Interferon-α/β Receptor as a Prognostic Marker in Osteosarcoma

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Background: A large-scale randomized trial of adjuvant interferon-α therapy for patients with osteosarcoma has been initiated as a joint protocol by the European and American Osteosarcoma Study Group. Because the expression of functional interferon-α/β receptor is necessary for interferon-α agents to interact with osteosarcoma cells, we examined the expression of interferon-α/β receptor in a series of osteosarcoma specimens.

Methods: Forty patients with high-grade resectable osteosarcoma, from whom surgical specimens had been obtained at the time of biopsy, were included in this retrospective study. Biopsy specimens were immunohistochemically stained with anti-interferon-α/β receptor antibodies. Survival was estimated with the Kaplan-Meier method. The Cox proportional hazards model was used for multivariate analysis to determine the independent prognostic factors. Furthermore, we used Holm and Benjamini-Hochberg procedures to adjust for multiple comparisons in setting the level of significance. The median follow-up period was five years and two months (range, four to 195 months).

Results: The expression of interferon-α/β receptor was positive in eighteen (45%) of the forty patients with high-grade osteosarcoma. American Joint Committee on Cancer surgical stage IIA, a good histologic response to chemotherapy, and expression of interferon-α/β receptor correlated significantly with better disease-free survival ($p < 0.05$). Multivariate analysis showed that interferon-α/β receptor expression alone retained its power to predict an improved prognosis ($p = 0.042$). There were no significant variables after corrections for multiple comparisons.

Conclusions: Interferon-α/β receptor may be a useful marker for assessing tumor prognosis in patients with osteosarcoma and may play an important role in tumor progression. These findings are encouraging and support the ongoing clinical trials of adjuvant interferon-α therapy by the multinational Osteosarcoma Study Group. Our pilot study was based on a small sample size, and larger trials are needed to confirm this finding.

Level of Evidence: Prognostic Level II. See Instructions to Authors for a complete description of levels of evidence.

Osteosarcoma is the most common primary malignant bone tumor and is characterized by a high metastatic potential. Chemotherapy protocols, beginning in the mid-1970s, have increased the five-year disease-free survival rate from approximately 15% to 20% to 60% to 70%. However, over the last two decades, attempts at further intensifying therapy with conventional chemotherapeutic drugs, including high-dose methotrexate, doxorubicin, cisplatin, and high-dose ifosfamide, have not improved survival substantially. Despite aggressive surgical and chemotherapy approaches, patients with an unresectable primary tumor and those with clinically evident metastases still have a poor prognosis[1-5]. Better prognostic factors and more effective therapeutic modalities are needed for patients with refractory osteosarcoma.

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TABLE I Univariate Survival Analysis of Disease-Free Survival

Variable	5-Year Survival* (%)	P Value† (Power)	Family-Wise Error Rate	False Discovery Rate
Age		0.594 (0.106)	>1.00	0.792
<40 yr	57.5 ± 8.5			
≥40 yr	50.0 ± 35.4			
Sex		0.355 (0.217)	>1.00	0.710
Male	49.1 ± 11.4			
Female	67.5 ± 11.3			
Site		0.943 (0.227)	0.943	0.943
Femur	63.5 ± 9.7			
Tibia or fibula	53.3 ± 17.6			
Surgical stage		0.033 (0.103)	0.198	0.088
IIA	85.7 ± 13.2			
IIB	45.9 ± 11.1			
Histologic type		0.854 (0.074)	>1.00	0.976
Osteoblastic	50.3 ± 10.6			
Fibroblastic	60.0 ± 21.9			
Surgery		0.438 (0.165)	>1.00	0.701
Amputation	50.5 ± 12.6			
Limb salvage	62.1 ± 10.8			
Response to chemotherapy		0.030 (0.589)	0.210	0.120
Poor	40.9 ± 11.1			
Good	73.3 ± 13.2			
Interferon-α/β receptor		0.022 (0.542)	0.176	0.176
Negative	41.8 ± 11.2			
Positive	75.2 ± 10.9			

*The values are given as the survival rate and the standard error. †Log-rank test.

Interferons are cytokines characterized by a wide variety of biologic properties, including antiviral, antiproliferative, and immunomodulatory effects[6,7]. Interferons are grouped into two families: type I and type II. Interferon-α, which belongs to the type-I family, interacts with interferon-α/β receptor on the cell surface to induce activation of JAK1 and TYK2, leading to phosphorylation and activation of signal transducers and activators of transcription 1 (STAT1). The activated STAT1 forms homodimers or heterodimers (STAT1/STAT2), which translocate into the nucleus and subsequently bind specific promoter regions, including interferon-stimulated response elements, to regulate transcription of the genes responsible for biologic effects.

