

# Expression of insulin-like growth factors and their receptors in malignant fibrous histiocytoma of soft tissues

Research Article

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**Key Words:** Malignant fibrous histiocytoma, soft tissue, immunohistochemistry, insulin-like growth factor, insulin-like growth factor receptor

**Abbreviations:** insulin-like growth factor (IGF), malignant fibrous histiocytoma (MFH), insulin-like growth factor receptor (IGF-R)

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## Summary

The insulin-like growth factor (IGF) family of ligands and receptors is an important cell growth factor involved in the development and progression of a variety of malignant tumors. Little information is currently available regarding the expression of IGFs and their receptors in soft tissue tumors of a fibrohistiocytic origin. We investigated the expression of IGFs and their receptors in 43 malignant fibrous histiocytoma (MFH) tissue specimens using immunohistochemical techniques. Furthermore, we examined the correlation between the IGF receptor (IGF-R) expression and proliferative activity assessed by MIB-1 immunostaining and mitotic indices. Positive cytoplasmic immunoreactivity for IGF-1 and IGF-2 was identified in tumor cells of all MFHs studied. Positive cytoplasmic immunoreactivity for IGF-1R and IGF-2R was identified in tumor cells of 6 (19%) and 30 (70%) respectively of the MFHs. There was no significant difference in the MIB-1 or mitotic indices between the IGF-1R positive and negative groups. There was no significant difference in the MIB-1 or mitotic indices between the IGF-2R positive and negative groups. The results might indicate that, in most MFHs, any direct autocrine effects of this ligand/receptor system on cell growth regulation are precluded. Further studies on the role of other IGF family members are required to determine the tumor growth mechanism of MFH.

## I. Introduction

Insulin-like growth factors (IGF) are polypeptide hormones which regulate the cell growth of normal and neoplastic tissues *in vivo* and *in vitro* (Zapf et al, 1978; Van Buul-Offers and Van den Brande 1981). IGFs are secreted in the liver and in a variety of other tissues (Froesch and Zapf, 1985). IGF-1 and IGF-2 are structurally related, and both ligands bind with different affinities to the IGF-1 receptor (IGF-1R). In addition, IGF-1R is known to mediate the variable biologic effects of both ligands through a tyrosine kinase activity (Ullrich et al, 1986). IGF-2 binds to the IGF-2 receptor (IGF-2R), but IGF-2 does not bind with high affinity to the IGF-1R. IGF-2R is a single transmembrane polypeptide which does not possess tyrosine kinase activity (Morgan et al, 1987). Previous studies have shown that both IGF-1 and IGF-2 are overexpressed in a variety of tumor cell lines and

tissues of epithelial and mesenchymal origin, and are involved in the promotion of cell growth, differentiation and motility (Cullen et al, 1991; LeRoith and Roberts, 2003). However, little information is currently available on the prevalence and distribution of IGFs and their receptors in soft tissue tumors with a fibrohistiocytic origin, including malignant fibrous histiocytoma (MFH) (Sekyi-Otu et al, 1995). Malignant fibrous histiocytoma is one of the most common soft tissue sarcomas in adult life, and has an aggressive behavior and a high metastatic potential (Enzinger and Weiss, 1995). The aims of the present study were to investigate the endogenous expression of IGF-1, IGF-2 and their receptors in human MFH tissues using immunohistochemical techniques, and discuss its significance in tumor progression. Furthermore, we aimed to examine the correlation between the IGF-Rs

expression in MFHs and proliferative activity assessed by mitotic indices and MIB-1 immunohistochemical staining.

## II. Materials and methods

### A. Tissue samples

Tissue samples from 43 cases of soft tissue MFH were selected from the files of the pathology departments in the current authors' institutes. All the specimens were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. There were 21 female and 22 male patients. The patients ranged in age from 17 to 83 years (mean, 61 years). The tumors were located in the upper extremity (n=10), lower extremity (n=28), and in the trunk (n=5). The specimens were obtained from biopsies or resections prior to chemotherapy. Four-micrometer serial sections were prepared for hematoxylin and eosin staining, and for immunohistochemical studies. After evaluating the specimens stained with hematoxylin and eosin, all cases were confirmed to conform to the diagnostic histologic criteria of MFH proposed by Enzinger and Weiss (Enzinger and Weiss, 1995). The specimens were classified based on their histology into 31 spindle-pleomorphic, 8 myxoid, 2 inflammatory, and 2 giant cell MFHs according to Enzinger and Weiss's classification (Enzinger and Weiss, 1995). In each specimen, the mitotic index was scored by observing 10 high power fields (HPF) of viable tumor tissue areas using a standard light microscope equipped with a 40X objective.

