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Analysis of serum Insulin Growth Factor-1 concentrations in localized osteosarcoma: A Children's Oncology Group Study

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Abstract

To investigate the role of Insulin-like growth factor-1 (IGF-1), in localized osteosarcoma, serum levels of IGF-1, IGFBP-2, and IGFBP-3 were measured in 224 similarly treated, newly diagnosed patients. We demonstrated that younger patients had lower concentrations of IGF-1 and IGFBP-3 compared to older ($p < 0.001$) along with lower IGFBP-3:IGF-1 and IGFBP-2:IGF-1 ratios ($p < 0.001$). IGFBP-2 did not correlate with age ($p = 0.16$), yet IGFBP-2:IGF-1 ratios were higher in the younger population ($p<0.001$). These findings show that older patients have higher concentrations of free IGF-1. None of IGF-1, IGFBP-2, nor IGFBP-3 concentrations correlated with event-free nor overall survival.

INTRODUCTION

The insulin growth factor-1 (IGF-1) signaling pathway, hypothesized to play an important role in the development of clinical osteosarcoma, plays a significant role in laboratory models of osteosarcoma (1). Osteosarcoma primary tumors express both IGF-1 and the IGF-1 receptor (IGF-1R), and supplementation of osteosarcoma cell lines with IGF-1 increases their growth (2–4). IGF-1R antibody treatment or receptor level reduction by siRNA results in decreased invasiveness and slows growth of osteosarcoma xenografts (3, 5–7).

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Regulation of IGF-1 signaling is controlled in part by limiting the amount of free IGF-1 through interaction with IGF-1 binding proteins. Two of the better characterized binding proteins are IGFBP-3 and IGFBP-2, both of which have been shown to sequester IGF-1, thus decreasing the amount of free IGF-1 ligand signaling. However, the roles of IGFBP-3 and IGFBP-2 are unclear in osteosarcoma. To prospectively investigate the IGF-1 pathway in newly diagnosed, similarly treated, localized osteosarcoma, serum levels of IGF-1, IGFBP-2, and IGFBP-3 were measured and the analysis compared these biomarker levels to patient age and correlated with patient outcomes, measured by both event-free survival (EFS) and overall survival (OS).

MATERIALS AND METHODS

Patients were eligible if they were enrolled on the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG) therapeutic clinical trial P9754 and/or the tumor banking study P9851 (8) and received neo-adjuvant and adjuvant chemotherapy per clinical trial guidelines or institutional preference. Patients diagnosed with secondary osteosarcoma or metastatic disease were excluded from this analysis. Both of these studies were approved by both local and central institutional review boards and informed consent and assent was obtained from the patient or parent/legal guardian for patients less than 18 years old and minors. Serum samples were collected at diagnosis, prior to staring treatment and levels of IGF-1, IGFBP-2, and IGFBP-3 were measured, stratified into quartiles (Supplemental Table 1), and analyzed as described (9). Event-free survival (EFS) and overall survival (OS) were defined as described and calculated using the method of Kapan and Meier (9, 10). Equality of risk of event across groups defined by patient characteristics was assessed using the logrank test (10). Data received by the COG as of noon PST on March 21, 2011 were used for analysis. The measured variables were checked for association with each other and with the demographic characteristics of the patients within each of the analytic populations by the exact conditional test of proportions (11).

RESULTS

There were 224 samples available for analysis from eligible patients with localized osteosarcoma (Supplemental Fig. 1). Nine patients did not have follow up data, four patients had only IGF-1 and IGFBP-3 measurements, and sixty patients had only IGFBP-2 measurements. Therefore, IGF-1 and IGFBP-3 levels were determined in 142 patients and IGFBP-2 was measured in 198 patients. Since thirteen samples were replicates (obtained at the same point), a total of 202 unique patient samples were available for analysis. When multiple samples were obtained from the same patient, the values were averaged. IGF-1, IGFBP-2, and IGFBP-3 concentrations were correlated to patient age at diagnosis. Patients were stratified into three groups: Age ≤ 10 years, 10–17 years, and greater than 18 years. Younger patients had lower concentrations of IGF-1 and IGFBP-3 compared to older patients ($p < 0.001$), shown in Supplemental Table 2 and Fig. 1. Older patients had lower IGFBP-3:IGF-1 and IGFBP-2:IGF-1 ratios ($p < 0.001$), Interestingly, IGFBP-2 did not correlate with age ($p = 0.16$) but IGFBP-2:IGF-1 ratios were higher in the younger population ($p<0.001$). These findings demonstrate that older patients have higher concentrations of free IGF-1.

As shown in Fig. 2, IGF-1 concentration did not correlate with EFS or OS ($p = 0.98$ and 0.98, respectively). Similar findings were observed for IGFBP-2 ($p= 0.54$ and 0.70), IGFBP-3 ($p = 0.43$ and 0.63), and IGFBP-2:IGF-1 ($p = 0.88$ and 0.91). Patients with lower IGFBP-3:IGF-1 ratios were associated with reduced risk for EFS-event and death, although these were not considered statistically different from no relationship ($p= 0.21$ and 0.12, respectively).

