Notch Signaling Differentially Regulates Proliferation of Cultured Mouse Ovarian Granulosa Cells Dependent on Exogenous Gonadotropin Exposure.

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Ovarian follicle development relies on a complex interplay of signaling events that coordinate granulosa cell specification and proliferation and also support oocyte health. Early stages of folliculogenesis are influenced by myriad local signaling factors. During the pubertal transition in the mouse, these local factors prepare the activated follicle to be receptive to circulating pituitary gonadotropins. Among the many signaling systems involved in these important processes is Notch signaling, an evolutionarily conserved juxtacrine pathway that has pleiotropic effects on tissue patterning and regulates the balance between proliferation and differentiation in many cell types. Our group and others have shown that Notch ligands, receptors, and downstream effectors are expressed in the ovary throughout follicle development. We previously demonstrated that siRNA knockdown of the Notch ligand, *Jag1*, in cultured granulosa cells harvested from PMSG-primed prepubertal mice suppressed steroidogenesis and enhanced proliferation. In support of this, work presented here shows that knockdown of other Notch components, including the *Jag2* ligand and the Notch receptor, *Notch3*, in PMSG-primed granulosa cells likewise result in increased cell proliferation. However, we have paradoxically found that Notch signaling is positively associated with granulosa cell proliferation in the absence of gonadotropin treatment of the pre-pubertal mice. Using a Transgenic Notch Reporter (TNR) line of mice, we observed that granulosa cells that are Notch active are more proliferative than those that that are Notch inactive. We postulate that Notch activation promotes proliferation in using primary cultures of non-PMSG primed granulosa cells co-cultured in the presence of recombinant Jag1 (rJag1), an increase in proliferation was observed. Gene expression analysis of the cell cycle regulators, *Ccnd2* and *Cdk1*, show an increase in their expression as well. When cultured in the presence of DAPT, a Notch signaling inhibitor, granulosa cell proliferation is suppressed, further supporting a pro-proliferative role for Notch in this context. Currently, we are investigating if the same Notch receptors or ligands are responsible for promoting proliferation in granulosa cells naïve to exogenous gonadotropin stimulation as those involved in more differentiated response in cells from PMSG-primed mice. Overall, this study indicates that Notch regulation of granulosa cell proliferation is highly context dependent, adding an important nuance to our current understanding of the network of regulatory mechanisms that drive granulosa cell proliferation and differentiation in the ovarian follicle.

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