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ORIGINAL ARTICLE

Serum levels of growth factors HGF (Hepatocyte Growth Factor), TGFβ1 (transforming growth factor β1) and IGF-I (Insulin Like Growth Factor I) in parathyroid tumors

Jaromír Astl [1,2], David Veselý [1], Petr Matucha [3], Jindrich Martínek [5], Tomáš Kucera [5], Petr Laštuvka [1], Jan Betka [1,2], Ivan Šterzl [3,4]

[1] Department of Otorhinolaryngology, Head and Neck Surgery. The First Medical Faculty of the Charles University in Prague, University Hospital Motol, CZECH REPUBLIC.
[2] ENT Department, Postgraduate Medical School. Prague, CZECH REPUBLIC.
[3] Institute of Endocrinology. Prague, CZECH REPUBLIC.
[4] Institute of Immunology and Microbiology. The First Medical Faculty of the Charles University in Prague, General University Hospital in Prague, CZECH REPUBLIC.
[5] Institute of Histology and Embryology. The First Medical Faculty of the Charles University in Prague, General University Hospital in Prague, CZECH REPUBLIC.

Correspondence to: Jaromír Astl, M.D., PhD.
Department of Otorhinolaryngology and Head and Neck Surgery.
The First Medical Faculty of the Charles University.
University Hospital Motol, VÚvalu 84,
Prague 5, 150 06, CZECH REPUBLIC
TEL: ++420 22443 4300, FAX: ++420 22443 4318,
EMAIL jaromir.astl@fnmotol.cz

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Abstract

INTRODUCTION: HGF (Hepatocyte Growth Factor), TGFβ1 (Transforming Growth Factor β1) and IGF-I (Insulin Like Growth Factor I) are cytokines that are involved in the parathyroid tumors formation and growth. We tried to determine, if there are changes and relationships in the production of these cytokines by tumor cells of parathyroid tumors. **MATERIAL AND METHODS**: We determined concentrations of HGF, TGFβ1 and IGF-I in serum from peripheral blood of 16 patients with parathyroid adenoma and of 8 patients with parathyroid secondary hyperplasia before and after parathyroidectomy. Results were compared with serum levels in healthy people. **RESULTS**: Both preoperative and postoperative HGF serum levels in patients with parathyroid adenoma and secondary hyperplasia are significantly higher than in healthy people. Preoperative and postoperative serum levels of TGFβ1 in parathyroid adenoma and postoperative TGFβ1 serum levels in parathyroid secondary hyperplasia are higher, compared with those in the healthy population and in parathyroid secondary hyperplasia preoperatively. There are no significant differences of IGF-I serum levels among the all investigated groups of patients. **CONCLUSIONS**: Changes in the growth factors production by parathyroid tumor cells are reflected by their concentrations in peripheral blood. The elevation of HGF serum levels in patients with parathyroid adenoma and hyperplasia can be explained by very high HGF production by tumor cells. Nevertheless, there is no decrease of HGF serum levels after the parathyroidectomy. That may be the result of the extratumoral production of this cytokine. Also TGFβ1 and IGF-I serum levels indicate high possibility of the extratumoral production of these cytokines. Higher postoperative IGF-I serum levels (but not significantly) in parathyroid secondary hyperplasia are in accordance with its bone production.

Introduction

Cytokines participate in the tumorigenesis of the parathyroid gland [1]. Many works are focused on the influence of growth factors HGF, TGFβ1 and IGF-I. Their effect on the PTH (parathormone) production has not been described yet. Dynamic cell culture systems can play an important role in the understanding of synergic and antagonistic effects of cytokines on the parathyroid gland. Every new knowledge about the role of growth factors in the physiology and pathology of the parathyroid tissue may be used for the diagnosis and therapy of parathyroid diseases in the future [2,3,4,5,6].

Mechanisms of the parathyroid cells tumor transformation are not described in detail. Hsi et al. 1996 [7] and Vasef et al. 1999 [8] describe overproduction of cyclin D1 protein in parathyroid tumors, in 91% of carcinomas and in only 39% of adenomas. Tominaga et al. 1999 [9] proved, that overproduction of PRAD1/cyclin D1 induced by the PTH gene is not responsible for a tumorigenesis.

