

Primary versus Radiation-Associated Craniofacial Osteosarcoma

Biologic and Clinicopathologic Comparisons

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BACKGROUND. Craniofacial osteosarcoma differs from long bone osteosarcoma in that patients are older, tumors are often low grade, and prognosis is more favorable. Although most are sporadic, some tumors occur in association with prior radiation therapy. The purpose of the current study was to compare clinicopathologic and prognostic features of primary and radiation-associated osteosarcoma.

METHODS. The study group consisted of 15 primary and 6 radiation-associated osteosarcomas. Clinical and follow-up data were obtained in every case. Tissue microarrays were immunohistochemically stained for p53, pRB, Ki-67 (MIB-1), and ezrin. DNA was sequenced for TP53 mutations.

RESULTS. All radiation-associated osteosarcomas were high grade and half were fibroblastic. In contrast, 47% of primary craniofacial osteosarcomas were high grade and only 1 was fibroblastic. All radiation-associated osteosarcomas recurred, half the patients died of disease, 2 were alive with unresectable tumors, whereas only 1 was alive without disease. In contrast, 80% of patients with primary tumors were alive without disease, 33% had local recurrences, and 13% died of disease. Radiation-associated tumors overexpressed p53 more often (33% vs. 13%), more often had TP53 mutations (33% vs. 8%), had higher proliferative activity (67% vs. 0% showing >50% MIB-1 staining), and expressed ezrin more frequently (83% vs. 40%) than primary tumors. Compared with a control group of 24 high- and 7 low-grade primary extremity osteosarcomas, radiation-associated tumors marked as the high-grade tumors.

CONCLUSIONS. Craniofacial radiation-associated osteosarcomas are high-grade tumors that behave more aggressively than most primary craniofacial osteosarcomas. In addition, they demonstrate higher expression rates of adverse prognostic indicators, further highlighting the distinction. *Cancer* 2006;107:554–62.

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Craniofacial osteosarcoma accounts for 6% to 10% of all osteosarcomas and usually occurs in the mandible or maxilla.^{1–3} Patients tend to be older than those with osteosarcoma of the long bones, with a peak incidence in the fourth decade.⁴ These osteosarcomas are more frequently low grade and half are chondroblastic.^{2,4–6} Osteoblastic osteosarcoma is also common in this location, but fibroblastic osteosarcoma uncommon.⁷

The prognosis is more favorable compared with conventional osteosarcoma of the extremities and the mainstay of therapy is surgical resection.⁸ Morbidity and mortality are primarily due to local recurrence, hence prognosis is largely dependent on margin status

at resection.⁹ Distant metastases are uncommon.⁸ Unlike its counterpart in the long bones, the role of chemotherapy in the treatment of craniofacial osteosarcoma remains to be proven.³

Most cases of craniofacial osteosarcoma are sporadic.² However, known predisposing factors include fibrous dysplasia, Paget disease, retinoblastoma, Li-Fraumeni syndrome, and previous radiation therapy.¹⁰ Secondary osteosarcomas of the head and neck are uncommon and, accordingly, radiation-associated craniofacial osteosarcoma is rare. Osteosarcoma is a common phenotype of radiation-associated sarcoma, accounting for half the cases,¹¹ with approximately 10% occurring in the head and neck region.⁹ The latent period after radiation averages 11 years (range, 4–23 years) in the head and neck,⁹ and its length is inversely proportional to radiation dosage.¹³

Compared with primary craniofacial osteosarcoma, craniofacial radiation-associated osteosarcoma tends to be higher grade,^{8,14,15} more often fibroblastic,^{10,14} shows higher levels of p53 expression,² and has a less favorable prognosis.^{9,12,14} In contrast to conventional osteosarcoma and similar to primary craniofacial osteosarcoma, radiation-associated craniofacial osteosarcomas has been found to demonstrate a low incidence of metastases.^{3,4} A significant factor impacting survival is locally aggressive growth.⁹ However, reports of craniofacial radiation-associated osteosarcoma are limited and not all studies were able to demonstrate behavioral differences with primary craniofacial osteosarcoma.^{16–18}

The purpose of this study was to better understand biologic and clinicopathologic differences between primary and radiation-associated craniofacial osteosarcoma. We analyzed a series of 21 cases, comparing patient characteristics and outcomes, histopathology, and immunohistochemical and molecular data on a number of prognostic indicators. In addition, we compared the prognostic markers in 31 extremity osteosarcomas, both high- and low-grade tumors, as a control group.

