YALE JOURNAL OF BIOLOGY AND MEDICINE 85 (2012), pp. 415-416.

Yale School of Medicine Thesis Award Recipient — 2012

Leptin: A Novel Hormone of the Parathyroid Gland. Don Hoang.

Purpose: To investigate the expression and exogenous effect of leptin hormone in diseased parathyroid glands at a basic science and clinical level.

Introduction: Hyperparathyroidism (HPTH) is a common endocrine problem affecting 1 in 500 individuals who are typically female and over the age of 60. Combined effects of low calcium and vitamin D stimulating parathyroid activity and excess PTH secretion are well established, especially in secondary HPTH. Unclear is the mechanism behind the development of hyper secreting parathyroid adenomas, responsible for nearly 90 percent of primary HPTH. Discovered in 1994 as a class I cytokine receptor, leptin has mounted considerable interest as a mitogenic factor involved in the stimulation and growth of tumors. Also, leptin has been implicated in multiple calcium-related metabolic processes, which have led to numerous evaluations of the connections between vitamin-D, PTH, and obesity. Ultimately, these scientific findings, the prevalence of higher leptin levels in women with HPTH, and our hypothesis that leptin found with increased body weight contributes to the pathogenesis of HPTH has led to our study on endogenous leptin production and its exogenous effect in diseased parathyroid glands. Furthermore, with routine serum calcium screening now, many patients are treated with minimally invasive parathyroidectomy (MIP) before hypercalcemic sequelae develop; we also wished to clarify the clinical relationship between PTH, leptin, and BMI in a cohort of patients with HPTH undergoing surgery.

Methods: We enrolled 96 patients with HPTH undergoing MIPs in a prospective study to collect tissue and co-localize leptin hormone and PTH by in situ hybridization (ISH), immunohistochemistry (IHC), immunofluorescence (IF), and electronmicroscopy (EM). Cell culture and whole organ explant experiments in which surgically removed human parathyroid tissue were exposed to recombinant human leptin to functionally characterize the effect of leptin on PTH secretion. Parathyroid adenomas and hyperplasia were xenografted into nude rats and evaluated for the production of human leptin. Blood was also collected to assess subsequent perioperative changes to serum leptin levels. Patients without HPTH undergoing hemithyroidectomies under identical surgical conditions were enrolled as control subjects. Wilcoxon signed-rank test, non-parametric version of paired t-test, and Pearson correlation were used to compare leptin level changes with different clinical variables using SAS 9.2.

Results: Leptin, leptin receptor, and PTH mRNA transcripts and protein were detected in an overlapping fashion in secretory (chief) cells in parathyroid hyperplasia and adenoma samples by ISH, IHC, IF, and EM studies. Immunofluorescence studies under spinning disc confocal microscopy and electron microscopy imaging confirm co-localization of endogenous leptin and PTH as well as active exogenous leptin uptake in cultured parathyroid cells. Tissue explant experiments show that PTH-secretion responds to recombinant leptin exposure in a dose dependent manner. Nude rats had positive human leptin at 2, 4, and 8 weeks in increasing trend for adenoma and decreasing trend for hyperplasia xenografts. Our study subjects included 71 (76 percent) adenoma, 13 (14 percent) hyperplasia, and 12 (13 percent) control patients. The median age was 59 years old, of whom 76 percent were females. This population was overweight (median BMI: 28) and pre-hypertensive (median SBP:131). Comparing measurements pre and post surgery, BOTH serum leptin and PTH levels decrease significantly in the whole cohort ($p<0.001$). Leptin decreases significantly

in adenoma ($p<0.001$) and hyperplasia subgroups ($p=0.002$); it increases in controls ($p=0.007$). Leptin decrease was significantly associated with a decrease in PTH pre-post surgery ($r=0.32$, $p=0.003$) ($r=0.55$, $p<0.001$ for adenoma subgroup). Bivariate analysis revealed several variables associated with changes in perioperative leptin and PTH levels. In multivariate analysis, parathyroid disease subtype, starting leptin levels, age, BMI, and calcium at diagnosis were significantly associated with changes in leptin.

Conclusion: Our results provide interesting insight and implications into the relationship between leptin, obesity, and primary hyperparathyroidism (adenomas) as there is no previous research data elucidating the direct relationship between leptin, PTH, and the parathyroid gland. Our results provide very strong evidence of a new functional and physiologically relevant parathyroid hormone. Until now, no previous research data identified the presence of leptin hormone in parathyroid glands; it remains unclear as to whether high exogenous leptin levels initiate hyperparathyroid disease, possibly acting mitogenically on parathyroid leptin receptors, or whether hyperparathyroid glands and elevated PTH serum levels precede elevated leptin production, either by endogenous parathyroid production or through downstream endocrine signaling of adipose tissue production. Based on our results and published reports, future studies will test our hypothesis that leptin play a central tumorigenic role in the etiology of parathyroid neoplasms.