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Do Insulin-like Growth Factor Associated Proteins Qualify as a Tumor Marker? Results of a Prospective Study in 163 Cancer Patients*

C. Matuschek[1], M. Rudoy[1], M. Peiper[3], P. A. Gerber[2], N. P. Hoff[2], B. A. Buhren[2], B. Flehmig[11], W. Budach[1], W. T. Knoefel[3], H. Bojar[4], H. B. Prisack[4], G. Steinbach[5], V. Shukla[6], A. Schwarz[7], K. Kammers[8], A. Erhardt[9], A. Scherer[10], E. Bölke[1], M. Schauer[3]

[1]Department of Radiation Therapy and Radiooncology, Universitätsklinikum Düsseldorf, Heinrich Heine Universität, Duesseldorf, Germany
[2]Department of Dermatology, Universitätsklinikum Düsseldorf, Heinrich Heine Universität, Düsseldorf, Germany
[3]Department of General-, Visceral-, and Pediatric Surgery, Universitätsklinikum Düsseldorf, Heinrich Heine Universität, Düsseldorf, Germany
[4]Institute of Molecular Medicine, Düsseldorf, Germany
[5]Department of Clinical Chemistry, University of Ulm, Germany
[6]Methodist Hospital, Houston, Texas, USA
[7]Department of Surgery, Hospital Biberach, Germany
[8]Department of Statistics, University of Dortmund, Germany
[9]Department of Gastroenterology, Universitätsklinikum Düsseldorf, Heinrich Heine Universität, Düsseldorf, Germany
[10]Institute of Diagnostic and Interventional Radiology, University Hospital, Düsseldorf, Germany
[11]Childrenshospital, University of Tübingen, Tübingen, Germany

Abstract

Objective: Insulin-like growth factor (IGF)-1, -2 and Insulin like growth factor binding proteins (IGFBP) are involved in the proliferation and differentiation of cells. It has never been evaluated, if the IGF-system can serve as a tumor marker in neoplasms.

Methods: In our prospective study 163 patients with colorectal cancer (22), prostate cancer (21), head and neck tumors (17), lymphomas (20), lung cancer (34) and other entities (49) were analysed for their IGF and IGFBP serum levels at the beginning and the end of radiotherapy and compared to 13 healthy people. Subgroups of patients with local tumor disease versus metastatic disease, primary and recurrent therapy and curative versus palliative therapy were compared.

Results: The serum levels of IGF-2 were significantly elevated in patients with prostate and colorectal cancer. However, sensitivity and specificity were only 70%. IGFBP-2 serum levels were elevated in patients with head and neck tumors. Again sensitivity and specificity were only 73%. A difference between local disease and metastatic disease could not be found. A difference between IGF serum levels before and after radiotherapy could not be detected.

Conclusion: The IGF-system cannot serve as a new tumor marker. The detected differences are very small, sensitivity and specificity are too low. IGF measurement is not useful for the evaluation of the success of radiotherapy in malignancies.

Key words: Insulin-like growth factor, tumor marker, insulin like growth factor binding protein;, prognostic factors

Introduction

Insulin-like growth factors (IGF)-1 (somatomedin C) and -2 (somatomedin A), IGF-receptors (IGF1R, IGF2R) and IGF-binding proteins (IGFBP1-7) participate in the proliferation, differentiation and apoptosis during embryogenesis and development [1, 2]. Therefore, IGF blood levels depend on patients' age and liver function with an effective half-life from seven minutes to several hours [3]. Linked to the binding protein, the complex cannot leave the blood system and half-life is accordingly longer [4, 5].

In malignant tumors, autocrine and paracrine loops in the IGF-system contribute to the inhibition of apoptosis [6, 7]. A high concentration of this growth factor and its binding proteins could be detected in sarcomas and many different carcinomas, such as prostate, breast, colon and other cancers [8-12]. In these neoplasms especially a high expression rate and serum level of IGFBP-2 could be shown by different studies [13, 14]. IGFBP-2 seems to play a major role in proliferation and cell-adhesion. Assumable, high levels of acid labile subunits (ALS) of IGFBPs protect from fast tumor growth by an associated higher affinity [3]. The regulating effect of the IGF-system on proliferation, apoptosis, cell-migration and metastases could be demonstrated in cell cultures and also in a tumor animal model [15, 16].

