Reduced Growth of Human Sarcoma Xenografts in Hosts Homozygous for the *lit* Mutation

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Background and Objectives: Prior studies have shown that sarcoma growth can be stimulated by insulin-like growth factor-I (IGF-I). To extend this line of research, we carried out in vivo growth studies of primary human sarcoma in immunosuppressed control and IGF-I–deficient mice.

Methods: Human sarcoma specimens (one osteosarcoma and seven soft tissue sarcomas) were harvested in the operating room and implanted in immunosuppressed mice. Second-generation sarcomas were transplanted to control (GH replete *lit*/+ mice) and to experimental (GH/IGF-I–deficient *lit*/*lit*) animals. When tumors reached 1,000 mm^3 in one group, average tumor size was compared in the two groups. IGF-I receptor expression was measured by RT-PCR and IGF-I receptor binding sites were assayed by radiolabeled IGF-I.

Results: Five of eight sarcomas demonstrated reduced growth in the GH/IGF-I–deficient *lit*/*lit* animals. In four of the five sarcomas that demonstrated growth inhibition, IGF-R was elevated relative to placenta or a positive control cell line (MCF-7, which is known to be responsive to IGF-I in vitro and in vivo). In three of the five sarcomas that demonstrated growth suppression, IGF-R was elevated twofold after implantation in the experimental IGF-I–deficient animals.

Conclusions: The GH–IGF axis may be an important stimulator of tumor growth in sarcomas. These experiments suggest that IGF suppression may inhibit sarcoma growth in vivo.

KEY WORDS: growth factor; animal models; insulin-like growth factor

INTRODUCTION

The observation that insulin-like growth factor-I (IGF-I) is a potent mitogen for osteosarcoma cell lines in vitro initiated this group's interest in the IGF signaling pathway in bone and soft tissue sarcoma [1]. Using the Massachusetts General Hospital-Osteogenic Sarcoma (MGH-OGS) murine osteosarcoma as an in vivo model, it was shown that the tumor had high specific binding of IGF-I, that it responded to IGF-I as a mitogen in vitro, and that tumor growth and metastases in this model were inhibited by implantation in mice that had undergone hypophysectomy (lowering circulating IGF-I to less than 20% of normal) [2]. Similar results were observed in a second murine sarcoma model (RIF-1); experiments in this murine model showed that metastatic potential was partially restored when hypophysectomized mice were treated with human growth hormone (GH) [3].

A large proportion of human bone and soft tissue sarcomas express IGF receptor and ligand at a similar level to that in cell lines known to be responsive to IGF in vitro [4,5]. The current experiments were designed

to determine whether human sarcomas implanted in immunosuppressed murine hosts deficient for serum IGF-I would have a growth rate that differed from tumors implanted in animals with normal serum IGF-I.

MATERIALS AND METHODS

Fresh sarcoma specimens were obtained in the operating room, and adjacent frozen sections were examined to ensure that the specimens were viable. The specimens were processed for transplantation by mincing in Dulbecco's modified Eagle's medium (DMEM), which was chilled on ice. The first-generation tumors were implanted in NOD/LtSz-scid (nod-scid) hosts [6] and allowed to grow to 500-mm^3 size before being used as source tumor for the experiments described below. These first-generation source tumors were minced in chilled DMEM to 1-mm size; fragments were injected subcutaneously into B6:CB17-Ghrhr^*lit*Prkdc^scid mice. All mice were homozygous for scid (i.e., scid/scid) while segregating for *lit* (i.e., *lit/lit* or *lit/+*).

lit/lit animals are homozygous for a mutation in the growth hormone–releasing hormone receptor. These animals are growth retarded and have serum IGF-I levels that average about 5% of the serum levels of *lit/+* mice [7]. In the present experiments, 8-week-old littermates were used in the experimental (GH/IGF–deficient *lit/lit*) and control (GH replete *lit/+*) groups. To minimize the immune response to tumor transplantation in the animals, the experimental and control mice received sublethal total body irradiation before implantation. After implantation, both groups of animals received weekly injections of anti-Asialo anti-natural killer (NK) cell polyclonal antibody, as previously described [8].