### B. Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections by the indirect immunoperoxidase method. Briefly, the sections were deparaffinized with xylene and routinely dehydrated through a series of graded alcohols. Antigen unmasking in the sections was performed by autoclaving pretreatment for 15 min. Following elimination of endogenous peroxidase activity with a 10-min incubation in 3% H<sub>2</sub>O<sub>2</sub>, the sections were incubated at 4°C overnight with primary antibodies against IGF-2 (monoclonal, 1:50, Genzyme-Techne, Minneapolis, MN, USA), IGF-1R (monoclonal, 1:50, Quertett GMBH, Berlin, Germany), and MIB-1 (monoclonal, prediluted, Dako Japan, Japan), or at room temperature for one hour with primary antibody against IGF-1 (monoclonal, 1:50, Upstate Biotechnology, Lake Placid, NY, USA) and IGF-2R (polyclonal, 1:80, Santa Cruz Biotechnology, Santa Cruz, CA, USA). After washing with Triton-X-100/trisbuffered saline (TBS), for IGF-1, IGF-1R and MIB1 immunostaining, the sections were then incubated at room temperature for 40 min with goat antimouse immunoglobulins conjugated to peroxidase-labeled dextran polymer (EnVision<sup>+</sup>, HRP<sup>TM</sup>, Dako Japan). For IGF-2 and IGF-2R immunostaining, the sections were developed using the streptavidine-biotin-peroxidase complex technique (Dako LSAB+kit/HRP, Dako Japan). 3,3-diaminobenzidine was used for color development, and the sections were counterstained with hematoxylin. Negative controls were obtained by substituting the primary antibodies with non-immune mouse serum.

### C. Evaluation of immunohistochemistry

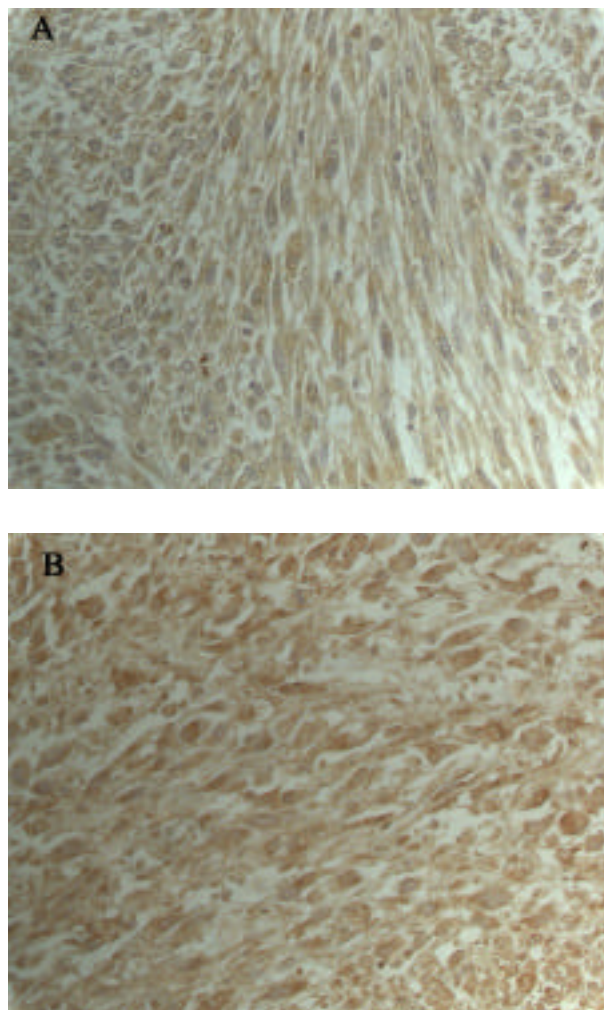
A semi-quantitative system was employed to evaluate the level of antigen expression: immunoreactivity was scored as either negative (0), focal (1+; less than 10% of positive tumor cells), moderate (2+; 10-50% of positive tumor cells), or diffuse (3+; more than 50% of positive tumor cells). The percentage of MIB-1 positive cells (MIB-1 proliferative index) was determined by examining 20 HPFs in representative areas. In each case, at

least 500 viable tumor cells were counted. The specimens were evaluated by two observers.