MATERIALS AND METHODS

Twenty-one patients with craniofacial osteosarcoma were identified from the files of the Department of Pathology at the University of Michigan (15 cases) and Wayne State University (6 cases) between 1986–2004. Clinical and follow-up data were obtained from medical records, referring clinicians, and the Social Security death registry.

All sections from each case were reviewed by 2 pathologists (J.B.M. and D.R.L.) to confirm the diagnosis and to determine subclassification and grade.

Tumors were subclassified as osteoblastic, chondroblastic or fibroblastic according to the predominant histologic element, and were graded using a 2-tiered system of low and high grade. After pathologic review, a tissue microarray (TMA) was constructed from the most representative areas using the methodology of Nocito et al.¹⁹ Representative sections from 24 high-grade and 7 low-grade extremity osteosarcomas were used as a comparative control group.

Immunohistochemistry

Immunohistochemical staining was performed on a DAKO Autostainer (DAKO, Carpinteria, CA) using DAKO LSAB+ and diaminobenzadine as the chromogen. Deparaffinized sections of the TMA at 5- μ thickness were labeled with antibodies to p53 (rabbit polyclonal antibody, 1:100, NCL-p53-CM1 Novocastra, Newcastle, UK), retinoblastoma protein (pRB) (mouse monoclonal antibody, 1:50, NCL-RB-358, Novocastra), ezrin (mouse monoclonal antibody, 1:200, 3C12, Zymed, San Francisco, CA), or Ki-67 (mouse monoclonal antibody, 1:100, MIB-1, DAKO). p53, pRB, and ezrin immunohistochemistry required microwave citric acid epitope retrieval. Staining with Ki-67 required microwave antigen retrieval in high pH buffer. Appropriate negative (no primary antibody) and positive controls were stained in parallel with each set of tumors studied.

TMA cores stained with anti-p53 and anti-pRB antibodies were scored as positive if strong nuclear staining was identified in >50% of cells for p53 and in any positive cells for pRB. TMA cores stained with anti-ezrin antibody were scored as positive when cytoplasmic and/or membranous staining was present. TMA cores stained with anti-Ki-67 antibody were scored as <10, 10–25, 25–50, or >50% based on the percentage of cells demonstrating nuclear staining.

Sequence Analysis of p53

DNA was extracted from 3 5- μ m thick sections of each tissue block using a Nucleon HT DNA extraction kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. Genomic exons 5, 6, 7, and 8 of the p53 gene were separately amplified according to the methods of de Vos et al.²⁰ Amplified product was purified using a Wizard SV PCR clean-up kit (Promega, Madison, WI) and sequenced directly within the University of Michigan Medical Center DNA Sequencing Core using an ABI 377 DNA sequencer (ABI, Foster City, CA). Chromatograms were downloaded directly to CodonCode Aligner software (v. 1.3.4, Dedham, MA) and the sequence compared with reference sequence

TABLE 1
Clinicopathologic Features in Craniofacial Osteosarcoma

Patient	Age/Sex	Radiation/ Interval, years	Site	Histologic type	Grade	Recurrence	Metastasis	Follow-up, months
1	22/F	No	Mandible	Osteoblastic	Low	No	No	NED/36
2	29/F	No	Mandible	Osteoblastic	Low	No	No	NED/41
3	16/F	No	Mandible	Chondroblastic	Low	No	No	NED/108
4	13/F	No	Maxilla	Osteoblastic	Low	Yes	No	NED/96
5	37/F	No	Mandible	Osteoblastic	Low	No	No	NED/12
6	37/M	No	Maxilla	Chondroblastic	Low	No	No	NED/156
7	62/F	No	Maxilla	Chondroblastic	Low	No	No	NED/24
8	48/F	No	Maxilla	Osteoblastic	Low	No	No	NED/216
9	12/M	No	Mandible	Osteoblastic	High	No	No	NED/14
10	64/M	No	Maxilla	Osteoblastic	High	No	No	NED/168
11	30/M	No	Maxilla	Chondroblastic	High	Yes	No	DOD/24
12	19/F	No	Mandible	Chondroblastic	High	No	No	NED/51
13	20/M	No	Mandible	Fibroblastic	High	Yes	Lymph node	NED/38
14	29/M	No	Mandible	Osteoblastic	High	Yes	No	AWD/14
15	64/F	No	Maxilla	Osteoblastic	High	Yes	No	DOD/15
16	53/M	Yes/?	Mandible	Osteoblastic	High	Yes	Unknown	DOD/21
17	71/M	Yes/9	Oropharynx	Osteoblastic	High	Yes	No	AWD/12
18	26/M	Yes/22	Maxilla	Osteoblastic	High	Yes	Brain	DOD/24
19	19/M	Yes/18	Maxilla	Fibroblastic	High	Yes	No	AWD/84
20	33/F	Yes/18	Maxilla	Fibroblastic	High	Yes	No	NED/48
21	73/M	Yes/24	Mandible	Fibroblastic	High	Yes	No	DOD/19

