Review Article

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 year[1)]. Prematurity is one of the direct causes of neonatal deaths and often leads to developmental impairment and long-term disabilities in those who survive[1)]. 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 (P_4) resistance, uterine over-distension and cervical aberration are identified as contributors to preterm birth[2)] (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 P_4 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 problem[2)].

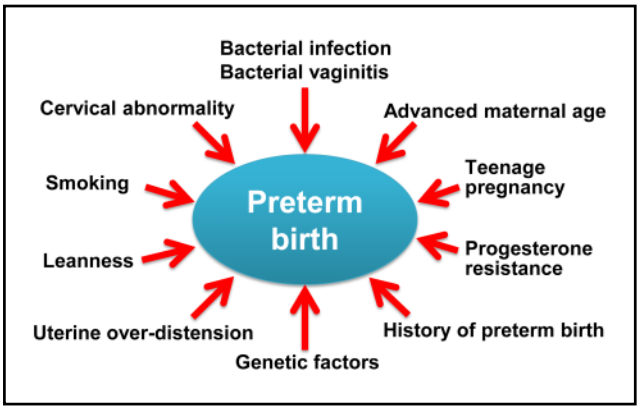

Fig.1 Multiple factors are associated with the pathogenesis of preterm delivery.

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[3], these models are not ideal because the onset of labor in these models is induced by ovarian luteolysis with a drop in P_4 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 P_4 withdrawal[4]. The mice with conditional deletion of uterine *p53* ($p53^{d/d}$) showed increased levels of endometrial cyclooxygenase-2 (Cox2) and $PGF_{2\alpha}$ that induce premature delivery with neonatal death without early luteolysis, mimicking an aspect of human preterm delivery[4]. The merits of this model are further described in the next section.

Mice with conditional deletion of uterine p53

We generated $p53^{d/d}$ mice by crossing *p53*-floxed ($p53^{f/f}$) mice with progesterone receptor-Cre ($Pgr^{Cre/+}$) mice[4]. Although embryo implantation was normal in $p53^{d/d}$ mice, their deciduae, differentiated endometrial stromal tissues whose differentiation starts at the attachment of embryo on the endometrium, grew restrictedly. According to the compromised formation with downregulation of phosphorylated Akt, senescence-associated β-galactosidase (SA-β-gal) activity, the most popular marker of cellular senescence, was increased in $p53^{d/d}$ deciduae[4]. In addition to SA-β-gal activity, p21, a possible regulator of cellular senescence, and γH2AX, a DNA damage marker, were upregulated in the $p53^{d/d}$ endometrium[4, 5]. Since p21 and γH2AX 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 $p53^{d/d}$ endometrium[6]. In spite of the restricted endometrial growth, the incidence of resorption did not change. Excitingly, about half of $p53^{d/d}$ dams showed preterm delivery and neonatal death without a drop in serum P_4 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[4]. 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 $p53^{d/d}$ females. This hypothesis was clearly supported by the observation of increased uterine levels of $PGF_{2\alpha}$ 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[4]. Collectively, these findings provide evidence that uterine deficiency of *p53* evokes cellular senescence and elevates Cox2-derived $PGF_{2\alpha}$ levels.

Cellular senescence

Cellular senescence is defined as an irreversible replication arrest. Although it was discovered in 1961 by Hayflick and Moorhead[7], 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[8, 9]. The latter occurs prior to the stage at which cellular senescence is induced by

telomere shortening, and is alternatively stimulated by acute stress caused by overactive oncogenes, DNA damage, oxidative stress, and metabolic stress[10-13]. Because our findings indicate that both DNA damage and oxidative stress are increased in the *p53*$^{d/d}$ endometrium, we strongly believe that the endometrial cellular senescence in our mouse model is premature senescence.

Apart from irreversible cell cycle arrest, senescent cells are usually larger and flattened out with a vacuole-rich cytoplasm and display increased lysosomal β-galactosidase activity which is detected by SA-β-gal staining. They also exhibit global changes in chromatin structure that often can be visualized as so called senescence-associated heterochromatin foci (SAHF). Cell cycle regulators such as p21 and p16, the molecules involved in the DNA damage response such as γH2AX, and those involved in the formation of SAHF such as the heterochromatin protein HP1γ are often used as the surrogate markers of cellular senescence. Further, senescent cells are characterized by global reprogramming of gene expression, including expression and secretion of pro-inflammatory cytokines and tissue remodelling enzymes referred to as the senescence-associated secretory phenotype (SASP), indicating that senescent cells communicate with each other and with non-senescent cells in the environment[10-12]. For example, known SASP cytokines interleukin-6 (IL-6) and IL-8 have a cell autonomous function that reinforces senescence in a paracrine manner[14]. In addition, it is speculated that SASP factors promote the malignant phenotypes of neighboring cells[15]. Evidence has accumulated during recent years clearly showing that cellular senescence plays an important role in the pathophysiology of several diseases such as cancers and metabolic syndrome[8-10, 16, 17].

