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"Trophoblast islands of the chorionic connective tissue" (TICCT): A novel placental histologic feature

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ABSTRACT

Introduction: We found isolated or clustered trophoblasts in the chorionic connective tissue of the extraplacental membranes, and defined this novel histologic feature as the "trophoblast islands of the chorionic connective tissue" (TICCT). This study was conducted to determine the clinical significance of TICCT.
Methods: Immunohistochemistry for cytokeratin-7 was performed on the chorioamniotic membranes ($N = 2155$) obtained from singleton pregnancies of 1199 uncomplicated term and 956 preterm deliveries. The study groups comprised 1236 African–American and 919 Hispanic women. Gestational age ranged from 24^{+0} weeks to 41^{+6} weeks. Multiple logistic regression analysis was performed to investigate the magnitude of association between patient characteristics and the presence of TICCT.
Results: The likelihood of TICCT was significantly associated with advancing gestational age both in term (OR: 1.29, 95% CI: 1.16–1.45, $p < 0.001$) and preterm deliveries (OR: 1.19, 95% CI: 1.07–1.32, $p = 0.001$) . Hispanic women were less likely than African–American women to have TICCT across gestation in term (OR: 0.23, 95% CI: 0.18–0.31, $p < 0.001$) and preterm pregnancies (OR: 0.41, 95% CI: 0.29–0.58, $p < 0.001$). Women with a female fetus were significantly more likely to have TICCT than women with a male fetus, in both term (OR: 1.64, 95% CI: 1.28–2.11, $p < 0.001$) and preterm gestations (OR: 2.04, 95% CI: 1.46–2.85, $p < 0.001$). TICCT was 40% less frequent in the presence of chronic placental inflammation [term (OR: 0.60, 95% CI: 0.45–0.81, $p = 0.001$) and preterm gestations (OR: 0.58, 95% CI: 0.40–0.84, $p = 0.003$)] and in parous women at term (OR: 0.60, 95% CI: 0.44–0.81, $p = 0.001$).
Conclusions: Our findings suggest that the duration of pregnancy, fetal sex, and parity may influence the behavior of extravillous trophoblast and placental mesenchymal cells.

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1. Introduction

The chorioamniotic membranes play an important role in the maintenance of pregnancy and parturition [1–6]. The chorion laeve is composed of a trophoblast layer harboring stratified extravillous trophoblasts and a connective tissue layer containing myofibroblasts and macrophages [7–9]. Chorionic trophoblasts serve as a fetal immune barrier against maternal immunocytes and predominantly express and produce non-classical, non-polymorphic HLA-G, but do not express classical HLA class I and II molecules [10–12]. The

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trophoblasts of the chorion laeve have also been implicated in the regulation of prostaglandin metabolism and, therefore, in the regulation of myometrial contractility [13–16]. These cells express high levels of 15-hydroxy-prostaglandin dehydrogenase, which can inactivate prostaglandins by oxidation of these compounds to inactive 15-keto metabolites [17,18]. In addition to the conventional roles, phagocytic activity in the chorionic trophoblasts has been reported when chorioamnionitis is diagnosed, an observation that suggests another important role of the trophoblast in the chorion laeve for the innate immune response [19].

Integrins and CD9 (surface molecules) have been implicated in the regulation of extravillous trophoblast invasion [20,21]. Chorionic trophoblasts normally display a unidirectional invasion toward the decidua where they intermingle with decidual cells of maternal origin [22,23]. Therefore, the chorionic connective tissue, which is surrounded by the basal lamina from the chorionic trophoblast layer, is not expected to contain any trophoblasts [2]. Yet, we found some oval to polygonal cells in the chorionic connective tissue layer of the chorioamniotic membranes. These cells had glassy, eosinophilic or amphophilic cytoplasm, and were morphologically different from myofibroblasts or macrophages. Some of these cells had cytological features indistinguishable from those of chorionic trophoblasts. Subsequent immunostaining for cytokeratin-7 and HLA-G demonstrated co-localization of strong immunoreactivity in these cells. Based on their characteristic morphological and immunohistochemical features, we termed this novel histological finding of unknown significance as the “trophoblast islands of the chorionic connective tissue” (TICCT).

The aim of this study was to determine the clinical significance of TICCT by analyzing the chorioamniotic membranes obtained from women who presented with different obstetrical conditions such as normal delivery at term, preterm delivery, and pre-eclampsia. We conducted an immunohistochemical screening of a large number of cases across gestation for the detection of TICCT in two different study populations. The relationship between the frequency of TICCT and various placental pathologies was also assessed.

2. Materials and methods

2.1. Tissue materials

To evaluate for TICCT, one chorioamniotic membrane roll section from each pregnant woman was examined. Paraffin blocks of the membrane rolls were retrieved from the Bank of Biological Materials of Wayne State University, which has been assembled in collaboration with the Perinatology Research Branch of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U.S. Department of Health and Human Services (NICHD/NIH/DHHS). The study groups included 1236 African–American women who delivered at Hutzel Women's Hospital, Detroit, Michigan, USA, and 919 Hispanic women who delivered at the Sótero del Río Hospital, Santiago, Chile. All women provided written informed consent, and the Institutional Review Boards of the participating institutions approved the collection and use of biological samples and clinical data for research purposes.

