Sustained YAP Activity Induces Transdifferentiation of Luteal and Müllerian Mesenchymal Cells in Mice

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The Hippo pathway plays a pivotal role in regulating cell growth and differentiation in the reproductive system throughout embryonic and postnatal stages of development. Deletion of the Hippo pathway kinases Large tumour suppressor (LATS) *1* and *2* in the ovary and Müllerian duct has been shown to lead to the overactivation of the downstream transcriptional coactivators Yes-associated protein (YAP) and the transcriptional coactivator with a PDZ-binding motif (TAZ) and the transdifferentiation of granulosa and Müllerian mesenchymal cells. However, the relative contribution of YAP and/or TAZ (and/or additional effectors downstream of *Lats1/2*) to these phenotypic changes remains unclear.

To assess the impact of YAP on the transcriptome in granulosa cells, RNA-seq analyses were conducted on granulosa cells from eCG-primed immature *Rosa26*$^{Yap5SA/Yap5SA}$ mice transduced with adenoviruses to drive Cre or eGFP (control) expression. In this model, Cre recombinase permits the expression of a transgene encoding a constitutively active, stable and nuclear mutant YAP protein (YAP5SA), co-expressed with the reporter gene *LacZ*. The analyses revealed that 129 genes were upregulated and 147 downregulated when the transgene was expressed (P corrected < 0.05; fold-change > |1.5|), most of which were associated with biological processes related to cell differentiation and angiogenesis.

To gain a deeper understanding of YAP's role in gonadotropin-dependent folliculogenesis, *Rosa26*$^{Yap5SA/+}$; *Cyp19-cre* were generated to drive YAP5SA expression in antral follicles. Unexpectedly, X-GAL staining of *Rosa26*$^{Yap5SA/+}$; *Cyp19-cre* ovaries revealed that *Yap5SA* was expressed only in a fraction of luteal cells. These *LacZ*-positive cells later formed roughly spherical nests of poorly-differentiated, vimentin-positive cells embedded in an abundant collagen matrix. BrdU incorporation showed that some of these cells were proliferative, and PECAM1 staining showed the lesions to be highly vascularized. Furthermore, seminiferous tubule-like structures containing cells positive for SOX9 were observed in the ovaries of older mice. Surprisingly, the presence of these lesions did not significantly impact fertility or serum progesterone levels in *Rosa26*$^{Yap5SA/+}$; *Cyp19-cre* mice. Although the *Rosa26*$^{Yap5SA/+}$; *Cyp19-cre* model was not found to be useful for studying the role of YAP during folliculogenesis, it showed that luteal cells are capable of undergoing dedifferentiation and transdifferentiation in response to sustained YAP activity, thereby partially recapitulating the effects of loss of *Lats1/2* in granulosa cells.

To determine the impact of YAP overactivation on reproductive tract development and function, the *Rosa26*$^{Yap5SA/}$; *Amhr2*$^{cre/+}$ mouse model was generated in order to target the Müllerian mesenchyme. Transgene expression was (indirectly) detected by X-GAL staining starting at embryonic day 17.5. Expression was initiated at the rostral end of the Müller ducts and the ovary, and extended caudally to the antimesometrial side of the ducts. Histological analyses revealed disorganization of the Müllerian duct mesenchyme, with the presence of a population of spindle-shaped, X-GAL-positive cells and increased abundance of extracellular matrix. Postnatally, these cells were present in endometrial mesenchyme and myometrium, causing a disorganization of myometrial layers and the deposition of collagen. The development of oviduct was also impeded by the proliferating X-GAL-positive cells, interfering with elongation and coiling, and causing dilations to occur. Ongoing studies are aimed at further characterizing the histopathologic changes observed in the uterus, and to identify the changes in the expression of YAP's target genes that underlie them.