Endometrial cellular senescence and preterm birth

To investigate whether endometrial cellular senescence triggers preterm birth, we performed further analyses using *p53*$^{d/d}$ mice. We found that endometrial cellular senescence in *p53*$^{d/d}$ females is associated with heightened signaling of mammalian target of rapamycin complex 1 (mTORC1)[5], 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*$^{d/d}$ mice was rescued by an oral gavage of a low dose of rapamycin, an mTORC1 inhibitor[5]. In the *in-vitro* experiment using *Tsc1*$^{-/-}$ 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[5]. 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*$^{d/d}$ mice[4, 5] (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.

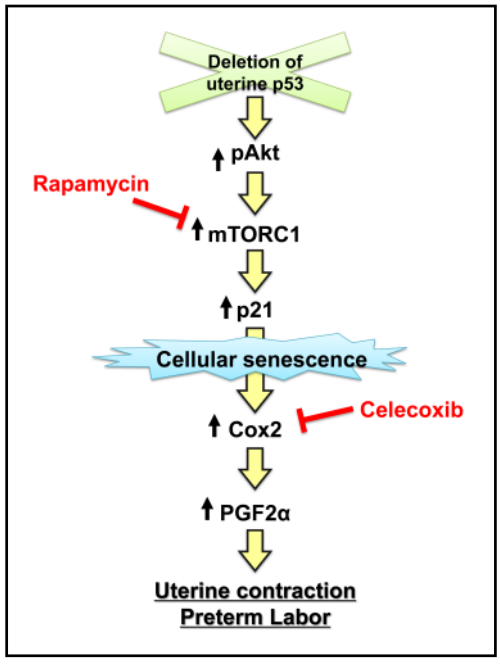

Fig.2 A scheme illustrating potential contribution of cellular senescence in the preterm birth of uterine p53-deleted mice.

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*$^{d/d}$ dams subject to even low grade immunological insults by LPS remarkably increases their predilection towards preterm birth[18], suggesting that a close relationship between ge-

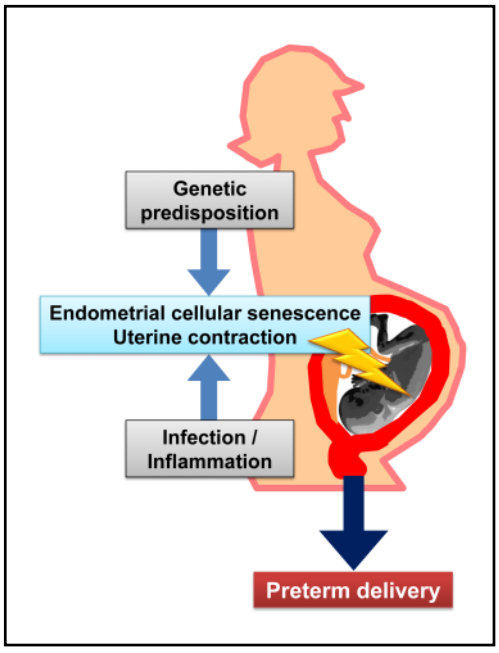

Fig.3 A scheme showing gene-environment interactions aggravate preterm delivery.

netic predisposition and environmental insults in exacerbating preterm delivery (Fig.3). These findings have raised many more questions. How and why is the pregnant endometrium programmed to undergo cellular senescence during pregnancy? How does endometrial cellular senescence occurring early in pregnancy triggers preterm birth? With infection/inflammation known to amplify secretion of SASP cytokines IL-6 and IL-8[19, 20], does the SASP contribute to premature birth? Can diets or endocrine disruptors influence endometrial cellular senescence to alter timing of birth? Answering these questions will require intensive research. If these questions are answered, it may be possible to develop efficient preventive strategies by handling key regulators in the labor pathway.

Conclusions

Our results emphasize a new role of endometrial cellular senescence involving the p53-mTORC1-p21-Cox2 signaling axis in determining the timing of birth[4, 5, 18] (Fig.2). Since aging is a contributing factor to cellular senescence[21] and since advanced maternal age is epidemiologically associated with preterm birth[22-24], it is possible that endometrial cellular senescence due to maternal aging can increase the risk of preterm birth. *p53* polymorphisms have been implicated with aging and life span in humans[25, 26]. Although there is evidence that certain *p53* polymorphisms in women correlate with recurrent pregnancy failure[27], 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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