NED indicates no evidence of disease; AWD, alive with disease; DOD, died of disease.

Boldfaced type (16–21) designates radiation-associated.

downloaded from the National Center of Biotechnology Information (NCBI; p53 reference sequence number: NM_000546, <http://www.ncbi.nlm.nih.gov> [Accessed February 27, 2006]).

RESULTS

Patient Characteristics (Table 1)

The median age at diagnosis was 37 years (range, 16–73 years). Patients with radiation-associated osteosarcoma had a higher median age at diagnosis than those with primary osteosarcoma (43 vs. 33 years). Half the patients were men, including 5 of 6 with radiation-associated and 6 of 15 with primary osteosarcoma. The mandible and maxilla were equally involved (10 cases for each site) and 1 patient had an oropharyngeal osteosarcoma.

Six (29%) patients had a history of radiation in the field where the osteosarcoma arose. The median latency period was 18 years (range, 9–24 years). In 1 patient the interval was unknown. Two patients had radiation for squamous cell carcinoma, 1 involving the oral mucosa (Patient 16) and the other involving the larynx (Patient 17). Two patients received radiation for retinoblastoma (Patients 18 and 19), 1 for

Ewing sarcoma (Patient 20), and 1 for parotid adenocarcinoma (Patient 21).

Pathology

All tumors demonstrated classic light microscopic features of osteosarcoma. Evidence of malignant osteoid production, albeit focal in some, was present in every case. Of the primary osteosarcomas, 9 (60%) were osteoblastic (Fig. 1) and 5 (33%) were chondroblastic (Fig. 2). Only 1 primary osteosarcoma was fibroblastic. In contrast, half the radiation-associated osteosarcomas were fibroblastic (Fig. 3), whereas 3 were osteoblastic and none chondroblastic. All radiation-associated osteosarcomas were high grade compared with only 7 (47%) of the primary osteosarcomas.

Immunohistochemistry and TP53 Mutational Analysis (Table 2)

More radiation-associated osteosarcomas overexpressed p53 protein than primary osteosarcomas, with 33% compared with 13% having strong nuclear staining in >50% of the cells (Fig. 4). Mutation of the TP53 gene was also more common in radiation-associated osteosarcoma. Two (33.3%) radiation-associated osteosarcomas had TP53 mutations (one involving exon 7 at codon 175 and 1 involving exon 5 at codon 248),

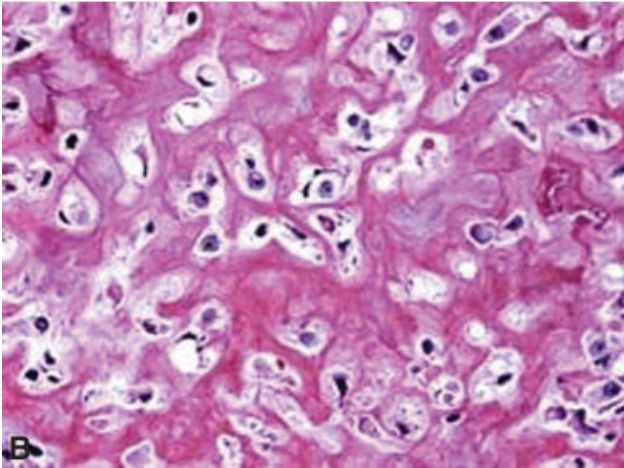


FIGURE 1. Osteoblastic osteosarcoma in a patient with primary mandibular osteosarcoma (Case 9). (A) Computed tomography scan shows a well-circumscribed, heavily mineralized tumor involving the body of the mandible, which was resected with negative margins. (B) Histologically, it had extensive malignant osteoid production. Osteoblastic osteosarcoma was the most frequent histologic type of primary craniofacial osteosarcoma (9 of 15 patients) in this study.

whereas only 1 primary osteosarcoma (8%) had a TP53 mutation (involving exon 7 at codon 248). In 3 primary osteosarcomas we were unable to amplify DNA due to technical restraints and these cases were excluded from mutational analysis.

