Thyroid cancer is characterized by the abnormal growth of cells within the thyroid gland often causing a lump and is more prevalent in females and Asians. Non-Medullary Thyroid Cancer (NMTC) refers to all types of thyroid cancers that originate from follicular cells [1] [2]. While NMTC may occur due to a variety of genetic and environmental factors, recently identified single nucleotide polymorphisms (SNP) in the Forkhead Box E1 (FOXE1) gene causes an increased susceptibility to the disease [3] [4] [5]. FOXE1 is a transcription factor that plays a crucial role in thyroid morphogenesis, yet it is unclear how FOXE1 regulates the growth and morphogenesis of thyroid cells during development. [6] Gaining insight into the mechanisms of how FOXE1 functions to mediate thyroid morphogenesis will provide valuable information for determining future drug targets and prevention.

My **primary goal** is to identify how FOXE1 is responsible for the uncontrolled growth of follicular cells within the thyroid gland. The role of FOXE1 in morphogenesis was first identified in mice and there currently exist mouse lines with mutations in genes that exhibit the thyroid abnormalities associated with various types of thyroid cancers [6] [7]. This indicates that mice may be a good model organism for characterizing the association between FOXE1 mutations and NMTC development. My **hypothesis** is that the FOXE1 is responsible for the expression of downstream thyroid growth factors that stimulate follicular cell growth and proliferation. My **long term goal** is to determine the mechanisms of how FOXE1 controls thyroid morphogenesis.

**Aim 1: Identify conserved amino acid sequences in FOXE1 that help regulate cell growth/ proliferation.**

**Approach:** Use Ensembl to retrieve FOXE1 homologs across species with and without thyroid glands, then align the sequences using MEGA to identify the conserved sequences. After conserved sequences are identified, use CRISPER-Cas9 to make amino acid substitutions that are specific to species with thyroids, grow model organisms until maturity, and screen for thyroid abnormalities.

**Rationale:** Different SNPs within the FOXE1 gene have been associated with different subtypes of NMTC. By screening for homologous sequences between humans and model organisms, then introducing corresponding human-NMTC associated SNPs, the acute effects of FOXE1 mutations can be studied.

**Hypothesis:** Mice with mutated conserved FOXE1 amino acids specific to species with thyroid glands will lead to thyroid abnormalities.

**Aim 2:** **Identify enriched genes important for thyroid morphogenesis in FOXE1 mutants**.

**Approach:** From wild type and mutant FOXE1 mice, follicular cells will be isolated from the thyroid and put through single-cell RNA-seq. RNA-seq data will be sorted using GO terminology and compared between the wild type and mutant mice to identify changes in molecular function.

**Rationale:** By analyzing the RNA-seq data and GO analysis between WT mice and FOXE1 mutant mice, we can determine which mutations are associated with changes in expression levels of genes associated with thyroid morphogenesis.

**Hypothesis:** Genes responsible for molecular functions such as growth factor binding will be over or under expressed in FOXE1 mutant mice.

**Aim 3: Characterize the effects of FOXE1 mutations in the phosphorylation pattern of proteins.**

**Approach:** I will perform a phosphoproteomic study using mass spectrometry on thyroid follicular cells of wild type and mutant FOXE1 mice. Phosphorylation patterns will be compared and differences between the wild type and mutant will be searched for.

**Rationale:** Cell growth and proliferation are dependent on a variety of cell signaling pathways. As phosphorylation of proteins can either activate or deactivate certain signaling pathways, FOXE1 may alter the normal cell signaling pathways responsible for cell growth and proliferation.

**Hypothesis:** Proteins responsible for cell growth/proliferation signaling pathways will be over-phosphorylated.

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