Glucose Metabolism as a Driver for Embryonic Sertoli Cell Differentiation

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The gonadal primordium, unlike many other tissues in the body, has the unique ability to differentiate into two completely different organs, testes in the male and ovaries in the female. During gonadal sex differentiation, every cell in the gonad must commit to an ovarian or testicular fate. This process is orchestrated by the supporting cell lineage. The supporting cells are the first ones to differentiate into either Sertoli cells in the testes or pre-granulosa cells in the ovary. Once they differentiate, they control the fate of the rest of the gonadal cells. Sex determination of the supporting cells is therefore critical for proper establishment of sex-specific reproductive organs. In the fetal testis, sex determination of the supporting lineage into Sertoli cells involves the upregulation of thousands of genes over a short period of time, suggesting a genome-wide chromatin remodeling process. One uncharted mechanism for such regulation is cell metabolism, which has gained interests as a novel regulator of cell fate and differentiation. Glucose metabolites play important roles in chromatin modification, linking cellular metabolism to epigenetic control of gene expression. We hypothesized that a change in the cell metabolic state is required to achieve genome-wide modification during sex determination of gonadal supporting cells. We first performed single-cell RNA-sequencing analysis of the supporting cell lineage in the mouse embryos to identify sex specific differences in genes involved in glucose metabolism. Sertoli cells exhibit a higher expression of enzymes associated with glucose metabolism, including glycolysis, the hexosamine biosynthetic pathway and mitochondrial oxidative phosphorylation, than the pre-granulosa cells. We used a combination of specific chemical inhibitors and ex-vivo gonadal cultures to evaluate the role of each pathway in supporting cell differentiation. When gonads were cultured with 2-Deoxy-D-glucose, an inhibitor of the second step of glycolysis, Sertoli cells failed to differentiate. However, perturbation of the last step of glycolysis (Shikonin), and mitochondrial glucose oxidation (UK-5099) had no impacts on Sertoli cell differentiation. This suggests that glucose is used a non-ATP-generating pathway that branches from glycolysis. With this in mind, we examined the hexosamine biosynthetic pathway, a side-branch of glycolysis that results in post-translational modification (O-GlcNAcylation) of histone or non-histone proteins. Notably, Sertoli cells showed higher cytoplasmic and nuclear protein O-GlcNAcylation compared to pre-granulosa cells. The Inhibition of nuclear O-GlcNAcylation (OSMI-1) disrupted Sertoli cell differentiation, mirroring the perturbations observed in the 2-Deoxy-D-glucose inhibition. Moreover, Sertoli cells expressed higher levels of *Gfat1*, the rate-limiting enzyme of the hexosamine biosynthetic pathway, than the pre-granulosa cells. Based on these results, we propose that male supporting cells shuttle glucose into the hexosamine biosynthetic pathway, which then facilitate nuclear O-GlcNAcylation to induce Sertoli cell differentiation. These findings shed new light on the pivotal role of the hexosamine biosynthetic pathway in the intricate process of gonadal sex differentiation.