DISCUSSION

In this study, we investigated IGF-1 concentrations in patients with newly diagnosed non-metastatic osteosarcoma. Serum samples from 202 newly diagnosed patients with localized osteosarcoma enrolled on prospective clinical trials were analyzed to determine concentrations of IGF-1, IGFBP-3, and IGFBP-2. A major strength of this study is that serum samples were collected prospectively from a large number of newly diagnosed patients, without the knowledge of IGF status. We demonstrated that older patients had increased serum concentrations of IGF-1 and decreased IGFBP-3:IGF-1 ratios, showing that older patients have increased free IGF-1. IGF-1 concentrations are low at birth and slowly rise through childhood and peak during puberty, and remain elevated until the fourth decade of life, when they begin to decrease (12). Increased IGF-1 levels have been observed previously in older patients newly diagnosed with Ewing Sarcoma and is more likely due to normal physiology than malignancy (9, 13). Furthermore, when IGF-1, IGFBP-2, and IGFBP-3 levels were evaluated based upon patient outcome, there were no significant correlations (EFS and OS).

The determination of how free IGF-1 levels respond to treatment could provide insight on how to best use agents that target the IGF-1 signaling pathway. In addition, IGFBP-3 has IGF-1 independent functions that contribute to cancer pathogenesis. Several studies have shown that IGFBP-3 promotes apoptosis and suppresses metastasis in prostate cancer cell lines and can inhibit cell cycle progression in skin, kidney, and breast cancers (14, 15). The IGF-1 independent effects of IGFBP-3 in osteosarcoma are poorly understood, but may have anti-proliferative effects and warrant further investigation. While IGFBP-1, -4, -5, and -6 are found in the circulation, they generally each represent less than 10% of circulating IGFBP, thus were not assayed for in this project (16).

Despite very promising preclinical data suggesting efficacy of IGF-1 receptor antagonists, these agents have not demonstrated robust clinical activity (17, 18). Prior to directly targeting the IGF-1R, the somatostatin analog Oncolar (octreotide pamoate long-acting release) was used to lower ligand levels in the circulation. Mansky *et al* demonstrated the ability of Oncolar to lower serum concentrations of IGF-1, but there was no clinical response to this agent (19). Furthermore, a recent report by Schwartz et al revealed that only 3/24 patients with relapsed osteosarcoma treated with cixutumumab and temsirolimus had a partial response to therapy (20). To enhance the likelihood of success in future clinical trials that target the IGF-1 pathway in osteosarcoma, patients should be stratified based upon novel patient or tumor biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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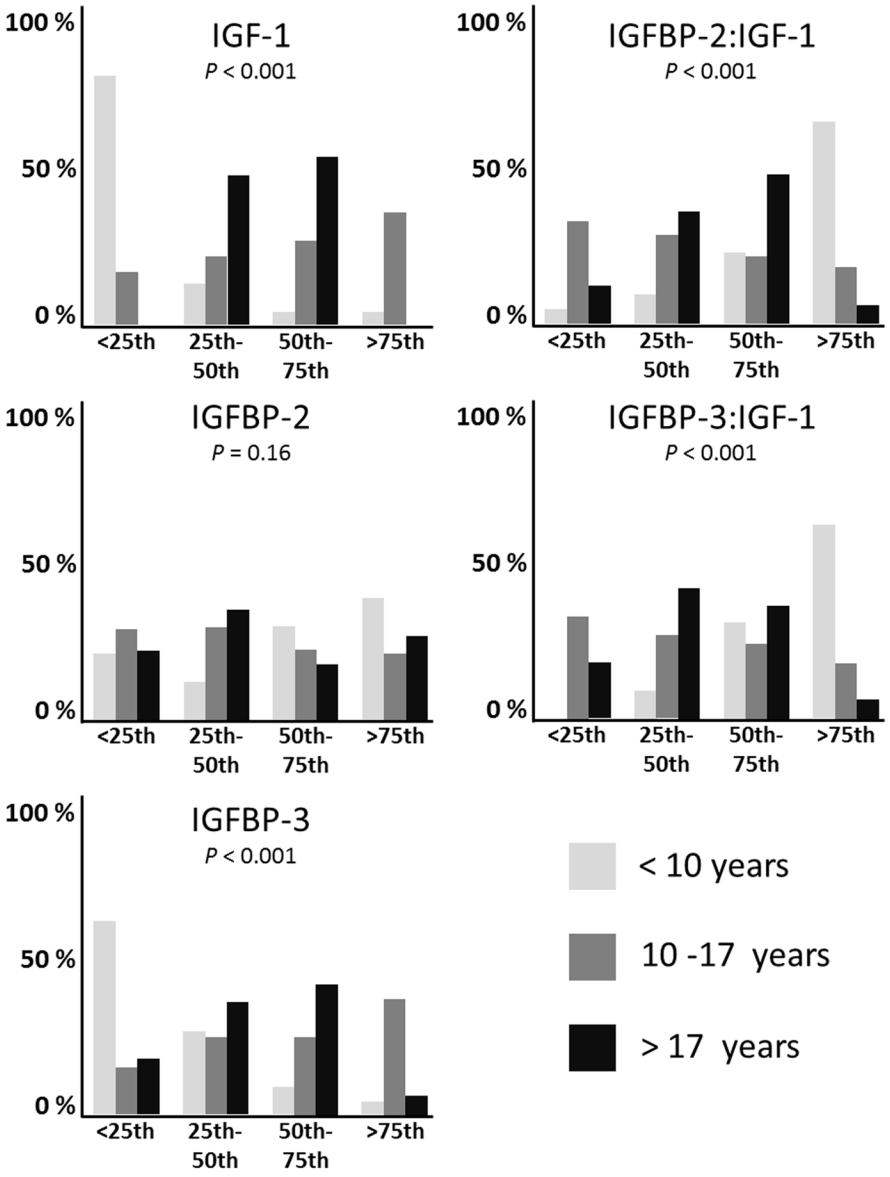

