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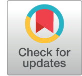
Human predecidual stromal cells have distinctive characteristics of pericytes: Cell contractility, chemotactic activity, and expression of pericyte markers and angiogenic factors



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ABSTRACT

Introduction: Human decidual stromal cells (DSCs) play a key role in maternal–fetal interactions. Precursors of DSCs (preDSCs) localize around vessels in both the endometrium and decidua. Previous studies suggested a relationship between preDSCs and pericytes because these cells share a perivascular location, alpha smooth muscle actin (α-SM actin) expression and the ability to contract under the effects of cytokines.
Methods: To further study this relationship, we established 15 human preDSC lines and 3 preDSC clones. The preDSC lines and clones were tested by flow cytometry with a panel of 29 monoclonal antibodies, 14 of which are pericyte markers. The expression of angiogenic factors was determined by RT-PCR, chemotactic activity was studied with the migration assay, and cell contractility was evaluated with the collagen cell contraction assay. Confocal microscopy was used to study decidual sections.
Results: Under the effect of progesterone and cAMP, these lines decidualized in vitro: the cells became rounder and secreted prolactin, a marker of physiological DSC differentiation (decidualization). The antigen phenotype of these preDSC lines and clones was fully compatible with that reported for pericytes. PreDSC lines displayed pericyte characteristics: they expressed angiogenic factors and showed chemotactic and cytokine-induced contractile activity. Confocal microscopic examination of decidual sections revealed the expression of antigens detected in preDSC lines: α-SM actin colocalized with CD146, CD140b, MFG-E8, nestin, and STRO-1 (all of which are pericyte markers) in cells located around the vessels, a distinctive location of preDSCs and pericytes.
Discussion: Taken together, our results show that preDSCs are pericyte-like cells.
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1. Introduction

The human decidua or gestational endometrium is in close contact with the trophoblast (fetal tissue), forming the maternal–fetal interface. Physiological interrelations between the mother and fetus at this interface are important for embryo development, e.g. nutrition and expansion of the trophoblast, vascular homeostasis, and immunoregulation. Decidual stromal cells (DSCs), the main cellular component of human decidua, exert activities that are thought to play a key role in embryo implantation [1], trophoblast expansion [2], and the development of maternal–fetal immune tolerance [3–7]. Decidual stromal cells originate from fibroblastic precursors located around the vessels, and are detected in both the endometrium and decidua [8,9]. During the luteal phase of the menstrual cycle, under the effect of the ovarian

Abbreviations: α-SM actin, alpha smooth muscle actin; DSC, decidual stromal cell; MSC, mesenchymal stem cell; preDSC, predecidual stromal cell; P4, progesterone; PRL, prolactin.

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hormones estradiol and progesterone (P4), predecidual reaction begins around the vessels and spreads through the upper two-thirds of the endometrium. Precursors of DSCs (preDSCs) leave the vessels and differentiate into decidualized cells, which exhibit a rounder shape and secrete prolactin (PRL) and other factors such as insulin-like growth factor-binding protein 1 and IL-15 [10–12]. When menstruation occurs, these differentiated cells are discarded; however, if pregnancy takes place, this process of differentiation (decidualization) continues through the effect of pregnancy hormones [8,9]. Although in earlier publications, the terms used for these perivascular precursors of DSCs were "endometrial stromal (predecidual) cells" [9], "predecidual cells" [8] or "precursor decidual cells" [13], Olivares et al., 1997 [14] introduced the term "predecidual stromal cells" (preDSCs), and preDSCs was later used by other authors [10,15–17]. PreDSCs can be isolated from both the endometrium and decidua and cultured in vitro, and under the effects of P4 and cAMP they decidualize, changing, as in vivo, to a rounder cell morphology and secreting PRL, which is considered a distinctive marker of decidualization [14,18]. However, the cell lineage and functions of DSCs have remained elusive until recently. The isolation and maintenance of highly purified human DSC lines in culture allowed us to study the antigen phenotype and immune activities of these cells [3–5]. Based on evidence from earlier work [4,19,20], in which we reported the perivascular location, α-SM actin expression and cell contractility of preDSCs, we suggested a relationship between these cells and pericytes.