Tumor volumes were measured with calipers, three times weekly, in both groups. Volume was estimated by assuming spherical proportions for the lesions. Measurements were continued until tumor size in one or more animals reached 1,000 mm^3 in either group. The size of tumors in the two groups was then compared using the Kruskal-Wallis test.

At the original tumor harvest in the operating room, specimens were obtained for quantitation of type I IGF-I receptor, using either measurement of gene expression or determination of membrane receptor binding, or both. IGF-R expression was determined by semiquantitative polymerase chain reaction (PCR) as previously described [4,5]. Levels of IGF-R expression were compared with the MCF-7 cell line, which is known to be responsive to exogenous IGF-I in vitro. Membrane binding of IGF ligand was undertaken as described previously [1,2]. Human placenta was used as a positive control for membrane binding. In each case, receptor levels were assayed a second time after the specimens had been harvested

from control and experimental animals. At harvesting, histological sampling was used to ensure that the tumor remained viable.

RESULTS

Eight different high-grade human sarcomas (one osteosarcoma and one pleomorphic liposarcoma, five malignant fibrous histiocytomas, and one malignant peripheral nerve sheath tumor) were tested. In five of these sarcomas, growth in the GH/IGF-I–deficient *lit/lit* experimental group was significantly slower than growth in the GH replete *lit/+* control animals (Figs. 1 and 2). In three of the sarcomas, growth was the same in both groups. These results are summarized in Table I.

In four of the five tumors that demonstrated significant growth retardation in the GH/IGF-I–deficient *lit/lit* experimental group, type 1 IGF-I receptor expression was equal to or greater than expression in the positive controls (MCF-7). In one of these five sarcomas (osteosarcoma 1), IGF-I receptor expression was lower than MCF-7.

In the three sarcomas that failed to demonstrate growth retardation in the GH/IGH–deficient animals, two had receptor levels lower than positive control values. In one case (malignant peripheral nerve sheath tumor) receptor binding was 130% of positive control placenta levels and the sarcoma failed to demonstrate growth inhibition.

In three sarcomas (all of which were growth inhibited in homozygote animals, i.e, liposarcoma, MFH-2, MFH-6), expression of IGF-I receptor was higher after implantation in the IGF–deficient animals compared with implantation in control animals. These results raise the possibility that expression of IGF-I receptor was increased in the experimental animals in response to lower circulating serum IGF-I.
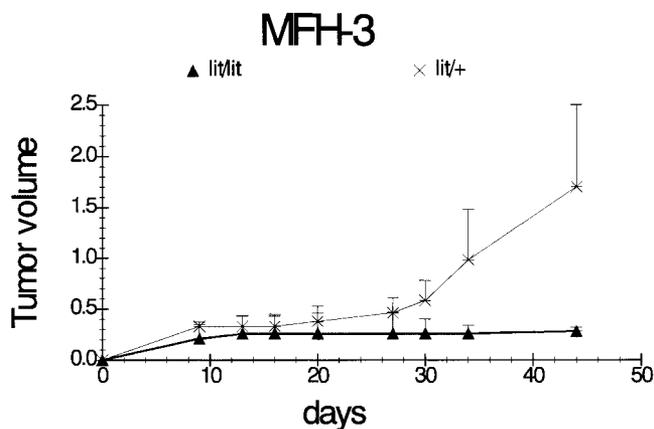