The variation of the IGF system serum levels, especially of IGFBP, on tumor growth, metastases and under antiproliferative therapy has not sufficiently been explored in vivo. A possible benefit from IGF as a new tumor marker has never been analyzed so far. Therefore we compared serum levels of IGF-1 and -2 and IGFBP-2 and -3 of patients with different malignant diseases with tumor staging and further tumor progress after chemotherapy, radiotherapy or resection.

*This work is dedicated to Kim Speer.

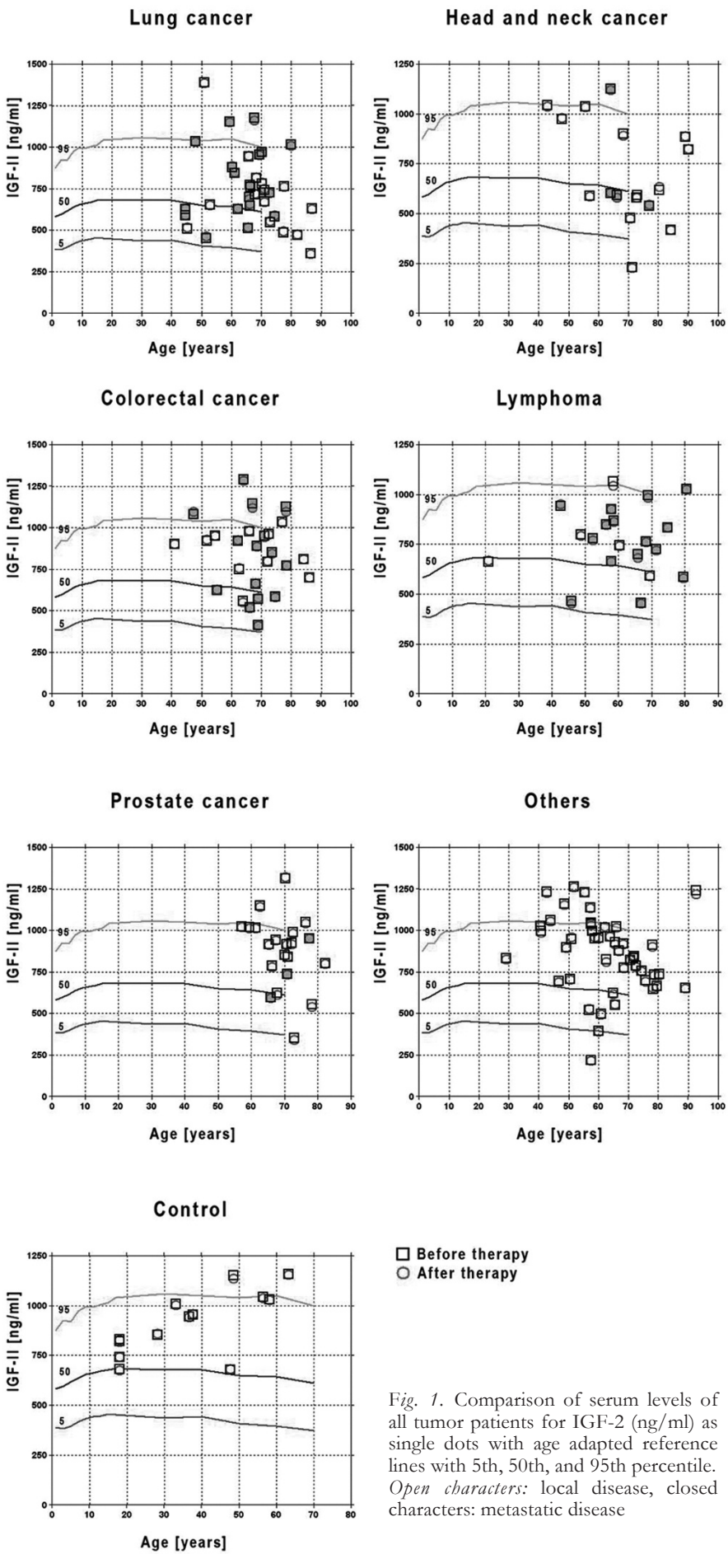

Fig. 1. Comparison of serum levels of all tumor patients for IGF-2 (ng/ml) as single dots with age adapted reference lines with 5th, 50th, and 95th percentile. *Open characters:* local disease, closed characters: metastatic disease

