RESEARCH HIGHLIGHT

The androgen receptor is a key component of myometrium phenotype programming during pregnancy

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The economic burden associated with preterm birth is significant to our society. While research efforts on preterm birth over past decades had been directed toward understanding the late stage of parturition, recent discoveries have indicated that the timing of labour is delicately programmed as early as the initiation of pregnancy. There exists a myometrium phenotype programming during pregnancy consisting three characteristic phases that can be referred to as the proliferative phase in the early pregnancy, the hypertrophic phase in the mid-term pregnancy and the contractile phase in the late stage of pregnancy. This remarkable plasticity of myometrium allows it not only provides containment for fetus to be fully developed within the womb during pregnancy, but also performs coordinative contractions at the onset of labour to expel the fetus from the uterus to extra-uterine environment. Our two recent studies further demonstrate that the androgen receptor plays an important role in determining the phenotypes of myometrium throughout the pregnancy progression. Here we summarize our endeavor in characterizing the androgen receptor signaling in myometrial smooth muscle cells.

Keywords: androgen receptor; pregnancy; myometrium phenotype programming

The Societal and Economic Impacts of Preterm Labour

The incidence of preterm birth is counted at approximately 5-10% over all pregnancies [1]. However, it is accompanied by ~70% of neonatal mortality and up to 75% of neonatal morbidity [1]. Preterm neonates are 40 times more likely to die than term infants [2, 3] and are at increased risk of neuron-developmental complications, motor and mental disabilities including deafness, blindness, cerebral palsy as well as respiratory illness [3, 4]. The cost of preterm birth in 2005 was estimated over $26.2 billion in the USA [5]. Although decades of research have been aiming at developing drugs to inhibit myometrium contraction at the late stage of pregnancy, the incidence of premature birth still remains at ~5-7% in many developed countries and significantly higher in developing countries [6, 7]. Clearly new approaches are needed. To achieve this goal, full understanding the mechanisms by which control

women parturition is required.

Although our knowledge on the mechanisms of onset of labour has been significantly enhanced, research has been extensively focused on the late stage of pregnancy where myometrial smooth muscle cells are transformed from a relative quiescent status into a highly contractile phenotype. This myometrium phenotype switch is believed to be modulated by two signal cascades: **i**) the stretching forces induced by the growing fetus against the uterine wall and **ii**) the fetal hypothalamic–pituitary–adrenal–placental axis [8]. Recent studies discover that the myometrial smooth muscle cells undergo continuous phenotype changes throughout the pregnancy process. Three distinct types of phenotypes had been observed and classified based upon myometrial cell morphology and gene signatures of myometrial cell. The early proliferative stage of myometrium is characterized by high proliferative indexes such as proliferating cell nuclear antigen (PCNA) and BrdU incorporation and augmented expressions of anti-apoptotic protein factors such as B-cell CLL/Lymphoma 2 (Bcl2) and Bcl-2-Like Protein 1 (Bcl2L1) [9]. The mid-term synthetic stage of myometrium is featured with increased myometrial cell size and deposition of extracellular matrix that integrates myometrial muscle cells and empowers the contraction capacity of the myometrium [10-12]. The late contractile phenotype of myometrium presents increased expressions of contraction-associated proteins such as connexin 43, receptors for oxytocin and prostaglandins that are required for the activation of the myometrium to commit coordinative contractions for labour [13-15]. Since myometrium has to gain enough myometrial cell numbers thereby contraction ability to engage in labour, disruption of myometrial cell proliferation would therefore affect the outcome of pregnancy and labour.

The Androgen Receptor Signaling in myometrium during pregnancy

In our recent two studies, we demonstrate that the androgen receptor (AR) plays critical roles in myometrium phenotype programming during pregnancy [16, 17]. In the early stage of pregnancy, AR exerts both proliferative and anti-apoptotic functions. It have been previously reported that the AR is expressed in non-pregnant myometrium and its levels fluctuated during the estrous cycle [18]. Using pregnant rat model, we profiled AR protein levels in myometrium by immunoblotting assays and showed that the AR was highly expressed in the proliferative stage of pregnancy, started to reduce in the synthetic stage and reached its extremely low levels in the contraction stage of pregnancy. Immunohistochemistry further confirmed these findings and indicated that the AR was mainly localized in the nuclei of both longitudinal and circular myometrial smooth muscle cells.