The statistical significance of the individual findings and their association indices were evaluated by Mann-Whitney U test. Probability (P) values less than 0.05 were considered to be significant.

## III. Results

The results of immunohistochemical staining for IGFs and their receptors are shown in **Table 1**. Positive cytoplasmic immunoreactivity for IGF-1 and IGF-2 was identified in tumor cells of all 43 MFH cases analyzed (**Figure 1**). All the MFHs coexpressed both ligands. Most MFHs showed intense and diffuse immunoreactivity for both ligands (IGF-1: 93%, IGF-2: 91%). Most MFHs analyzed (81%) showed negative immunoreactivity for IGF-1R. Only six of the 43 cases (19%) showed focally positive immunoreactivity for IGF-1R, and number of positive tumor cells was very limited. Positive immunoreactivity for IGF-2R was identified in tumor cells of 30 (70%) of the 43 MFHs studied. Coexpression of IGF-1R and IGF-2 was observed in five cases (12%).



**Figure 1** Tumor cells of MFH show diffusely positive immunoreactivity for IGF-1 (A) and IGF-2 (B) (immunostain, original magnification x 400).

**Table 1.** Expression of IGFs and their receptors in soft tissue MFH

staining	IGF-1	IGF-2	IGF-1R	IGF-2R
number of cases (%)				
0	0 (0%)	0 (0%)	37 (81%)	13 (30%)
1+	0 (0%)	1 (2%)	6 (19%)	12 (28%)
2+	3 (7%)	3 (7%)	0 (0%)	7 (16%)
3+	40 (93%)	39 (91%)	0 (0%)	11 (26%)

Total: n=43

IGF: insulin-like growth factor, MFH: malignant fibrous histiocytoma

Overall, in the MFH cases analyzed, the MIB-1 proliferative indices ranged from 2.5 to 74% (mean; 26.0). Because all the MFHs expressed both ligands, the cases were divided into two groups: those with the receptors negative and those with the receptors positive. The mean MIB-1 index was 23.5% in the IGF-1R negative cases and 39.7% in the positive cases. The mean MIB-1 index was 18.3% in the IGF-2R negative cases and 29.8% in the positive cases. There were no significant differences in the MIB-1 indices between the IGF-1R or IGF-2R negative and positive cases. Overall, the mitotic indices ranged from one to 37 per 10 HPFs (mean; 8.1). The mean mitotic index was 7.8 in the IGF-1R negative cases and 9.7 in the positive cases. The mean mitotic index was 6.0 in the IGF-2R negative cases and 9.0 in the positive cases. There were no significant differences in the mitotic indices between the IGF-1R or IGF-2R negative and positive cases.

#### IV. Discussion

There have been many *in vivo* and *in vitro* studies indicating that IGFs regulate a variety of epithelial and mesenchymal cells, and influence the development and progression of human malignant neoplasms (Cullen et al, 1991; LeRoith et al, 1995; Zumkeller et al, 1996). It has been suggested that this receptor/ligand system might play an important role in tumor growth regulation in an autocrine or paracrine manner. Many investigators demonstrated expression of IGFs and their receptors in various tumor tissues and tumor cell lines of mesenchymal origin (Blatt et al, 1984; Burrow et al, 1998; El-Badry et al, 1990; Toretsky et al, 2001; Van der Ven et al, 1997, van Valen et al, 1992; Yun, 1992).