Patients and Methods

We analysed the IGF-1, -2, IGFBP-2, -3 and ALS-serum levels at the beginning and at the end of radiotherapy of 163 patients with malignant diseases treated in our university hospital in 2006 and 2007. The clinical profiles, age, sex and malignant diseases of all patients analysed are detailed in Table 1. Patients were treated for localized neoplasms and metastatic disease, as a primary or recurrent therapy, in curative or palliative intention. Therefore, we measured serum levels of a very heterogeneous patient collective with many different diseases and different therapies. For the statistical work-up, patients with glioblastoma, melanoma, breast, cervical, endometrial, renal, pancreatic, gastric, esophageal and anal cancer were combined into one group. Serum levels of IGF-1, -2, IGFBP-2, -3 and ALS were age adapted by calculating the standard deviation score. All statistical work-up was performed using PASW Statistics, Version 18. For the comparison of IGF-2 and IGFBP-2 a Students' t-test was used. The prospective study was IRB approved.

Radioimmunassay for IGF-1, -2 and IGFBP-3 Analysis

Serum levels were measured in blood samples taken from the cubital vein. After centrifugation the serum was frozen at -70 °C. For the analyses a competitive radioimmunoassay (RIA) (Mediagnost Tübingen, Germany) was used. All samples were measured several times according to the manufacturer's instructions with positive and negative controls via gamma-counter. The serum levels were finally calculated by means of the standard deviation of all measurements for each sample.

Enzyme-linked Immunosorbent Assay (ELISA) for IGFBP-2 and ALS Analysis

Serum levels of IGFBP-2 and ALS were analysed with an ELISA (Diagnostic Systems Laboratories Sinsheim, Germany). According