Recombinant interferon-α was one of the first cytokines widely available for treating infectious and malignant diseases. Interferons have been evaluated for the treatment of several human malignant tumors, and objective responses have been documented in patients with multiple myeloma, chronic myeloid leukemia, non-Hodgkin's lymphoma, renal cell carcinoma, and melanoma[8-15].

Compared with other malignant tumors, scant data are available on the effects of interferon-α in patients with osteosarcoma. A large-scale randomized trial of adjuvant interferon-α therapy was initiated as a joint protocol by the European and American Osteosarcoma Study Group. Because the expression of functional interferon-α/β receptor is necessary for interferon-α agents to interact with osteosarcoma cells, we examined the expression of interferon-α in a series of osteosarcoma specimens.

Materials and Methods

Patients

Forty patients with high-grade resectable osteosarcoma, from whom surgical specimens had been obtained at the time of biopsy at our institute between 1980 and 2006, were included in this retrospective study. The included patients had a newly diagnosed primary osteosarcoma, had not received chemotherapy before biopsy, and had completed the treatment protocols consisting of tumor excision and multiagent chemotherapy. Patients were excluded if representative biopsy material and follow-up data were not available. The histologic subtypes included thirty-five conventional, three small cell, and two telangiectatic osteosarcomas. Twenty-one patients received conventional chemotherapy, basically including high-dose methotrexate, doxorubicin, and cisplatin. Combinations of these agents were made empirically. After 1997, we used two established chemotherapy regimens. The NECO-95J[16] and K2[17] protocols

