ANOVA was employed to compare values among multiple groups. If the ANOVA revealed significant differences, the Tukey-Kramer post-hoc test was employed to compare values between two specific groups. Dunnett's post-hoc test was employed to compare values with the control group. Statistical significance was defined as $p < 0.05$. **Results:** 1. NMB and NMBR expression levels were significantly higher in human corticotroph adenoma (13 and 33 times higher than non-functioning adenoma, respectively) than in somatotroph adenoma (2 and 3 times higher than non-functioning adenoma, respectively) and non-functioning adenoma in the qPCR analyses. Immunostaining confirmed higher expression of NMB and NMBR in corticotroph adenoma than in somatotroph and non-functioning adenoma. 2. Treatment with 100 nM PD168368 significantly suppressed Pomc mRNA and protein expression in AtT-20 cells by 22%±3% and 25%±10%, respectively. 3. Treatment with 1 μM PD168368 significantly suppressed POMC mRNA expression in human corticotroph adenoma cells by 18%±1%. **Conclusions:** NMB and NMBR were both expressed in human corticotroph adenoma, suggesting that NMB may stimulate adenoma cell proliferation and hormone secretion in autocrine or paracrine manners. Because the NMBR antagonist suppressed Pomc expression in both AtT-20 cells and human corticotroph adenoma cells, it may represent a potential treatment for Cushing disease. **Reference:** (1) Kameda H et al., Endocrinology 2014;155(7):2492-9.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Overactive Reproductive Axis Due to Fragile X Gene Mutation

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Women carrying a pre-mutation or mutation of the Fragile X mental retardation gene (FMR1) comprise the largest portion of premature ovarian failure (POF) cases due to known genetic factors. FMR1 mutation causes Fragile X syndrome, the most common cause of inherited mental impairment. The mutation inhibits the expression of the fragile X mental retardation protein (FMRP), a ubiquitously expressed mRNA binding protein. The specific molecular mechanism(s) leading to premature ovarian failure in Fragile X carriers are not known. Here, we utilize the complete KO mouse model, to mimic the lack of FMRP in Fragile X mutations and analyze the hypothalamic-pituitary-gonadal axis to uncover causes of POF due to FMR1 mutation. Consistent with mutations in human population, KO females experience early cessation of reproductive function and stop having litters at 150 days of age, compared to controls that stop reproducing at 250 days of age. Since POF can be caused by either insufficient pool of primordial follicles or by increased recruitment in each cycle and early depletion, we analyzed ovaries at 3 weeks of age and determined that the FMR1 KO mice had the same number of primordial follicles when compared to the controls, suggesting that POF is not due to a deficit in primordial follicles. However, at 8 weeks of age, FMR1 KO ovaries had higher number of corpora lutea, and KO females had larger litters, indicating that FMR1 KO mice have more follicles recruited in each estrous cycle. FMR1 KO mice have higher FSH, which corresponds to the high FSH in women. Serum estradiol levels and inhibin b expression levels were unaffected by FMR1 mutation suggesting normally functioning negative feedback signals from the ovaries. Analyses of hypothalamic gene expression demonstrated elevated *GnRH* mRNA in KO mice. To further investigate alterations is hypothalamic protein levels, western blot analyses determined that FMR1 KO mice have higher levels of NMDAR1 and higher levels of $GABA_A$ receptor G2 subunit. Dual label immunofluorescence analyses revealed higher number of NMDAR1 and $GABA_A$ receptors specifically in GnRH neurons of FMR1 KO mice when compared to control, suggesting that GnRH neurons themselves are affected by FMR1 mutation. Given that both glutamate and GABA can activate GnRH neurons, alterations in the number of these receptors can potentially cause hyperactivity in the HPG axis at the hypothalamic level leading to elevated FSH and the subsequent POF. In summary, our results reveal a potential mechanism of premature ovarian failure in Fragile X mutation carriers.

Neuroendocrinology and Pituitary

NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Paracrine Signalling From SOX2-Expressing Pituitary Embryonic Cells Is Required for Terminal Differentiation of Hormone-Producing Cells

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The pituitary gland is the master regulator of the endocrine system, housing six major hormone producing cell types. This gland is derived from Rathke's Pouch, an invagination of the oral ectoderm. Hormone-producing pituitary cell lineages are derived from a population of embryonic cells expressing SOX2. ZFP36L1/Butyrate Response Factor 1 (BRF1) is an RNA binding protein that binds and targets mRNAs of various cytokines and chemokines for degradation prior to translation, attenuating secretion of inflammatory factors (Herranz et al. 2015). Here, we show that BRF1 is a novel marker expressed in SOX2+ cells in human and mouse pituitaries, suggesting that these cells may have a secretory profile. To investigate this possibility, we have combined molecular and genetic studies *in vivo*. We have used a novel mouse model, $R26^{lsl\text{-}mBRF1}$ that allows the expression of a mutant, constitutively active BRF1 protein upon *Cre*-mediated recombination, alongside our lab's models ($Hesx1^{Cre/+}$ and $Sox2^{CreERT2/+}$), to express mutant BRF1 in HESX1+ and SOX2+ cells during development and postnatally. This approach results in pituitary hypoplasia and severe hypopituitarism due to a failure of cell-lineage specified cells to differentiate into hormone-producing cells.

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.