OBSTETRICS

Preterm labor in the absence of acute histologic chorioamnionitis is characterized by cellular senescence of the chorioamniotic membranes

Nardhy Gomez-Lopez, PhD; Roberto Romero, MD; Olesya Plazyo, PhD; George Schwenkel, BSc; Valeria Garcia-Flores, MSc; Ronald Unkel, BSc; Yi Xu, PhD; Yaozhu Leng, MSc; Sonia S. Hassan, MD; Bogdan Panaitescu, MD, PhD; Jeeyeon Cha, PhD; Sudhansu K. Dey, PhD

BACKGROUND: Decidual senescence has been considered a mechanism of disease for spontaneous preterm labor in the absence of severe acute inflammation. Yet, signs of cellular senescence have also been observed in the chorioamniotic membranes from women who underwent the physiological process of labor at term.

OBJECTIVE: We aimed to investigate whether, in the absence of acute histologic chorioamnionitis, the chorioamniotic membranes from women who underwent spontaneous preterm labor or labor at term exhibit markers of cellular senescence.

STUDY DESIGN: Chorioamniotic membrane samples were collected from women who underwent spontaneous preterm labor or labor at term. Gestational age-matched nonlabor controls were also included. Senescence-associated genes/proteins were determined using reverse transcription quantitative polymerase chain reaction (n = 7-9 each for array; n = 26-28 each for validation), enzyme-linked immunosorbent assays (n = 7-9 each), immunoblotting (n = 6-7 each), and immunohistochemistry (n = 7-8 each). Senescence-associated β-galactosidase activity (n = 7-11 each) and telomere length (n = 15-22 each) were also evaluated.

RESULTS: In the chorioamniotic membranes without acute histologic chorioamnionitis: (1) the expression profile of senescence-associated genes was different between the labor groups (term in labor and preterm in labor) and the nonlabor groups (term no labor and preterm no labor), yet, there were differences between the term in labor and preterm in labor groups; (2) most of the differentially expressed genes among the groups were closely related to the tumor suppressor protein 53 pathway; (3) the expression of *TP53* was down-regulated in the term in labor and preterm in labor groups compared to their nonlabor counterparts; (4) the expression of *CDKN1A* (gene coding for p21) was up-regulated in the term in labor and preterm in labor groups compared to their nonlabor counterparts; (5) the expression of the cyclin kinase *CDK2* and cyclins *CCNA2*, *CCNB1*, and *CCNE1* was down-regulated in the preterm in labor group compared to the preterm no labor group; (6) the concentration of tumor suppressor protein 53 was lower in the preterm in labor group than in the preterm no labor and term in labor groups; (7) the senescence-associated-β-galactosidase activity was greater in the preterm in labor group than in the preterm no labor and term in labor groups; (8) the concentration of phospho-S6 ribosomal protein was reduced in the term in labor group compared to its nonlabor counterpart but no differences were observed between the preterm in labor and preterm no labor groups; and (9) no significant differences were observed in relative telomere length among the study groups (term no labor, term in labor, preterm no labor, and preterm in labor).

CONCLUSION: In the absence of acute histologic chorioamnionitis, signs of cellular senescence are present in the chorioamniotic membranes from women who underwent spontaneous preterm labor compared to those who delivered preterm in the absence of labor. However, the chorioamniotic membranes from women who underwent spontaneous labor at term did not show consistent signs of cellular senescence in the absence of histologic chorioamnionitis. These results suggest that different pathways are implicated in the pathological and physiological processes of labor.

Key words: acute histologic chorioamnionitis, cell cycle, cyclin-dependent kinase inhibitor 1 (p21), cyclin kinases, cyclins, decidua parietalis, human, intraamniotic infection, parturition, pregnancy, preterm birth, preterm labor, senescence-associated β-galactosidase, sterile inflammation, telomere length, tumor suppressor protein 53

Introduction

Cellular senescence is a process in which cells stop dividing and undergo alterations in their phenotype, chromatin, secretome, and tumor suppressor activation.[1] Such a process is described as an irreversible cell cycle arrest, which is a result of the overexpression of the cyclin kinase inhibitors, cyclin-dependent kinase inhibitor 1 ($p21^{CDK1}$), cyclin-dependent kinase inhibitor 2A ($p16^{INKT/6/CDKN2}$), as well as alterations in the tumor suppressor protein 53 (TP53) pathway.[1,2] Senescent cells are generally nonproliferative and can be identified by their enlarged nuclei with aberrant distribution of heterochromatin and prominent nucleoli, flattened morphology with marked actin stress fibers, and chronic DNA damage.[2-5]

In the placenta, cellular senescence was first described using morphological and histological characteristics,[6-11] where it is considered a physiological process of aging in this organ.[10,12] Decidual senescence, however, is proposed to be a mechanism of disease for spontaneous preterm labor,[13,14] a syndrome of multiple pathological processes[14] that frequently leads to preterm birth,[15-19] the leading cause of perinatal morbidity and mortality worldwide.[20-23]

Cite this article as: Gomez-Lopez N, Romero R, Plazyo O, et al. Preterm labor in the absence of acute histologic chorioamnionitis is characterized by cellular senescence of the chorioamniotic membranes. Am J Obstet Gynecol 2017;volume:x.ex-x.ex.