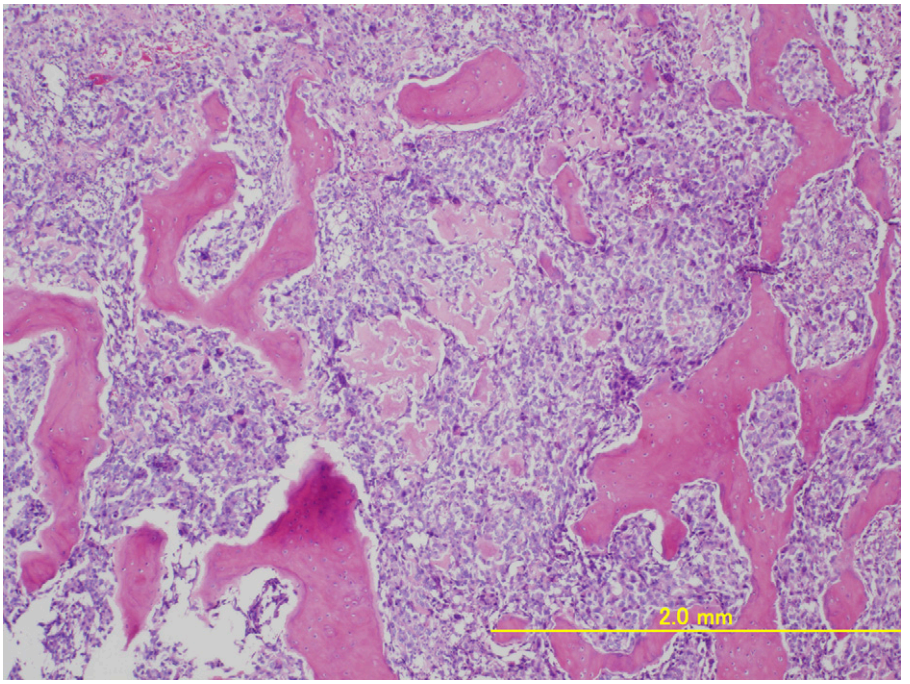

Fig. 1-A

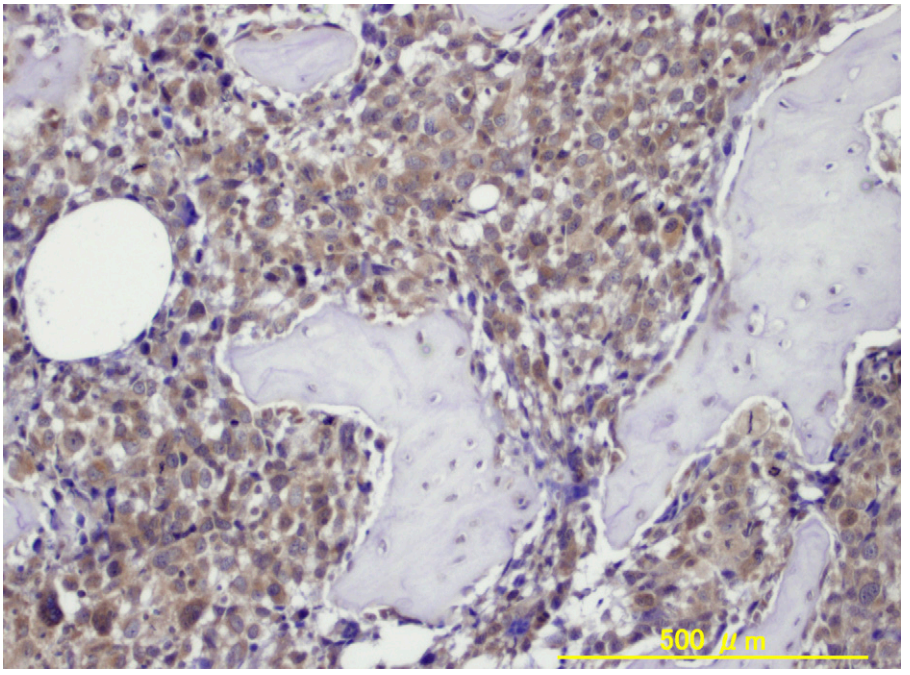

Fig. 1-B

Figs. 1-A and 1-B Photomicrographs showing staining with hematoxylin and eosin and immunohistochemical staining with anti-interferon-α/β receptor antibodies in a high-grade osteoblastic osteosarcoma specimen. **Fig. 1-A** Tumor cells producing osteoid and osseous trabeculae (original magnification, ×40). **Fig. 1-B** There is strong dark-brown staining of the cytoplasm in >50% of the cells (×100).

were used for twelve patients between 1997 and 2002 and for seven patients between 2002 and 2006. All biopsy samples used for the immunohistochemical analysis were reviewed to confirm the diagnosis of high-grade osteosarcoma. Surgical resection specimens were used to evaluate surgical margins and histologic response to preoperative chemotherapy. The surgical margins were classified with the method described by Kawaguchi et al.[18], in

which surgical margins are evaluated according to the distance of the margin from the tumor's reactive zone and are divided into four categories: curative, wide, marginal, and intralesional. The reactive zone is composed of hemorrhagic tissue, scar tissue, degenerated muscle, edema, or the tumor capsule. If the surgical margin is >5 cm outside the reactive zone, we grade the margin as curative. The surgical margin is considered to be wide if the margin is <5 cm outside, but still outside, the reactive zone. A margin in the reactive zone is graded as marginal, and a margin passing through the tumor, as intralesional. Histologic evidence of necrosis following preoperative chemotherapy was determined according to the grading system described by Huvos[19]. Each case was defined as a good responder (Huvos grade III or IV) or a poor responder (grade I or II). Disease-free survival was defined as the interval from diagnosis to relapse or the last follow-up evaluation, and metastasis-free survival was defined as the interval from diagnosis to discovery of a distant metastasis or the last follow-up evaluation. Overall survival was calculated from the day of diagnosis to death or the last follow-up evaluation. There were no treatment-related deaths or deaths due to causes other than the disease.

Immunohistochemical Staining

We used archival paraffin-embedded open biopsy specimens to analyze the expression of interferon-α/β receptor immunohistochemically. A paraffin-embedded tissue block was cut into 5-μm sections, transferred to glass slides coated with Matsunami adhesive silan (Matsunami, Osaka, Japan), deparaffinized in xylene, rehydrated in a graded series of decreasing ethanol concentrations, and then rinsed in Tris-buffered saline solution with Tween-20 (50 mM Tris/HCl, pH 7.6, containing 0.3 M sodium chloride and 0.1% Tween-20). Tissue sections were immersed in Target Retrieval Solution (DakoCytomation,

