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SJSU Undergraduate Research Grants

The Role of Sirtuin 4 in Modulating Metabolism in Mammary Epithelial Cells

Albert A. Nguyen[1,2], Fiara M.S. Llaguno[1,2], Thi-Tina N. Nguyen[1], and Frank K. Huynh[1]
[1]San Jose State University, College of Science, Department of Biological Sciences
[2]SJSU Undergraduate Research Grant Recipient

Abstract

Sirtuin 4 (SIRT4) is a mitochondrial deacylase protein that regulates nutrient metabolism by removing different post-translational modifications (PTMs). Our previous work shows that SIRT4 has effects on regulating leucine metabolism and insulin sensitivity. The absence of SIRT4 in mice (SIRT4KO mice) leads to decreased leucine metabolism and elevated leucine-induced insulin levels. Long-term hyperinsulinemia in SIRT4KO mice increases insulin resistance, thus promoting obesity and diabetes.

Unexpectedly, our preliminary data suggests that SIRT4 also plays a role in female reproductive system development. While female SIRT4KO mice appear to have normal pregnancies and births, nearly 100% of their pups, regardless of pup or sire genotype, do not survive past postnatal day 2. We found that SIRT4KO females had impaired mammary ductal development during puberty and therefore do not develop enough milk-secreting alveoli during pregnancy. Furthermore, treatment with the ovarian hormones 17β-estradiol and progesterone rescues this phenotype. Since SIRT4 has been shown to regulate nutrient metabolism, the overall goal of our current research is to investigate how SIRT4's metabolic regulatory activities affect mammary ductal development.

Research Goals

1) In order to complete our overall goals, we need to generate SIRT4KO mice. Thus, we aim to optimize breeding and genotyping protocols for generating SIRT4KO mice.

2) In order to specifically assess metabolism in SIRT4KO mammary ducts, we aim to optimize a procedure for isolation and culture of mammary epithelial cells, which comprise the mammary ducts, from SIRT4KO mice.

Breeding Strategy

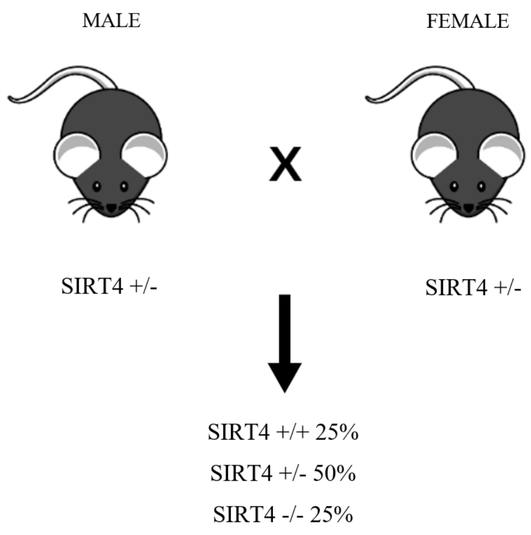
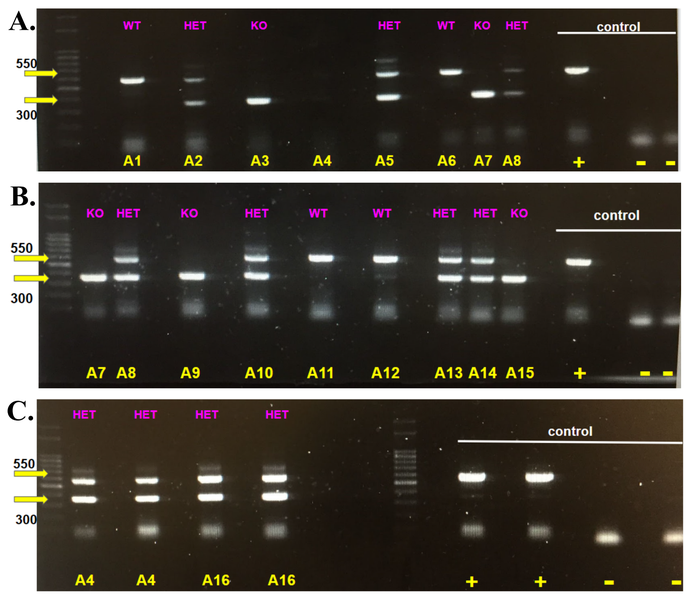


Figure 1. Generation of SIRT4KO mouse line. Mice with the SIRT4 heterozygous genotype were obtained from Duke University and paired. This pairing results in three possible genotypes: SIRT4+/+ (WT), SIRT4+/- (HET), SIRT4-/- (KO).

Results



D.

Genotype	Males	Females	Total
WT (+/+)	1	0	1
Het (+/-)	7	3	10
KO (-/-)	2	3	5

Figure 2: (A-C) Genotyping results for SIRT4KO mouse line. Samples were run on 1.5% agarose gel after endpoint PCR with SIRT4 primers. WT ear samples were used as positive control (+). Distilled water served as negative control (-). Arrows to the left mark the predicted product sizes on the 1000bp ladder. The expected sizes are 300 bp for KO (SIRT4-/-) and 550 bp for WT (SIRT4+/+). **(D)** Summary of genotyping results.

Future Directions

1) Continue breeding SIRT4KO mice to obtain enough wild type and SIRT4KO mice for our studies.

2) Optimize mammary epithelial cell isolation protocol.

3) Determine SIRT4 expression levels in mammary epithelial cells in response to varying nutrient availability. We will measure SIRT4 expression levels in fasted mice, mice fed a regular diet, and mice fed a high-fat diet.

4) Investigate how mammary epithelial cells from SIRT4KO mice respond to the hormone insulin. Insulin is a master regulator of nutrient metabolism and SIRT4KO mice develop resistance to insulin as they age. However, it is unknown if mammary epithelial cells specifically become unresponsive to insulin in SIRT4KO mice and whether insulin resistance might contribute to impaired mammary development.

Poster Presentations at the 2020 CSUPERB Biotechnology Symposium.

Top: "Expression of Sirtuin 4 in mammary glands and ovaries reveals a potential role in female development" (Fiara Llaguno, Tina Nguyen).

Bottom: "Estrogen and Progesterone Rescues Defective Mammary Development in SIRT4KO Mice" (Albert Nguyen, Michael Ouyang).