Hormone production in these mutant cells, however, can be rescued *in vitro* through co-culture with WT pituitaries and *in vivo* in chimeric pituitaries, highlighting a cell non-autonomous mechanism underlying the phenotype. Single cell RNA sequencing of WT and $Sox2^{CreERT2/+};R26^{lsl\text{-}mBRF1}$ murine embryonic pituitaries, as well as use publicly available human pituitary single cell datasets, have allowed us to identify specific cytokines and chemokines secreted by SOX2+ cells, as well as downstream intracellular signalling pathways in differentiating cells (Zhang et al. 2020), which may be responsible for controlling terminal differentiation of hormone-producing cells within the developing pituitary. Together with our recently published data, these results support the notion that SOX2+ pituitary stem cells play a critical paracrine role in controlling progenitor cell proliferation and terminal differentiation (Russell et al. 2021). References: Herranz, Nicolás et al. 2015. "MTOR Regulates MAPKAPK2 Translation to Control the Senescence-Associated Secretory Phenotype." *Nature Cell Biology* 17(9): 1205–17. http://www.nature.com/doifinder/10.1038/ncb3225. Russell, John P et al. 2021. "Pituitary Stem Cells Produce Paracrine WNT Signals to Control the Expansion of Their Descendant Progenitor Cells." *eLife*. Zhang, Shu et al. 2020. "Single-Cell Transcriptomics Identifies Divergent Developmental Lineage Trajectories during Human Pituitary Development." *Nature Communications*.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

PI3K Inhibition by BKM120 Results in Antiproliferative Effects on Corticotroph Tumor Cells

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Purpose: Cushing's disease is associated with significant morbidity, thus additional tumor-directed drugs with the potential to exert antineoplastic effects on corticotroph adenoma cells are desired. The PI3K (phosphoinositide-3-kinase)/AKT (protein kinase B) pathway, which plays regulatory roles in cell survival and proliferation, is activated in pituitary adenomas. The present study evaluated the effects of BKM120 (Buparlisib), an oral PI3K inhibitor, in corticotroph tumor cells. **Methods:** AtT-20/D16v-F2 mouse pituitary corticotroph tumor cells were treated with increasing concentrations of BKM120 or vehicle. Cell viability was measured using MTS-based assay. Apoptosis was evaluated by Annexin V staining. ACTH levels were measured in the culture supernatants by chemiluminescent immunometric assay. Cell cycle analysis was performed by propidium iodide DNA staining and flow cytometry. Gene expression of cell cycle regulators (*Cdkn1b*, *Rb1*, *Ccnd1, Cdk4*, *Cdk2,* and *Myc*) was assessed by qPCR. Protein expression of p27, p70 S6 Kinase, p85 S6 Kinase, and phosphorylated AKT was assessed by Western blot. **Results:** Treatment with BKM120 decreased AtT-20/D16v-F2 cell proliferation and ACTH levels in the cell culture supernatants. Furthermore, BKM120 treatment diminished the phosphorylation of AKT at residue 473, increased p27 expression and induced a G0/G1 cell cycle arrest. **Conclusion:** *In vitro* inhibition of PI3K/AKT pathway by BKM120 resulted in antiproliferative effects on corticotroph tumor cells, decreasing cell viability and ACTH production. These encouraging findings shape the path for further experiments with the inhibition of PI3K/AKT pathway in Cushing's disease.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

***Polyciliation of GnRH Neurons** in Vivo **and** in Vitro*

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Puberty and reproduction are initiated and controlled through the hypothalamic-pituitary-gonadal (HPG) axis. A critical surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH) are released from the anterior pituitary upon release of gonadotrophins from gonadotrophin releasing hormone (GnRH) neurons. Thus, GnRH neurons are key regulators of the HPG axis. GnRH neurons become active when kisspeptin (Kiss1) neuropeptides are released from neurons in the arcuate nucleus. Kiss1 binds to the Kiss1 receptor (Kiss1R), a G-protein coupled receptor (GPCR) which localizes to the primary cilia of GnRH neurons. Loss-of-function mutations of Kiss1R cause hypogonadism in mouse and human models while gain-of-function mutations are associated with precocious puberty. Interestingly, the subset of GnRH neurons that express Kiss1R are observed to be polyciliated, possessing more than one primary cilia, an uncommon property as most neurons only possess a single, primary cilium. The mechanism and conditions leading to GnRH neuron polyciliation are unknown. It is also unclear if multiple cilia impact Kiss1R or other GPCR signaling in these neurons. Here, we utilize cultured mouse primary hypothalamic neurons to begin addressing some of these questions. We have confirmed with qPCR that the ligands GnRH and Kiss1, as well as Kiss1R, are all expressed in these cultures. Surprisingly, when treated with Kiss1 and GnRH ligands we observed a small subset of polyciliated neurons compared to vehicle treated neurons. These observations mirror what is seen during sexual maturation *in vivo* and suggest that our model system may help elucidate fundamental questions about how ciliary localization of Kiss1r and other GPCRs participate in initiation of puberty and regulation of reproduction. Future studies will focus on the mechanisms of polyciliation and the conditions needed to induce the formation of new cilia in GnRH neurons. Investigating neuronal polyciliation could provide insights into new signaling paradigm in hypogonadism and HPG signaling.