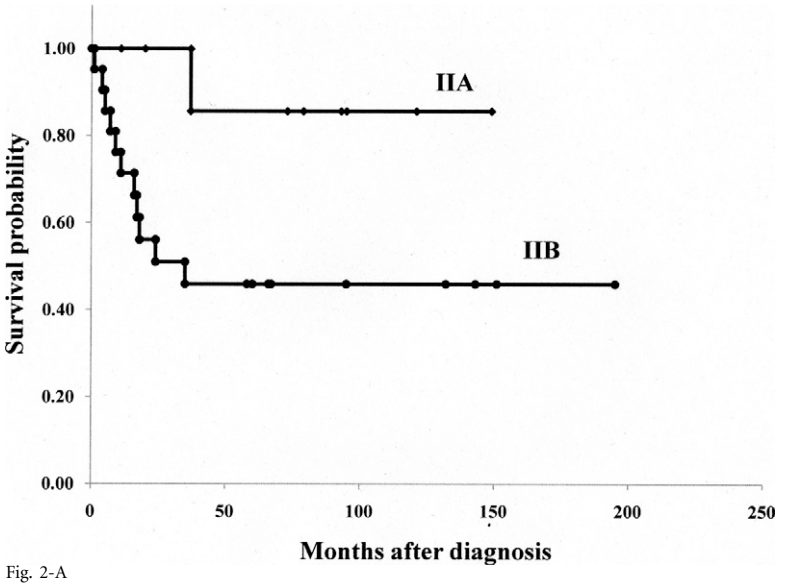

Fig. 2-A

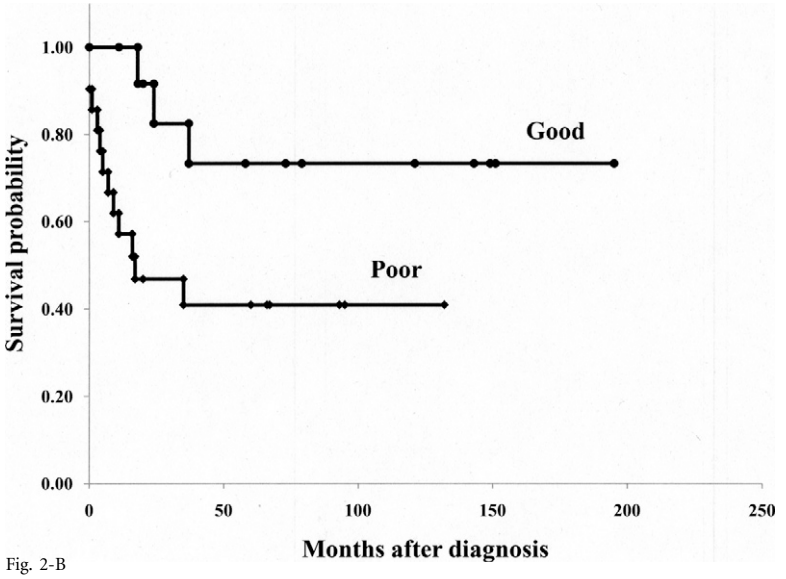

Fig. 2-B

Kaplan-Meier plots of disease-free survival of patients with osteosarcoma according to the AJCC surgical stage (Fig. 2-A), histologic response to chemotherapy (Fig. 2-B), and expression of interferon-α/β receptor (Fig. 2-C).

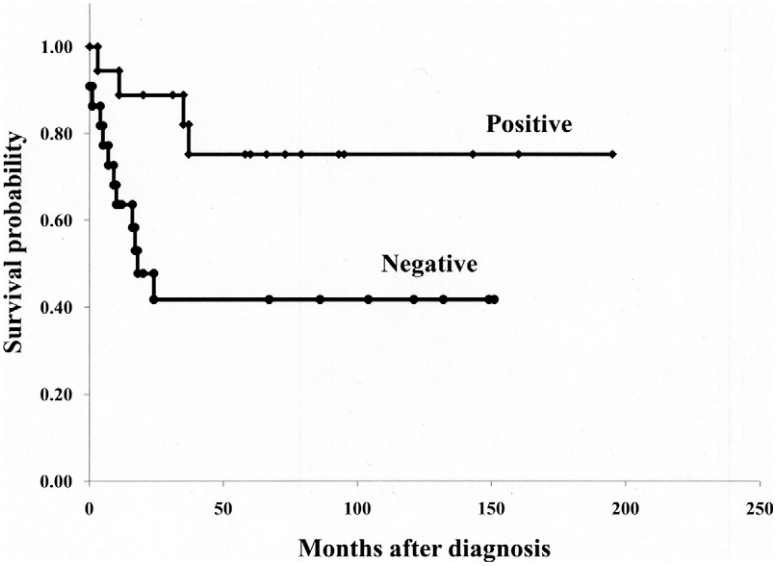

Fig. 2-C

Carpinteria, California)and subjected to a hot water bath for twenty minutes, cooled for twenty minutes, then incubated with a mouse anti-human interferon-α/β receptor antibody (1:200 dilution; R&D Systems, Minneapolis, Minnesota) or control mouse IgG2a (DakoCytomation) for sixty minutes. The polymer-peroxidase method was used for visualization according to the manufacturer's instructions (EnVision+/HRP; DakoCytomation). Nuclei were lightly counterstained with Gill's hematoxylin. Two independent observers evaluated the specimens in a blinded fashion. Each score was discussed by all authors to reach an overall consensus. The extent of staining was scored as: (−), indicating a negative reaction of the tumor cells; (±), indicating staining of <10% of the cells; (+), indicating staining of 10% to 50% of the cells; or (++), indicating staining of >50% of the cells. For analyses, the tumors in which stained cells made up >10% of the tumor were regarded as positive.

