14-year old white girl with severe obesity (BMIz +3.00), autistic behavior, pituitary dysfunction and central hypoventilation. This gene is known to cause autosomal recessive hydrolethalis and acroscallosal syndromes with mutations also noted in Bardet-Biedl, Meckel and Joubert syndromes. **Conclusion:** While no unifying genetic cause has been identified in ROHHAD syndrome, it is possible that the phenotype represents a collection of complex genetic syndromes.

Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

Heterodimerization and Subcellular Distribution of Melatonin and Cannabinoid Type 1 Receptors

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Membrane receptors belonging to the G proteins coupled receptors (GPCRs) form the largest family of proteins in the human genome with more than 800 members. Until recently GPCRs functions were thought to occur only at the plasma membrane after activation upon binding of their cognate ligand. However evidences show that many functional GPCRs are found in intracellular compartments opening new direction of research to understand their roles in a cellular context. Among these intracellular compartments mitochondria are the latest organelle in which some GPCRs were identified. Melatonin receptor type 1 (MT1) and cannabinoid receptor type 1 (CB1) were identified in mouse neuronal mitochondria where they were shown to exert an inhibitory action on cytochrome c release (MT1) or on the respiratory chain (CB1). Using several techniques my current results describe a new cross-talk between MT1 and CB1 receptors. Confocal analysis of immunofluorescence experiments of cells coexpressing both receptors showed a high degree of colocalisation. A combination of coimmunoprecipitation experiments performed on extracts of transfected HEK293T or HeLa cell lines and immunodetection of receptors by Western-blot revealed that MT1 and CB1 receptors can physically interact to form heterodimers in absence of ligand. Heterodimers formation was also confirmed by Proximity Ligation Assay (PLA) experiments in live HEK293T and HeLa cells. Confocal analysis revealed a colocalisation of PLA staining with the mitochondrial marker TOMM20. Experiments in relation with the functional role of MT1 and CB1 in mitochondria are ongoing.

Genetics and Development (including Gene Regulation)

FROM BENCH TO BEDSIDE: GENETICS, DEVELOPMENT AND CELL SIGNALING IN ENDOCRINOLOGY

Histone Lysine Demethylase 1A Is a Master Regulator of Genes Necessary for Trophoblast Cell Proliferation

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Histone lysine demethylase 1A is a master regulator of genes necessary for trophoblast cell proliferation.

A proper functioning placenta is critical for pregnancy, fetal growth and development and postnatal health. Trophoblast cell proliferation and differentiation is critical for placental development and function. Recently we demonstrated that the histone lysine demethylase KDM1A binds to androgen receptor (AR) in human and sheep trophoblast cells, and targets the same promoter region of vascular endothelial growth factor A (VEGFA), suggesting a role for KDM1A and AR in early placental angiogenesis. The goal of this study was to determine the function of KDM1A during early placental development. We hypothesized that KDM1A regulates genes that are necessary for trophoblast cell proliferation, and early placental development. To this end, both in vitro and in vivo approaches were used in this study. ACH-3P cells (human first trimester trophoblast cells (CT and EVT) fused with the choriocarcinoma cell line AC1-1) were used, and a KDM1A knock out (KO) cell line was generated using CRISPR-Cas 9 based genome editing. KDM1A KO in ACH-3P cells led to significant ($P<0.05$) reduction in AR and VEGFA. Furthermore, factors important for cell proliferation and trophoblast cell development high mobility group AT-hook 1 (HMGA1), LIN28, and MYC protooncogene (cMYC) were significantly ($P<0.05$) lower in KDM1A KO ACH-3P cells. Cell proliferation assays revealed a significant ($P<0.05$) reduction in KDM1A KO ACH-3P cells compared to scramble controls. An *in vivo* experiment was conducted to demonstrate a role for KDM1A in placental development, using the sheep as a model. Day 9 hatched blastocysts were flushed and infected with a Lenti-CRISPRv2 KDM1A target construct (n=4) to knockout KDM1A specifically in the trophectoderm, or with SC (n=5). Infected embryos were transferred to recipient ewes and embryos were collected at gestational day 16. Data suggests that KDM1A KO in trophoblast cells is necessary for conceptus elongation. Current experiments are ongoing to determine the effects of KDM1A and AR knockdown using shRNA lentiviral target vectors on conceptus elongation and pregnancy. Collectively these results indicate that KDM1A plays a central role in regulating genes necessary for trophoblast cell proliferation.

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