characteristics derived from the embryonic cord, secrete factors that induce apoptosis in different tumor cell lines (Ganta et al., 2009; Fong et al., 2011; Lin et al., 2017). Accordingly, we also investigated the antitumoral effects of preDSCs and dDSCs.

2. Materials and methods

2.1. Samples

Samples from elective vaginal terminations of first-trimester pregnancies (6–11 weeks) were collected from 12 healthy women aged 20–30 years. We excluded women who were using any medication or with infectious, autoimmune or other systemic or local disease. None of the abortions was pharmacologically induced. The specimens were obtained by suction curettage at the Clínica El Sur in Málaga or the Clínica Ginegranada in Granada. Blood samples for PBLs isolation were obtained from healthy volunteers aged 20–35 years. All donors provided their written informed consent. The Research and Ethics Committee of the University of Granada approved the study.

2.2. Isolation and culture of preDSC lines

To establish preDSC lines from decidua, we used the method described by Kimatrai and colleagues (Kimatrai et al., 2005). Briefly, decidua samples were minced between two scalpels in a small volume of PBS. The suspension was mixed with a solution of 5 mg/mL Collagenase V (Sigma-Aldrich, St. Louis, MO) for 30 min at 37 °C. The suspension was diluted in PBS and centrifuged at 425 *g* for 10 min. The cell pellet was suspended in PBS and centrifuged on Ficoll-Paque (Sigma-Aldrich) for 20 min at 600 g. Decidual cells were collected from the interface, suspended in PBS and washed. The resulting suspension was incubated in culture flasks for 24 h at 37 °C with 5% CO_2 in Opti-MEM (Invitrogen, Grand Island, NY) supplemented with 3% FCS (Invitrogen), 100 IU/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin (Sigma-Aldrich). After overnight incubation to allow adherent cells to attach to the flask, nonadherent cells in the supernatant were discarded. The medium was then replaced and changed thereafter twice a week. After 1–3 weeks, adherent cells exhibited a uniform fibroblastic morphology and covered the entire surface of the 25-cm^2 culture flask. Cells were split with trypsin-EDTA solution 0.25 % (Sigma-Aldrich) when they were 90–100 % confluent. For this study 12 preDSC lines were obtained (each from a different sample), and were used between 3 and 8 weeks after collection (up to 5 passages). The maternal origin of each preDSC line was confirmed by comparison with their corresponding trophoblasts obtained from the same sample using short tandem repeat markers and quantitative-fluorescent PCR (Devyser AB, Hägersten, Sweden). As under in vivo conditions, preDSC lines exhibited a fibroblastic morphology and consisted of a highly purified, uniform, adherent cell population with a characteristic antigen profile (Muñoz-Fernandez et al., 2018) in which almost all cells expressed the endometrial stromal cell marker CD10 (Sumathi and McCluggage, 2002), and CD29 (Supplementary Fig. 1A).

2.3. Decidualization

To induce decidualization, preDSC lines were treated with 300 nM P4 and 500 μM dibutyryl cAMP (Sigma-Aldrich) for 14 days. Decidualization was verified by PRL secretion and changes in cell morphology from a fibroblastic shape in preDSCs to a round shape in dDSCs, as observed by light microscopy (Supplementary Fig. 1B). Conditioned media (CM) were collected from cultures of undifferentiated pre-DSCs (preDSC-CM) and dDSCs (dDSC-CM), and kept frozen until use. The presence of PRL was verified with an electrochemiluminescence immunoassay (Roche, Indianapolis, IN). The assays were performed according to the manufacturer's instructions, and all samples were tested in duplicate.

2.4. Isolation and culture of lymphocytes

PBLs were obtained by density gradient centrifugation on Ficoll-Paque (Sigma-Aldrich) and incubation with RPMI 1640 medium supplemented with 10 % FCS (Invitrogen), 100 IU/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich) for 1 h at 37 °C to eliminate adherent cells. 2×10^6 PBL/mL were activated with 5 μg/mL phytohemagglutinin (Sigma-Aldrich) and 1 μg/mL anti-CD28 (eBioscience, San Diego, CA) for 20 h. To activate PBLs, the cells were then washed and incubated in complete RPMI medium supplemented with 25 U/mL rIL-2 (National Institute of Health AIDS Reagent Program, Rockville, MD) for an additional 6 days.

2.5. Tumor cell lines

The following human tumor cell lines were used: HeLa (cervical carcinoma, ATCC), SKBR3 (breast cancer, ATCC), Raji (Burkitt's lymphoma, ATCC), A375 (melanoma, ATCC), CAL33 (tongue squamous cell carcinoma, Leibniz Institute, Braunschweig, Germany), and THP-1 (monocytic leukemia, ATCC). The HeLa, SKBR3, A375, and THP-1 lines were cultured in RPMI 1640 medium (Gibco, Carlsbad, CA), and Raji and CAL33 lines were cultured in DMEM (Gibco). Both media were supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin (Sigma-Aldrich) and 10 % FCS (Invitrogen).