Pericytes are contractile cells that surround microvascular endothelial cells, and regulate vessel structure and vascular homeostasis [21,22]. Many lines of evidence indicate that pericytes also display immune properties and are involved in leukocyte trafficking [23]. To carry out their functions, pericytes produce angiogenic factors [24], and display contractile [25], chemotactic [26], phagocytic [27], and immunoregulatory activities [28]. Furthermore, pericytes play a role in immune-privileged sites such as the brain, where they are involved in maintaining the integrity of the blood–brain barrier and in the immune control of brain inflammation [29]. Some reports have confirmed that pericytes are involved in diseases or disorders such as fibrosis and cancer [21], and in gynecological processes such as idiopathic heavy menstrual bleeding [30], Asherman syndrome [31] and endometrial cancer [32].

In earlier work we demonstrated that preDSCs 1) express vimetin and α-SM-actin [20], two intracellular protein associated to pericytes [22], 2), show phagocytic activity [33], a function exerted by pericytes [27], and 3) have contractile activity [4,19], a characteristic function of pericytes [25]. Furthermore, like pericytes [28], preDSCs exert different immune activities [3–7]. These similarities led us to suggest a relationship between pericytes and preDSCs. In the present work we extend the study of pericyte phenotype characteristics and functions of preDSC lines to shed additional light on this relationship.

2. Materials and methods

2.1. Samples

For the preDSC lines, samples from elective vaginal terminations of first-trimester pregnancies (6–11 weeks) were collected from 15 healthy women aged 20–30 years, of whom 13 were nulliparous and 2 were primiparous. Three women were smokers and 12 were nonsmokers. 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 Clínica El Sur in Malaga or Clínica Ginegranada in Granada. Informed consent was obtained from each donor. This study was approved by the Research and Ethics Committee of the University of Granada.

2.2. Isolation and culture of preDSCs

To establish preDSC lines, we used the method described by Kimatrai and colleagues [19]. Briefly, tissues 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, USA) 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% CO2 in Opti-MEM (minimum essential medium) (Invitrogen, Grand Island, NY, USA) supplemented with 3% fetal calf serum (FCS) (Invitrogen), 100 IU/ml penicillin, 10 μg/ml streptomycin μg/ml 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 were morphologically uniform and covered the whole surface of the 25-cm2 culture flask. Although the different cell lines are referred to generically as preDSCs, in experiments in which several lines of the same type of cell were included, we used a specific designation for each line (e.g. preDSC1, preDSC2, etc.). For this study 15 preDSC lines were obtained (each from a different sample) and were always used between 3 and 8 weeks after collection (up to 5 passages). For flow cytometry analysis, preDSCs were detached from the culture flask with 0.04% EDTA at 37 °C.

2.3. Cell cloning

Predecidual stromal cell clones were obtained from preDSC lines by limiting dilution in 96-well plates, using complete Opti-MEM supplemented with 10% FCS. Three days after cell seeding, the plates were checked and wells with only one cell were selected. After 2 weeks, single cells had formed colonies which we then trypsinized and seeded into 24-well plates for culture in complete Opti-MEM supplemented with 3% FCS. The clones were expanded and the phenotype was determined by flow cytometry.

2.4. Decidualization

To induce decidualization, preDSC lines or clones were treated with 300 nM P4 and 500 μM 8-bromo-cAMP (Sigma-Aldrich) for 15 days. Decidualization was verified by PRL secretion and changes in cell morphology from a fibroblastic to a round shape, as observed with light microscopy. The presence of PRL was verified with an electrochemiluminescence immunoassay (Roche, Indianapolis, IN, USA). The assays were performed according to the manufacturer's instructions, and all samples were tested in duplicate.

2.5. Collagen gel contraction assay

Cellular collagen gel contraction assays were performed as previously described [19]. A sterile solution of purified, pepsin-solubilized bovine dermal collagen (Vitrogen, Cohesion Technologies Inc., Palo Alto, CA, USA) was prepared according to the manufacturer's instructions and combined with 25×10^4 preDSCs. The collagen/cell mixture (100 μl/well) was dispensed into culture plates and allowed to polymerize at 37 °C for 30 min. Immediately after polymerization, 2 ml Opti-MEM with 3% FCS with or without

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