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Functional roles of the bone morphogenetic protein system in thyrotropin signaling in porcine thyroid cells☆

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Abstract

We uncovered a new regulation of thyrocyte function by bone morphogenetic protein (BMP) under the influence of thyrotropin (TSH) using primary culture of porcine thyrocytes. The BMP type I receptors, ALK-2 (ActRIA), -3 (BMPRIA), and -6 (BMPRIB), were expressed in porcine thyrocytes, while ALK-6 was not detected in human thyroid. Treatment with BMP-2, -4, -6, -7, and TGF-β1 exhibited a dose-dependent suppression of DNA synthesis by porcine thyrocytes. BMP-2, -4, -6, -7, and TGF-β1 suppressed TSH receptor mRNA expression on thyrocytes, which was consistent with their suppressive effect on TSH-induced cAMP synthesis and TSH-induced insulin-like growth factor-1 expression. Activin exhibited minimal suppression of thyrocyte DNA synthesis and did not exhibit suppressive effects on TSH receptor mRNA expression. Phosphorylated Smad1/5/8 was detected in the lysates of porcine thyrocytes treated with BMP-2, -4, -6, and -7. However, in the presence of TSH, BMP-6 and -7 failed to activate Smad1/5/8 phosphorylation and 3TP-reporter activity, whereas BMP-2 and -4 maintained clear activation of the BMP signaling regardless of the presence of TSH. This diverged regulation of thyroid BMP system by TSH is most likely due to the reduction of ALK-6 expression caused by TSH. Thus, the thyroid BMP system is functionally linked to TSH actions through modulating TSH receptor expression and TSH, in turn, selectively inhibits BMP signaling. Given that BMP system is present in human thyroid and the expression pattern of ALK-2 and BMPRII is different between follicular adenomas and normal thyroid tissues, the endogenous BMP system may be involved in regulating thyrocyte growth and TSH sensitivity of human thyroid adenomas.

Keywords: Activin receptor-like kinase; Bone morphogenetic protein; Thyroid follicular adenoma; Thyroid papillary carcinoma; Thyrotropin; Transforming growth factor-β

There is accumulating evidence that several growth factors and cytokines can act as local autocrine/paracrine regulators for the migration, proliferation, and differentiation of thyroid cells. A number of factors, including hormones, cellular proteins, and genetic aberrancies, are likely involved in the initiation of thyroid tumorigensis [1]. Specifically, abnormal production of growth factors that stimulate cellular proliferation, or constitutive activation of component receptors and signal transduction pathways, has been implicated in thyroid tumor formation.

Among these, thyrotropin (TSH) is the principal regulator of thyrocyte growth and hormone production.

☆ *Abbreviations:* ActRII, activin receptor type II; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BMPRII, BMP receptor type II; FS, follistatin; IGF-1, insulin-like growth factor-1; TGF, transforming growth factor; TSH, thyrotropin; TSH-R, TSH receptor.

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TSH transduces its signaling pathway through its receptor (TSH-R) and results in stimulation of the adenylate cyclase (AC)/cyclic AMP (cAMP) and phospholipase C (PLC) pathways [2]. In the presence of TSH, a number of growth factors act as mediators of thyrocyte growth. For instance, insulin and insulin-like growth factor-1 (IGF-1) acting via the IGF-1 receptor promote follicular cell growth synergistically with TSH [3]. Additionally, IGF-1 binding proteins (IGFBP)-1 and -4 in thyroid tumors modulate the IGF-1 signaling pathway in thyrocytes [4]. A number of other factors, such as transforming growth factor (TGF)-α, nerve growth factor (NGF), basic fibrobroblast growth factor (bFGF), and various cytokines, have also been shown to stimulate thyroid epithelial growth [5–7], while TGF-β is linked to thyroid cell differentiation and growth inhibition. Epidermal growth factor (EGF), as well as phorbol esters, induces the protein kinase C (PKC) pathway and may either stimulate thyrocyte growth or antagonize the effects of TSH [8].

The significance of different TGF-β superfamily members in the regulation of thyroid growth and function remains poorly understood. TGF-β has been shown to inhibit thyrocyte growth and function, in part by down-regulating thyroid specific gene expression such as decreased synthesis of sodium iodide symporter and thyroglobulin [9–11]. TGF-β signaling results in the induction of apoptosis and alteration of cell-cycle regulatory pathways in the thyroid [12], in which TGF-β can selectively abrogate cAMP pathways, inhibit growth, and regulate differentiation [13,14]. Increased production of TGF-β has been implicated in the development of nodular goiter while normal thyrocytes produce and secrete TGF-β [13]. Alterations can occur at the ligand level, with altered TGF-β expression seen in malignant thyroid lesions as compared with normal thyroid epithelial cells [15], or can occur at the level of TGF-β type II receptor (TGF-βRII) expression with an indirect correlation with the size of thyroid papillary carcinoma [16]. Interestingly, another member of TGF-β superfamily, activin, has been reported to be expressed in thyroid epithelial cells [17] and to have an inhibitory effect on thyrocyte function and stimulatory effect on thyroid cell growth [18,19].