Expression of pRB was similar in both groups, with nuclear staining identified in 50% of the radiation-associated and 53% of the primary osteosarcomas. Immunoreactivity for the membrane cytoskeletal organizer protein ezrin was higher in radiation-associated osteosarcoma, identified in 5 of 6 (83%) com-

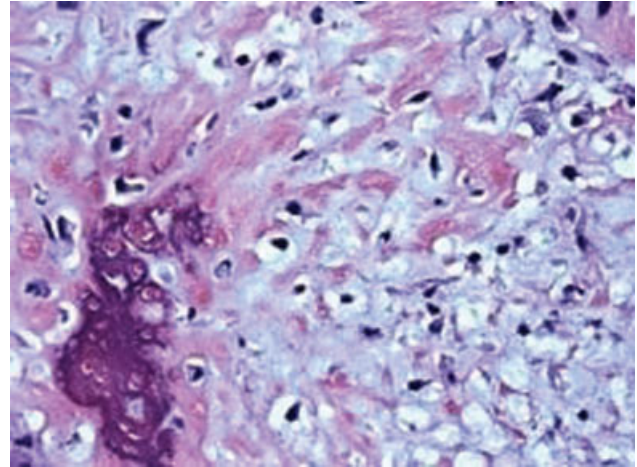


FIGURE 2. Chondroblastic osteosarcoma in a patient with primary mandibular osteosarcoma (Case 7). Chondroblastic osteosarcoma was a frequent variant in primary craniofacial osteosarcoma (5 of 15 patients) in this study, exemplified by this low-grade tumor that produced both chondroid and osteoid matrix.

pared with only 6 of 15 (40%) of the primary osteosarcomas (Fig. 5). Ezrin expression was independent of grade among the primary craniofacial osteosarcoma group, with 4 of 6 (67%) low-grade tumors being positive compared with 2 of 9 (22%) high-grade tumors. Proliferative activity was higher in radiation-associated osteosarcoma, with 4 of 6 (67%) showing >50% Ki-67 nuclear staining compared with none with >50% staining in primary osteosarcoma.

In a control group of 31 extremity osteosarcomas (Table 3), the high-grade tumors ($n = 24$) had a higher rate of p53 expression (18% vs. 0%), higher rate of TP53 mutation (21% vs. 14%), more ezrin-positivity (72% vs. 57%), and greater proliferative activity (50% with Ki-67 staining in >50% of cells vs. 0%) compared with the low-grade tumors ($n = 7$). The TP53 mutation incidences reported herein are consistent with those reported elsewhere for high-²¹ and low-grade osteosarcomas.²²

Follow-up (Table 1)

Follow-up information was available for all patients, with a mean follow-up time of 57 months (range, 12–216 months). Chemotherapy with or without radiation was given to 67% of primary craniofacial osteosarcoma patients compared with 80% of radiation-associated osteosarcoma patients. All six patients with radiation-associated osteosarcomas developed local recurrences, which were characterized by bulky, unresectable disease in all but 1 (Patient 17). Two

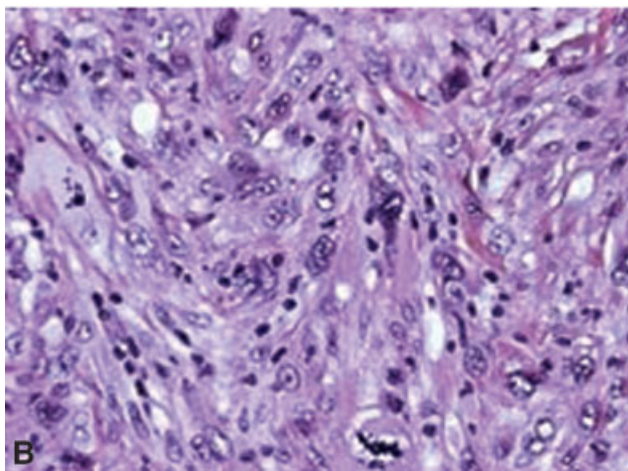


FIGURE 3. Fibroblastic osteosarcoma in a patient with a radiation-associated maxillary osteosarcoma (Case 19). (A) Computed tomography scan shows a poorly circumscribed and destructive tumor involving the maxilla and paranasal sinuses in a patient with a history of retinoblastoma. The tumor was unresectable. (B) Histologically it was a high-grade, pleomorphic, spindle-cell sarcoma that had only focal malignant osteoid production. Fibroblastic osteosarcoma was a frequent finding in craniofacial radiation-associated osteosarcoma (3 of 6 patients) in this study. In contrast, only 1 primary craniofacial osteosarcoma was fibroblastic.