IGF-I mediate effects of growth hormone somatotropine to the cells in the fetal and growth period of an organism [10]. IGF-I is connected with higher cell activity and malignant transformation. This risk is increased mainly in the DNA damage by mutagenes. IGF-I inhibits an apoptosis. This antiapoptotic activity of IGF-I is suppressed by the specific bound protein 3 (IGFBP 3) in the cell culture.

Tanaka et al. 1994 [11] found direct proliferative effect of IGF-I on parathyroid tumor cells. They demonstrate the presence of EGF receptors. Jehle et al. 2000 [12] say, that IGF-I play an important role in the bone remodelation and in a development of the renal osteodystrophy. There are high IGFBP, IGF-I and II levels in these patients. Ueland et al. 1999 [13] show the IGF-I and II increase in bones of acromegalic people. Kaplanski et al. 1995 [14] present the parathyroid cells stimulation by IGF-I and somatotropine hormone in the cell suspension.

Driman et al. 1992 [15] proved, that TGFβ1 is an iniciator of the reversible transformation of cell phenotype. It takes part in the parathyroid tumorigenesis and physiology. Detailled mechanism of particular interactions has not been described yet. There exist an synergism between TGFβ1 and EGF receptors. Gogusev et al. 1996 describe high TGFβ1 levels in parathyroid adenoma and secondary hyperplasia. There is also increased EGF production in normal parathyroid tissue. Therefore EGF detection is not significant for the diagnosis of parathyroid pathology [16].

There is evident, but not well described, relationship between growth factors HGF, TGFβ1, IGF-I and parathyroid gland tissue. There is not described the detailled mechanism of these cytokines effects, their production by the parathyroid cells and their receptors presence in the parathyroid tissue.

Patients and methods

We investigated serum levels of growth factors in 24 patients with parathyroid gland disease, 16 patients with parathyroid adenoma and 8 patients with parathyroid secondary hyperplasia. Patients were operated on the ENT Department of the University Hospital Motol in Prague, an exstirpation of the pathological parathyroid tissue (parathyroidectomy) was carried out. There was anamnestically no other tumor or inflammatory disease in these patients.

From every patient, we obtained blood from cubital vein just before the operation and three weeks after the parathyroidectomy. After 30 minutes, the peripheral blood was centrifuged for 10 min. at 2600 turns/min. The serum was frozen in liquid nitrogen and stored in a closed plastic tube at –70 °C. Measurements of serum concentrations of HGF, TGFβ1 and IGF-I were executed by ELISA method using RD Systems Labs kits.

Serum levels of mentioned growth factors were compared between groups of patients with parathyroid adenoma and hyperplasia before and after the operation. These serum levels were also compared with serum levels in a group of healthy people.

Results

Hepatocyte Growth Factor (HGF)

Preoperative and postoperative serum levels of HGF in patients with parathyroid adenoma (preoperative 1552 ± 592 pg/ml; postoperative 1991 ± 1920 pg/ml) and secondary hyperplasia (preoperative 2719 ± 1383 pg/ml; postoperative 2847 ± 2069 pg/ml) are significantly higher compared with the healthy population (653 ± 145 pg/ml). (Table 1, 2) There is surprisingly no decrease of HGF serum levels after the operation in both groups of patients.

Transforming Growth Factor β1 (TGFβ1)

Both preoperative and postoperative TGFβ1 serum levels in patients with parathyroid adenoma (preoperative 29,7 ± 12,7 ng/ml; postoperative 31,9 ± 10,3 ng/ml) are significantly higher compared with those in healthy people (13,6 ± 5,8 ng/ml). Also postoperative TGFβ1 serum levels in patients with parathyroid secondary hyperplasia (28,8 ± 11,8 ng/ml) are significantly higher than in healthy people. Preoperative TGFβ1 serum concentrations in parathyroid secondary hyperplasia (14,8 ± 5,0 ng/ml) are also higher compared with the healthy population, but not significantly. (Table 3, 4)

Insulin Like Growth Factor I (IGF-I)

There are no significant differences of IGF-I serum levels among the all investigated groups of patients. (Table 5, 6)

Table 1: HGF serum levels in patients with parathyroid adenoma and secondary hyperplasia before and after the operation (values in pg/ml).

