The Oral Hypothalamic–Pituitary–Thyroid Axis Equivalent

Subjects: Dentistry, Oral Surgery & Medicine

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The hypothalamic–pituitary–thyroid (HPT) axis is crucial in regulating thyroid hormone levels that contribute to the development and homeostasis of the human body. Studies supports the presence of a local HPT axis equivalent within keratinocytes, with thyroid hormones playing a potential role in cancer progression.

Keywords: head and neck cancer ; oral squamous cell carcinoma ; thyroid hormones

The hypothalamic–pituitary–thyroid (HPT) axis is primarily responsible for maintaining normal circulating levels of thyroid hormones (TH)—thyroxine (T4) and triiodothyronine (T3)—essential to the development and homeostasis of all body tissue [1]. TH themselves exert negative feedback effects on their upstream HPT axis components, creating a complex negative neuroendocrine feedback system [2]. However, advances in research have shown that synthesis and direct target effects of neuroendocrine machinery like the HPT axis are not restricted to their primary system. For example, human skin has its own local neuroendocrine systems equivalent to the HPT axis, hypothalamic–pituitary–adrenal (HPA) axis, and more [3]. Human skin has been shown to express genes for HPT axis components, including thyroid-stimulating hormone (TSH) and TH-regulating molecules [4]. Furthermore, expression of thyrotropin-releasing hormone (TRH) and TSH receptors occurs within skin keratinocytes, melanocytes, fibroblasts, and hair follicles, enabling skin to be a direct target of thyroid hormone effects locally and systemically [5][6][7]. These HPT hormone effects on skin keratinocytes include proliferation, differentiation, and regulation of skin immunity and inflammation [7][8]. In hair follicles, changes in TH and TRH lead to stimulation of hair follicle proliferation, inhibition of apoptosis, and pigmentation [6][9].

Within the recent literature, TH are increasingly acknowledged for their role in cancer. Previous rodent models have demonstrated TH promoted growth and metastasis of tumor transplants, while epidemiological studies suggest that hyperthyroidism increases the risk of some solid malignancies [10][11]. In skin, loss of TH receptors is a common feature in some tumors and can increase aggressiveness of skin tumors [12]. Thus, the increasing evidence that other organs like the skin have local neuroendocrine systems not only deepens the understanding of human pathophysiology but also presents an opportunity for further research and potential therapeutics.

Here this entry present published evidence for the presence of a local HPT axis in the oral cavity.

1. Loss of Heterozygosity (LOH) in THRB Gene Loci

Loss of heterozygosity involving the THRB gene loci in human samples was reported in four studies [13][14][15][16] summarized in **Table 1**. El-Naggar et al. [13] reported that three of nine informative primary head and neck squamous carcinoma specimens exhibited LOH at the THRB locus, with all three of these carcinomas showing DNA aneuploidy. Miyashita et al. [14] reported two of seven informative OSCC tissue samples exhibiting LOH on 3p24.1-22 (THRB) loci. Patridge et al. [15] found that 10 of 36 informative primary OSCC samples exhibited LOH at the 3p24-26 chromosomal region (which includes THRB loci). Rowley et al. [16] reported that 2 of 11 informative squamous cell carcinoma of the head and neck specimens exhibited LOH at the 3p24 chromosomal position.

Table 1. LOH in THRB gene loci.

Author (Year)	Human Carcinoma Sample	Chromosomal Location	LOH/Informative Sample (%)
el-Naggar et al. (1993) [13]	HNSCC	3p24	3/9 (33%)
Miyashita et al. (2008) [14]	OSCC	3p24.1-22	2/7 (28.6%)
Patridge et al. (1999) [15]	OSCC	3p24-26	10/36 (28%)
Rowley et al. (1996) [16]	Head and neck tumours	3p24	2/11 (18%)

Patridge et al. [15] additionally reported that allelic imbalance (AI) at one or more loci involving the THRB gene loci was associated with reduced survival (HR = 4.21) (p-value = 0.0002). AI at 3p24-26 was also found to predict poor prognosis independently of other loci (HR = 3.93) (p = 0.002), and that AI at the 3p24-26 chromosomal region was found to be a better predictor of outcome than TNM staging.

2. Methylated CpG Site of Thyrotropin-Releasing Hormone Gene Sequence

Methylation of CpG site in the TRH gene sequence in human samples was reported in one study [17]. Through bioinformatics, CpG site cg01009664 in the TRH gene sequence was found to have the highest difference in methylation level between healthy and cancerous cells. Pyrosequencing of microdissected samples revealed five CpG sites surrounding cg01009664, with the average methylation percentage in cancerous cells (52.96% ± 5.36%) being significantly higher than that in healthy cells (5.7% ± 0.85%) ($p < 0.001$).

Real time PCR at cg01009664 in oral rinse and swab samples was used as a screening marker in two cohorts. In both cohorts, the average TRH methylation levels of oral rinse from SCC subjects were significantly higher than that of healthy controls. There were no significant differences in TRH methylation levels between sexes, ages, stages, or grades in any cancer samples.

Using receiver-operating characteristic analysis and a TRH methylation cutoff value of 3.31 ng/μL, the detection of OSCC from oral rinse had 86.15% sensitivity, 89.66% specificity, and 0.93 area under curve (AUC). With the same cutoff, the detection of oropharyngeal SCC from oral rinse had 82.61% sensitivity, 92.59% specificity, 0.93 AUC. For detecting OSCC, oral swab samples (91.30% sensitivity, 84.85% specificity, 0.97 AUC) were superior to oral rinse samples.

