OBSTETRICS

Chorioamniotic membrane senescence: a signal for parturition?

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OBJECTIVE: Senescence is an important biological phenomenon involved in both physiologic and pathologic processes. We propose that chorioamniotic membrane senescence is a mechanism associated with human parturition. The present study was conducted to explore the association between senescence and normal term parturition by examining the morphologic and biochemical evidences in chorioamniotic membranes.

STUDY DESIGN: Chorioamniotic membranes were collected from normal term deliveries; group 1: term labor and group 2: term, not in labor. Senescence-related morphologic changes were determined by transmission electron microscopy and biochemical changes were studied by senescence-associated (SA) β-galactosidase staining. Amniotic fluid samples collected from both term labor and term not in labor were analyzed for 14 SA secretory phenotype (SASP) markers.

RESULTS: Morphologic evidence of cellular senescence (enlarged cells and organelles) and a higher number of SA β-galactosidase-stained amnion and chorion cells were observed in chorioamniotic membranes obtained from women in labor at term, when compared to term not in labor. The concentration of proinflammatory SASP markers (granulocyte macrophage colony-stimulating factor, interleukin-6 and -8) was significantly higher in the amniotic fluid of women in labor at term than women not in labor. In contrast, SASP factors that protect against cell death (eotaxin-1, soluble Fas ligand, osteoprotegerin, and intercellular adhesion molecule-1) were significantly lower in the amniotic fluid samples from term labor.

CONCLUSION: Morphologic and biochemical features of senescence were more frequent in chorioamniotic membranes from women who experienced term labor. Senescence of chorioamniotic membranes were also associated with amniotic fluid SASP markers.

Key words: aging, delivery, fetal signals, labor, pregnancy, premature birth, senescence-associated secretory phenotype, sterile inflammation, β-galactosidase

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Understanding the initiation signals and sequential set of events culminating in normal labor is integral to deciphering the mechanism of alterations in the timing of labor, especially preterm labor.[1] The role of fetal endocrine signals functioning as the biologic clock of organ maturation and as triggers for labor at term has been well documented.[2] However, knowledge gaps still exist in our understanding of the initiator and effecter signals of normal term labor.

In this study, we propose that senescence may play a role in term labor. The term "senescence" refers to the physiologic and biomolecular mechanisms that are normal and natural associated with aging of a living organism.[3] Senescence involves irreversible arrest of cell growth.[4,5] The importance of senescence have been demonstrated in age-related pathologies such as Alzheimer disease, cardiovascular diseases, metabolic disorders such as diabetes, and chronic inflammatory conditions.[6-10] Lack of senescence or failure of cell cycle arrest is associated with cancer.[7,11,12] Therefore, senescence has been implicated in normal physiologic aging of

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living organisms; however, premature senescence may also lead to pathologic states.

Morphologic evidence of senescence is characterized by enlargement of cells, often doubling in volume, and biochemically by the presence of senescence-associated (SA) β-galactosidase (gal) and formation of DNA-damaged foci with chromatin alterations and phosphorylation of histone 2AX.[13,14] Unlike apoptosis (programmed cell death), senescent cells persist, alter their function, and change the tissue environment with a unique inflammatory milieu. Recent studies by our laboratory have reported markers of SA with term labor chorioamniotic membranes compared to term not in labor.[15]

Senescence is also associated with changes in a set of biomarkers that are termed as SA secretory phenotype (SASP). SASP is marked by differential production of various natural compounds. These compounds include, but are not limited to, cytokines, chemokines, angiogenic and other growth factors, matrix-degrading enzymes, as well as inhibitors, cell adhesion molecules, apoptotic inducers and their ligands may constitute the inflammation associated with term labor.[16-18] Coppé et al[16,17] reported SASP factors secreted by senescent fibroblasts, epithelial cells, and epithelial tumor cells after genotoxic stress in culture, and in epithelial tumor cells in vivo, after treatment with DNA-damaging chemotherapy.