	PTApre	PTApost	PTHpre	PTHpost	Healthy
mean	1551,9	1991,0	2718,6	2846,5	652,7
σ	592,3	1919,9	1383,4	2069,3	145,2
median	1434,3	1359,7	2676,0	2263,2	630,9
lower quartill	1224,3	1188,7	1569,0	1379,2	561,7
upper quartill	1702,8	1846,3	3347,3	3375,1	710,9

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative

Table 2: Analysis of HGF serum levels in patients with parathyroid pathology by Kruskal-Wallis Z-test.

	PTApre	PTApost	PTHpre	PTHpost	Healthy
PTApre	0	0,1539	1,7292	1,1864	**3,2620**
PTApost	0,1539	0	1,5839	1,0613	**3,3527**
PTHpre	1,7292	1,5839	0	0,3347	**4,3225**
PTHpost	1,1864	1,0613	0,3347	0	**3,6671**
Healthy	**3,2620**	**3,3527**	**4,3225**	**3,6671**	0

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative
Regular Test: Medians significantly different if z-value>1,9600
Bonferroni Test: Medians significantly different if z-value>2,8070

Table 3: TGFβ1 serum levels in patients with parathyroid adenoma and secondary hyperplasia before and after the operation (values in ng/ml).

	PTApre	PTApost	PTHpre	PTHpost	Healthy
mean	29,7	31,9	14,8	28,8	13,6
σ	12,7	10,3	5,0	11,8	5,8
median	28,6	29,3	14,7	31,3	13,9
lower quartill	22,9	24,7	12,6	21,0	8,0
upper quartill	40,9	38,8	18,3	34,2	18,7

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative

Table 4: Analysis of TGFβ1 serum levels in patients with parathyroid pathology by Kruskal-Wallis test.

	PTApre	PTApost	PTHpre	PTHpost	Healthy
PTApre	0	0,3998	**3,0330**	0,1620	**3,1825**
PTApost	0,3998	0	**3,3280**	0,4580	**3,4759**
PTHpre	**3,0330**	**3,3280**	0	**2,2881**	0,1295
PTHpost	0,1620	0,4580	**2,2881**	0	**2,4081**
Healthy	**3,1825**	**3,4759**	0,1295	**2,4081**	0

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative
Regular Test: Medians significantly different if z-value>1,9600
Bonferroni Test: Medians significantly different if z-value>2,8070

Table 5: IGF-I serum levels in patients with parathyroid adenoma and secondary hyperplasia before and after the operation (values in ng/ml).

	PTApre	PTApost	PTHpre	PTHpost	Healthy
mean	101	122	99	151	98
σ	42	56	47	56	34
median	103	113	90	149	86
lower quartill	71	77	64	95	71
upper quartill	130	128	112	207	121

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative

Discussion

Growth factors are proteins involved in many physiological and pathological processes. The presence of growth factors receptors is necessary for their effects. HGF and its receptor are regulators of the cell migration. Their expression may be important for the metastatic processes. HGF also stimulates synthesis of chemokines by dendritic cells. HGF production depends on the presence of met receptor [17].

HGF is like a mitogene for epithelial cells. HGF receptor expression is under the paracrine control of activated tyrosine kinase. HGF has strong mitogenic effect. [1,10,18]

HGF effect in parathyroid gland tissue has not been described yet. We found significantly higher HGF serum levels in patients with parathyroid adenoma and secondary hyperplasia (preoperatively and postoperatively) compared with the healthy population [19]. We can say, that pathological parathyroid tissue produces HGF and can maintain its tumoral autonomic activity by that mechanism. Nevertheless, there was no decrease of HGF serum levels after the operation. There may be extraparathyroid HGF production. Therefore the direct HGF effect on parathyroid cells has to be proved in dynamic system of tissue culture. Immunohistochemical detection of HGF receptor on parathyroid cells also can explain the HGF role in this tissue.

TGFβ1 directly stimulates an apoptosis. TGFβ1 inhibits the cell proliferation in the most of tissues, except of mesenchymal tissues. TGFβ1 also regulates the cell differentiation. The growth of parathyroid cells is stimulated by high levels of TGFβ1 and phosphates [20]. Parathyroid cells product TGFβ1 and EGF (Epidermal Growth Factor) [21]. TGFβ1 is produced by parathyroid adenoma cells, but not in the normal parathyroid tissue. Driman et al. show, that TGFβ1 can not be used like the tumor marker [15].