Fig. 1.

IGF-1, IGFBP-2, IGFBP-3, IGFBP-2:IGF-1, and IGFBP-3:IGF-1 ratios compared to age. Serum levels and ratios were stratified into quartiles and compared. P values are demonstrated.

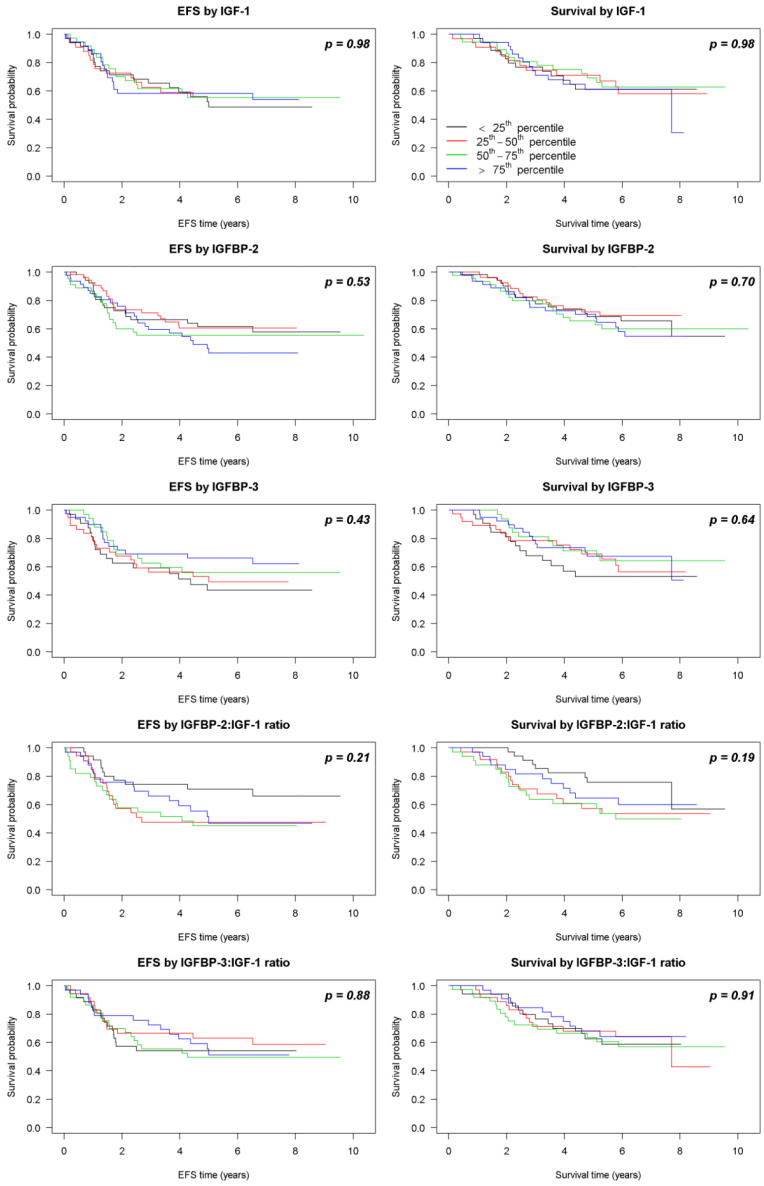

Fig. 2.

EFS and OS as demonstrated by Kaplan-Meier Analysis compared to IGF-1, IGFBP-2, IGFBP-3, IGFBP-2:IGF-1, and IGFBP-3:IGF-1 ratios. Serum levels and ratios were stratified into quartiles and compared. P values are demonstrated.