2.6. Monoclonal antibodies for flow cytometry

The mAbs used in flow cytometry were against CD10-PE, CD44-FITC, CD140b-PE, CD146, SUSD2 (W5C5)-allophycocyanin (APC) (Biolegend, San Diego, CA), CD29-APC, C-X-C motif chemokine 12 (CXCL12) (Caltag Laboratories, Burlingame, CA), nestin (R&D Systems, Minneapolis, MN), α-smooth muscle actin (α-SM actin)-FITC or cyanine 3 (Cy3) (Sigma-Aldrich). The isotype controls used were immunoglobulin IgM, IgG1-FITC, IgG1-PE, IgG1-APC and IgG2-PE (Biolegend). The secondary antibodies were FITC-labeled goat anti-mouse IgM and AlexaFluor 488-labeled goat anti-mouse IgG (Invitrogen). CD10, CD29, CD44, CD140b, CD146, and SUSD2 are extracellular molecules, while α-SM actin, CXCL12 and nestin are intracellular molecules.

2.7. Flow cytometric analysis

Cells were detached from the culture flask with 0.04 % EDTA at 37 °C and were then centrifuged. The supernatant was discarded and the pellet was suspended in PBS at 10^6 cells/mL. One hundred microliters of the cell suspension was incubated with 5 μL of the appropriate mAb for 30 min at 4 °C in the dark. The cells were washed, suspended in 0.5 mL PBS and immediately analyzed in a fluorescence-activated cell sorter (FACScan Cytometer, BD Biosciences, San Jose, CA). The percentage of antibody-positive cells was calculated by comparison with the appropriate isotype control. For double labeling we followed the same procedure as for single labeling but added a second mAb with a different fluorescent marker. For intracytoplasmic labeling, cells were fixed with 4% paraformaldehyde (PFA) (Merck, Darmstadt, Germany) for 20 min at 4 °C and permeabilized with cold 0.05 % PBS saponin (Merck) before the mAb was added. For indirect labeling, FITC-labeled goat anti-mouse Ig was added after the first mAb.

2.8. Reverse transcription polymerase chain reaction

Total RNA was extracted from cells with the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA). cDNA was synthesized at an RNA starting concentration of 0.5 μg with the Access RT-PCR System kit (Promega, Madison, WI) and oligo-dT primers according to the manufacturer's protocol. The PCR Mastermix 2x kit (Promega) along with appropriate primers and cDNA samples were mixed and placed in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA). The PCR products were size-separated on Gel Red-stained 1.5 % agarose gels (Biotium, Fremont, CA), and a 100-bp DNA ladder (Promega) was included in each run. Bands were further quantified with Adobe Photoshop CS3 software (Adobe San Jose, CA). Intensity values were normalized against the respective intensity values of the housekeeping gene *GAPDH* (Zhou et al., 2019; Jiang et al., 2020). mRNA expression levels were represented as fold change, calculated as normalized intensity values from dDSCs relativized against normalized intensity values from preDSCs. To maintain graph symmetry, fold change values less than 1 (negative fold change, NFC) were represented with the formula NFC = −1/FC. Primer sequences are listed in Table 1.

Table 1
Primer sequences used for RT-PCR.

Gene	Primer sequence	Product size (bp)	Tm (°C)
Angiopoietin 1 (ANGPT1)	F: 5'-ACT AAA GGG AGG AAA AAG AGA GG-3' R:5'-TAC AGT CCA ACC TCC CCC AT-3´	166	59
Fibroblast growth factor 2 (FGF2)	F: 5'-GGG TGC CAG ATT AGC GGA C-3' R: 5'-GGT TCA CGG ATG GGT GTC TC-3'	112	58
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F: 5'-TGC ACC ACC AAC TGC TTA GC-3' R: 5'-GGC ATG GAC TGT GGT CAT GAG-3'	87	59
Hepatic growth factor (HGF)	F: 5'-CAATGCCTCTGGTTCCCCTT-3' R :5'-GGGCTGACATTTGATGCCAC-3'	171	59
Placental growth factor (PGF)	F: 5'-TGT TCA GCC CAT CCT GTG-3' R: 5'-ACG TGC TGA GAG AAC GTC AG-3'	163	59
Vascular endothelial growth factor (VEGF)	F: 5'-TCT CTA CCC CAG GTC AGA CG-3' R: 5'-AGC AAT GTC CTG AAG CTC CC-3'	98	58

2.9. Confocal microscopy

Decidua samples were harvested and snap-frozen in an OCT-filled mold (Tissue-Tek, Sakura Finetek, Torrance, CA) immersed in isopentane (Merck) on a liquid nitrogen-cooled metal surface. Samples were stored at −80 °C until the sections were cut. Briefly, 10 μM-thick cryosections were made and fixed in 3.7 % PFA for 7 min, or in ice-cold acetone (Merck) for 3 min for PFA-sensitive epitopes (CD140b). The slides were rehydrated in PBS for 15 min and then blocked with 0.1 % Tween, 5 % bovine serum albumin, 0.2 % triton X-100 and 5 % goat serum (all from Merck) in PBS solution for 45 min. Immunostaining was performed overnight (4 °C) with the direct mAbs FITC-CD10, FITC-CD146, FITC-W5C5 (SUSD2), Brilliant Violet 421-CD34 (Biolegend) or Cy3-α-SM actin (Sigma-Aldrich). The indirect mAbs nestin (R&D Systems) and CD140b (Biolegend) were additionally incubated for 2 h at room temperature with an AlexaFluor 488-labeled goat anti-mouse IgG (Thermo Fisher Scientific). The slides were counterstained with Hoechst 33258 (Merck) and mounted in Fluoroshield mounting media (Merck). Images were acquired with a Nikon A1 confocal microscope (Nikon Instruments Europe BV, Amsterdam, The Netherlands) and processed with NIS-Elements AR 3.2 imaging software (Nikon Instruments Europe BV).

