CLINICAL IMPLICATIONS OF BASIC RESEARCH

How Does Progesterone Relax the Uterus in Pregnancy?

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Most of us owe our existence to the calming influence of progesterone on our mother's uterine muscle (the myometrium). By the third trimester

of pregnancy, the myometrium is akin to a sleeping giant. Once awakened, it becomes one of the strongest muscles in the human body to facilitate birth. How progesterone calms the myometrium for most of pregnancy is a major unanswered question in obstetrics.

The actions of progesterone are mediated by two progesterone receptors, PR-A and PR-B, which function as ligand-activated modulators of gene expression. Progesterone appears to relax the myometrium by repressing the expression of genes that encode factors collectively referred to as contraction-associated proteins (CAPs), which promote labor. Unraveling the molecular mechanisms by which progesterone and progesterone receptors coordinately repress CAP expression, however, has been difficult because these receptors do not interact with the regulatory elements of most CAP genes.

Renthal et al. have recently described a novel pathway in which progesterone coordinately represses the expression of two critical CAP genes, connexin43 (CNX43), which encodes a major gap-junction protein that helps synchronize contractile activity, and the oxytocin-receptor gene (OXTR), which determines the responsiveness of myometrial cells to oxytocin, a potent stimulator of contraction.¹ These researchers obtained data from experiments in mice, human myometrium, and various mouse and human cell cultures to construct a model that explains how progesterone coordinately represses CAP expression in myometrial cells. First, they used bioinformatic and array-based approaches to explore the hypothesis that micro-RNAs (miRNAs) (short RNA molecules that bind to complementary sequences in target messenger RNAs [mRNAs] and inhibit translation) regulate CAP production in myometrial cells. They found that levels of two miRNAs belonging to the mi-RNA-200 family increase in the mouse and human myometrium with advancing gestation and in parallel with CNX43 and OXTR. These data suggested

Figure 1. Progesterone and Pregnancy.

The combined actions of inhibitory transcription factors ZEB1 and ZEB2 (zinc finger E-box binding homeobox proteins 1 and 2) and members of the microRNA (miRNA)-200 family mediate the effect of progesterone on key contraction-associated proteins (CNX43 and OXTR) in the uterus during pregnancy. A recent study by Renthal et al.¹ has shown that during pregnancy, these proteins and miRNAs coordinately form a negative-feedback loop in the myometrium through mutual suppression (purple arrows with minus signs). The pre-pregnancy hormone progesterone stimulates ZEB1 expression (green arrows with plus signs), shifting the steady state toward high levels of ZEB and low levels of miRNA-200. ZEB1 and ZEB2 inhibit the CNX43 and OXTR genes (blue arrows with minus signs), mediating the inhibitory effect of progesterone on the expression of the two key contraction-associated proteins. The action of progesterone diminishes at the time of labor, and the steady state of the feedback loop drifts toward low ZEB levels and high miRNA-200 levels. ZEB1 and ZEB2 no longer inhibit CNX43 and OXTR, which increases myometrial contractility and stimulates the onset of labor. The weight of the arrows indicates the relative strength of the effects.

that the immediate miRNA-200 targets were factors that down-regulate CAP levels. Subsequent experimental and bioinformatic analysis identified two major miRNA-200 targets in the mouse uterus: the repressive transcription factors ZEB1 and ZEB2 (zinc finger E-box binding homeobox proteins 1 and 2). Renthal et al. found that ZEB1 and ZEB2 repressed the expression of CXN43 and OXTR in mouse and human myometrial cells. In addition, they found that ZEB1 and ZEB2 inhibited expression of members of the miRNA-200 family, suggesting that these proteins and miRNAs form a mutually repressive negative-feedback loop in myometrial cells — as is also the case in some human cancers.²

A critical observation made by Renthal et al. was that progesterone directly up-regulates ZEB1 expression in various mouse and human cell lines, suggesting that progesterone promotes a ZEB-dominant state in myometrial cells. Because ZEB1 inhibits expression of miRNA-200, expression of ZEB2 would also be increased, leading to ZEB-mediated inhibition of CXN43 and OXTR expression. When the action of progesterone weakens at the end of pregnancy, ZEB1 levels would be expected to decrease, causing the ZEB-miRNA-200 steady state to shift toward high miRNA-200 levels and low ZEB levels, in turn leading to the withdrawal of ZEB-mediated inhibition of CXN43 and OXTR and a coordinated increase of CXN43 and OXTR levels (Fig. 1). Renthal et al. found that ZEB levels decreased and miRNA-200 levels increased in two mouse

models of preterm birth and that artificial over-expression of ZEB1 and ZEB2 in human myometrial cells inhibited oxytocin-induced contraction.

Could this novel pathway for progesterone action in the myometrium during pregnancy have clinical relevance, especially for the development of therapies to prevent or repress preterm labor? It will be important to determine whether the ZEB-miRNA-200 system is involved in human preterm birth. Even if it is, however, the fact that the ZEB-miRNA-200 loop is involved in cancer progression² raises a red flag in considering its potential as a target for therapeutic intervention during pregnancy. Like all good research, however, the study by Renthal et al. raises exciting questions and tantalizing possibilities that should be explored; preterm birth is a huge and persistent public health problem that requires bold new approaches.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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