Statistical Analysis

The disease-free survival, overall survival, and metastasis-free survival were estimated with the Kaplan-Meier method. The log-rank test was used to evaluate the differences between survival curves. Factors influencing the disease-free survival rate were then analyzed with a multivariate Cox regression analysis to determine independent variables predicting survival. The associations of interferon-α/β receptor expression with clinicopathologic parameters were analyzed with use of the chi-square test. These analyses were performed with Ekuseru Statistics 2008 (Social Survey Research Information, Tokyo, Japan). The statistical power

TABLE II Multivariate Cox Regression Analysis of Disease-Free Survival

	3 Variables			5 Variables		
Variable	Relative Risk	95% Confidence Interval	P Value	Relative Risk	95% Confidence Interval	P Value
Surgical stage			0.159			0.130
IIA	1.00			1.00		
IIB	4.54	0.55-37.29		5.59	0.60-51.76	
Response to chemotherapy			0.235			0.743
Poor	1.00			1.00		
Good	0.44	0.11-1.70		0.76	0.14-4.01	
Interferon-α/β receptor			0.042			0.091
Negative	1.00			1.00		
Positive	0.25	0.07-0.95		0.31	0.08-1.20	
Sex						0.951
Male				1.00		
Female				0.95	0.21-4.28	
Surgery						0.251
Amputation				1.00		
Limb salvage				0.38	0.07-1.97	

TABLE III Associations of Interferon-α/β Receptor Expression with Clinicopathologic Parameters

	Interferon-α/β Receptor *(no. of cases)*		
	Negative	Positive	P Value*
Age			0.560
<40 yr	20	18	
≥40 yr	2	0	
Sex			1.000
Male	11	9	
Female	11	9	
Site			0.886
Femur	13	12	
Tibia or fibula	6	4	
Others	3	2	
Surgical stage			0.276
IIA	4	6	
IIB	12	9	
IV	2	0	
Histologic type			0.538
Osteoblastic	16	11	
Fibroblastic	3	2	
Others	3	5	
Surgery			0.289
Amputation	11	6	
Limb salvage	11	12	
Response to chemotherapy			0.533
Poor	12	9	
Good	6	7	
Metastases			0.038
No	10	14	
Yes	12	4	

*Chi-square test.

analysis was performed according to the power and sample size calculation described by Dupont and Plummer[20]. $P < 0.05$ was considered significant. Furthermore, to adjust for multiple comparisons when setting the level of significance, we used Holm's family-wise error rate[21] and Benjamini's and Hochberg's false discovery rate[22].

Source of Funding
There was no external funding source for the study.

Results

We reviewed clinical data from the patients' medical charts after this study was approved by the institutional review board. The median age at the time of surgery was 18.3 years (range, ten to fifty-five years). There were twenty male and twenty female patients. The tumor site was the pelvis in one patient and the extremities in thirty-nine patients (twenty-five femora, ten tibiae or fibulae, and four humeri). According to the sixth edition of the American Joint Committee on Cancer (AJCC) Staging Manual[23], ten tumors were stage IIA, twenty-one were stage IIB, and two were stage IV. (The tumor stage based on size was missing from the charts of seven patients.) Two patients had lung metastases at the initial presentation. Operative treatment consisted of twenty-three limb salvage operations and seventeen amputations. The surgical margins of all limb salvage operations were wide, and the surgical margins of the amputations were wide or curative. Histologic subtypes included osteoblastic (twenty-seven tumors), fibroblastic (five), chondroblastic (three), small cell (three), and telangiectatic (two). There were thirteen good responders to preoperative chemotherapy and twenty-one poor responders. (The data with regard to response was missing for six patients.) The median duration of follow-up was five years and two months (range, four to 195 months). There was one local recurrence, and there were sixteen distant metastases. Thirteen patients died of disease, twenty-six showed no evidence of disease, and one was alive with disease. The five-year actuarial disease-free and overall survival rates were 57.3% and 63.2%, respectively.

In eighteen (45%) of the forty patients with high-grade osteosarcoma, the expression of interferon-α/β receptor was positive, with six patients (15%) showing strong expression of protein. A representative immunostain of interferon-α/β receptor is shown in Figures 1-A and 1-B. AJCC surgical stage IIA, a good histologic response to chemotherapy, and the expression of interferon-α/β receptor correlated significantly with favorable disease-free survival ($p < 0.05$) (Table I and Figs. 2-A, 2-B, and 2-C). Multivariate analysis, with only the three significant variables that had been detected with the log-rank test, was performed with use of the Cox proportional hazards model, with the result that interferon-α/β receptor expression alone retained its power to predict an improved prognosis ($p =$

TABLE IV Overall and Metastasis-Free Survival According to Interferon-α/β Receptor Expression

Variable	5-Year Overall Survival* *(%)*	P Value†	5-Year Metastasis-Free Survival* *(%)*	P Value†
Interferon-α/β receptor		0.043		0.023
Negative	47.2 ± 11.8		41.3 ± 11.2	
Positive	81.5 ± 9.7		75.2 ± 10.9	

*The values are given as the survival rate and the standard error. †Log-rank test.

0.042) (Table II). However, these data were underpowered to detect significant associations because of the small sample size (Table I). Furthermore, we considered the p value with respect to multiple hypothesis testing. There were no significant variables after the corrections for the family-wise error rate and the false discovery rate (Table I). Five variables for which the univariate tests resulted in a Benjamini-Hochberg procedure-adjusted p value of <0.75 were reconsidered in the Cox proportional hazards regression model. No parameters in the multivariate analysis were found to be significant (Table II).