2.10. Migration assay

Migration assays were done in 24-Transwell chemotaxis chambers with a pore size of 5 μM (Corning Incorporated, New York, NY). The bottom of each well was covered with 600 μL of control fresh complete Opti-MEM with or without P4 and dibutyryl cAMP, or preDSC-CM, or dDSC-CM. Then 2.5 × 10^5 THP-1 cells in 100 μL complete Opti-MEM were added to each insert. After 24 h at 37 °C, the inserts were removed and the bottom cells were counted. The total number of migrated cells was determined with a hemocytometer, and the results were expressed as the percentage of cell migration, calculated with the formula: (number of migrated cells/total number of cells) × 100.

2.11. Detection of apoptotic cells

Hypodiploid apoptotic cells were detected by flow cytometry according to a published procedure (Gong et al., 1994). Briefly, after incubation with fresh Opti-MEM, preDSC-CM or dDSC-CM, cells were washed with PBS, fixed in cold 70 % ethanol and then stained with propidium iodide (PI) during treatment with RNase (Sigma-Aldrich). Quantitative analysis of sub-G1 apoptotic cells was carried out in a FACSCalibur cytometer using Cell Quest software (BD Biosciences).

2.12. Statistical analysis

The figures illustrate the results for a single experiment representative of 3 or more separate assays. All experiments were done in tripli-

cate or quadruplicate. The Mann Whitney *U* test was used to compare the effects of decidualization on cell morphology, PRL secretion, antigen expression, chemotactic activity and antitumoral activity. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Changes in the antigen expression by DSCs during decidualization

In decidual sections, preDSCs exhibited a fibroblastic morphology and were located perivascularly, while dDSCs, easily identifiable by their round shape and large size, were located in the extravascular space (Fig. 1A). In their perivascular location, preDSCs expressed the endometrial stromal cell marker CD10, together with the MSC/pericyte antigens α-SM actin, nestin, CD140b, CD146 and SUSD2, as seen with confocal microscopy (Fig. 1B). The expression of CD10 was similar in both perivascular preDSCs and extravascular dDSCs. However, all five MSC/pericyte markers detected in perivascular preDSCs were downmodulated by dDSCs in the extravascular space (Fig. 1B).

In vitro, DSC lines mimicked the changes in antigen expression detected in vivo. Under decidualization conditions, the proportion of CD10+, CD29+ and CD44+ dDSCs, as determined by flow cytometry, did not vary compared to preDSCs. However, in dDSCs the expression of CD140b, CD146, SUSD2, α-SM actin and nestin was significantly downmodulated, while the expression of CXCL12 (a chemokine with angiogenic activity (Ao et al., 2020)) increased significantly (Fig. 2A and B). The downmodulation of α-SM actin was quite remarkable when fluorescence intensity was compared (Fig. 2C).

On the other hand, decidualization did not modify the expression of the angiogenic factors HGF, FGF2, ANGPT1 or VEGF in dDCSs, although it significantly increased the expression of PGF, as determined by RT-PCR (Fig. 3).