Using *in situ* hybridization, Minniti et al. demonstrated that all embryonal and alveolar rhabdomyosarcoma specimens examined expressed the gene for IGF-2 and this expression was localized to the tumor cells themselves (Minniti et al, 1994). El-Badry et al. showed that most rhabdomyosarcoma tissues express high levels of IGF-2 mRNA and IGF-1R mRNA, and exogenous IGF-2 was able to stimulate cellular motility in rhabdomyosarcoma cell lines (El-Brady et al, 1990). In addition, Stewart et al. found that, in muscle cell lines, autocrine secretion of IGF-2 plays a critical role in stimulating spontaneous myogenic differentiation *in vitro* (Stewart et al, 1886), while other investigators have found that high levels of IGF-1 RNAs and IGF-2 RNAs were detected in leiomyomas and leiomyosarcomas, compared

to those of normal smooth muscle tissues of the uterus (Hoppener et al, 1988; Gloude-mans et al, 1990). Xie et al. reported that IGF-1R was detectable by Western blot analysis in 18 of 35 synovial sarcomas examined, and the IGF-1R positive tumors were associated with a high incidence of lung metastasis and a high tumor cell proliferative rate (Xie et al, 1999). Sekyi-Otu et al. evaluated expression of IGF-1R, IGF-1 and IGF-2 in a variety of bone and soft tissue sarcomas by reverse-transcription polymerase chain reaction (RT-PCR), and found that IGF-1R, IGF-1 and IGF-2 were expressed in 41%, 79%, and 63% of sarcomas examined, respectively (Sekyi-Out et al, 1995). Roholl et al. showed that most leiomyosarcomas, malignant schwannomas, and synovial sarcomas immunohistochemically expressed IGF-1 (Roholl et al, 1990). Pollak et al. reported that the IGF-1R in human MG-63 osteosarcoma cells and IGF-1 is a potent stimulator of proliferation for MG-63 cells *in vitro* (Pollak et al, 1990). Several other investigators also demonstrated expression of IGF-1, IGF-2 and their receptors in many osteosarcoma cell lines and tissues, suggesting that autocrine stimulation might be an important mechanism for stimulation of osteosarcoma proliferation (Blatt et al, 1984; Mohan et al, 1990; Fournier et al, 1993; Burrow et al, 1998).

However, there have been only a few reports on the prevalence and distribution of IGFs and their receptors in soft tissue tumors of a fibrohistiocytic origin including MFH. Roholl et al. reported that 10 of 18 MFHs analyzed expressed IGF-1, and suggested that positive immunoreactivity for IGF-1 in the 10 MFHs appeared to be related to the coexpression of smooth muscle actin (Roholl et al, 1990). Sekyi-Otu et al. evaluated five MFHs for the ligands and IGF-1R by RT-PCR and found that four and two of the MFHs studied expressed high levels of IGF-1 and IGF-1R, respectively, but levels of IGF-2 expression were low in all the MFHs, compared with control cell lines (Sekyi-Otu et al, 1995).

In the present study, we immunohistochemically examined the expression of IGFs and their receptors in the human MFH tissue specimens. Our data revealed that, of 43 MFH cases studied, all cases showed positive immunoreactivity for both IGF-1 and IGF-2. More than 90% of the MFHs showed intense and diffuse immunoreactivity for both ligands. In contrast, only 19% of the MFHs studied showed positive immunoreactivity for IGF-1R, and staining was weak and focal. There were no significant differences in the MIB-1 indices or mitotic indices between IGF-1R negative and positive groups.

Most of the actions of both IGF-1 and IGF-2 are mediated by their activation of the IGF-R (Roberts, 1996). These results might preclude the possibility of any direct autocrine effects of IGF-1 or IGF-2 through IGF-1R on the tumor cell growth in most MFHs.

Our results showed that IGF-2R was expressed in 70% of the MFHs studied. Recent studies have shown that the IGF-2R has tumor suppression function, while ligand binding to IGF-1R provokes mitogenic and anti-apoptotic effects (Pavelic et al, 2002; LeRoith and Roberts, 2003). Chen et al. reported that decreased expression of IGF-2R promotes growth of breast cancer cells (Chen et al, 2002). However, in the present study, there were no significant differences in the MIB-1 and mitotic indices between IGF-2R negative and positive MFH groups.

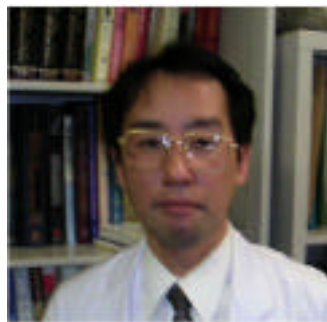
In MFH, there have been some reports on autocrine/paracrine stimulation systems of growth factor receptors by their ligands. It has been suggested that autocrine/paracrine growth stimulation is related to the cell proliferation of MFH through platelet-derived growth factor (PDGF) receptors (Taniuchi et al, 1997; Abdiu et al, 1998). The current authors have found that the transforming growth factor (TGF)- ligand/receptor system plays an important role in promoting cell proliferation of MFH (unpublished data). Nakatani et al. reported a MFH cell line expressed both stem cell factor (SCF) and c-kit (Nakatani et al, 2003).