Fig. 1. Growth curve comparing tumor volume of a malignant fibrous histiocytoma implanted in *lit/lit* and *lit/+* mice.
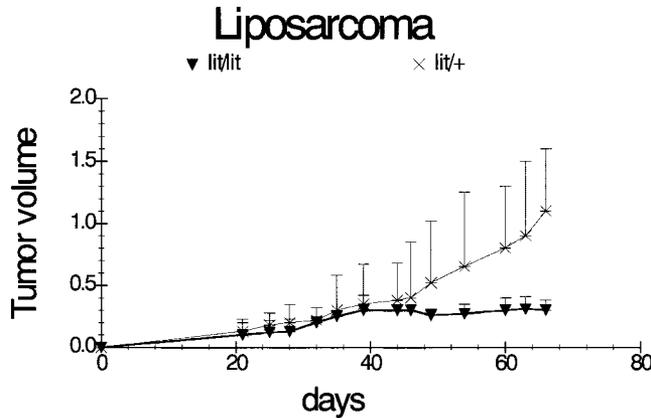
Liposarcoma

▼ lit/lit × lit/+

Fig. 2. Growth curve comparing tumor volume of a liposarcoma implanted in *lit/lit* and *lit/+* mice.

DISCUSSION

Prior investigations have demonstrated that the IGF signal transduction system is an important factor in several different mechanisms of mammalian cell transformation to a malignant phenotype and in the development of human cancers [9–12]. Cells derived from mouse embryos with a targeted disruption of the IGF-I receptor gene cannot be transformed by the SV40 T antigen, activated Ha-ras, bovine papillovirus, v-src, Raf, or overexpressed platelet-derived growth factor (PDGF) [13–17]. This resistance of receptor-negative cells to transformation can be reversed by transfection of a plasmid expressing wild-type type 1 IGF-I receptor (IGF-R) c-DNA [9]. Similar experiments have demonstrated that tumorgenicity of a rat glioblastoma model was inhibited by stable transfection with an anti-sense IGF-R c-DNA construct [9]. The strategy of transfecting tumor cell lines with IGF-IR anti-sense c-DNA is also effective

in inhibiting growth in MCF-7 breast cancer lines [18]. In addition to its role in promoting carcinogenesis, the IGF system is likely also important in preventing spontaneous and drug induced apoptosis [19–22]. These studies have all suggested that IGF signal transduction is important for development and maintenance of the transformed phenotype by a variety of different mechanisms.

Elevated levels of serum IGF-I levels have been shown to be a risk factor for the development of prostate [23], lung [24], colorectal [25], and breast cancer [26]. The level of free IGF-I in the tumor microenvironment has been associated with tumor progression in breast cancer [27] and colorectal cancer.

Control of expression of the IGF-I receptor may be an important mechanism in the development of the transformed phenotype. The type I IGF-I receptor promoter is suppressed by both the *p53* and *WT1* tumor suppressor genes [28,29] and is activated by the oncogenic EWS-WT1 fusion protein found in desmoplastic round cell tumors [4]. In addition to the proliferative effect of the IGF signal transduction system, IGF-I has been shown to activate expression of VEG-F in colorectal cell lines [30], potentially accounting for the lower risk of tumor metastases seen in some experimental models with serum IGF-I suppression [2,3].

Experiments that have tested the effect of IGF suppression on the growth of tumors in vivo have generally used established cell lines [7]. Pinski et al. [31] used osteosarcoma and Ewing's sarcoma cell lines implanted in immunosuppressed animals to show that tumor growth could be inhibited by a somatostatin analogue; a similar approach was used in prostate cancer cell lines by Lamharzi et al. [32]. Conzemius et al. [33] showed that administration of either recombinant GH or somatostatin enhanced the effect of carboplatin in treating orthotopic osteosarcoma in rats. Butler et al. [34] demonstrated that

TABLE I. Summary of Eight Experiments Undertaken With Second-Generation Human Sarcomas Implanted in GH/IGF–Deficient *lit/lit* and GH-replete *lit/+* mice*