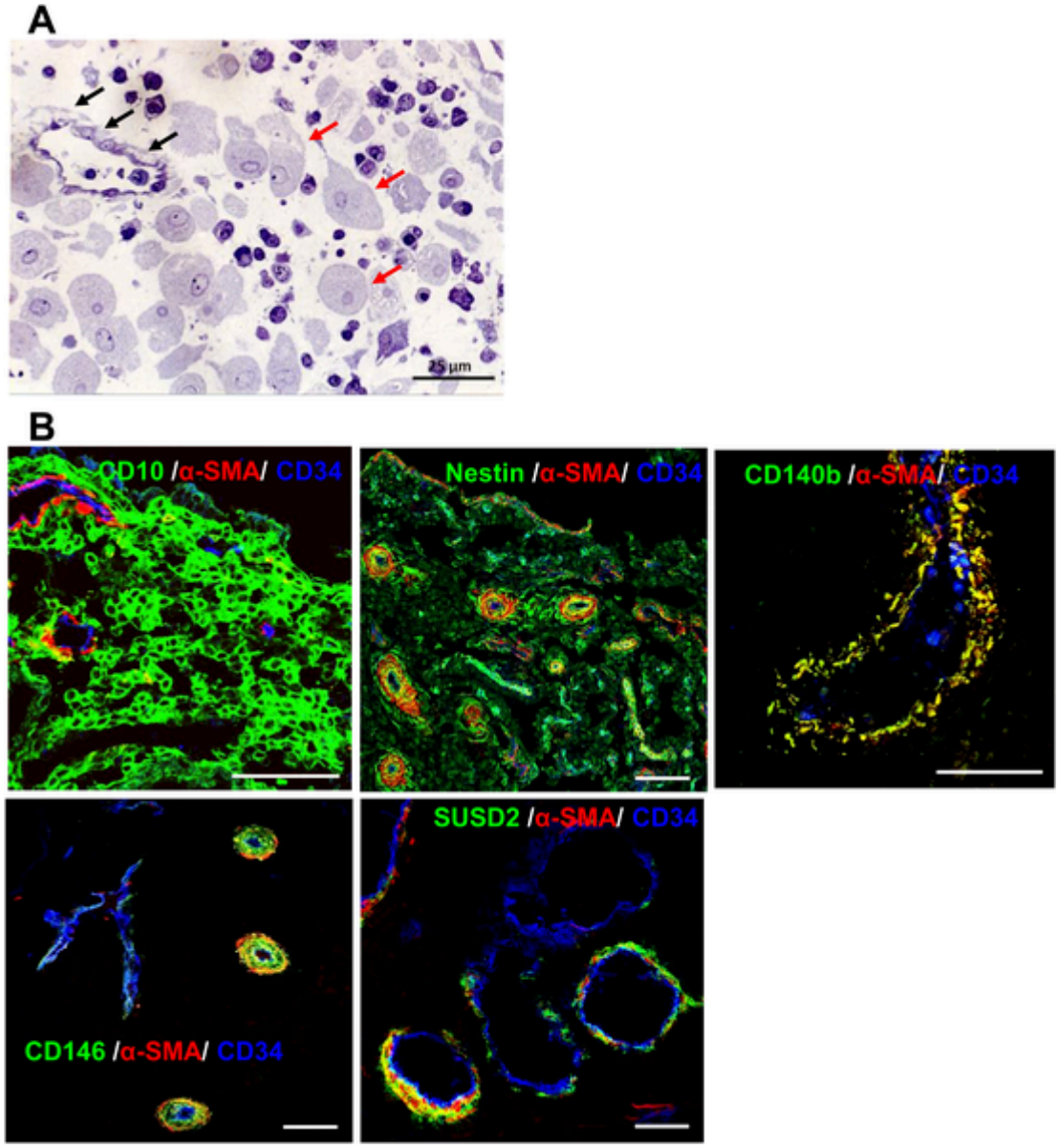

Fig. 1. Decidual stromal cells of first trimester human decidua. A) Semithin cryostatic section of early human decidua stained with toluidine blue, showing preDSCs (black arrows) and dDSCs (red arrowheads) Scale bar: 25 µm. B) Perivascular α-SM actin-positive cells coexpressed (yellow) CD10, nestin, CD140b, CD146, and SUSD2. Anti-CD34-stained endothelial cells. Scale bars: 100 µm. These experiments were done in 4 independent samples .

