Physiol. Res. 53: 83-89, 2004

Serum Levels of IGF-I, HGF, TGFβ1, bFGF and VEGF in Thyroid Gland Tumors

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Received April 4, 2002
Accepted January 10, 2003

Summary

IGF-I, HGF, TGFβ1, bFGF and VEGF are involved in the pathogenesis of thyroid gland tumors and their growth. We decided to find whether changes in the production of these cytokines by thyroid tumor cells are reflected by changes of their peripheral blood. Using ELISA kits, we measured the concentrations of growth factors in the peripheral blood serum in 28 patients with thyroid gland tumors (14 adenomas, 14 papillary carcinomas) and compared these concentrations with those in healthy people. We found significantly lower serum levels of IGF-I in patients with thyroid adenoma compared to the healthy population. Serum levels of HGF and bFGF were significantly higher in patients with thyroid adenoma and papillary carcinoma compared with those in healthy subjects. Serum concentrations of TGFβ1 and VEGF were not significantly different in any groups of investigated subjects. Changes in the production of these cytokines by thyroid gland tumor cells are reflected in their peripheral blood levels, but these levels also depend on a number of other physiological and pathological processes in the organism. However, significant differences of HGF and bFGF serum levels can be explained by their very high production by thyroid tumor cells and by their strong effect on the follicular and endothelial cell proliferation.

Key words
Growth factors • Thyroid gland • Thyroid adenoma • Thyroid papillary carcinoma • Angiogenesis

Introduction

Growth factors and their receptors are products of oncogenes. Most of them have proliferative and dedifferentiating effects. They ensure autonomic growth of the tumor cell population by their autocrine and paracrine effects. Growth factors affect tumor growth not only by their direct effect upon cell proliferation, but also indirectly by influencing immunity and angiogenesis.

Vascularization of enlarging tumor mass is ensured by higher production of angiogenic growth factors, such as bFGF (basic Fibroblast Growth Factor), PDGF (Platelet Derived Growth Factor), EGF (Epidermal Growth Factor) and VEGF (Vascular Endothelial Growth Factor). Their production may rise owing to growing

hypoxia in the center of the tumor. Of course, there are also inhibitors of angiogenesis, for example TGFβ (Transforming Growth Factor β), interferons, interleukine-6, trombospondin. TNFα. (Tumor Necrosis Factor α) is responsible for hemocoagulation and tumor necrosis. It has an opposite effect in the extravascular space, it leads to a degradation of extracellular matrix and to angiogenesis (Patel *et al.* 1996, Klener 1997, Šterzl 1999).

The production of IGF-I (Insulin-like Growth Factor I, known as somatomedin C) is influenced by STH (Somatotropic Hormone). IGF-I has a mitogenic effect and stimulates the function of many cell types. It forms complexes with IGF-binding proteins (IGFBP) that regulate free IGF levels (Bachrach *et al.* 1989, Klener 1997, Šterzl 1999). In the thyroid gland, IGF-I stimulates fibroblasts, follicular cells and endothelial cells. It does not inhibit specific thyroidal functions, in contrast to most of other growth factors. The synergy between IGF-I and TSH is necessary for the stimulation of follicular cell growth and function, because IGF-I is ineffective alone (Bachrach *et al.* 1988, Eggo *et al.* 1990, Beere *et al.* 1991). TSH also inhibits secretion of IGFBP. Inhibitors of thyroid gland function (thyreostatics) mostly raise IGFBP synthesis (Bachrach *et al.* 1989, 1991, Eggo *et al.* 1996).

Mitogenic effect of HGF (Hepatocyte Growth Factor) is more expressive than that of other growth factors. HGF affects proliferation of epithelial cells, hepatocytes, keratinocytes and epidermal melanoblasts (Klener 1997, Šterzl 1999). HGF is a potential mitogen for follicular cells and C-cells of the thyroid gland. Its proliferative and dedifferentiating effect seems to take part especially in the phase of rapid growth of the tumor mass (Dremier *et al.* 1994, Eccles *et al.* 1996).