We detected significantly higher preoperative and postoperative serum levels of TGFβ1 in patients with parathyroid adenoma compared with the healthy population. We also detected significantly higher postoperative TGFβ1 serum levels in patients with parathyroid secondary hyperplasia. Our results are in harmony with the works of other authors. Nevertheless, the role of TGFβ1 in para-

Table 6: Analysis of IGF-I serum levels in patients with parathyroid pathology by Kruskal-Wallis test.

	PTApre	PTApost	PTHpre	PTHpost	Healthy
PTApre	0	0,8781	0,5842	1,6784	0,2851
PTApost	0,8781	0	1,2987	1,0100	1,0029
PTHpre	0,5842	1,2987	0	1,9562	0,2591
PTHpost	1,6784	1,0100	1,9562	0	1,7164
Healthy	0,2851	1,0029	0,2591	1,7164	0

PTA=parathyroid adenoma, PTH=parathyroid hyperplasia, pre=preoperative, post=postoperative
Regular Test: Medians significantly different if z-value>1,9600
Bonferroni Test: Medians significantly different if z-value>2,8070

Table 7: Comparison of preoperative and postoperative serum levels of HGF, TGFβ1 and IGF-I in patients with parathyroid pathology, evaluated by Mann-Whitney test.

	HGF	TGFβ1	IGF-I
PT adenoma	0,9842	0,7972	0,3952
PT hyperplasia	0,9485	**0,0454**	0,0814

Medians significantly different, if z-value<0,0500

thyroid tissue is still not described. TGFβ1 is supposed to have an inhibitory effect on the parathyroid cell proliferation. On the other side, TGFβ1 may also have stimulatory effect in this tissue. Therefore the TGFβ1 effect on parathyroid cells has to be clear up in the dynamic system of tissue culture [1,10,22].

IGF-I mediates effects of growth hormone (STH) on cells in the fetal and growing period. IGF-I increases cell activity and possibility for the malign cell transformation. IGF-I inhibits cell apoptosis. This antiapoptotic effect could be used in the therapy of tumors. It is proved in vitro, that antiapoptotic effect of IGF-I can be blocked by specific bounding protein 3 (IGFBP3). Osteoblasts produce IGF-I during their differentiation, IGF-I is stored in a bone matrix [23].

IGF-I has a direct effect on the parathyroid tumors growth regulation [11,24]. In the cell suspension, IGF-I stimulates growth of parathyroid cells, like growth hormone (STH) [14,25]. IGF-I and II have important role in the regulation of the bone metabolism. There are described decreased serum concentrations of IGF-I and II and increased levels of IGFBP5 in patients with a bone remodelation. [1, 12]

Conclusions

The removing of pathological parathyroid tissue does not lead to the decrease of HGF, TGFβ1 and IGF-I serum levels. We suppose, these cytokines are not produced in parathyroid tissue in the sufficient amount, that could influence theirs serum concentrations. The production of these growth factors in the parathyroid tissue was not significant even in immunohistochemical detection. Nevertheless, pathological parathyroid cells can be affected by these growth factors.

The increased TGFβ1 serum levels after the operation (near total parathyroidectomy), mainly in parathyroid secondary hyperplasia, can be explained by the searching for "a reserve mechanism" of the PTH production or by the stimulation of the reimplantated parathyroid tissue.

The increase of postoperative IGF-I serum levels in parathyroid hyperplasia (not significant) can be explained by its production in the bone marrow. There occurs a bone remodelation after the near total parathyroidectomy in organisms owing to a changes in a bone metabolism after the decrease of PTH production. The removing of the bone matrix leads to the releasing of IGF-I. Higher IGF-I levels then rise a risk of early recidives, mainly in the synergy with the other cytokines.

We found higher preoperative and postoperative HGF serum levels in patients with parathyroid adenoma and secondary hyperplasia compared with the healthy population. There was no decrease of HGF serum levels after the operation. This result can not be explained in our study.

More knowledges about effects of HGF, TGFβ1 and IGF-I can be obtained by the studies with dynamic systems of tissue cultures.

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