There was a significant association between the expression of interferon-α/β receptor and distant metastases ($p = 0.038$), whereas no association was detected between the expression of interferon-α/β receptor and the other clinicopathologic features, which included age, sex, site, AJCC surgical stage, surgery, histologic subtype, and histologic response to chemotherapy (Table III). The expression of interferon-α/β receptor correlated significantly with favorable overall and metastasis-free survival ($p < 0.05$) (Table IV).

Discussion

An increased understanding of the molecular events in the malignant cell signal transduction machinery, including cell proliferation, invasion, apoptosis, cell-cycle control, and tumor-related angiogenesis, have led to the development of novel agents for anticancer therapy. In recent years, several targeted agents have become available and have improved the outcomes of patients with solid tumors. For example, inhibition of human epidermal growth factor receptor (ErbB/HER) pathways blocks cell-cycle progression, inhibits the production of pro-angiogenic factors, and induces apoptosis in numerous in vitro and xenograft models. Accordingly, anti-ErbB/HER monoclonal antibodies (cetuximab, panitumumab, and trastuzumab) and tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib) have been used for the treatment of advanced colorectal cancer, squamous cell carcinoma of the head and neck, advanced non-small-cell lung cancer, and pancreatic and breast cancer. Furthermore, overexpression of HER2 in breast cancer is associated with aggressive clinical behavior and poor clinical outcome, and trastuzumab, a monoclonal antibody against the human HER2, has proven effective in the treatment of women with HER2-positive breast cancer[24,25]. These encouraging data led us to the identification of novel molecular targets in osteosarcoma.

Recent studies have suggested that assessment of the expression of interferon-α/β receptor may be a useful prognostic test for patients with pancreatic adenocarcinoma, renal cell carcinoma, or hepatocellular carcinoma[26-28]. However, few studies of interferon-α/β receptor have been performed on sarcomas. Rosolen et al.[29] found that all of the tumor specimens that they studied with immunohistochemical analysis, including neuroblastoma, primitive neuroectodermal tumor, and rhabdomyosarcoma, stained positive for the interferon-α/β receptor antibody with no apparent correlation between the degree of immunoreactivity and the histologic subtype. This finding is in contrast with the data reported by Navarro et al.[30], who found little or no reactivity to the monoclonal antibody in some sarcomas. Regarding osteosarcoma, little is known about the role of the interferon-α pathway, and we are not aware of any previous data on the expression of interferon-α/β receptor in osteosarcoma. In our study, multivariate analysis before the adjustment for multiple comparisons showed that interferon-α/β receptor expression alone was an independent prognostic factor ($p < 0.05$). In addition to previously reported prognostic factors, such as the surgical stage and the histologic response to chemotherapy, the expression of interferon-α/β receptor may help to predict a group of patients with osteosarcoma who have a poor prognosis before treatment.

Interferon-α can affect tumor cell functions by multiple mechanisms. Recent findings have provided new information on the molecular mechanisms of the direct antitumor activity. Interferon-α appears to block cell proliferation, at least in part, through the induction of apoptotic effects. This cytokine can also regulate the progression of tumor cells through the different phases of the cell cycle, inducing an increase in the expression of the cyclin-dependent kinase inhibitors p21 and p27. An important additional mechanism of the interferon-α-mediated antitumor activity seems to rely on the interference with tumor-mediated angiogenesis[7]. With regard to osteosarcoma, interferon-α has been shown to inhibit cell growth in in vitro and animal models and to enhance the sensitivity of human osteosarcoma cells to conventional chemotherapeutic agents, such as doxorubicin and etoposide, by p53-dependent apoptosis[31-33]. Our qualitative immunohistochemical analysis suggests that interferon-α/β receptor may play an important role in osteosarcoma progression. Our findings should prompt further investigation with quantitative analysis of interferon receptors and the molecular mechanism of intracellular interferon signaling with use of fresh osteosarcoma tissues or cell cultures.

Interferon-α improved the survival rate of patients with non-metastatic high-grade osteosarcoma in nonrandomized clinical trials and induced temporary partial tumor regression in patients with metastatic osteosarcoma[33]. Furthermore, Todesco et al. reported a case in which the combination of interferon-α and all-trans retinoic acid was well tolerated and the patient was in stable complete remission fourteen months after the end of therapy[34]. A large-scale randomized trial of adjuvant interferon-α therapy for patients with osteosarcoma has been initiated as a joint protocol named EURAMOS 1 by the multinational European and American Osteosarcoma Study Group[33]. A primary objective of EURAMOS 1 is to examine whether the addition of interferon-α as maintenance therapy after postoperative chemotherapy improves event-free survival for patients with resectable osteosarcoma. In brief, all patients receive a standard induction chemotherapy regimen (cisplatin, doxorubicin, and high-dose methotrexate). The good responders (those with <10% viable tumor) continue to receive the same regimen as they had received before their operation, but they are then randomized to either receive or not receive pegylated recombinant interferon-α 2b in addition. Our findings suggest that interferon-α/β receptor may play an important role in tumor progression. Therefore, adjuvant interferon-α therapy may be a promising strategy for patients with osteosarcoma, especially interferon-α/β receptor-expressing osteosarcoma.