TGFβ1 usually inhibits cell proliferation, but under some conditions it can also stimulate cell proliferation. It increases the intensity of the secretion of collagen and protease inhibitors and takes part in the production of extracellular matrix. TGFβ1 has also immunosupressive effects. It inhibits the production of immunoglobulines and NK-cell activity. It suppresses mitotic activity of endothelial cells and acts as an inhibitor of angiogenesis (Patel *et al.* 1996, Klener 1997, Šterzl 1999). TGFβ1 inhibits the proliferation of follicular thyroid cells, it further raises the production of IGFBP (Bachrach *et al.* 1991, Beere *et al.* 1991). TGFβ1 in the thyroid gland is activated by the plasminogen/ plasminogen activator system in response to TSH stimulation. This may be the mechanism involved in the stabilization of goiter volume (Cowin and Bidey 1994, 1996, Cowin *et al.* 1996, Franzen *et al.* 1999).

bFGF stimulates follicular cell growth and has mitogenic and dedifferentiating effects. It is a very strong activator of angiogenesis, it activates fibroblasts, endothelial proliferation, and migration. Higher production of bFGF was found in follicular cells of thyroid gland carcinomas (Eggo *et al.* 1995, Patel *et al.* 1996, Shingu *et al.* 1998).

VEGF is a strong mitogen for endothelial cells and raises vascular permeability. It takes part in the neovascularization of the tumor tissue (Klener 1997, Šterzl 1999). It has a similar effect on the proliferation of follicular cells as TGFβ1. Owing to TSH, the production of VEGF is activated in thyrocytes that leads to the end of mitogenic TSH stimulation and to the initiation of angiogenesis (Sato *et al.* 1995, 1997, Viglietto *et al.* 1997, Wang *et al.* 1998). The VEGF production is increased in thyroid gland adenomas and especially carcinomas so that histological type of the tumor thus regulates its own vascularization intensity (Fellmer *et al.* 1999, Katoh *et al.* 1999). VEGF also takes part in the lymphatic vessel formation and affects tumor cell dissemination into regional lymphatic nodes (Fellmer *et al.* 1999).

The aim of our study was to find out whether differences in the growth factors production by the cells of thyroid gland adenoma and papillary carcinoma described in the literature are reflected by their peripheral blood levels.

Methods

The study involved 28 patients (23 women and 5 men) with a thyroid gland tumor, who were operated at the Clinic of Otorhinolaryngology and Surgery of the Head and Neck in Faculty Hospital Motol from October 2000 until March 2001. In all these patients, total thyroidectomy was carried out and then the thyroid gland tissue was histologically investigated. In 14 persons a benign thyroid adenoma (12 women, 2 men) and in other 14 subjects papillary carcinoma (11 women, 3 men) was found.

From every patient, 20 ml of blood were withdrawn from cubital vein in the operating-room before the start of the operation. After 30 min this peripheral blood was centrifuged for 10 min at 2600 rpm. The serum thus obtained was frozen in liquid nitrogen and stored in a closed plastic tube at –80 ºC. Measurements of serum

concentrations of IGF-I, HGF, TGFβ1, bFGF and VEGF were performed by the ELISA method.

Serum levels of mentioned growth factors were compared among groups of patients with benign thyroid adenomas and papillary carcinomas. These serum levels were also compared with serum levels in a group of healthy people.

Table 1. Particular serum concentrations of growth factors in patients with thyroid adenoma and papillary carcinoma and in healthy subjects.