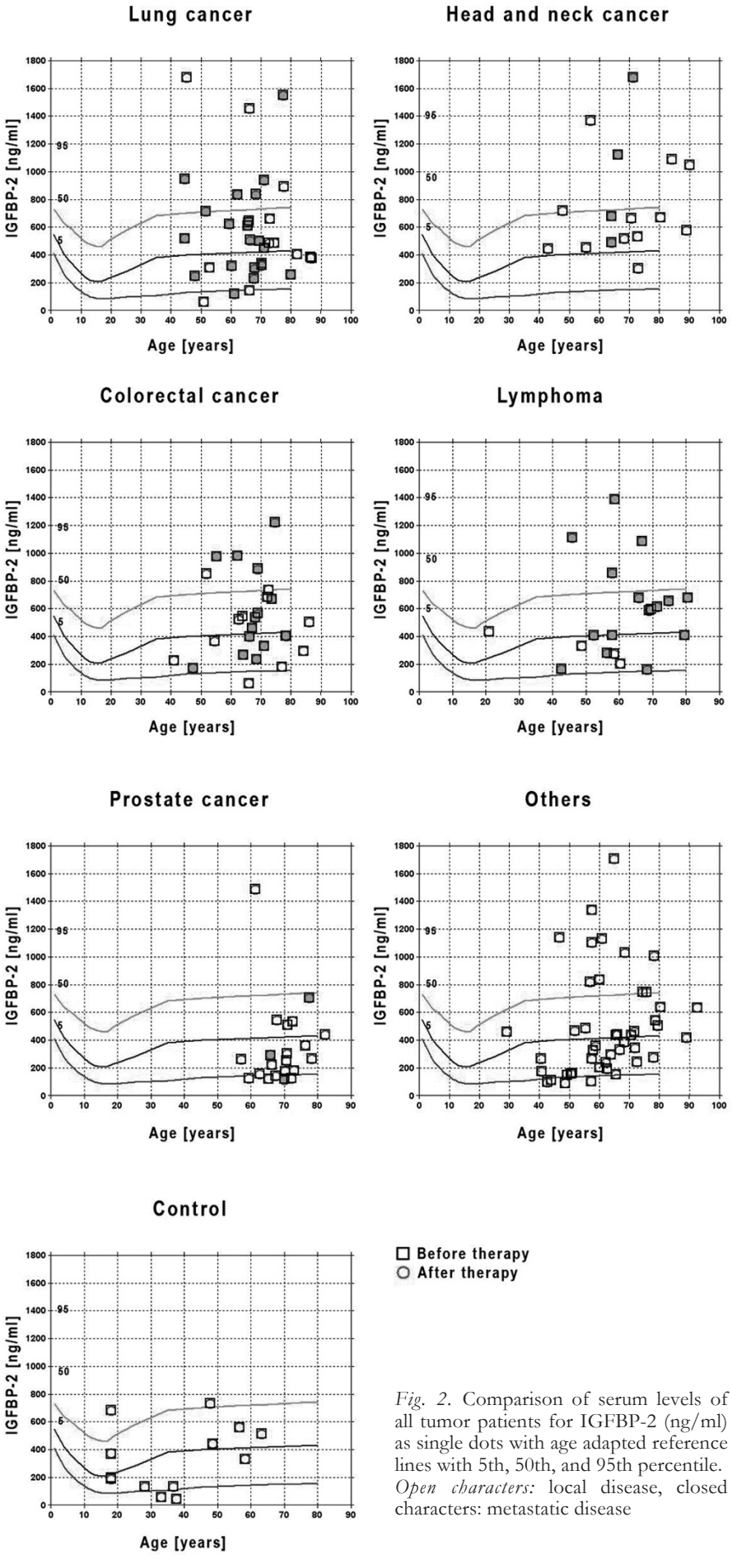

Fig. 2. Comparison of serum levels of all tumor patients for IGFBP-2 (ng/ml) as single dots with age adapted reference lines with 5th, 50th, and 95th percentile. *Open characters:* local disease, closed characters: metastatic disease

to the manufacturer's instructions the ELISA-kit was used for multiple measurements of each patient sample, positive and negative controls. The IGFBP-2 and ALS concentrations were analyzed with an ELISA-reader at 450nm wave length and calculated after generating a standard curve.

In order to assess the concentration of the unbound and therefore effective IGF-1 the quotient of IGF-1 and IGFBP-3 of each sample was calculated. Since 98% of IGF-1 builds a complex with IGFBP-3 and ALS and only 2% of the IGF-1 serum concentration present in an active form, the equilibrium can help to define the active protein. A decline of the IGFBP-3 serum level results in an increase of soluble IGF-1.

Results

176 patients were included in this study, 67 female and 109 male patients with a median age of 65 years (range from 45 to 85). Patients were treated for colorectal carcinoma (n = 22), prostate cancer (n = 21), head and neck tumors (n = 17), lung tumors (n = 34), lymphomas (n = 20), other carcinomas (renal, pancreas, gastric cancer, esophageal cancer,(n = 49) and controls.(n = 13)

In these groups, patients' serum levels of the IGF-system were correlated with a control group (13 healthy patients). Furthermore, patients were differentiated according to metastatic disease and treatment status.

IGF-1 did not show different serum levels for patients with malignant disease (pre-treated levels) versus a healthy control group. The serum levels at the beginning and in the end of radiotherapy did not differ either.

IGF-2 showed a significantly elevated serum level only in patients with prostate and colorectal cancer ($p<0,01$). A comparison between metastatic disease and local disease did not show different serum levels. IGF-2 did not show different values before and after radiotherapy either (Fig. 1).

IGFBP-2 showed significantly different serum levels for the

Table 1. Patients' epidemiology, diagnosis, age.

Diagnosis	Patient number	Mean age	Sex male	Sex female
Colorectal cancer	22	67.1	13	9
Prostate cancer	21	69.4	21	0
Glioblastoma	12	54.8	8	4
Breast cancer	9	63.3	1	8
Cervical cancer	5	58.6	0	5
Endometrial cancer	1	65.9	0	1
Head and neck	17	69.1	7	10
Lymphoma	20	60.3	16	4
Lung cancer	34	66.0	25	9
Renal cancer	3	59.7	2	1
Pancreatic cancer	3	65.4	2	1
Urinary bladder	1	60.0	1	0
Malignant melanoma	5	77.2	3	2
Gastric cancer	1	70.6	0	1
Esophageal cancer	5	66.2	4	1
Anal cancer	4	58.4	1	3
controls	13	37.1	5	8
Sum	176	63.2	109	67

head and neck tumors ($p<0,001$; sensitivity and specificity 73% and 75%). Patients with other entities showed normal serum levels.

