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Alteration in the serum concentrations of FGF19, FGFR4 and βKlotho in patients with thyroid cancer

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ABSTRACT

Introduction: βKlotho (βKL) is known to act as co-receptor for fibroblast growth factor receptor 4 (FGFR4) which is the main cognate receptor for fibroblast growth factor 19 (FGF19). Dysregulation of this FGF19/FGFR4/βKL signaling axis has been implicated in the pathogenesis of several cancers. However, its role in the pathogenesis of thyroid cancer has not been determined.

Materials and methods: The aim of this study was to assess FGF19, FGFR4 and βKL concentrations in a group of 36 patients with papillary thyroid cancer (PTC), 11 patients with follicular thyroid cancer (FTC), 9 patients with anaplastic thyroid cancer (ATC) and a group of 19 subjects with multinodular nontoxic goiter (MNG). The control group consisted of 20 healthy volunteers. Serum FGF19, FGFR4 and βKL concentrations were measured using specific ELISA methods.

Results: Significantly lower concentrations of βKL and higher concentrations of FGF19 were found in patients with PTC, FTC and ATC as compared with MNG group and controls. An elevation of FGFR4 serum concentration was observed in all thyroid cancer groups in comparison to MNG group and controls; however, in FTC group it was statistically insignificant. A positive correlation was found between βKL and FGFR4 concentrations in PTC patients. The levels of βKL, FGF19 and FGFR4 did not differ significantly between MNG group and healthy controls.

Conclusions: Our results indicate that a disrupted FGF19/FGFR4/βKL signaling pathway may play a role in the development of thyroid cancers. However, further studies are needed to elucidate the molecular mechanism of the neoplastic transition of thyroid epithelial cells.

1. Introduction

Thyroid cancer is the most common endocrine malignancy with rising incidence, occurring in ~2.1% of patients with all cancer diagnoses worldwide [1]. Based on the differentiation degree, thyroid cancers are divided into well-differentiated thyroid cancers (90% of all thyroid cancers) including papillary thyroid cancer (PTC) (the most common histological type) and follicular thyroid cancer (FTC), followed by medullary thyroid carcinoma and undifferentiated or anaplastic thyroid cancer (ATC) [2].

The human fibroblast growth factor family is composed of 22 structurally related polypeptides divided into three groups, i.e. intracrine, paracrine, and endocrine fibroblast growth factors (FGFs), based on their mechanisms of action. Paracrine and endocrine FGFs are signaling molecules which act via cell-surface FGF receptors (FGFRs). However, while paracrine FGFs require heparan sulfate as a cofactor for FGFR activity, the endocrine FGFs, comprising FGF19, FGF21 and FGF23, have lost their heparan sulfate-binding affinity and instead use a systemic signaling system with α-Klotho (KL) or βKlotho (βKL), two related transmembrane proteins, as a cofactor for FGFRs [3].

Klotho was originally identified as an anti-aging gene [4] which is expressed predominantly in renal distal convoluted tubules and brain choroid plexus. It encodes a type I single pass transmembrane protein composed of intracellular, transmembrane and extracellular domains with two internal repeats (KL1 and KL2). The extracellular domain can be cleaved by proteases, and released into the blood, urine or cerebrospinal fluid, where it acts as soluble circulating hormone (secreted Klotho, sKL). Hence, there are two forms of Klotho protein: membrane

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and sKL [5]. Membrane Klotho protein functions as an obligate coreceptor for FGF23, while sKL functions as humoral factor regulating the activity of oxidative stress, ion channels, ion transporters and multiple growth factor receptors on the cell surface [5,6]. sKL binds to a putative cell-surface receptor and represses intracellular signals of insulin and insulin-like growth factor 1 (IGF1) [7]. sKL is also reported to bind to Wnt3 and inhibit the activation of canonical Wnt3 signaling [8].

The *βKlotho* gene was identified based on sequence similarity with the *klotho* gene and shares 41.2% amino acid identity with Klotho. It also encodes the single pass transmembrane protein [9]. However, its tissue distribution differs from that of Klotho. βKL is expressed mainly in the liver, pancreas and in adipose tissue. It modulates FGF19 and FGF21 signaling [5].

The endocrine FGFs exhibit diverse biological activity. FGF23 is a bone-derived hormone that regulates phosphate and vitamin D metabolism. FGF21 is secreted from the liver upon fasting and acts on adipose tissue, promoting lipolysis and glucose uptake. FGF19 is secreted from the intestine upon feeding and suppresses bile acid synthesis in the liver [10,11]. FGF19 seems to also have oncogenic potential [12]. FGF19 signaling is mediated mainly by FGFR4 and its coreceptor βKL [11]. Therefore, the FGF19/FGFR4/βKL signaling axis is of particular interest as its deregulation at the ligand or receptor levels has been implicated in the pathogenesis of several cancers [13]. However, its role, and especially the role of βKL, in thyroid cancer remains unknown. Therefore, the aim of the present study was to determine the serum concentrations of FGF19, FGFR4 (its main cognate FGFR) and coreceptor βKL in patients with PTC, FTC and ATC.