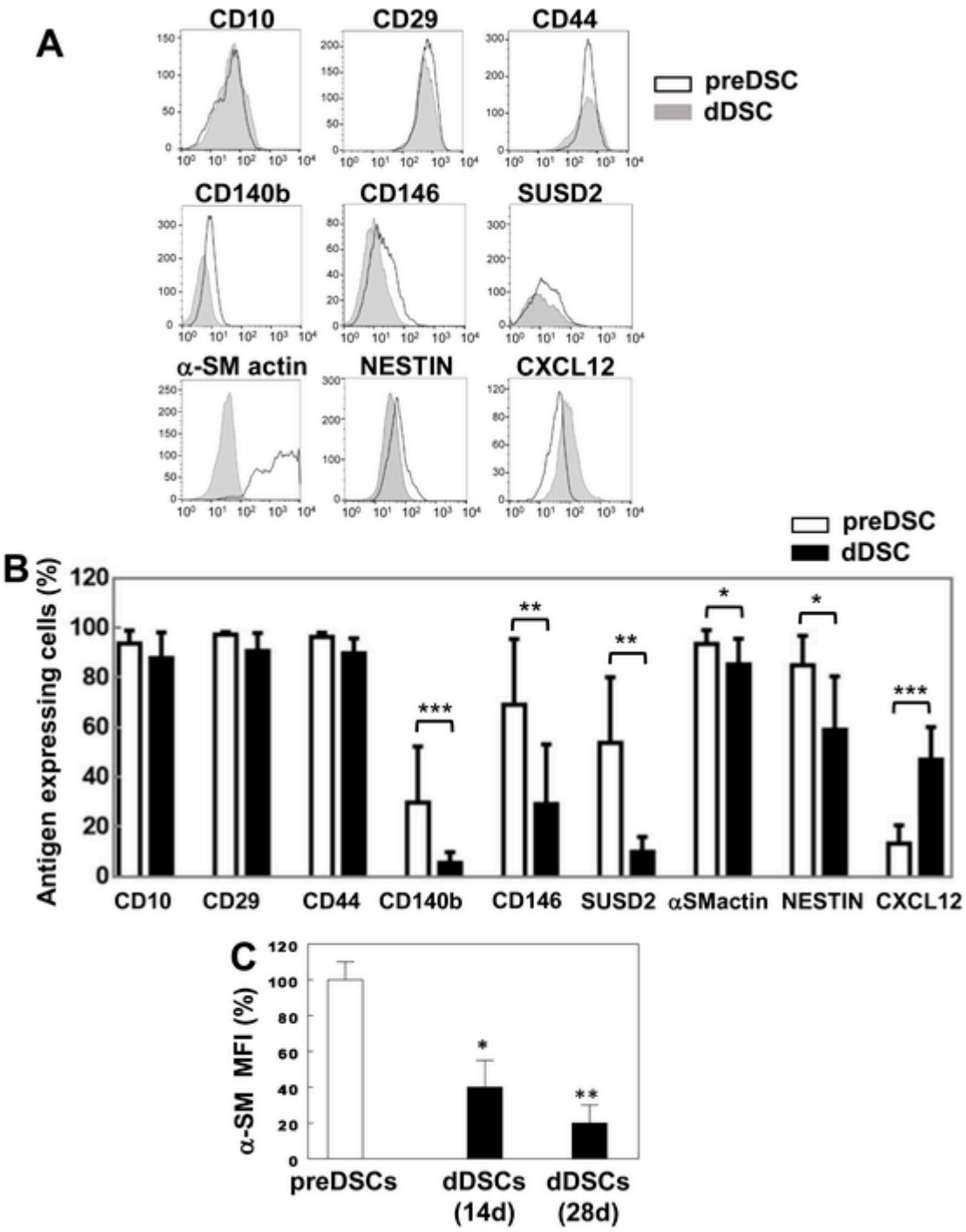

Fig. 2. Flow cytometric analysis of the antigen expression of DSC lines before (preDSCs) and after decidualization (dDSCs). A) Histograms of a representative DSC line. B) Mean percentage ± SD of antigen-expressing cells in DSC lines. C) Mean percentage ± SD of mean fluorescence intensity (MFI) of α-SM actin expression by DSC lines. These experiments were done in 4 independent samples. *P < 0.05, **P < 0.01, ***P < 0.001.

3.2. Effects of decidualization on the chemotactic activity of DSCs on monocytes

We studied the chemotactic activity of our DSC lines on monocytic THP-1 cells cultured in Transwell plates. PreDSC-CM attracted a significantly higher number of THP-1 cells than non-conditioned medium. This activity was significantly higher with dDSC-CM (Fig. 4A), showing that decidualization increased the secretion by DSCs of chemotactic factors for monocytes.

3.3. Effects of decidualization of DSCs on induction of apoptosis in tumor cell lines

In this study, we used PI staining and flow cytometry to investigate the effect of preDSC-CM and dDSC-CM on apoptosis in different tumor cell lines. We found that dDSC-CM but not preDSC-CM significantly induced apoptosis in 4 out of 6 different tumor cell lines tested, with monocytic leukemia THP-1 being the only unaffected line. In the carcinoma CAL33 line, apoptosis was also observed although it was not significant in comparison to the effect of control medium (Fig. 4B). These results are consistent with previous findings that dDSC-CM, but not

