Abstract

Feline hyperthyroidism (FH) is the most common endocrinopathy with over 10% of cats greater than 10 years of age expected to develop the disorder (1, 2) Clinical signs result from over-production of thyroid hormones (predominately T4) by thyroid adenomas or adenomatous hyperplasia. Studies have identified risk factors for FH including some canned food diets, eating fish-based diets, living indoors, environmental contaminants, and exposure to cat litter (which may be related to the indoor status of cats) (3-7) These factors have resulted in further investigation into goiterogens associated with diet or the environment that may result in the pathogenesis of feline hyperthyroidism. We are developing primary cell cultures from thyroid tissue of cats to test the effects of small molecules, chemicals, and supplements on thyroid hormone production. Currently we have established the technique to culture both euthyroid and hyperthyroid-derived cells. **Specific Aim 1** will characterize the euthyroid and hyperthyroid primary cell line using immunohistochemistry and/or gene expression using specific markers indicative of feline adenomatous hyperthyroid cells including TSH receptor, thyroid hormone receptor, and inhibitor G proteins regulating thyroid hormone receptor activity (8, 9). *The objective is* to characterize *in vitro* receptor expression for these two different primary cell cultures to improve data interpretation in light of cell response to treatments with food additives designed to improve abnormal thyroid cell function. *The hypothesis is that* hyperthyroid cells will express higher levels TSH receptors and lower expression of inhibitory G-protein subunits compared to euthyroid cells. **Specific Aim 2** will test one small molecule (proprietary and "unknown" to researchers, provided to us by Royal Canin) of interest for its ability to modify thyroid hormone synthesis in primary cell culture. The hypothesis is that the small molecule will inhibit T4 production from hyperthyroid-derived cells. *The hypothesis is that* the small molecule will reduce the production of thyroid hormones (T4) in culture in both hyperthyroid and euthyroid cats. *Significance of research:* Primary cell culture experiments act as an initial screening tool for modification of thyroid hormones and offers a mechanism by which to optimize supplements that may control feline hyperthyroidism, as well as improve understanding of the etiology of the disease that afflicts so many individuals. The diagnostic laboratory in the College of Veterinary Medicine has successfully measured thyroid hormones in our primary cell culture media. This project is expected to develop and optimize a useful functional primary cell line for both feline euthyroid and hyperthyroid cells for testing new supplements, as well as environmental chemicals that can affect hormone output (i.e., for example the relationship between flame retardants and hyperthyroidism in cats). Molecular characterization of feline euthyroid vs. hyperthyroid primary cells, and discerning signaling pathways related to supplemental effects, can be potentially used to identify environmental and dietary factors that drive feline hyperthyroidism, as well as test potential therapeutic agents.

The significance for Veterinary Medicine and the student. The student will learn the basics of *in vitro* cell culture, critical for any mechanistic study in veterinary medicine. The student will be exposed to fundamental concepts in endocrinology, toxicology, and nutrition. Understanding how these variables intersect to exacerbate or cause disease in animals will be highly valuable for the student. The student will be responsible for maintaining primary cell cultures and conducting immunohistochemistry (or gene expression analysis) in culture with and without treatments with the small molecule. Our team is currently growing primary cells from both euthyroid vs. hyperthyroid cats; cells are slow growing, but we anticipate an adequate supply for the summer. We also have thyroid tissue from cats in freezers that can be used to optimize assays. We encourage trainees to present their research at the UF Genetics Institute symposium in the Fall. Students will develop their own