For IGFBP-3 variations compared to normal serum levels could not be found for any tumor entity. During the ongoing course of treatment no further variations occurred.

Serum levels of ALS did not show a significant difference to controls. In patients with lung cancer, head and neck tumors and prostate cancer ALS-levels were under the 5%-percentile, but these results did not show statistical significance.

Corresponding to our results for IGF-1 and IGFBP-3 the quotient of both parameters was slightly elevated in some patients with colorectal cancer without any statistical significance. We could not detect a difference for metastatic disease or during the course of our treatment either.

We found differences only for IGF-2 and IGFBP-2 for colorectal, prostate and head and neck tumors. However, these differences did not gain with metastatic disease or decline during further treatment.

Discussion

Tumor markers are important to monitor the therapeutic effect in cancer patients [32-35]. The insulin-like growth factor system is known for regulating cell growth and apoptosis. Taking into account its effect on these essential effectors a potential role for IGF-signaling associated proteins in cancer biology is discussed controversially.

Notably, stimulating effects of IGFs on the growth of tumor cells have been demonstrated in vitro. Until now, changes of the serum levels in the IGF-system in patients with malignant disease have not been fully elucidated. Strikingly, low IGF-1, -2 and IGFBP-3 serum levels and elevated IGFBP-2 levels in tumor patients have been reported by different authors. The role of ALS is unknown so far [17, 18].

In our prospective study serum levels of the IGF system were analysed in patients with different malignant diseases, in order to evaluate the relationship between this protein family, malignant tumors, metastatic potential and their development during radiotherapy. Since our patients' diagnosis encompass seventeen different malignant diseases, the study group is very heterogeneous and was initially analysed from a general perspective. Subsequently, we differentiated our analyses with respect to the type of cancer. Due to the vast number of different tumor entities some of these subgroups are small.

In our study we could not detect elevated IGF-1 serum levels for patients compared to levels of a healthy control group. This is contrary to our knowledge that endocrine cells react to IGF-1 as a growth factor. Endocrine cells are able to overexpress IGF-1 by an autocrine/paracrine loop [19, 20]. Supposing that the production of IGF-1 by tumor cells is too small to detect an increase of the serum level, the local level might be high enough for an autocrine/paracrine effect with an accelerated tumor growth. However, other authors described increased serum levels and could even correlate the levels with tumor load [21]. We could not confirm these results in our patients.

On the other side serum levels of the IGF-system underlie many different control mechanisms such as growth hormones, nutrition status, liver-, kidney-function and specific tumor therapy [30, 31]. These factors were not taken into account in our study. Patients in a poor general condition and a catabolic status on basis of the malignant disease and the radio-/ chemotherapy can develop a resistance against growth hormones, which results in low serum levels of the IGF-system.

IGF-2 serum levels were only significantly increased in patients with prostate cancer (Fig. 1). Because of a low sensitivity and specificity IGF-2 cannot be used as a tumor marker though. Other authors could also show an elevated serum level in patients with breast cancer even with a correlation to tumor size and a decrease after tumor resection [21]. Another study could show elevated serum levels in patients with a well differentiated colon cancer [22]. However, we found many partially contradictory results for the IGF-2 serum level in patients with a malignant disease. This suggests that just like the IGF-1 serum levels, the IGF-2 serum levels do not correlate with the tumor size, but that other factors influence the findings. The small case numbers in all studies might account for the contradictory results, too.

IGFBP-2 serum levels were significantly elevated in patients with head and neck tumors and patients with prostate cancer in our analyses. Since sensitivity and specificity are 73% and 75%, respectively, IGFBP-2 does not qualify as a tumor marker. Similar to our results other authors could also find elevated serum levels in patients with prostate [9], lung [23], colon cancer

[22], head and neck tumors, nephroblastoma and acute lymphatic leukaemia [17, 24, 25]. In some tumor entities even a correlation with the stadium could be detected. Corresponding to the knowledge that especially malignant lymphatic cells produce IGFBP-2, we could find remarkably high serum levels in patients with leukaemia.