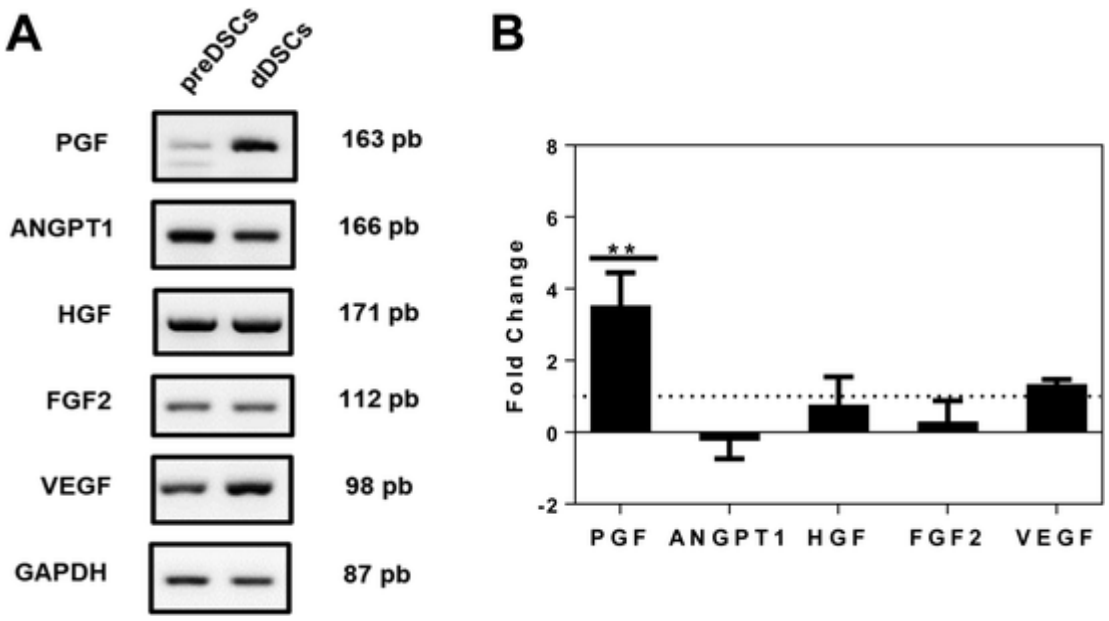

Fig. 3. Decidualization induces changes in angiogenic factor expression of DSCs. A) Detection of angiogenic factors by RT-PCR in a DSC line before and after decidualization, showing ANGPT1, FGF2, HGF, PGF and VEGF expression. B) Fold change in mRNA expression levels of angiogenic factors. Intensity values were normalized against the respective intensity values of the housekeeping gene GAPDH. Normalized intensity values from dDSCs were relativized against normalized intensity values from preDSCs. These experiments were done in 4 independent samples. $^{**}P < 0.01$.

preDSC-CM, induced apoptosis in preDSCs (Leno-Duran et al., 2014). In contrast, both preDSC-CM and dDSC-CM protected resting PBL from spontaneous apoptosis. As expected, and in contrast to resting PBLs, activated PBLs did not show spontaneous apoptosis. Nevertheless, like resting PBLs, activated PBLs did not undergo apoptosis upon incubation with either preDSC-CM or dDSC-CM (Fig. 4B). Together, these results show that factors secreted by dDSCs induced apoptosis in DSCs and in tumor cell lines, but had no effect on PBLs, or even protected resting PBLs from apoptosis.

4. Discussion

We previously showed that preDSCs are phenotypically and functionally related to pericytes and MSCs, two closely related or identical cell types (Caplan and Correa, 2011; da Silva Meirelles et al., 2016). The MSC/pericyte characteristics are not associated with different subpopulations of cells, since studies with preDSC clones demonstrated that all characteristics are displayed by the same cells (Muñoz-Fernandez et al., 2018; Muñoz-Fernández et al., 2019). In the present study, we show that decidualization changed the MSC/pericyte properties of DSCs. In vivo, preDSCs exhibited a fibroblastic morphology, were located around the vessels (Wynn, 1974; Ferenczy and Guralnick, 1983) (Fig. 1A), expressed the endometrial stromal cell marker CD10 (Sumathi and McCluggage, 2002) and the MSC/pericyte markers α-SM actin, nestin, CD140b, CD146 and SUSD2 (Munoz-Fernandez et al. 2018) (Fig. 1B). During decidualization, dDSCs migrated from the vessels toward the extravascular zone, became rounder (Wynn, 1974), and maintained CD10 expression, but downmodulated the expression of all five MSC/pericyte markers (Fig. 1B). In previous work (Munoz-Fernandez et al. 2018), we found that other antigens associated with MSCs and pericytes, such as podoplanin and STRO-1, were also downmodulated by dDSCs in vivo. The DSC lines assayed here reproduced these changes: in vitro, decidualization changed DSC morphology toward a rounder shape, induced the secretion of PRL (Supplementary Fig. 1), and inhibited the expression of all five MSC/pericyte markers, whereas the expression of CD10 did not vary (Fig. 2). Our results are also consistent with findings from a single-cell transcriptomic analysis of first-trimester human decidua, which showed that the cellular composition of human decidua comprises subsets of perivascular and nonperivascular stromal cells. Perivascular PRL-negative stromal cells exhibited an antigenic phenotype equivalent to that of preDSCs, i.e., expression of α-SM actin, CD140b, CD146, and SUSD2, and no expression of PRL, whereas nonperivascular PRL-positive stromal cells, which correspond to our dDSCs, did not express α-SM actin or CD140b, CD146, or SUSD2 (Vento-Tormo et al., 2018). Similar conclusions can be drawn from a second single-cell transcriptomic study of first-trimester human decidua (Suryawanshi et al., 2018). The expression of CD140b and CD146 by preDSCs is probably related to their perivascular location, since these molecules are involved in interactions with endothelial cells during angiogenesis (Wiater et al., 2018), a process that is crucial for normal pregnancy (Torry et al., 2007). The expression of these molecules, however, was downmodulated by dDSCs in the extravascular zone, where they cease to interact with endothelial cells (Fig. 1B). As observed in DSCs, downmodulation of CD140b by lymphoid perivascular precursors of stromal cells (also related to MSCs and pericytes) that differentiate toward the extravascular space was also observed in the human tonsil and mouse spleen (Krautler et al., 2012). Other antigens such as α-SM actin, SUSD2, nestin, podoplanin and STRO-1, which are also involved in vascular homeostasis and angiogenesis, and are expressed by perivascular preDSCs, were also downmodulated by extravascular dDSCs (Fig. 1B, (Munoz-Fernandez et al. 2018)). The expression of other angiogenic factors, however, was unaffected (HGF, FGF2, ANGPT1, VEGF) or even increased (PGF, CXCL12) in dDSCs (Figs. 2B and 3), probably to counterbalance, in the extravascular space, the downmodulation of angiogenic molecules in the perivascular zone. Regarding PGF, apart from angiogenesis, this factor also plays other relevant roles in, for example, trophoblast growth and differentiation, and monocyte migration and activation (Albonici et al., 2019). CXCL12 is also involved in trophoblast homeostasis and NK cell chemotaxis to the decidua (Ao et al., 2020). As we previously reported, the contractility and fibroblastic shape of preDSCs are determined by the incorporation of α-SM actin into the stress fibers (Kimatrai et al., 2005). Therefore, the downmodulation of α-SM actin expression in dDSCs (Fig. 2) is probably associated with their rounded morphology and decreased cell contractility (Kimatrai et al., 2005). At-