3. THRA in Tongue Squamous Cell Carcinoma (TSCC) Progression

Isolation of 7SK chromatin by RNA purification (ChIRP)-Seq data and bioinformatic analysis was performed to identify interactions with 5' regulatory regions and 5 motifs were obtained. Of the 5 motifs, motifs 3, 4, and 5 had similar binding motifs to PAX5, THRA, and FOXJ3 [18]. RT-qPCR revealed that FOXJ3 and THRA was found abundantly in SCC15 cells compared to PAX5. The results showed that 21 out of 27 genes had either knockdown of FOXJ3 or THRA. Amid these genes, there were 9 genes in common and 12 genes oppositely regulated by 7SK and FOXJ3/THRA. In particular, CXCL1, SYDE1, COL5A1, and HIF1A were identified to be negatively regulated by 7SK and positively regulated by FOXJ3 and THRA.

4. TSH and Antithyroid Antibody Expression in OLP Patients

Two studies [19][20] looked at the expression of thyroid-stimulating hormone (TSH) and antithyroid antibodies in oral lichen planus (OLP) patients. Vehviläinen et al. [20] obtained negative results from both qRT-PCR and ddPCR for TSH from OLP lesions from both patients with hypothyroidism and without. However, Robledo-Sierra et al. [19] found that more patients in the OLP+/LT4+ group had low levels of FT3 (p = 0.0.387), while more patients from the OLP-/LT4+ group had high levels of FT4 (p = 0.142). There were no significant differences in the TSH levels (p = 0.5773), and no correlation between increased levels of antithyroid antibodies and clinical types of OLP lesions. The levels of TgAb (p = 0.2450), TPOAb (p = 0.1366) andTRAb (p = 1.0000) were similar in OLP+/LT4+ and OLP-/LT4+ groups [19]. Immunohistochemical analyses showed positive staining for TSHR in all OLP+/LT4+ patients in the basal layer of epithelium, while there were negative results for healthy controls [19]. The qPCR analysis presented higher expression of TSHR in patients than in healthy controls (p = 0.0008). The expression of Tg and TPO was not different between the groups [19].

5. Effects of HPT Axis Components on Oral Cell Lines

All studies showed that T4 induces PD-L1 expression. Proliferative genes (BTLA and CCND1) were shown to increase in the presence of T4. The proapoptotic gene BAD was shown to decrease in the presence of T4. T4 also upregulated TNF-β1 and IL-1β expression, and downregulated COX-2 expression.

One study [21] explored the effects of T4 at other concentrations. For OEC-M1 cells, relative mRNA expression and protein abundance increased in a dose-dependent manner. Compared to controls, T4 induced a 1.9-fold increase in PD-L1 expression (n = 3, $p < 0.001$) and a 1.5-fold increase in protein abundance (n = 3, $p < 0.01$). In SCC-25 cells, maximal mRNA expression was induced by 10^{-7} M concentration of T4, which induced a 2.7-fold increase in PD-L1 expression (n = 3, $p < 0.001$) and a 1.8-fold increase in protein abundance (n = 3, $p < 0.001$) compared to controls. For both cell lines,

T4 induced pPI3K proteins that increased in a dose-dependent manner in SCC-25 cells and exhibited maximal protein abundance, with 10^{-7} M of T4 in OEC-M1 cells. This was also found in another study (18), where T4 showed an increase in protein abundance for pSer-STAT3, pPI3K and pERK 1/2 ($n = 4$, $p < 0.05$ for each protein) in OEC-M1 cells.

Formanek et al. [22] investigated the effects of various media additives on growth of human oral keratinocytes, including hydrocortisone, epidermal growth factor, insulin, bovine pituitary extract, transferrin, cholera toxin, adenine, and triiodothyronine, in varying concentrations and combinations, with cell proliferation measured by ^{3}H-labeled thymidine incorporation. Results were expressed as a percentage of an additive-free control. Bovine pituitary extract and triiodothyronine, both showed slightly stimulatory effects only at their lowest concentrations (2 µg/mL and 10^{-9} M respectively). In combination additive experiments containing triiodothyronine, the highest proliferation was found in combination with insulin and hydrocortisone, with 166.5% at 48 h.

6. TSH Levels and Association with Disease Outcome in Head and Neck Squamous Cell Carcinoma (HNSCC)

Jank et al. [23] reported on the association of TSH and CRYM (µ-crystallin) with disease outcome in HNSCC patients in a retrospective observational cohort study. At a 5-year follow-up, patients with low preoperative TSH levels had worse overall survival (OS) rates than patients with normal TSH (20% vs. 58%, $p = 0.035$). The study found an association between low TSH and OS (HR 2.99, $p = 0.047$), but not with disease-free survival (DFS).

Using antibody staining in a tissue microarray, it was found that CRYM+ was associated with better OS than CRYM- in a 5-year follow-up (78.6% vs. 56%, $p = 0.027$). No statistically significant improvement in DFS was found. No correlations were found between TSH and CRYM levels ($p = 0.289$). The study was not able to investigate whether high CRYM could abrogate effects of low TSH, as no CRYM+ TSH- patients were in the cohort.

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