Group	Pacient	IGF-I (ng/ml)	bFGF (ng/ml)	TGFb1 (ng/ml)	HGF (pg/ml)	VEGF (pg/ml)
Adenoma	1	52	6.78	10.14	1759	9
	2	93	13.37	33.9	2749	89
	3	78	8.15	26.73	508	204
	4	50	4.2	31.17	1072	40
	5	124	2.02	38.61	2803	66
	6	146	3.24	25.29	2106	46
	7	89	7.47	49.17	1629	735
	8	55	3.24	49.53	1292	219
	9	123	9.25	33.93	616	292
	10	189	1.34	40.59	915	511
	11	107	3.65	46.47	887	73
	12	118	2.43	46.98	575	147
	13	141	2.02	37.86	1192	334
	14	112	1.89	31.74	2844	221
Papillary carcinoma	1	133	2.97	43.17	440	276
	2	88	21.67	6.63	3295	0
	3	101	4.74	17.19	2285	0
	4	227	5.83	50.61	807	140
	5	89	0.8	46.56	1487	311
	6	213	1.89	36.87	277	26
	7	128	6.1	55.02	512	232
	8	91	2.02	50.49	427	599
	9	112	0.26	37.41	378	91
	10	117	6.78	43.29	707	312
	11	79	6.38	37.32	866	465
	12	108	0.67	35.37	712	20
	13	152	13.64	32.55	1811	352
	14	201	5.97	35.76	1910	111
Healthy	1	123	0.39	47.64	348	293
	2	161	0	11.67	347	39
	3	164	0	27.15	330	127
	4	234	5.83	52.86	380	732
	5	207	1.48	15.72	419	196
	6	89	1.07	16.89	334	27
	7	184	1.89	55.05	523	400
	8	147	1.07	4.83	210	0

Results

Table 1 shows individual data of all patients. All patients had a nodal goiter for several months or years from the first diagnosis and there was a growth progression of nodules in the last months. There was anamnestically no other tumor disease, or any acute or chronic inflammation (including autoimmune diseases) where production of cytokines can be changed (Hrdá *et al.* 2003).

Average values of serum concentrations of individual growth factors are shown in Table 2 and Figure 1. The results were statistically evaluated by one-way analysis of variance (IGF-I, TGFβ1) and by Kruskal-Wallis test (HGF, bFGF, VEGF).

