Endometrial cellular senescence contributes to preterm birth

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Preterm birth is a major global health issue, and its causes and underlying mechanism remain obscure. We recently established a mouse model of spontaneous preterm birth. In this model, endometrial cellular senescence early in pregnancy via mTORC1-p21 signaling is a major contributor of preterm birth and fetal death, and these adverse phenotypes are restored by the inhibition of mTORC1 or p21. This role of endometrial cellular senescence in determining the timing of birth in mouse models may help us better understand the mechanism of the timing of birth in humans and develop new and improved strategies against preterm birth.

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Preterm birth

Parturition is an intricate process. Under the protection of the mother, the fetus safely grows for 37-41 weeks until the right combination of signals stemming from both endocrine and mechanical stimulation induces parturition. If this process goes wrong, the child may be born premature or become the victim of stillbirth. There are nearly 15 million premature births with more than 3 million stillbirths worldwide each year¹. Prematurity is one of the direct causes of neonatal deaths and often leads to developmental impairment and long-term disabilities in those who survive¹. In humans, preterm birth is defined by birth occurring earlier than 37 weeks of gestation. Many factors, including genetics, infection/inflammation, maternal age, life style, progesterone (P₄) resistance, uterine over-distension and cervical aberration are identified as contributors to preterm birth² (Fig.1). However, a comprehensive understanding of this aberration in pregnancy remains elusive. The incidence of preterm birth remains high even with continued clinical efforts to reduce the occurrence, such as with tocolytic drugs, antibiotics, surgical cerclage and P₄ supplementation, since the targets of these therapies are not fundamental but symptomatic. Therefore, there is a crucial need for an intensive effort from basic research using different preclinical models to target this global problem².
Animal models of preterm birth

Animal models that spontaneously develop preterm delivery are powerful tools to better understand the underlying mechanism, and to develop prevention and treatment strategies. Although rodent models of preterm birth induced by pro-inflammatory agents, such as lipopolysaccharide (LPS), are generally used, these models are not ideal because the onset of labor in these models is induced by ovarian luteolysis with a drop in P4 levels to trigger labor, which does not occur in human parturition. Furthermore, LPS induces both uterine and systemic inflammation making the interpretation difficult as to the cause of preterm birth. Thus, rodent models of preterm labor without luteolysis are more desirable; however, such models have scarcely existed. Remarkably, we have developed a novel mouse model of spontaneous preterm birth without P4 withdrawal. The mice with conditional deletion of uterine p53 showed increased levels of endometrial cyclooxygenase-2 (Cox2) and PGF2α that induce premature delivery with neonatal death without early luteolysis, mimicking an aspect of human preterm delivery. The merits of this model are further described in the next section.

Mice with conditional deletion of uterine p53

We generated p53-Δmice by crossing p53-floxed (p53fl) mice with progesterone receptor-Cre (PgrCre+) mice. Although embryo implantation was normal in p53-Δ mice, their decidua, differentiated endometrial stromal tissues whose differentiation starts at the attachment of embryo on the endometrium, grew restrictedly. According to the compro-mised formation with downregulation of phosphorylated Akt, senescence-associated β-galactosidase (SA-β-gal) activity, the most popular marker of cellular senescence, was increased in p53-Δ females. In addition to SA-β-gal activity, p21, a possible regulator of cellular senescence, and H2AX, a DNA damage marker, were upregulated in the p53-Δ endometrium. Since p21 and H2AX are often used as surrogate markers of cellular senescence, these findings indicate that uterine p53 deletion promotes endometrial cellular senescence. The proteomic analyses showed the downregulation of a cluster of antioxidant enzymes in the p53-deficient endometrium, suggesting increased oxidative stress in the p53-Δ endometrium. In spite of the restricted endometrial growth, the incidence of resorption did not change. Excitingly, about half of p53-Δ dams showed preterm delivery and neonatal death without a drop in serum P4 levels, mimicking aspects of human parturition. In pursuit of the underlying cause of premature birth, we found persistent signs of increased endometrial cellular senescence with increased levels of pAkt and Cox2. Since pAkt is known to upregulate Cox2 levels in various cell types including uterine cells and Cox-derived prostaglandins (PGs) are crucial for the initiation of labor, we hypothesized that an early rise in uterine pAkt and Cox2 levels initiated premature labor in p53-Δ females. This hypothesis was clearly supported by the observation of increased uterine levels of PGF2α in the face of unaltered levels of Cox1 and other PGs in these mice, and the prevention of their preterm births by oral administration of the Cox2 selective inhibitor celecoxib. Collectively, these findings provide evidence that uterine deficiency of p53 evokes cellular senescence and elevates Cox2-derived PGF2α levels.

Cellular senescence

Cellular senescence is defined as an irreversible replication arrest. Although it was discovered in 1961 by Hayflick and Moorhead, its molecular aspects have not been well-known compared to apoptosis. Recently, many researchers are focusing on cellular senescence, and this research field starts to move forward. Cellular senescence can be divided into two different forms: replicative and premature senescence. The former is the growth arrest after a period of apparently normal cell proliferation due to end replication problem, and is considered to be part of a cellular aging process limiting its lifespan. The latter occurs prior to the stage at which cellular senescence is induced by...
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Review Article

Cellular senescence induces prematurity

Endometrial cellular senescence and preterm birth

To investigate whether endometrial cellular senescence triggers preterm birth, we performed further analyses using p53<sup>−/−</sup> mice. We found that endometrial cellular senescence in p53<sup>−/−</sup> females is associated with heightened signaling of mammalian target of rapamycin complex 1 (mTORC1)<sup>9</sup>, which is known to be a downstream signaling of Akt. Heightened mTORC1 signaling is a significant contributor to preterm birth, since the phenotype of prematurity and the increase of uterine Cox2 in p53<sup>−/−</sup> mice was rescued by an oral gavage of a low dose of rapamycin, an mTORC1 inhibitor<sup>26</sup>. In the in-vitro experiment using Tsc<sup>−/−</sup> mouse embryonic fibroblasts where mTORC1 is activated, the inhibition of mTORC1 signaling negatively regulates the status of p21 and Cox2. We further established the mice with double deletion of p53 and p21, and these mice did not show any symptoms of preterm deliveries<sup>5</sup>. In addition, the superimposition of p21 deletion repressed endometrial cellular senescence and Cox2 expression induced by deletion of uterine p53. These findings suggest that the Akt-mTORC1-p21-Cox2 signaling axis is a critical component in the timing of birth and an intervention of any of these three targets is capable of rescuing preterm delivery in p53<sup>−/−</sup> mice<sup>4, 5</sup> (Fig.2). This study also reveals that progressive endometrial cellular senescence approaching term birth is a normal occurrence in mice. These studies may help to elucidate the mechanism of human birth and develop new strategies to combat this global problem.

This mouse model of endometrial cellular senescence-induced preterm labor can be used to explore interactions between genetic predisposition and infection/inflammation during pregnancy. We have shown that p53<sup>−/−</sup> dams subject to even low grade immunological insults by LPS remarkably increases their predilection towards preterm birth<sup>16</sup>, suggesting that a close relationship between ge-
there is evidence that certain p53 polymorphisms in women correlate with recurrent pregnancy failure, there have not been any reports about relationship between p53 polymorphisms and the incidence of preterm birth. It also remains to be seen whether the patients with high risk of preterm birth retain genetic alterations of any members of this signaling axis. Further investigations are needed to elucidate this issue.

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Conflict of Interest

The author has no conflict of interest.

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