Histology	No. of *lit/lit* mice (GH/IGF deficient)	No. of *lit/+* mice (GH replete)	*lit/lit* mean vol. at harvest 10^3 mm (SD)	*lit/+* mean vol. at harvest 10^3 mm (SD)	P	IGF-R in *lit/lit* (% of ctrl)	IGF-R in *lit/+* (% of ctrl)
Liposa	4	3	0.4 (0.1)	1.1 (0.5)	<0.05	400	160
Osteosa	12	15	0.2 (0.1)	1.2 (0.8)	<0.05	10	10
MFH-2	4	4	0.3 (0.2)	0.9 (0.5)	<0.05	170	120
MPNS	11	6	0.7 (0.4)	0.7 (0.5)	ns	130	160
MFH-3	3	9	0.3 (0.04)	1.7 (0.8)	<0.05	140	130
MFH-4	7	3	1.2 (0.3)	1.1 (0.4)	ns	30	30
MFH-5	6	5	0.4 (0.3)	0.4 (0.3)	ns	10	10
MFH-6	5	6	0.3 (0.1)	0.8 (0.3)	<0.05	180	70

IGF-I, insulin-like growth factor I; liposa, liposarcoma; osteosa, osteosarcoma; MFH, malignant fibrous histiocytoma; MPNS, malignant peripheral nerve Sheath tumor; SD, standard deviation.
*The mean tumor size in each group was calculated at the time that tumors first reached 1,000 mm^3 and mean volumes compared using the Kruskal-Wallis test. At tumor harvest the level of IGF-I receptor was compared in *lit/lit* and *lit/+* animals.

fibrosarcoma growth in cell lines implanted in nude mice could be stimulated by exogenous IGF only in tumors that overexpressed the type I IGF-I receptor. Alpha IR-3 antibody to IGF-I receptor inhibited tumor growth in NSCLC cell lines implanted in nude mice [35]. To date, no experiments have been reported to test the effect of IGF suppression on growth or progression of early-generation human cancers.

In the current experiment, second-generation human sarcoma tumors were transplanted to animals with normal serum IGF-I (*lit/+* animals) or markedly decreased serum IGF-I (*lit/lit* animals). Unlike previous experiments in our laboratory that have used hypophysectomy as a method for lowering serum IGF-I, the *lit/lit* mouse is characterized by normal pituitary function, except for GH synthesis and secretion, with the consequent secondary deficiency in serum IGF-I levels. Using this model, five of eight human sarcomas demonstrated marked inhibition of growth in the IGF−deficient hosts.

In general, the results in the human early-generation sarcomas paralleled the findings of Butler et al. that alteration of growth characteristics was dependent on the level of IGF-I receptor expressed by the tumor [13]. Four of five sarcomas that demonstrated growth inhibition in the homozygote little animals had IGF-R levels greater than MCF-7 positive control cell lines. One sarcoma was growth-suppressed despite IGF-R levels markedly lower than positive control cell lines (osteosarcoma sample with IGF-R levels 10% of positive control). Of the three sarcomas that failed to show growth retardation, two had IGF-R levels lower than positive control (MFH-4 30%; and MFH-5 10%). The final specimen that failed to show growth retardation (a malignant peripheral nerve sheath tumor) had elevated levels of IGF-R in tumors implanted in both groups of animals (130% in experimental group vs 160% in controls).

CONCLUSIONS

The results reported suggest that a pharmacological approach to lowering serum IGF-I might be effective in some patients as adjuvant therapy after sarcoma resection. Although IGF-I suppression therapy might be useful, it is important to recognize that expression of IGF-I receptor was increased in three of eight tumors implanted in IGF-I−deficient animals as compared with tumors implanted in controls. This finding suggests that although IGF-I−suppressing therapeutic strategies may have useful clinical activity, molecular mechanisms of resistance to these therapies may limit their efficacy. Nevertheless, results with a unique in vivo human sarcoma xenograft model indicate that at least a subset of tumors might respond. It is conceivable that a combined IGF-I−lowering and chemotherapy approach might improve

the effect of current adjuvant therapy because other data [36] indicate that, in a deficient IGF-I environment, the induction of apoptosis by cytotoxic agents is facilitated.

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