In conclusion, this study indicates that the expression of interferon-α/β receptor is associated with improved disease-free and metastasis-free survival rates, suggesting that interferon-α/β receptor may play an important role in tumor progression. Therefore, the expression of interferon-α/β receptor may be a reliable marker to assess tumor prognosis in patients with osteosarcoma. Our findings are encouraging and support the ongoing large-scale randomized trial of adjuvant interferon-α therapy for patients with osteosarcoma. Since there was no significant prognostic variable after the corrections for the family-wise error rate and the false discovery rate, more research with a larger number of patients is needed to confirm our finding. ■

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References

1. Meyers PA, Gorlick R, Heller G, Casper E, Lane J, Huvos AG, Healey JH. Intensification of preoperative chemotherapy for osteogenic sarcoma: results of the Memorial Sloan-Kettering (T12) protocol. J Clin Oncol. 1998;16:2452-8.

2. Bacci G, Briccoli A, Ferrari S, Longhi A, Mercuri M, Capanna R, Donati D, Lari S, Forni C, DePaolis M. Neoadjuvant chemotherapy for osteosarcoma of the extremity: long-term results of the Rizzoli's 4th protocol. Eur J Cancer. 2001;37:2030-9.

3. Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, Helmke K, Kotz R, Salzer-Kuntschik M, Werner M, Winkelmann W, Zoubek A, Jürgens H, Winkler K. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. J Clin Oncol. 2002;20:776-90.

4. Lewis IJ, Nooij MA, Whelan J, Sydes MR, Grimer R, Hogendoorn PC, Memon MA, Weeden S, Uscinska BM, van Glabbeke M, Kirkpatrick A, Hauben EI, Craft AW, Taminiau AH; MRC BO06 and EORTC 80931 collaborators; European Osteosarcoma Intergroup. Improvement in histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: a randomized phase III trial of the European Osteosarcoma Intergroup. J Natl Cancer Inst. 2007;99:112-28.

5. Eselgrim M, Grunert H, Kühne T, Zoubek A, Kevric M, Bürger H, Jürgens H, Mayer-Steinacker R, Gosheger G, Bielack SS. Dose intensity of chemotherapy for osteosarcoma and outcome in the Cooperative Osteosarcoma Study Group (COSS) trials. Pediatr Blood Cancer. 2006;47:42-50.

6. Ferrantini M, Capone I, Belardelli F. Interferon-alpha and cancer: mechanisms of action and new perspectives of clinical use. Biochimie. 2007;89:884-93.

7. Tagliaferri P, Caraglia M, Budillon A, Marra M, Vitale G, Viscomi C, Masciari S, Tassone P, Abbruzzese A, Venuta S. New pharmacokinetic and pharmacodynamic tools for interferon-alpha (IFN-alpha) treatment of human cancer. Cancer Immunol Immunother. 2005;54:1-10.

8. Fritz E, Ludwig H. Interferon-alpha treatment in multiple myeloma: meta-analysis of 30 randomised trials among 3948 patients. Ann Oncol. 2000;11:1427-36.

9. Myeloma Trialists' Collaborative Group. Interferon as therapy for multiple myeloma: an individual patient data overview of 24 randomized trials and 4012 patients. Br J Haematol. 2001;113:1020-34.

10. Lipton JH, Khoroshko N, Golenkov A, Abdulkadyrov K, Nair K, Raghunadharao D, Brummendorf T, Yoo K, Bergstrom B; Pegasys CML Study Group. Phase II, randomized, multicenter, comparative study of peginterferon-alpha-2a (40 kD) (Pegasys) versus interferon alpha-2a (Roferon-A) in patients with treatment-naïve, chronic-phase chronic myelogenous leukemia. Leuk Lymphoma. 2007;48:497-505.

11. Kloke O, Opalka B, Niederle N. Interferon alfa as primary treatment of chronic myeloid leukemia: long-term follow-up of 71 patients observed in a single center. Leukemia. 2000;14:389-92.

12. Tsavaris N, Baxevanis C, Kosmidis P, Papamichael M. The prognostic significance of immune changes in patients with renal cancer, melanoma and colorectal cancer, treated with interferon alpha 2b. Cancer Immunol Immunother. 1996;43:94-102.

13. Medical Research Council Renal Cancer Collaborators. Interferon-alpha and survival in metastatic renal carcinoma: early results of a randomised controlled trial. Lancet. 1999;353:14-7.