In the present study, we investigated the role of the BMP system as a new regulatory system that governs TSH-induction of thyrocyte function and proliferation. BMP ligands belong to the TGF-β superfamily and were originally identified as the active components in bone extracts capable of inducing bone formation at ectopic sites. BMPs regulate cell growth, apoptosis, differentiation, and cell patterning and specification in numerous tissues [20,21]. Moreover, recent studies have shown that BMPs exhibit multifunctional activities in endocrine tissues including the ovary, pituitary [22,23], and adrenal gland [24]. We here demonstrate novel roles of BMPs in controlling thyroid functions in accordance with TSH. The present study also provides a possible involvement of the thyroid BMP system in development of thyroid adenoma.

Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin solution, fetal calf serum (FCS), 3-isobutyl-1-methylxanthine (IBMX), and bovine pituitary TSH were purchased from Sigma–Aldrich (St. Louis, MO). Human TGF-β1 was from PeproTech EC (London, UK). Recombinant human BMP-2, -4, -6, -7, and activin A were from R&D Systems (Minneapolis, MN). Human adult ovary total RNA was purchased from Stratagene (La Jolla, CA).

Cell culture of porcine thyroid cells and human thyroid tissues. Primary porcine thyroid follicle cells were provided by Yamasa Corp. (Choshi, Japan). In brief, fresh porcine thyroid glands were obtained from a slaughterhouse, immediately washed with sodium hypochlorite, and rinsed with PBS containing antibiotics. After removal of connective tissue, glands were minced and treated with PBS containing collagenase and trypsin at 37 °C. The digest was filtered through metallic meshes (800–250 μm) and washed repeatedly by Ham's F12 medium with centrifugation. The final pellet was resuspended into RPMI-1640 with 25% FCS and 10% DMSO, and then the collected thyroid cells were slowly frozen and kept at −80 °C until experiments. The cells were grown in DMEM supplemented with 1% FCS and antibiotics in humidified 5% CO_2 at 37 °C. Human thyroid tissues were obtained from patients who had been diagnosed with follicular adenoma (10 patients: M/F 2/8, age 52 ± 6) or papillary carcinoma (4 patients: M/F 0/4, age 44 ± 9), using clinical and pathological criteria. Normal portions of thyroid and tumor tissues were separated during surgical procedure. The tissues were immediately frozen in liquid nitrogen and stored at −80 °C until extraction of the RNA. Total tissue RNAs were extracted by isothiocyanate–acid–phenol–chloroform methods using TRIzol (Invitrogen, Carlsbad, CA) after the tissue homogenization. All human subject protocols were approved by our Institutional Committee and written permission from each individual regarding the experimental use of the tissues was obtained in advance to the surgery.

RNA extraction, RT-PCR, and quantitative real-time PCR analysis. Porcine thyroid cells (2×10^6 viable cells) were cultured in DMEM containing 1% FCS in 6-well plates. After 6 h preculture, the cells were treated with or without BMP-2, -4, -6, -7 (100 ng/ml), activin A (100 ng/ml), TGF-β1 (10 ng/ml), and TSH (1 mU/ml). After 24 h culture, the medium was removed and total cellular RNA was extracted using TRIzol (Invitrogen), quantified by measuring the absorbance at 260 nm, and stored at −80 °C until assay. The expression of BMP/activin ligands, receptors, and follistatin mRNAs were detected by reverse transcription-polymerase chain reaction (RT-PCR) analysis. Oligonucleotides used for RT-PCR were custom-ordered from Kurabo Biomedical (Osaka, Japan). PCR primer pairs were selected from different exons of the corresponding genes to discriminate PCR products that might arise from possible chromosome DNA contaminants as we have earlier reported [23]. The extracted RNA (1 μg) was subjected to a RT reaction using First-Strand cDNA Synthesis System (Invitrogen) with random hexamer (2 ng/μl), reverse transcriptase (200 U), and deoxynucleotide triphosphate (dNTP; 0.5 mM) at 42 °C for 50 min, 70 °C for 10 min. Subsequently, hot-start PCR was performed using $MgCl_2$ (1.5 mM), dNTP (0.2 mM), and 2.5 U *Taq* DNA polymerase (Invitrogen) under the conditions we have previously reported [23]. All the PCR product sizes are as follows: ALK-2, 706 bp; ALK-3, 510 bp; ALK-4, 529 bp; ALK-6, 456 bp; ActRII, 492 bp; BMPRII, 522 bp; follistatin, 188 bp; and TSH-R, 300 bp (574–593 and 854–873 from GenBank Accession No. AY082015): IGF-1, 219 bp (142–162 and 340–360 from GenBank