2. Materials and methods

2.1. Study design and patient characteristics

Seventy-five patients aged from 18 to 75 years (53.72 ± 12.62) (mean ± standard error of the mean) treated by surgery in the Clinic of Endocrinological and General Surgery, Copernicus Memorial Hospital, Lodz, Poland between 2012 and 2015 were enrolled to the study. The examined group was composed of 36 subjects diagnosed with PTC, 11 with FTC, 9 with ATC and 19 patients with multinodular nontoxic goiter (MNG).

Selected cases were diagnosed by fine-needle biopsy and confirmed by postoperative histopathologic examination. Other thyroid gland pathologies were excluded on the basis of familial and clinical history, clinical examination, ultrasonography and thyroid function tests (aTPO, aTG TSH, fT3, fT4 and calcitonin serum concentrations). The control group (CG) included 20 healthy, age-matched volunteers with no history of any thyroid disease, confirmed by clinical, hormonal, thyroid ultrasound scan, and the presence of thyroid autoantibodies. Demographic and clinical characteristics of the examined groups and healthy controls are presented in Table 1. All patients diagnosed with PTC, FTC and ATC were treated with total thyroidectomy, and therapeutic neck dissection was performed with standard indications. The MNG patient group was treated by bilateral subtotal thyroidectomy. The histopathological diagnosis and clinical staging of thyroid cancer patients included to the study is presented in Table 2. The multinodular nontoxic goiter group was comprised of 19 cases with benign postoperative histopathological diagnoses, including 8 follicular adenomas and 11 adenomatous nodules.

Table 1
Demographic and clinical characterization of the studied groups (papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC), multinodular nontoxic goiter (MNG)) and healthy controls.

Group	Number of patients	Gender F/M	Age (years) Mean ± SEM	Clinical status
Control	20	10/10	55.15 ± 9.80	Euthyreosis
MNG	19	10/9	54.71 ± 10.23	Euthyreosis
PTC	36	20/16	52.73 ± 11.20	Euthyreosis
FTC	11	6/5	52.42 ± 9.11	Euthyreosis
ATC	9	4/5	57.12 ± 12.36	Euthyreosis

Table 2
Histopathological diagnosis and clinical staging of thyroid cancer patients (papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer (ATC)) included to the study, recommended by the 2010 TNM system edition by the Union for International Cancer Control.

Diagnosis (n = number of patients)	Stage			
	I	II	III	IVA
PTC (n = 36)	12	12	10	2
FTC (n = 11)	5	4	1	1
ATC (n = 9)	0	0	4	5

The project was approved by the Bioethics Committee of the Medical University of Lodz.

2.2. Measurements of βKlotho, FGF19 and FGFR4 serum levels by ELISA

Blood samples were collected from the antecubital vein between 7:00 and 8:00 am after an overnight fast, one day before surgery. Blood samples were processed within one hour after collection, and serum aliquoted and stored at −80 °C until analysis. Serum concentrations of FGF19 (R&D Systems), FGFR4 and βKlotho (Shanghai Sunred Biological Technology Ltd.) were evaluated using enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's instructions. All measurements were taken in duplicate and averaged.

2.3. Statistical analysis

The results were presented as mean ± standard error of the mean (SEM). The Shapiro-Wilk test was applied to analyze the data distribution. ANOVA followed by Fisher's protected Least Significant Difference was used to calculate differences between investigated groups; $p < 0.05$ was considered significant. The independent relationship between serum βKlotho, FGF19 and FGFR4 concentration was examined using Pearson's linear correlation analysis. All statistical analyses were performed using StatSoft statistical software v.12.0. (Statistica PL).

3. Results

The quantitative determination of the βKL, FGF19 and FGFR4 concentrations in the serum and the statistical evaluation of these results are presented in Figs. 1–4. The mean serum level of FGF19 in PTC, FTC and ATC patients (145.7 ± 9.6 pg/mL; 260.3 ± 55.6 pg/mL; 269.3 ± 51.6 pg/mL; respectively) was significantly higher than that obtained in the MNG group (89.8 ± 17.7 pg/mL) or controls (97.5 ± 16.4 pg/mL) (Fig. 1). Similarly, the mean serum level of FGFR4 in PTC, FTC and ATC patients (29.6 ± 2.9 ng/mL; 27.8 ± 8.0 ng/mL; 32.3 ± 7.9 ng/mL; respectively) was also higher than the MNG group (14.0 ± 3.6 ng/mL) or controls (13.8 ± 3.7 ng/mL) but the difference in FTC group was statistically insignificant (Fig. 2). However, the mean serum level of βKL in PTC, FTC and ATC patients (3964.8 ± 385.8 ng/L; 2223.2 ± 371.8 ng/L; 2645.8 ± 347.7 ng/L; respectively) was significantly lower than that observed in the MNG group (7419.0 ± 571.9 ng/L) or controls (7454.8 ± 602.1 ng/L) (Fig. 3). The FGF19, FGFR4 and βKL levels in patients diagnosed with MNG did not differ significantly from those of healthy controls ($p > 0.05$). A strong positive correlation was found between serum concentrations of βKL and FGFR4 ($r = 0.8$; $p < 0.001$) in the PTC group (Fig. 4).