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Tissue expression and serum levels of periostin during pregnancy: a new biomarker of embryo–endometrial cross talk at implantation

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ABSTRACT

Objective: The molecular aspects involved in human implantation include many elements that were first discovered due to their role in cancer cell metastasis. Periostin, a cell adhesion protein that allows the maintenance of cancer stem cells, may influence implantation. The objective of this experimental case–control study was to investigate tissue and serum expression of periostin during pregnancy, and evaluate the potential role of periostin in endometrial receptivity and embryo implantation.
Study design: Forty-five subjects were included in the final analysis: 15 women who had experienced spontaneous pregnancy loss were enrolled as cases, and 30 healthy pregnant women awaiting voluntary pregnancy termination were enrolled as controls. For both cases and controls, trophoblastic and decidual tissues were collected at 12 weeks of gestation. Periostin expression in decidual and trophoblastic tissues was evaluated by immunohistochemical staining and reverse transcription polymerase chain reaction in cases and controls, and periostin serum levels was analyzed by Western blotting assays in cases, controls and non-pregnant female volunteers.
Results: Periostin mRNA and protein levels were higher in decidual and trophoblastic tissues from women undergoing voluntary pregnancy termination compared with women who had experienced spontaneous pregnancy loss. This finding was also reflected at serum level.
Conclusions: Periostin may be a serum marker of good endometrial receptivity and embryo quality, and predictive of pregnancy evolution.

1. Introduction

At the time of implantation, intensive blastocyst–endometrial cross-talk is required for embryo attachment and development [1]. Embryo growth/differentiation and endometrial cell differentiation must be closely synchronized [2]. Therefore, implantation failure may be due to impaired embryo developmental potential and/or impaired uterine receptivity and endometrial–embryo interactions. There is a need for better understanding of implantation and the roles of its associated factors [3].

As endometrial–embryo interactions seem to represent the major limiting step for good implantation, the identification of new biomarkers [4–6] that closely represent this dialogue may be key to increasing pregnancy rates in both spontaneous and stimulated cycles.

Periostin, a cell adhesion protein, promotes cell adhesion and motility by interaction with integrin (αvβ3 and αvβ5) [7]. In addition, periostin overexpression has been observed in the stroma of the primary tumour and metastasis localization site of various cancers [8,9]. It has been shown that invasive tumour cells need to induce stromal periostin expression in the secondary target organ to initiate colonization [10].

The molecular aspects involved in human implantation include many elements that were first discovered due to their role in cancer cell metastasis [11]. Due to the critical role of periostin in metastatic processes, it is possible that this protein may influence some crucial steps in the implantation process, placentation and/or placental functions throughout pregnancy. In human endometrium, the presence of periostin protein in both stromal and epithelial cells is affected by the stage of the menstrual cycle and pregnancy [12]. A literature survey revealed that periostin is expressed in human and mouse endometrium, particularly in the early secretory phase [7,13]. In this endometrial cycle phase, when there is also increased expression of αVβ3 and αVβ5, periostin may activate the differentiation of the endometrium for implantation. Experimental data from animal studies [14–17] show that

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periostin may induce endometrial decidualization, attachment and/or migration of trophoblastic cells through AKT/PBK signalling [12,18].

As such, the aim of this study was to investigate the expression of periostin at endometrial and/or trophoblastic levels, and evaluate the role of periostin expression in endometrial receptivity and human implantation.

2. Materials and methods

2.1. Subjects

In total, 27 women referred to the Hospital 'Pugliese-Ciaccio', Catanzaro, Italy for dilatation and curettage following spontaneous pregnancy loss were enrolled as cases, and 33 healthy pregnant women awaiting voluntary pregnancy termination were selected consecutively as controls. Gestational age at the time of dilatation and curettage was required for both groups. Exclusion criteria were: age <18 years, pre-malignancies or malignancies, medical conditions or other concurrent medical illnesses, metabolic abnormalities, cigarette smoking, drug/alcohol use, non-compliance with the study protocol, multiple gestations, blighted ovum and absent cardiac activity in the embryo at the moment of voluntary pregnancy termination. Spontaneous pregnancy loss was diagnosed by transvaginal ultrasound and clinical assessment. A blood sample was collected immediately after hospital admission for all subjects. Serum and tissue samples were also collected for women who had experienced spontaneous pregnancy loss at an earlier gestational age.

3. Protocol

The procedures used in this study were in accordance with the Declaration of Helsinki. Ethical approval was obtained from the institutional review board. Clinical data and blood samples were collected for each patient after the receipt of written consent.

3.1. Tissue collection, periostin expression and localization evaluation

All collected tissues, obtained surgically from each patient under sterile conditions, were processed immediately. Chorionic villous tissue was separated from placental tissue and decidual tissue was separated from trophoblastic tissue by repeated washes with Hanks' balanced salts solution.