2.2. Immunofluorescence staining/immunohistochemistry

For double immunofluorescence staining, 5-μm-thick frozen tissue sections of the chorioamniotic membranes were used. The frozen sections were ethanol-fixed for 5 min, and used for immunofluorescence staining. A mouse monoclonal anti-HLA-G antibody (MEM-G1, 1:50 dilution; Abcam, Cambridge, MA, USA) and a rabbit polyclonal anti-cytokeratin 7 antibody (1:50 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were used as primary antibodies. Alexa Fluor® 488 donkey anti-rabbit IgG and Alexa Fluor® 594 donkey anti-mouse IgG were used as secondary antibodies (1:1000 dilution for both; Invitrogen, Carlsbad, CA, USA), and the slides were mounted in ProLong Gold antifade reagent with DAPI (Invitrogen). The immunofluorescence stained sections were evaluated with a Leica TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany).

For immunohistochemistry, formalin-fixed, paraffin-embedded, 5-μm-thick tissue sections of the chorioamniotic membranes were obtained on silanized slides. Deparaffinization, antigen retrieval, and immunostaining were done using a Ventana Discovery automatic staining system (Ventana Medical Systems, Tucson, AZ, USA). A mouse monoclonal anti-cytokeratin-7 antibody (OV-TL, 1:2000 dilution; Dako, Carpinteria, CA, USA) was a primary antibody, and the Discovery® DAB Map™ Kit (Ventana Medical Systems) was used for chromogen reaction.

2.3. Definition and grading of trophoblast islands of the chorionic connective tissue (TICCT)

We defined TICCT as the presence of cytokeratin-7-positive cells or cell clusters with cytological characteristics of trophoblasts in the chorionic connective tissue layer of the reflected chorion (rather than the placental amnion). The extent of TICCT was graded as follows: grade 1, when TICCT was observed in three or less isolated high-power fields (HPFs, X400); grade 2, when TICCT was observed in four to 10 isolated HPFs; grade 3, when TICCT was multifocal in more than 10 isolated HPFs; and grade 0, when none of these were observed. All cases ($n = 2155$) were reviewed by one pathologist (CJK) who was blinded to the clinical information while reviewing the immunostaining results. To determine intra- and inter-observer reliability in the diagnosis of TICCT, 398 cases which consisted of grade 0 ($n = 199$), grade 1 ($n = 119$), grade 2 ($n = 50$), and grade 3 ($n = 30$), selected by another author (JSH), were re-examined independently by two pathologists (CJK, JSK) who were blinded to the original results.

2.4. Transmission electron microscopy

The chorioamniotic membranes in the paraffin blocks were deparaffinized using xylene and rehydrated through serial incubations in graded ethanol. After rinsing with distilled water, the samples were incubated with 0.1 mol/L phosphate buffer (pH 7.4). Deparaffinized samples were stained with 1% OsO4 for 1 h and dehydrated with graded ethanol. Semi-thin sections of epoxy resin–embedded tissues were evaluated to locate the areas with trophoblasts in TICCT, and ultra-thin sections were examined using a JEM-1200EX II transmission electron microscope (JEOL Ltd., Tokyo, Japan).

2.5. Placental pathology

To determine whether the frequency of TICCT was associated with specific pathological changes of the placenta, we examined the relationship between this finding and the presence or absence of four categories of placental lesions: 1) amniotic fluid infection (acute chorioamnionitis); 2) maternal vascular underperfusion; 3) fetal vascular thrombo-occlusive disease; and 4) chronic placental inflammation (villitis of unknown etiology/chronic chorioamnionitis/chronic deciduitis). These samples were retrieved from the files maintained at Wayne State University and the Detroit Medical Center in collaboration with the Perinatology Research Branch, NICHD/NIH/DHHS.

2.6. Statistical analysis

Pearson's chi-square test for categorical variables and the *t*-test for continuous variables were performed to determine differences among maternal characteristics according to the TICCT results. Pearson's chi-square test and the linear by linear association were used to assess the magnitude of association between TICCT and gestational age at delivery. The association between TICCT and ethnicity across gestation was tested using the Mantel-Haenszel (M-H) test. Multiple logistic regression analysis was performed to evaluate the magnitude of association between selected patient characteristics and TICCT, adjusting for potentially confounding factors selected based on clinical knowledge. The association between high-grade TICCT (grade 2 or 3) and the related factors was tested by multinomial regression. To determine intra- and inter-observer reliability in the diagnosis of TICCT, quadratic weighted κ (κ_w) statistics [24] were calculated using R (A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org). All statistical analyses except κ_w were conducted using SPSS version 18.0 software (SPSS, Inc., Chicago, IL, USA). A 5% threshold was used to determine statistical significance.

3. Results

3.1. Patterns of trophoblast islands of the chorionic connective tissue (TICCT)

To confirm the extravillous trophoblast phenotype of the cells, double immunofluorescence staining was performed for cytokeratin-7 and HLA-G in frozen chorioamniotic membrane tissues of four cases of African–American origin with prominent TICCT. These cells showed variable degrees of HLA-G immunoreactivity along with strong cytoplasmic cytokeratin-7 immunofluorescent signals (Fig. 1a–d). Cytokeratin-7-positive TICCT was observed as an isolated single cell or clusters of cells with variable sizes (Fig. 2a–h).