In summary, we analyzed the expression of IGFs and their receptors in MFH tissue specimens using immunohistochemical techniques. All MFH cases analyzed demonstrated positive immunoreactivity for both ligands but 81% of the cases were negative for IGF-1R. Because most of the actions of both IGF-1 and IGF-2 on promoting cell growth are mediated by their activation of the IGF-R, the results indicate that any direct autocrine effects of this ligand/receptor system on cell growth regulation are precluded in most MFH cases. There was no significant difference of the cell proliferative indices between IGF-1R positive and negative groups. Further studies on the role of other cell growth factor are required to determine the tumor growth of MFH.

## References

- Abdiu A, Walz TM, Nishikawa BK, Wingren S, Larsson SE, Wasteson A (1998) Human malignant fibrous histiocytomas in vitro: growth characteristics and their association with expression of mRNA for platelet-derived growth factor, transforming growth factor-alpha and their receptors. **Eur J Cancer** 34, 2094-2100.
- Blatt J, White C, Dienes S, Friedman H, Foley TP Jr (1984) Production of an insulin-like growth factor by osteosarcoma. **Biochem Biophys Res Commun** 123, 373-376.
- Burrow S, Andrulis IL, Pollak M, Bell RS (1998) Expression of insulin-like growth factor receptor, IGF-1, and IGF-2 in primary and metastatic osteosarcoma. **J Surg Oncol** 69, 21-27.
- Chen Z, Ge Y, Landman N, Kang JX (2002) Decreased expression of the mannose 6-phosphate/insulin-like growth factor-II receptor promotes growth of human breast cancer cells. **BMC Cancer** 2, 18.
- Cullen KJ, Yee D, Rosen N (1991) Insulinlike growth factors in human malignancy. **Cancer Invest** 9, 443-454.
- El-Badry OM, Minniti C, Kohn EC, Houghton PJ, Daughaday WH, Helman LJ (1990) Insulin-like growth factor II acts as an autocrine growth and motility factor in human rhabdomyosarcoma tumors. **Cell Growth Differ** 1, 325-331.
- Enzinger FM, Weiss SW (1995) Malignant fibrohistiocytic tumors. In: Soft tissue tumors. (Enzinger FM, Weiss SW, eds) St. Louis, Mosby-Year Book Inc., 351-180.
- Fournier B, Ferralli JM, Price PA, Schlaeppi JM (1993) Comparison of the effects of insulin-like growth factors-I and -II on the human osteosarcoma cell line OHS-4. **J Endocrinol** 136, 173-180.
- Froesch ER, Zapf J (1985) Insulin-like growth factors and insulin: comparative aspects. **Diabetologia** 28, 485-493.
- Gloudemans T, Prinsen I, Van Unnik JA, Lips CJ, Den Otter W, Sussenbach JS (1990) Insulin-like growth factor gene expression in human smooth muscle tumors. **Cancer Res** 50, 6689-6695.
- Hirschfeld S, Helman L (1994) Diverse roles of insulin-like growth factors in pediatric solid tumors. **In Vivo** 8, 81-90.
- Hoppener JW, Mosselman S, Roholl PJ, Lambrechts C, Slebos RJ, de Pagter-Holthuizen P, Lips CJ, Jansz HS, Sussenbach JS (1988) Expression of insulin-like growth factor-I and -II genes in human smooth muscle tumours. **EMBO J** 7, 1379-1385.
- LeRoith D, Roberts CT Jr (2003) The insulin-like growth factor system and cancer. **Cancer Lett** 195, 127-137.
- LeRoith D, Werner H, Neuenschwander S, Kalebic T, Helman LJ (1995) The role of the insulin-like growth factor-I receptor in cancer. **Ann NY Acad Sci** 766, 402-408.
- Minniti CP, Tsokos M, Newton WA Jr, Helman LJ (1994) Specific expression of insulin-like growth factor-II in rhabdomyosarcoma tumor cells. **Am J Clin Pathol** 101, 198-203.
- Mohan S, Bautista CM, Herring SJ, Linkhart TA, Baylink DJ (1990) Development of valid methods to measure insulin-like growth factors-I and -II in bone cell-conditioned medium. **Endocrinology** 126, 2534-2542.
- Morgan DO, Edman JC, Standring DN, Fried VA, Smith MC, Roth RA, Rutter WJ (1987) Insulin-like growth factor II receptor as a multifunctional binding protein. **Nature** 329, 301-307.
- Nakatani T, Marui T, Yamamoto T, Hitora T, Akisue T, Kawamoto T, Nagira K,
- Fujita I, Matsumoto K, Yoshiya S, Kurosaka M (2003) Expression of stem cell factor and c-kit in human malignant fibrous histiocytoma cell line (TNMY1). **Anticancer Res** 23, 2329-2333.
- Pavelic K, Bukovic D, Pavelic J (2002) The role of insulin-like growth factor 2 and its receptors in human tumors. **Mol Med** 8, 771-780.
- Pollak MN, Polychronakos C, Richard M (1990) Insulinlike growth factor I: a potent mitogen for human osteogenic sarcoma. **J Natl Cancer Inst** 82, 301-305.
- Roberts CT (1996) Control of insulin-like growth factor (IGF) action by regulation of IGF-I receptor expression. **Endocr J** 43 Suppl: 49-55.
- Roholl PJ, Skottner A, Prinsen I, Lips CJ, Den Otter W, Van Unnik JA (1990) Expression of insulin-like growth factor I in sarcomas. **Histopathology** 16, 455-460.
- Sekyi-Otu A, Bell RS, Ohashi C, Pollak M, Andrulis IL (1995) Insulin-like growth factor 1 (IGF-1) receptors, IGF-1, and IGF-2 are expressed in primary human sarcomas. **Cancer Res** 55, 129-134.
- Stewart CE, James PL, Fant ME, Rotwein P (1996) Overexpression of insulin-like growth factor-II induces accelerated myoblast differentiation. **J Cell Physiol** 169, 23-32.