patients (Patients 18 and 19) had been treated for bilateral retinoblastomas in childhood and developed radiation-associated osteosarcomas 18 and 20 years afterwards. Both had unresectable tumors and 1 died of disease that included metastasis to the brain. At the time of last follow-up, 3 (50%) of the radiation-associated osteosarcoma patients had died of disease and 2 were alive with unresectable disease at 12 and 84 months. One was alive with no evidence of disease at 48 months.

TABLE 2
Immunohistochemical and TP53 Mutational Analysis of Prognostic Markers Comparing Primary and Radiation-Associated Craniofacial Osteosarcoma

	p53, %	TP53 mutation, %	Ezrin, %	>50% MIB-1, %
Primary OS (<i>n</i> = 15)	13	8	40	0
RAOS (<i>n</i> = 6)	33	33	83	67

OS indicates osteosarcoma; RAOS, radiation-associated osteosarcoma.

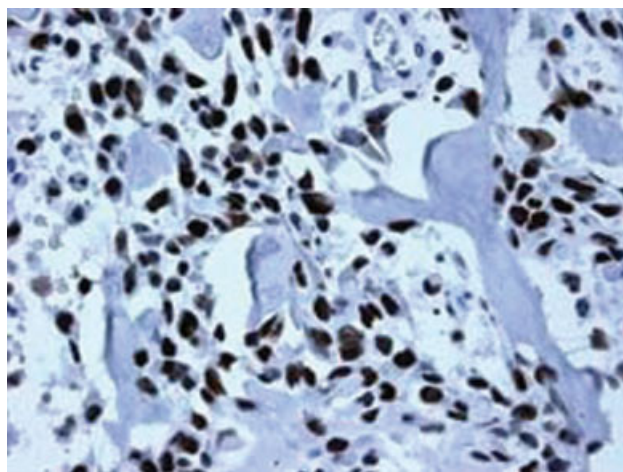


FIGURE 4. Immunohistochemical p53 staining in craniofacial radiation-associated osteosarcoma. Strong nuclear staining such as this was more frequent in radiation-associated than in primary craniofacial osteosarcoma (33% vs. 13%, respectively). In addition, mutations of TP53 tumor suppressor gene were also more frequent in craniofacial radiation-associated osteosarcoma (33% vs. 8%).

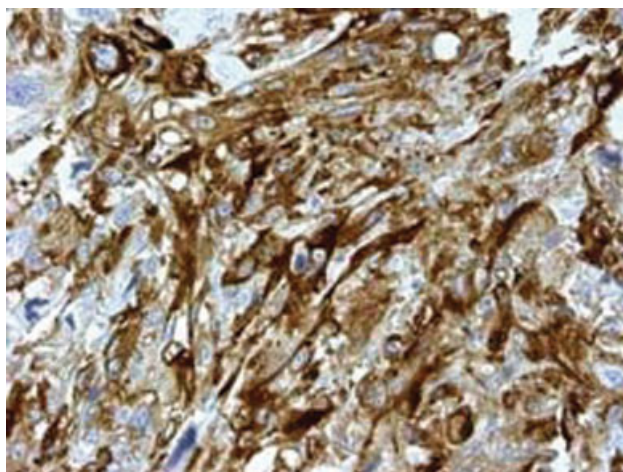


FIGURE 5. Immunohistochemical ezrin staining in craniofacial radiation-associated osteosarcoma. Cytoplasmic and membranous staining for the membrane cytoskeletal organizer protein ezrin was more common in craniofacial radiation-associated than primary craniofacial osteosarcoma (83% vs. 40%).

TABLE 3
Immunohistochemical and TP53 Mutational Analysis of Prognostic Markers Comparing Low- and High-Grade Extremity Osteosarcoma

	p53, %	TP53 mutation, %	Ezrin, %	>50% MIB-1, %
Low-grade OS (<i>n</i> = 7)	0	14	57	0
High grade OS (<i>n</i> = 22)	18	21	72	50

OS indicates osteosarcoma.