Regulation of AR expression in myometrium had also been studied [16]. Since both mechanical stretch and endocrine cascades determine myometrium phenotypes during pregnancy, we first used the unilaterally pregnant rat model to demonstrate that AR expression was effectively suppressed by the growing fetus that created stretching forces to the myometrium. Our non-pregnancy tubal ligation rat model also showed that the mechanical stretch induced by enlarged cylinders of laminaria can also inhibit AR expression in myometrium. Results from both rat models reached the consensus that the suppressive impacts of stretch were independent to circulating steroid hormones. Further experiments using the ovariectomized rat model showed that in addition to mechanical stretch, decreased ratio of progestin: estrogen also contributed to AR protein reduction.

Our studies on the functions of AR have been focused on how AR controls myocyte proliferation and myocyte responses to apoptotic stimuli. The rationale behind this is that the AR protein levels are at significantly high levels during the proliferative stage of pregnancy, when myometrium is experiencing a high speed of accumulation of cell numbers. Collaborative mechanisms between proliferation and anti-apoptosis have to be built to achieve a successful gain of myometrial cell numbers. We demonstrated that AR enhanced cell proliferation in a ligand-independent manner [16]. Knocking down endogenous AR expression in myometrial cells reduced cell proliferation rates and delayed G1-S and S-G2 phase transitions of myometrial cell cycling. Coincidently, reduced cyclin D, A and B1 and increased p27 protein expressions were also observed. In addition, we demonstrated that AR protein expression was required for myometrial cells to be invulnerable against apoptotic stimuli [17]. Under camptothecin, UV light or Fas ligand treatment, myometrial cells expressing higher levels of AR were more resistant to undergo apoptotic cell death. This AR action was in both ligand-dependent and ligand-independent fashions, as knocking down AR protein expression or treating cells with AR antagonist MDV3100 rendered myometrial cell more fragile when confronted by both endogenous and exogenous apoptotic stimuli.

The molecular mechanisms by which AR mediates the dual functions of proliferation and anti-apoptosis were further studied [17]. Gene microarray studies showed that the top ranked gene groups whose transcription were regulated by the AR were genes associated with cell cycling and apoptosis. Further analyses indicated that these AR targeted gene were divided into three major groups that are associated with: **i**) the signal pathway mediated by epidermal growth factor (EGF) and EGF

receptors; **ii**) RNA splicing processes; and **iii**) protein components within the DNA repair machinery[17]. The EGF signaling had been well studied for its anti-apoptotic action through its downstream effector, Akt. We further demonstrated that through the EGF/PI3K/Akt pathway, AR up-regulated the expression of myeloid cell leukemia 1 (Mcl-1) in myometrial cells in both ligand-dependent and ligand-independent manners [17]. AR agonist triggered the activation of protein tyrosine k**inase CYL (c-Src)** kinase, which further stimulated the transcriptional activity of signal transducer and activator of transcription 3 (STAT3) to up-regulate Mcl-1 gene expression [17].

We also defined the molecular mechanism by which AR mediated its proliferation action to myometrial cells [16]. The AR was required to sustain insulin-like growth factor-1 receptor (IGF-1R) stability ligand-independently. AR knockdown resulted in enhanced IGF-1R ubiquitination, followed by increased protein degradation through both the proteosomal and the lysosomal pathways. Therefore, the AR is required for the stimulation of the IGF-1 downstream PI3K/Akt signal cascade and myometrial cell proliferation. These were consistent with our previous observations that all IGF-1R downstream effectors including insulin receptor substrate 1 (IRS-1), phosphor-Akt, mechanistic target of rapamycin (mTOR) and ribosomal protein S6 kinase (S6K1) whose protein levels were significantly elevated during the early proliferative stage of pregnancy [19].

In summary, our studies profiled the specific expression pattern of the AR in myometrium during pregnancy, investigated the regulatory mechanisms that control AR expression, and characterized the dual proliferative and anti-apoptotic actions of the AR through AR regulated signal networks. Together, we conclude that the AR is an important regulator for the myometrium phenotype programming during pregnancy.

Conflict of interests

The authors declare that there is no conflict of interests.

Acknowledgements

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