Serum-levels of IGFBP-3 in our patients showed a normal distribution equivalent to the controls. In other studies low serum levels could be detected. Because IGFBP-3 binds the mitogenic IGF-1 and -2, it is supposed to have a protective effect. Correspondingly, it could be shown that high levels of IGFBP-3 are associated with a lower risk for prostate cancer [26, 27]. It has been shown that IGFBP-3 itself can induce apoptosis and can therefore inhibit carcinogenesis [28, 29]. In our study we could not reconfirm these results.

Summing up we could demonstrate that IGF-2 serum levels are slightly elevated in patients with head and neck tumors and with prostate cancer. IGFBP-2 is elevated in patients with head and neck tumors. A difference between metastatic and localized tumor growth could not be detected. Because subgroups were very small in our study, our results can only give an orientation. However, we can conclude that the IGF-system does not seem to be altered very much by the tumors that were analysed in our study. The IGF-associated proteins do not seem to qualify as reliable tumor markers.

References

1. Rinderknecht, E. and R. E. Humbel (1978). "The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin." J Biol Chem 253(8): 2769-76.
2. Rinderknecht, E. and R. E. Humbel (1978). "Primary structure of human insulin-like growth factor II." FEBS Lett 89(2): 283-6.
3. Barreca, A., P. Ponzani, et al. (1995). "Functions and regulation of the acid-labile subunit of the 150 K complex." Prog Growth Factor Res 6(2-4): 231-9.
4. Smith, W. J., L. E. Underwood, et al. (1995). "Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults." J Clin Endocrinol Metab 80(2): 443-9.
5. Twigg, S. M. and R. C. Baxter (1998). "Insulin-like growth factor (IGF)-binding protein 5 forms an alternative ternary complex with IGFs and the acid-labile subunit." J Biol Chem 273(11): 6074-9.
6. Gosteli-Peter, M. A., K. H. Winterhalter, et al. (1994). "Expression and regulation of insulin-like growth factor-I (IGF-I) and IGF-binding protein messenger ribonucleic acid levels in tissues of hypophysectomized rats infused with IGF-I and growth hormone." Endocrinology 135(6): 2558-67.
7. Han, V. K., A. J. D'Ercole, et al. (1987). "Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus." Science 236(4798): 193-7.
8. Scott, J., J. Cowell, et al. (1985). "Insulin-like growth factor-II gene expression in Wilms' tumour and embryonic tissues." Nature 317(6034): 260-2.
9. Cohen, P., D. M. Peehl, et al. (1993). "Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients." J Clin Endocrinol Metab 76(4): 1031-5.
10. Elmlinger, M. W., U. Rauschnabel, et al. (1999). "Secretion of noncomplexed 'Big' (10-18 kD) forms of insulin-like growth factor-II by 12 soft tissue sarcoma cell lines." Horm Res 52(4): 178-85.
11. Hoflich, A., H. Lahm, et al. (1998). "Insulin-like growth factor-binding protein-2 inhibits proliferation of human embryonic kidney fibroblasts and of IGF-responsive colon carcinoma cell lines." FEBS Lett 434(3): 329-34.
12. Wang, H., W. Shen, et al. (2003). "Insulin-like growth factor binding protein 2 enhances glioblastoma invasion by activating invasion-enhancing genes." Cancer Res 63(15): 4315-21.
13. Frommer, K. W., K. Reichenmiller, et al. (2006). "IGF-independent effects of IGFBP-2 on the human breast cancer cell line Hs578T." J Mol Endocrinol 37(1): 13-23.
14. Menouny, M., M. Binoux, et al. (1998). "IGFBP-2 expression in a human cell line is associated with increased IGFBP-3 proteolysis, decreased IGFBP-1 expression and increased tumorigenicity." Int J Cancer 77(6): 874-9.
15. Baxter RC, Martin JL, Tyler MI, Howden ME. Growth hormone-dependent insulin-like growth factor (IGF) binding protein from human plasma differs from other human IGF binding proteins. Biochem Biophys Res Commun. 1986 Sep 30;139(3):1256-61.
16. Daughaday WH, Rotwein P.Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. Endocr Rev. 1989 Feb;10(1):68-91.
17. Mohnike, K. L., U. Kluba, et al. (1996). "Serum levels of insulin-like growth factor-I, -II and insulin-like growth factor binding proteins -2 and -3 in children with acute lymphoblastic leukaemia." Eur J Pediatr 155(2): 81-6.
18. Ohlsson, C., B. A. Bengtsson, et al. (1998). "Growth hormone and bone." Endocr Rev 19(1): 55-79.
19. Yee, D. (1994). "The insulin-like growth factor system as a target in breast cancer." Breast Cancer Res Treat 32(1): 85-95.
20. Schutt, B. S., M. Langkamp, et al. (2004). "Integrin-mediated action of insulin-like growth factor binding protein-2 in tumor cells." J Mol Endocrinol 32(3): 859-68.
21. Holdaway, I. M., A. E. Lethaby, et al. (2001). "Effect of breast surgery on serum levels of insulin-like growth factors (IGF-I, IGF-II, and IGF binding protein-3) in women with benign and malignant breast lesions." Ann Surg Oncol 8(1): 25-31.
22. Renehan, A. G., J. Jones, et al. (2000). "Elevated serum insulin-like growth factor (IGF)-II and IGF binding protein-2 in patients with colorectal cancer." Br J Cancer 83(10): 1344-50.
23. Lee, D. Y., S. J. Kim, et al. (1999). "Serum insulin-like growth factor (IGF)-I and IGF-binding proteins in lung cancer patients." J Korean Med Sci 14(4): 401-4.
24. Zhan, S., D. N. Shapiro, et al. (1994). "Activation of an imprinted allele of the insulin-like growth factor II gene implicated in rhabdomyosarcoma." J Clin Invest 94(1): 445-8.
25. Elmlinger, M. W., K. Wimmer, et al. (1996). "Insulin-like growth factor binding protein 2 is differentially expressed in leukaemic B- and T-cell lines." Growth Regul 6(3): 152-7.
26. Kanety, H., Y. Madjar, et al. (1993). "Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen." J Clin Endocrinol Metab 77(1): 229-33.
27. Liu, F., D. R. Powell, et al. (1991). "Insulin-like growth factors (IGFs) and IGF-binding proteins in the developing rhesus monkey." J Clin Endocrinol Metab 72(4): 905-11.
28. Rajah, R., B. Valentinis, et al. (1997). "Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism." J Biol Chem 272(18): 12181-8.