SASP overlaps with inflammatory markers associated with term labor. A review of the literature prior to initiation of this study revealed that differences in the concentrations of 24 of the 74 SASP factors have been reported between term labor and term not in labor amniotic fluids and chorioamniotic membranes (Table 1). Some of the most notable SASP markers reported in amniotic fluid from women with term labor include cytokines, interleukin (IL)-1α and IL-1β,[19-22] IL-6,[20,23,24] IL-8,[25] tumor necrosis factor (TNF)-α,[26,27] and matrix metalloproteinases (MMPs).[26,28,29] Induction of prostaglandins in utero by these cytokines[22,30-39] and chorioamniotic membrane extracellular matrix degradation by MMPs facilitate parturition.[40-43] SASP-associated cytokines in maternal blood contributing to inflammation and parturition have also been reported recently.[44,45] Although many of the SASP markers have been reported to be different between term labor and term not in labor, none of them have been associated with senescence-related changes in the literature, but are mostly generalized as inflammatory changes during labor.

We postulate that senescence of the chorioamniotic membranes is a natural and physiological process that is initiated at the time of placentation, and term labor can be considered as an end stage of life for chorioamnion. The acceleration of chorioamniotic membrane senescence may be influenced by oxidative stress likely due to increased metabolic demands by the growing fetus. In vitro, we have shown induction of senescence by oxidative stress in fetal cells.[15,46] SASP markers generated from senescent fetal cells may constitute sterile intrauterine inflammation and function as a signal to promote labor. Therefore, the primary objective of this study is to investigate senescence-related morphologic and biochemical changes in chorioamniotic membranes in women who experienced term labor. We also examined the concentrations of 14 SASP-associated markers in the amniotic fluid of women with term labor and compared them to term not in labor.

Materials and Methods

Amniotic fluid samples used for this study were from the Nashville Birth Cohort Biobank established to study genetic and biomarkers differences contributing to racial disparity in preterm birth. Samples were collected at the Centennial Medical Center, Nashville, TN, from 2008 through 2011. The study protocols were approved by the Western Institutional Review Board, Seattle, WA, for recruitment and collection of amniotic fluid samples and the reuse of samples for preterm birth–related projects was approved by the Institutional Review Board at University of Texas Medical Branch, Galveston, TX. The study complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. Informed, written consent was obtained from subjects prior to sample collection. Enrollment occurred at the time of admission for delivery, either at term or preterm. Chorioamniotic membranes used for this study were obtained from John Sealy Hospital at the University of Texas Medical Branch from women delivering at term. Institutional review board approval as an exempt status for discarded tissues was obtained prior to collection of these samples.

Subjects

In this nested cross-sectional analysis, pregnant women between the ages of 18-40 years provided amniotic samples. Amniotic fluid samples were obtained from subjects in spontaneous term ($\geq 37^{0/7}$ weeks) labor (defined as the presence of strong, regular uterine contractions at a minimum frequency of 2 contractions/10 min, followed by changes in cervical effacement and dilatation that led to delivery at term ($\geq 37^{0/7}$). Amniotic fluid samples were also collected from women, not in labor, undergoing elective cesarean deliveries. Gestational age was determined by the last menstrual period, and was corroborated by ultrasound biometry. Subjects with multiple gestations, preeclampsia, placenta previa, fetal anomalies, gestational diabetes mellitus, and/or other medical/surgical complications of pregnancy were excluded. Subjects who were treated for preterm labor or for suspected intraamniotic infection and delivered at term were excluded from the study. Details of this cohort and samples can be found in our other publications.[47-51]

Collection of samples

For vaginal deliveries, amniotic fluid samples were collected during labor, immediately before artificial rupture of the membranes, by transvaginal amniocentesis of intact membranes, using a 22-gauge needle through the dilated cervical os. In cases undergoing cesarean delivery, samples were collected by transabdominal amniocentesis.