SOX9: A Novel Downstream of PGR and Regulator of Steroidogenesis in Granulosa Cells of Human Ovulatory Follicles

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Progesterone receptor (PGR) is found to be essential for successful ovulation in all species studied thus far. However, the specific action of PGR in the human ovulatory follicle remains largely elusive. We recently performed ChIP-Seq analysis to identify PGR-regulated genes in granulosa cells of human ovulatory follicles collected at the time of oocyte retrieval from patients undergoing IVF as well as primary human granulosa/lutein cells (hGLC) treated with hCG. ChIP-Seq analysis revealed a diverse array of genes directly bound by PGR. Of our particular interest, *SOX9* has emerged as a novel direct downstream target of PGR, suggesting its role as a downstream mediator of the P4/PGR-regulated pathway in the human periovulatory follicle.

SOX9, a member of the SRY-box transcription factor family, plays a crucial role in regulating a spectrum of genes across various tissues. Its roles have been extensively explored in embryonic development and sex determination in reproductive tissues. However, its expression, regulation, and function in human periovulatory follicles are unknown.

To address this, we first characterized the expression of *SOX9 in vivo* using dominant follicles collected from normally cycling women before the LH surge (preovulatory phase) and at defined times after hCG administration (early, late, and post-ovulatory phases). Following hCG administration, *SOX9* mRNA levels were transiently upregulated in granulosa cells of dominant follicles; the levels were highest during the early ovulatory phases. Consistently, the positive staining of SOX9 protein increased after hCG stimulation. Its localization was predominantly observed in granulosa cells of dominant follicles throughout all phases following hCG administration, including the early, late, and post-ovulatory phases.

Next, to dissect the regulatory mechanisms underlying SOX9 up-regulation in periovulatory granulosa cells, hGLCs were treated without or with hCG in the absence or presence of the PGR

antagonist, RU486, for various time points. We found that hCG induced transient increases in the expression of *SOX9* (mRNA and protein), mimicking *in vivo* expression patterns. The hCG-induced increase in *SOX9* expression was completely reduced by RU486, affirming PGR as an upstream regulator of SOX9. Lastly, to further elucidate the role of SOX9 in the periovulatory follicles, we employed a small interfering RNA (siRNA) approach to knockdown the expression of *SOX9* in hGLCs. siRNA-mediated attenuation of *SOX9* expression resulted in the reduction of progesterone production. In line with the reduced levels of progesterone, transfection with siRNA for *SOX9* decreased the expression of key steroidogenesis-related factors (*STAR*, *CYP11A1,* and *HSD11B1*). In summary, our study unveils, for the first time, that the expression of *SOX9* is transiently up-regulated in the granulosa cells of human periovulatory follicles *in vivo* and *in vitro*. ChIP-seq and PGR inhibition studies further demonstrated that PGR acts as a direct transcription mediator of hCG-induced *SOX9* expression in human luteinizing granulosa cells. In turn, SOX9 modulates progesterone production by regulating the expression of genes involved in steroidogenesis, thereby playing a crucial role in coordinating the ovulatory process and luteinization in human ovulatory follicles. Supported by R01HD096077.

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