Samples were fixed in 4% paraformaldehyde for at least 24 h and subsequently embedded in paraffin. Serial 4-μm sections were cut and placed in bioboxes. Histological sections were reviewed by an experienced pathologist who was blinded to the group assignment and any clinical details. From each surgical sample, slices <0.5-cm thick were stored separately into Nalgene tubes containing TRIzol (TRIzol ® Reagent, Life Technologies, Milan, Italy) reagent at −80 °C until reverse transcription polymerase chain reaction (RT-PCR) (RT-PCR Invitrogen, Life Technologies, Milan, Italy) analysis.

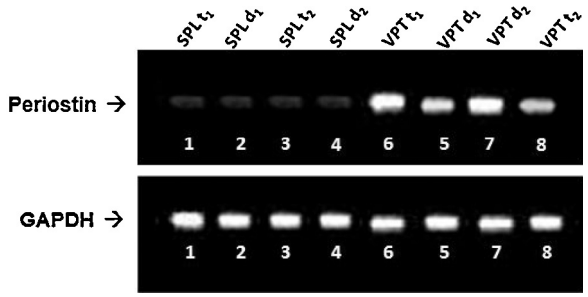

Fig. 1. Reverse transcription polymerase chain reaction (PCR) analyses of periostin from trophoblastic and decidual tissues. PCR analyses of periostin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using RNA from trophoblast (t) and decidual (d) tissues. Following nested PCR amplification, the products were separated with agarose gel and stained using ethidium bromide. Lanes 1–4, spontaneous pregnancy loss (SPL); Lanes 5–8, voluntary pregnancy termination (VPT).

3.1.1. RNA extraction and RT-PCR

At the time of analysis, tissues were disrupted using homogenizer (ULTRA-TURRAX, IKA ® Works, Inc, Wilmington, NC) at 11,000 rpm for 10–12 s keeping the Eppendorf tube (Invitrogen, Life Technology) in ice, and then left at 4 °C for 1 h. Total RNA was isolated by the TRIzol method (Invitrogen Life Technologies) according to the manufacturer's instructions. DNase-treated RNA (0.5 μg) was reverse transcribed into first-strand cDNA using the SuperscriptTM First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies) with random hexanucleotide primers. Periostin cDNA was amplified using appropriate primers (sequences and PCR conditions available on request) [19]. A human GAPDH cDNA fragment was amplified as the internal control for the amount of cDNA in the PCR (Fig. 1).

3.1.2. Immunohistochemical analysis for periostin expression

At the time of the histological and immunohistochemical evaluations, all paraffin-embedded sections were deparaffinized in xylene and rehydrated through a series of graded alcohol solutions.

Consecutive paraffin-embedded decidual and trophoblastic samples from women who had experienced spontaneous pregnancy loss and women undergoing voluntary pregnancy termination were studied.

Sections were cut and stained with haematoxylin and eosin. For each section, the presence or absence of implantation site fragments on the decidual side was determined, identified by the presence of through the detection of the endovascular trophoblast invasion. All implantation sites were examined and the expression of periostin was evaluated (Fig. 2).

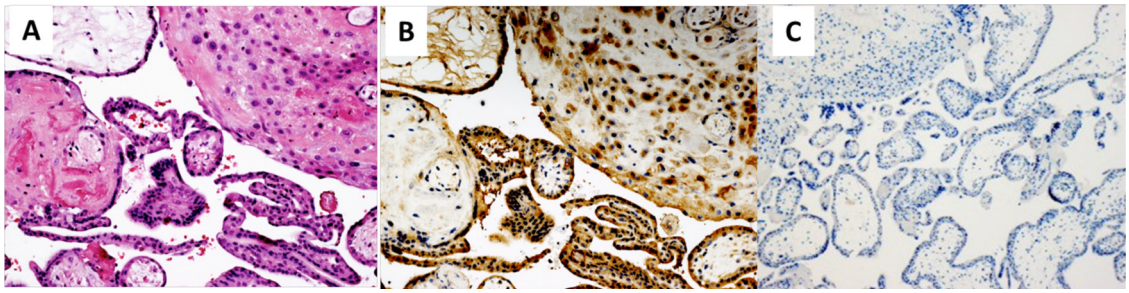

Fig. 2. Periostin immunohistochemical staining of decidual and trophoblastic samples. (A) Identification of implantation site fragments (original magnification ×20) with haematoxylin and eosin. (B) Determination of periostin immunoreactive cells (original magnification ×20) in decidual and trophoblastic samples of women undergoing voluntary pregnancy termination. (C) The immunohistochemistry images of samples tested with the secondary antibody only (as a negative control).