Among the primary craniofacial osteosarcoma patients, 5 (33%) developed local recurrences and 1 had metastatic disease to a regional lymph node. At last follow-up, 12 (80%) were alive with no evidence of disease, 2 (13%) had died of disease, and 1 was alive with disease at 14 months. Comparing low- and high-grade primary craniofacial osteosarcomas, patients with low-grade tumors had a better prognosis. Of the 8 patients with low-grade tumors, all were alive with no evidence of disease. In contrast, of the 7 patients with high-grade tumors, 2 had died of disease, 1 was alive with persistent disease, and four were alive without disease, including the 1 patient who had a lymph node metastasis.

DISCUSSION

Although uncommon, radiation-associated sarcoma of bone is a well-recognized entity associated with poor prognosis. In a review of 78 cases by Weatherby et al.,¹⁰ osteosarcoma was the most frequent histologic type (49%), followed by fibrosarcoma (41%). Sixteen (21%) of these cases occurred in the craniofacial bones: 7 in the maxilla, 4 in the mandible, and 5 in the cranium. Only 4 of the 16 patients with craniofacial radiation-associated osteosarcoma in that study were symptom-free survivors after 5 years, in addition to another patient who had a recurrence at 8 years and ultimately died of metastatic disease.

Other authors have reported similar findings regarding craniofacial radiation-associated osteosarcoma. Arlen et al.⁹ reported 7 (14%) head and neck tumors among 50 radiation-induced osteosarcomas. All but 1 developed local recurrences and all 7 patients died of disease within 3 years. In another study, Bennett et al.⁸ reported 22 cases of osteosarcoma of jaws including 4 with radiation-associated osteosarcoma. All 4 of these patients developed local recurrences and 3 died of disease within 2 years of diagnosis. In contrast, only half the patients with primary osteosarcoma developed recurrence.

Not all studies have demonstrated a worse prognosis for craniofacial radiation-associated osteosarcoma.^{3,16,17} For example, Oda et al.¹⁷ identified 2

cases of radiation-associated head and neck osteosarcoma among a series of 13 cases, and both patients were alive with no evidence of disease at 84 and 180 months. Ha et al.,¹⁶ in a series of 27 head and neck osteosarcoma, found no detectable effect of prior radiation exposure on survival.

In this study we found craniofacial radiation-associated osteosarcoma to have a poor prognosis compared with primary craniofacial osteosarcoma. All 6 patients with radiation-associated osteosarcoma in our study developed local recurrences with bulky, unresectable disease in all but 1. Three died, 2 had uncontrolled, locally aggressive tumors, and only 1 was alive with no evidence of disease. In contrast, 80% of the patients with primary craniofacial osteosarcoma were alive with no evidence of disease, and only 2 (13%) had died of disease. These survival data were not affected by different treatment regimens between the 2 groups, as similar rates of chemotherapy treatment were identified among the patients with high-grade tumors.

Similar to findings in prior studies,^{3,4,8} we found low rates of metastases compared with patients with conventional osteosarcoma of the long bones. Overall, only 2 patients had documented metastatic disease, including 1 primary and 1 radiation-associated tumor, both of which were high grade. Therefore, similar rates of metastases were identified among high-grade tumors in both groups (14% vs. 20% in primary and radiation-associated craniofacial osteosarcoma, respectively).

All craniofacial radiation-associated osteosarcomas in this study were high grade, compared with only 47% of the primary osteosarcomas. Although results have varied, a number of studies correlate high-grade histology with worse prognosis.^{16,23} For example, high-grade tumors and positive surgical margins were the only features impacting survival in a study of 27 head and neck osteosarcomas by Ha et al.¹⁶ Similarly, Slootweg and Muller²³ identified older age and high-grade histology as the only features affecting prognosis in a study of 18 osteosarcomas of the jaws. Our data show similar findings as these authors. Clearly the radiation-associated craniofacial osteosarcomas, which were all high grade, did worse. However, when we separately analyzed our cases of primary craniofacial osteosarcoma by grade, the high-grade tumors similarly did worse than the low-grade tumors. Thus, the preponderance of high-grade tumors among radiation-associated osteosarcomas appears to be an important prognostic variable.