14. Kirkwood JM, Ibrahim JG, Sosman JA, Sondak VK, Agarwala SS, Ernstoff MS, Rao U. High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIB-III melanoma: results of intergroup trial E1694/S9512/C509801. J Clin Oncol. 2001;19:2370-80.

15. Bottomley A, Coens C, Suciu S, Santinami M, Kruit W, Testori A, Marsden J, Punt C, Salès F, Gore M, Mackie R, Kusic Z, Dummer R, Patel P, Schadendorf D, Spatz A, Keilholz U, Eggermont A. Adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma: a phase III randomized controlled trial of health-related quality of life and symptoms by the European Organisation for Research and Treatment of Cancer Melanoma Group. J Clin Oncol. 2009;27:2916-23.

16. Iwamoto Y, Tanaka K, Isu K, Kawai A, Tatezaki S, Ishii T, Kushida K, Beppu Y, Usui M, Tateishi A, Furuse K, Minamizaki T, Kawaguchi N, Yamawaki S. Multi-institutional phase II study of neoadjuvant chemotherapy for osteosarcoma (NECO study) in Japan: NECO-93J and NECO-95J. J Orthop Sci. 2009;14:397-404.

17. Kimura H, Tsuchiya H, Shirai T, Nishida H, Hayashi K, Takeuchi A, Ohnari I, Tomita K. Caffeine-potentiated chemotherapy for metastatic osteosarcoma. J Orthop Sci. 2009;14:556-65.

18. Kawaguchi N, Matumoto S, Manabe J. New method of evaluating the surgical margin and safety margin for musculoskeletal sarcoma, analysed on the basis of 457 surgical cases. J Cancer Res Clin Oncol. 1995;121:555-63.

19. Huvos AG. Bone tumors: diagnosis, treatment, and prognosis. 2nd ed. Philadelphia: WB Saunders; 1991. p 85-155.

20. Dupont WD, Plummer WD Jr. Power and sample size calculations. A review and computer program. Control Clin Trials. 1990;11:116-28.

21. Holm S. A simple sequentially rejective multiple test procedure. Scand J Stat. 1979;6:65-70.

22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995;57:289-300.

23. Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, Morrow M, editors. AJCC cancer staging manual. 6th ed. Chicago: The American Joint Committee on Cancer; 2002.

24. Lurje G, Lenz HJ. EGFR signaling and drug discovery. Oncology. 2009;77:400-10.

25. Alvarez RH, Valero V, Hortobagyi GN. Emerging targeted therapies for breast cancer. J Clin Oncol. 2010;28:3366-79.

26. Kondo M, Nagano H, Sakon M, Yamamoto H, Morimoto O, Arai I, Miyamoto A, Eguchi H, Dono K, Nakamori S, Umeshita K, Wakasa K, Ohmoto Y, Monden M. Expression of interferon alpha/beta receptor in human hepatocellular carcinoma. Int J Oncol. 2000;17:83-8.

27. Kamai T, Yanai Y, Arai K, Abe H, Yamanishi T, Kurimoto M, Yoshida K. Increased interferon alpha receptor 2 mRNA levels is associated with renal cell carcinoma metastasis. BMC Cancer. 2007;7:159.

28. Saidi RF, Remine SG, Jacobs MJ. Interferon receptor alpha/beta is associated with improved survival after adjuvant therapy in resected pancreatic cancer. HPB (Oxford). 2007;9:289-94.

29. Rosolen A, Todesco A, Colamonici OR, Basso G, Frascella E. Expression of type I interferon receptor in solid tumors of childhood. Mod Pathol. 1997;10:55-61.

30. Navarro S, Colamonici OR, Llombart-Bosch A. Immunohistochemical detection of the type I interferon receptor in human fetal, adult, and neoplastic tissues. Mod Pathol. 1996;9:150-6.

31. Yuan XW, Zhu XF, Huang XF, Sheng PY, He AS, Yang ZB, Deng R, Feng GK, Liao WM. Interferon-alpha enhances sensitivity of human osteosarcoma U2OS cells to doxorubicin by p53-dependent apoptosis. Acta Pharmacol Sin. 2007;28:1835-41.

32. Yuan XW, Zhu XF, Liang SG, Fan Q, Chen ZX, Huang XF, Sheng PY, He AS, Yang ZB, Deng R, Feng GK, Liao WM. Interferonalpha enhances etoposide-induced apoptosis in human osteosarcoma U2OS cells by a p53-dependent pathway. Life Sci. 2008;82:393-401.

33. Strander H. Interferons and osteosarcoma. Cytokine Growth Factor Rev. 2007;18:373-80.

34. Todesco A, Carli M, Iacona I, Frascella E, Ninfo V, Rosolen A. All-trans retinoic acid and interferon-alpha in the treatment of a patient with resistant metastatic osteosarcoma. Cancer. 2000;89:2661-6.