0002-9378/$36.00
Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ajog.2017.08.008

FLA 5.5.0 DTD ■ YMOB11802_proof ■ 6 September 2017 ■ 6:21 pm ■ ce

Such a hypothesis is supported by the fact that, in pregnant mice with a uterine-specific deletion of transformation-related protein 53 (*Trp53*), the rate of preterm birth and decidual senescence (evidenced by senescence-associated [SA]-β-galactosidase [gal] activity[24]) was greater than in the control mice.[13] In addition, mice with a uterine-specific deletion of *Trp53* display an increased expression of p21,[13] pAkt[13] (also known as protein kinase B or PKB), and phospho-S6 ribosomal protein (pS6),[25] as well as a reduced expression of antioxidant enzymes, in the decidua.[26] The mechanisms whereby decidual senescence results in spontaneous preterm labor seem to be independent of severe acute inflammation since wild-type mice do not exhibit characteristics of cellular senescence upon injection with lipopolysaccharide,[25] which induces preterm birth.[27-30] In human beings, however, whether decidual senescence is a mechanism of disease for spontaneous preterm labor in the absence of severe acute inflammation is still under investigation.

Yet, cellular senescence is also considered a physiological mechanism for parturition at term.[31,32] Such a concept is supported by evidence demonstrating that the chorioamniotic membranes from women who underwent spontaneous labor at term display morphological characteristics of cellular senescence (enlarged cells and organelles) and have increased SA-β-gal activity compared to those from women who delivered at term without labor.[33] In addition, women who underwent spontaneous labor at term have an elevated amniotic fluid concentration of SA secretory phenotype markers (granulocytes macrophage colony-stimulating factor, interleukin-6 and -8),[33] a greater number of telomere fragments in the amniotic fluid,[34] and a shorter telomere length and reduced lamin B1 in the chorioamniotic membranes, as well as an up-regulation of p21,[35] compared to those who delivered at term without labor. Further, in the murine chorioallantoic membranes, the telomere length shortens as the presence of mitogen-activated kinase p38,[36] active TP53, and SA-β-gal activity increases gradually throughout gestation.[37]

The aim of the current study was to investigate whether, in the absence of acute histologic chorioamnionitis (severe acute inflammation), the chorioamniotic membranes from women who underwent spontaneous preterm labor and those who had undergone spontaneous labor at term exhibit markers of cellular senescence compared to gestational-age-matched nonlabor controls.

Materials and Methods

Human subjects

Chorioamniotic membrane samples were collected from women who delivered at term with or without spontaneous labor. Chorioamniotic membrane samples were also collected from women who underwent spontaneous preterm labor or delivered preterm in the absence of labor due to clinical indications. Sampling of the chorioamniotic membranes included the periplacental, middle and rupture zones[38-41] (spontaneous rupture zone in cases with labor and mechanical rupture zone in cases without labor). These samples were obtained from the Bank of Biological Specimens of the Detroit Medical Center, Wayne State University, and the Perinatology Research Branch, an intramural program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), National Institutes of Health, US Department of Health and Human Services (Detroit, MI). The institutional review boards of Wayne State University and NICHD approved the collection and use of biological materials for research purposes. All participating women provided written consent and samples were collected within 30 minutes after delivery. Demographic and clinical characteristics of the study population are displayed in Tables 1 to 3. Table 1 includes samples that were processed immediately after collection. Tables 2 and 3 contain samples that were preserved and used for future RNA and telomere length studies. All women in the study had singleton pregnancies and patients with neonates who had congenital or chromosomal abnormalities were excluded. Most of the chorioamniotic membranes samples were obtained from women without preterm prelabor rupture of membranes (pPROM) (>97%). Labor at term was defined as the presence of regular uterine contractions at a minimum frequency of 2 contractions every 10 minutes associated with cervical changes resulting in delivery >37 weeks of gestation.[42] Preterm labor was diagnosed by the presence of regular uterine contractions (at least 3 in 30 minutes) and documented cervical changes in patients with a gestational age between 20 and 36 6/7 weeks. Preterm delivery was defined as birth <37th week of gestation. In each case, tissue sections of the chorioamniotic membranes were evaluated for acute histologic chorioamnionitis, according to published criteria,[43,44] by pathologists who were blind to the clinical outcome. Samples collected from women with acute histologic chorioamnionitis were excluded from this study. The preterm no labor group included chorioamniotic membrane samples from women who delivered preterm due to clinical indications such as preeclampsia. The term no labor group included chorioamniotic membrane samples from women with a history of cesarean delivery, malpresentation, and/or cesarean delivery on maternal request.

RNA isolation, cDNA synthesis, and reverse transcription quantitative polymerase chain reaction analysis

Total RNA was isolated from the chorioamniotic membranes using TRIzol reagent (Life Technologies Corp, Grand Island, NY) and the RNeasy Kit (Qiagen, Gaithersburg, MD), according to the manufacturers' instructions. RNA purity and concentrations were assessed with the NanoDrop 1000 spectrophotometer (Thermo-Fisher Scientific Inc, Wilmington, DE) and RNA integrity was evaluated with the Bioanalyzer 2100 (Agilent Technologies, Wilmington, DE). The expression profile of 84 SA genes was initially determined in a small set of samples (n = 8-9 per group) (Table 1) using the RT[2] profiler polymerase chain reaction (PCR) array

FLA 5.5.0 DTD ■ YMOB11802_proof ■ 6 September 2017 ■ 6:21 pm ■ ce