- Taniuchi K, Yamada Y, Nonomura A, Takehara K (1997) Immunohistochemical analysis of platelet-derived growth factor and its receptors in fibrohistiocytic tumors. **J Cutan Pathol** 24, 393-397.
- Toretsky JA, Steinberg SM, Thakar M, Counts D, Pironis B, Parente C, Eskenazi A, Helman L, Wexler LH (2001) Insulin-like growth factor type 1 (IGF-1) and IGF binding protein-3 in patients with Ewing sarcoma family of tumors. **Cancer** 92, 2941-2947.
- Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, et al. (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. **EMBO J** 5, 2503-2512.
- van Buul-Offers S, Van den Brande JL (1981) The growth of different organs of normal and dwarfed Snell mice, before and during growth hormone therapy. **Acta Endocrinol (Copenh)** 96, 46-58.
- Van der Ven LT, Roholl PJ, Gloudemans T, Van Buul-Offers SC, Welters MJ, Bladergroen BA, Faber JA, Sussenbach JS, Den Otter W (1997) Expression of insulin-like growth factors (IGFs), their receptors and IGF binding protein-3 in normal, benign and malignant smooth muscle tissues. **Br J Cancer** 75, 1631-1640.
- van Valen F, Winkelmann W, Jurgens H (1992) Type I and type II insulin-like growth factor receptors and their function in human Ewing's sarcoma cells. **J Cancer Res Clin Oncol** 118,269-275.
- Xie Y, Skytting B, Nilsson G, Brodin B, Larsson O (1999) Expression of insulin-like growth factor-I receptor in synovial sarcoma: association with an aggressive phenotype. **Cancer Res** 59, 3588-3591.
- Yun K (1992) A new marker for rhabdomyosarcoma. Insulin-like growth factor II. **Lab Invest** 67, 653-664.
- Zapf J, Schoenle E, Froesch ER (1978) Insulin-like growth factors I and II: some biological actions and receptor binding characteristics of two purified constituents of nonsuppressible insulin-like activity of human serum. **Eur J Biochem** 87, 285-296.
- Zumkeller W, Groth O, Commentz J (1996) Regulation of insulin-like growth factors and IGF-binding proteins in bone tumours. **Growth Regul** 6, 10-15.



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