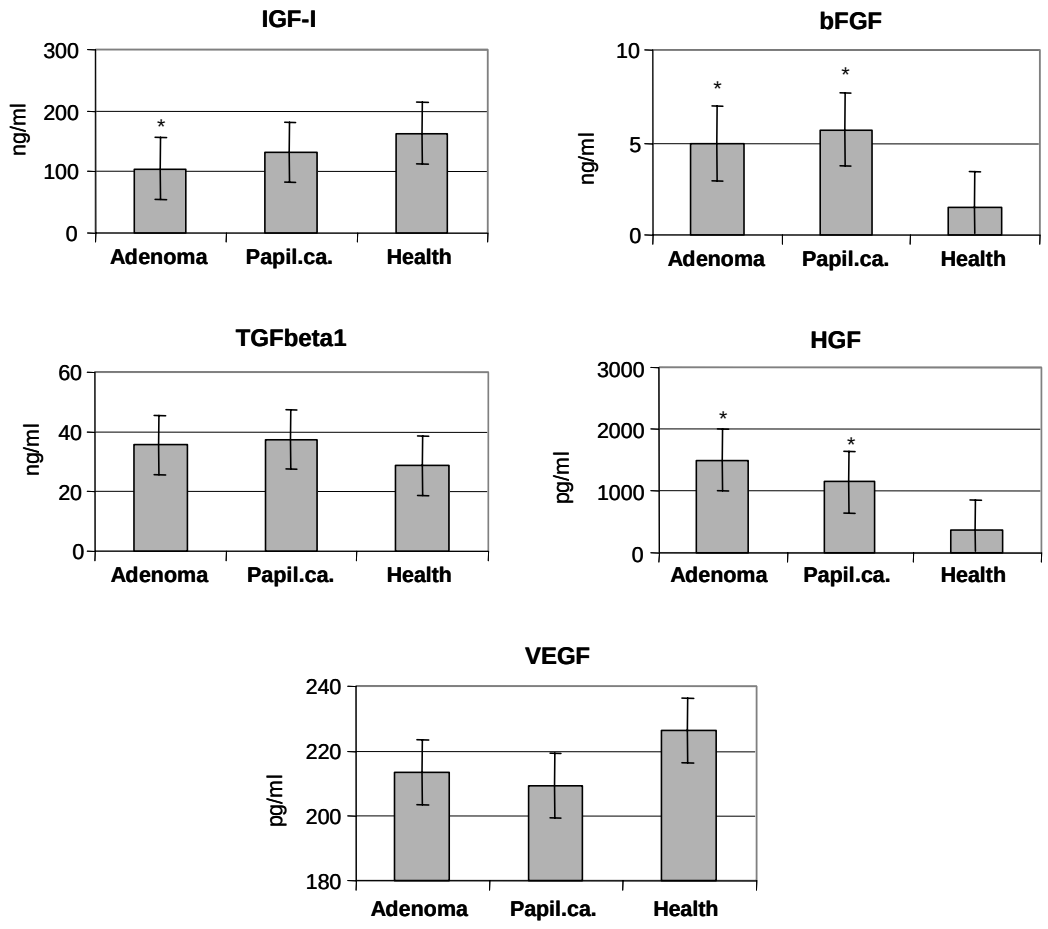

Fig. 1. Graphs with average serum growth factors concentrations.* denotes significant differences.

Table 2. Mean values of growth factors serum concentrations in patients with thyroid adenoma and papillary carcinoma and in healthy subjects.

Cytokine	Adenoma	Papillary carcinoma	Healthy
IGF-I (ng/ml)	105.5 ± 38.2	131.4 ± 47.2	163.6 ± 42.9
bFGF (ng/ml)	4.93 ± 3.42	5.69 ± 5.58	1.47 ± 1.77
TGFβ1 (ng/ml)	35.9 ± 10.5	37.7 ± 12.5	29.0 ± 18.7
HGF (pg/ml)	1496 ± 810	1137 ± 862	361 ± 83
VEGF (pg/ml)	213 ± 197	210 ± 179	227 ± 231

Data are means ± S.D.

We found significantly lower serum concentrations of IGF-I in patients with thyroid adenoma compared to the healthy population ($p=0.02$). There were no significant differences in serum IGF-I levels between the group of patients with thyroid papillary carcinoma and the healthy population and between groups of patients with thyroid adenoma and papillary carcinoma.

There were significantly higher serum concentrations of HGF in both groups of patients with thyroid adenoma and papillary carcinoma compared to the healthy population ($p<0.001$). HGF serum concentrations in patients with thyroid papillary carcinoma were higher (but not significantly) than in patients with thyroid adenoma.

We also found significantly higher serum levels of bFGF in both groups of patients with thyroid adenoma and papillary carcinoma compared to the healthy population ($p<0.01$). Similarly as HGF, bFGF serum levels were somewhat higher in patients with thyroid papillary carcinoma compared with thyroid adenoma, but the difference was also not significant.

TGFβ1 serum concentrations in healthy subjects were lower than in patients with thyroid adenoma and even lower than in patients with thyroid papillary carcinoma, but less significantly. We found a tendency to higher VEGF serum levels in the healthy population compared to patients with thyroid adenoma and papillary carcinoma.

Discussion

Several studies have described the production of particular growth factors in thyroid gland tumors. In these studies, the expression or occurrence of growth factors in thyroid gland was examined by their direct detection in the thyroid tissue, using PCR, immunohistological methods or *in situ* hybridization. We tried to elucidate, how these changes in the growth factor production by the thyroid gland tissue can be reflected by changes of their serum concentrations. Peripheral blood is still the most accessible biological material for routine screening investigation.

IGF-I has proliferative effects and takes part in many physiological processes in the organism. The serum levels express the functional state of many organ systems and it is therefore very difficult to relate such changes to a single particular tissue. Our measured IGF-I serum levels can be explained as follows: they are lower in thyroid adenoma and papillary carcinoma than in the healthy population. These results are in contrast with literary data about the IGF-I production examined directly in the thyroid tissue. This may be due to the fact that IGF-I takes part in a large number of various physiological processes in the organism (Klener 1997, Šterzl 1999) and its peripheral blood levels are not specific for a particular disease.