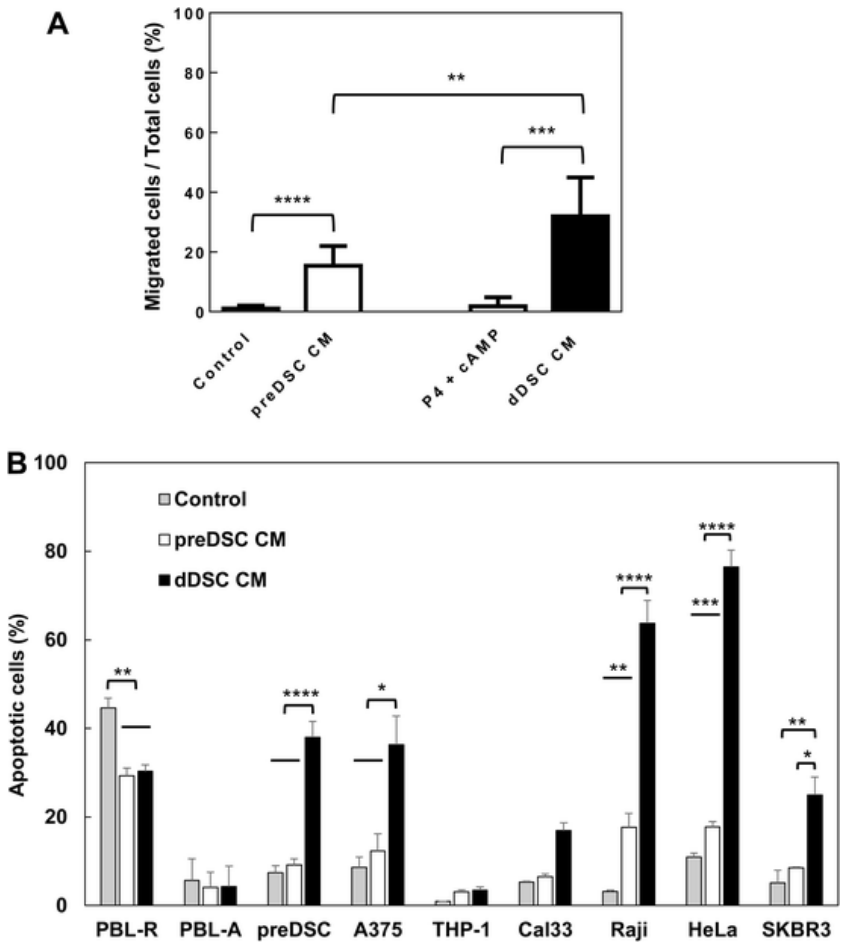

Fig. 4. Effects of preDSC and dDSC-conditioned culture media on monocyte migration and antitumoral activity. (A) PreDSC and dDSC-conditioned media induced migration in the THP-1 monocytic cell line. Migration index was calculated as (number of migrated cells/total number of cells) × 100. These experiments were done in four independent samples. (B) Effects of preDSC and dDSC- conditioned media on apoptosis in resting and activated PBLs, preDSCs, and 6 tumor cell lines. These experiments were done in 4 independent samples. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

tenuation of this activity may be crucial for apposition of the blastocyst on the luminal epithelium (Brosens et al., 1995).

In their perivascular location, preDSCs, like pericytes, may not only interact with endothelial cells, but may also regulate blood flow or control leukocyte entry into the decidua (Munoz-Fernandez et al. 2018). We and others previously demonstrated that preDSCs exert chemotactic activity on peripheral blood NK cells (Carlino et al., 2008; Muñoz-Fernandez et al., 2018). In human decidua, macrophages are the second most abundant leukocyte population after NK cells. Macrophages, apart from their antimicrobial activity, have a number of relevant functions in pregnancy, such as controlling trophoblast invasion and angiogenesis, and the phagocytosis of apoptotic cells (Yao et al., 2019). These roles led us to investigate the chemotactic activity of DSCs on monocytes, since pericytes have shown this activity on different cell types, especially monocytes (Stark et al., 2013). We found that both preDSC-CM and dDSC-CM exhibited chemotactic activity in the monocytic THP-1 line; however, this activity was significantly greater in dDSCs compared to preDSCs (Fig. 4A). As noted above, PGF as well as CXCL12, which were also increased in dDSCs (Figs. 2B and 3), may be responsible for this chemotactic activity. Other chemokines whose secretion by DSCs varies during decidualization are probably also involved (manuscript in preparation). In their perivascular location, preDSCs attract leukocytes from the blood to the decidua, whereas in the extravascular space, dDSCs increase the secretion of chemotactic factors, and the chemotactic gradient thus generated may enable leukocytes to disperse from the perivascular space throughout the extravascular space of the decidua. Nevertheless, upon decidualization, as shown in a mouse model, dDSCs downregulate the expression of chemokines that attract abortogenic Th1 and Tc1 lymphocytes, thus contributing to maternal–fetal tolerance (Nancy et al., 2012). Therefore, decidualization – among other effects – results in the selective expression of chemokines by dDSCs to attract only immune cells that favor pregnancy but not those that may attack the trophoblast (manuscript in preparation). Other lines of evidence confirm that decidualization drives DSCs toward a more immunoregulatory profile: dDSCs increase IL-10 production and HLA-G expression, and decrease IL-6 secretion (Blanco et al., 2008) – all of which are mechanisms that may contribute to immune tolerance. In addition, these properties appear to support the therapeutic effects of DSCs in immune-based diseases (Ringden et al., 2018; Muñoz-Fernández et al., 2019; Berishvili et al., 2020).