29. Rajah, R., K. W. Lee, et al. (2002). "Insulin-like growth factor binding protein-3 mediates tumor necrosis factor-alpha-induced apoptosis: role of Bcl-2 phosphorylation." Cell Growth Differ 13(4): 163-71.
30. Dai, J. and R. C. Baxter (1994). "Regulation in vivo of the acid-labile subunit of the rat serum insulin-like growth factor-binding protein complex." Endocrinology 135(6): 2335-41.
31. Brennan, B. M., M. Gill, et al. (1999). "Insulin-like growth factor I, IGF binding protein 3, and IGFBP protease activity: relation to anthropometric indices in solid tumours or leukaemia." Arch Dis Child 80(3): 226-30.
32. Halasova E, Adamkov M, et al (2010) "A. Lungcancer incidence and survival in chromium exposed individuals with respect toexpression of anti-apoptotic protein survivin and tumor suppressor P53 protein". Eur J Med Res. 15 (8):55-9.
33. Bölke E, Peiper M, et al. (2010) "Extranodal diffuse non hodgkin lymphoma in the thigh" Eur J Med Res. 15(8): 367-8
34. Juntermanns B, Radunz S et al. (2010). "Tumor markers as a diagnostic key for hilar cholangiocarcinoma". Eur J Med Res.15(8):357-61
35. Schuler PJ, Trellakis S et al. (2010) "In vitro chemosensitivity of head and neck cancer cell lines" Eur J Med Res. 15(8):337-44

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Address for correspondence:
PD. Dr. med. Edwin Bölke,
Universitätsklinikum Düsseldorf,
Heinrich Heine Universität,
Moorenstrasse 5,
40225 Duesseldorf,
Germany,
Tel.: +49-221-81-17990
Fax: +49-211-81-17051
Email: boelke@med.uni-duesseldorf.de