HGF is a very strong mitogen for follicular thyroid cells. Its proliferative and dedifferentiating effects take part especially in the phase of rapid goiter growth and this is just the most common indication for total thyroidectomy in patients with nontoxic goiter (Dremier *et al.* 1994, Eccles *et al.* 1996). According to this fact, we found high serum HGF concentrations in patients with thyroid adenoma and papillary carcinoma. Trovato *et al.* (1998) described very low concentration of HGF and low density of its receptor in the normal thyroid tissue, but also in benign thyroid adenomas and non-papillary carcinomas (including anaplastic carcinoma). In papillary thyroid carcinomas the authors found a higher production of HGF and its receptor (known as c-met) in 86 % and 93 % of patients, respectively (Trovato *et al.* 1998).

TGFβ1 and VEGF have antiproliferative effect on thyrocytes. They are involved in the stabilization of goiter volume, their production increases successively and at the final level the goiter stops to grow (Cowin and Bidey 1996, Cowin *et al.* 1996, Fellmer *et al.* 1999, Katoh *et al.* 1999). We found lower TGFβ1 serum concentrations in healthy people than in patients with thyroid tumors. However, these differences were not statistically significant. Patients with nontoxic goiter are often operated in the early phase of rapid goiter growth. That is the time, when TGFβ1 and VEGF concentrations are still low.

The role of VEGF in thyroid gland tumors is not yet quite clear. It inhibits follicular cells proliferation, but it also supports angiogenesis and tumor tissue vascularization (Sato *et al.* 1997, Viglietto *et al.* 1997, Wang *et al.* 1998). It is proved that VEGF is produced only in the isolated follicles of the normal thyroid gland. Its production rises in thyroid adenomas and especially in carcinomas (except of the follicular carcinoma) (Fellmer *et al.* 1999, Katoh *et al.* 1999). Our VEGF serum concentrations are not in accordance with these data. We have shown that VEGF serum levels are inversely related to its levels detected directly in the thyroid tumor tissue (Fellmer *et al.* 1999, Katoh *et al.* 1999). It can be explained by a higher growth factor consumption in the tissue, which is reflected by its lower serum concentration. This negative relationship might be

dependent on the expression of VEGF receptors in the tissue.

bFGF stimulates the proliferation of thyroid follicular cells, fibroblasts and endothelial cells. It has also dedifferentiating effects and it is a very strong activator of angiogenesis. Its production rises in the phase of a rapid goiter growth, similarly to HGF. Higher bFGF production has been described in thyroid adenomas and carcinomas, while this production is minimal in the normal thyroid gland tissue (Becks *et al.* 1994, Eggo *et al.* 1995, Patel *et al.* 1996, Shingu *et al.* 1998). We also found higher HGF serum levels in patients with thyroid adenoma and papillary carcinoma compared to the healthy population.

Growth factors, such as other cytokines, have primarily autocrine and paracrine effects. Their production in the organism is ubiquitous; they are not specific for particular tissues or diseases. Their serum concentrations express all physiological and pathological processes in the organism. This fact may explain the results of our measurements, because some of them are not in agreement with literary data describing growth factor production directly in the thyroid gland tissue. Significantly higher HGF and bFGF serum levels in patients with thyroid gland tumors, compared to healthy subjects, can be explained by their very high production in thyroid tumor cells. These two growth factors have a very strong effect on the thyrocytes and endothelial cell proliferation. If it will be confirmed in more extensive studies, HGF and bFGF could be accepted as sensitive (but perhaps not specific) peripheral markers of thyroid gland tumors.

Acknowledgements

This work was supported by the grant of the Internal Grant Agency of the Ministry of Health (IGA MZ) of the Czech Republic, Grant No. NK 6023-3/1999.

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