It was previously demonstrated that both preDSCs and dDSCs produce factors that inhibit spontaneous apoptosis in resting PBLs (Leno-Duran et al., 2014) (Fig. 4B). Although these factors remain unidentified, cytokines such as IL-15 and BAFF (B-cell activating factor), reportedly produced by DSCs, may be responsible for this antiapoptotic effect (Munoz-Fernandez et al. 2012). Taken together, our results show that DSCs not only selectively attract leukocytes from the blood to the decidua, but also favor survival of the lymphocytes they attract. In contrast to the protective effect on lymphocytes shown by preDSCs and dDSCs, we previously found that dDSCs, but not preDSCs, secrete as yet unidentified factors that induce apoptosis in both dDSCs and preDSCs (Leno-Duran et al., 2014). This in vitro finding is probably related to the gradual physiological thinning of the decidua in favor of the ex-

Table 2
Characteristics of preDSCs and dDSCs.

Characteristics	preDSC	dDSC	References
Location	Perivascular	Extravascular	Wynn, 1974; Muñoz-Fernandez et al. (2018)
Morphology	Fibroblastic	Round	Wynn (1974)
Secretion of PRL and IL-15	–	+	Wynn (1974); Braverman et al. (1984); Richards et al. (1995); Bergeron (2000)
Expression of MSC/pericyte markers: α-SM actin, CD140b, CD146, SUSD2, nestin, podoplanin, STRO-1	+	↓	Muñoz-Fernandez et al. (2018) This study
Expression of angiogenic factors	+	+/↑PGF	Muñoz-Fernandez et al. (2018) This study
Cell contractility	+	↓	Muñoz-Fernandez et al. (2018)
Chemotactic activity on monocytes	+	↑	This study
Phagocytic activity	+	↓	Ruiz et al. (1997)
Immunoregulatory activity	+	↑	Blanco et al., 2008; Muñoz-Fernandez et al. (2012); Muñoz-Fernández et al. (2019)
PBL apoptosis	–	-/↓	Muñoz-Fernandez et al. (2012); Leno-Duran et al. (2014) This study
DSC apoptosis	–	+	Leno-Duran et al. (2014)
Antitumoral activity	–	+	This study

panding trophoblast during normal human pregnancy (Sharma et al., 2016). Interestingly, the terms “decidua” and “decay” have a common etymological origin. Similarly, dDSC-CM but not preDSC-CM induced apoptosis in 4 out of 6 different tumor cell lines assayed in the present study (Fig. 4B). Although some findings in support of the effect of MSC-related cells on tumor cell growth are controversial (Galland and Stamenkovic, 2020), recent experimental evidence confirmed that WJ-MSCs, which have MSC characteristics, secrete factors that, like dDSCs, induce apoptosis in different tumor cell lines (Ganta et al., 2009; Fong et al., 2011; Lin et al., 2017). These pro-apoptotic molecules have yet to be identified, and proteomics studies will likely be needed for this. We believe that this apoptotic antitumoral effect does not represent a physiological activity of DSCs per se against tumor cells, but is rather the consequence of other physiological effects such as the self-induced DSC apoptosis discussed above, or a possible role in the control of trophoblast viability (Sharma et al., 2016). Nevertheless, our results suggest that these DSC factors hold potential for cancer therapy. In light of their protective role in resting lymphocytes, the absence of apoptosis induction in activated lymphocytes, and their antitumoral effect (Fig. 4B), these factors may also be useful in activated-lymphocyte-based immunotherapy against cancer (Waldman et al., 2020).

In conclusion, during decidualization, some pericyte/MSC characteristics of dDSCs are modulated, e.g. fibroblastic shape, perivascular location, expression of CD140b, CD146, SUSD2 and α-SM actin, and cell contractility, while functions such as angiogenesis, chemotaxis, lymphocyte protection, immunoregulatory activities, and antitumoral factor secretion are conserved or enhanced (Table 2). These changes may play a role in maternal–fetal immune tolerance and may prove to have potential therapeutic applications in inflammatory diseases and cancer.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2021.103326.

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