Another histologic feature that differed between the 2 groups was a disproportionately higher percent-

tage of fibroblastic osteosarcomas in the radiation-associated osteosarcoma group. Other studies have similarly identified a higher rate of fibroblastic osteosarcoma in radiation-associated osteosarcoma.^{14,24} For example, Huvos et al.²⁴ in a series of 66 postradiation osteosarcomas from all sites found fibroblastic osteosarcoma to be the most frequent histologic type, accounting for 49% of the tumors. Similarly, Shah et al.¹⁴ found high numbers of craniofacial osteosarcomas with fibroblastic histology in retinoblastoma patients who developed postradiation osteosarcomas. These findings are intriguing because in the long bones fibroblastic osteosarcoma has been shown to behave more aggressively.²⁵

p53 protein participates in regulation of the early G1 phase of the cell cycle and participates in apoptosis. TP53 gene mutations, which are commonly accompanied by immunohistochemical p53 overexpression,²⁶ appear to play an important role in the pathogenesis of postradiation sarcoma.²⁷ Junior et al.² identified p53 overexpression in 52% of tumors in a series of 25 head and neck osteosarcoma, with most tumors (32%) reported as strongly positive (>50% of nuclei with positive staining). Although they did not correlate p53 staining with radiation history or prognosis, they did find a correlation with grade in that 73% of high-grade tumors were positive compared with only 36% of low- and intermediate-grade tumors. In another study, Olivera et al.²⁸ found 47.5% of 17 of jaw osteosarcomas to overexpress p53, but also could not correlate staining with prognosis. Lopes et al.²⁹ identified overexpression in 88% of osteosarcomas of the jaws in their study of 9 cases, including 5 that were strongly positive.

In our study, p53 was overexpressed in 24% of cases, evidenced by strong nuclear staining in >50% of cells. Radiation-associated osteosarcomas more often overexpressed p53 than primary tumors (33% vs. 13%), and more often had detectable mutations of the TP53 gene (33% vs. 7%). Although most studies have shown p53 expression not to be prognostically useful, our data support a role for p53 in the pathogenesis of craniofacial radiation-associated osteosarcoma. However, further investigation with larger patient groups and molecular analyses is warranted.

Ezrin is a membrane-cytoskeleton linker protein belonging to the Band 4.1 protein superfamily and is the best-characterized ezrin-radixin-moesin protein.³⁰ Ezrin-radixin-moesin proteins are cytosolic proteins that when phosphorylated localize to the cell membrane where they link F-actin to the cell membrane.³⁰ In essence, this allows the cell to interact with its microenvironment while facilitating

intracellular signal transduction. These processes are thought to be responsible for its role in the development of metastases.³⁰ In fact, immunohistochemical staining for ezrin in metastatic osteosarcoma has been shown to be of membranous type, whereas primary and recurrent tumors demonstrate both membranous and cytoplasmic staining.³¹ Ezrin expression has been reported in osteosarcomas as well as in other malignancies.³¹ However, no prior studies on its expression or association with outcome in radiation-associated or primary craniofacial osteosarcoma have been reported.

The craniofacial radiation-associated osteosarcomas in our study more often expressed ezrin (cytoplasmic and/or membranous pattern) compared with the primary tumors (83% vs. 40%). Furthermore, ezrin expression appeared to be independent of grade. The expression pattern demonstrated in this study indicates that ezrin positivity may be a prognostic marker for craniofacial osteosarcoma and may shed light on the clinical and biologic differences between primary and radiation-associated craniofacial osteosarcoma.

A number of proteins expressed in actively proliferating cells have been identified. Among these, Ki-67 is commonly used and its prognostic value has been described in many tumor types. Unfortunately, little data exist on its use in osteosarcoma,³² especially craniofacial and radiation-associated osteosarcoma. Park and Park³² identified weak staining for Ki-67 in 64% of 67 osteosarcomas from all sites. In a study of 25 head and neck osteosarcomas by Junior et al.,² 88% were positive for Ki-67, including 48% that were strongly positive. However, they were unable to correlate Ki-67 positivity with prognosis, and no evaluation as to correlation with grade or association with radiation exposure was made. In our series, 4 of 6 of the radiation-associated osteosarcomas showed very high levels of Ki-67 expression (>50% nuclear staining) as compared with none with this degree of staining in the primary osteosarcoma group. In the primary osteosarcomas, the high-grade tumors tended to have higher proliferation rates, but did not approach the levels seen in the radiation-associated osteosarcoma.

Retinoblastoma results from deletion of the 13q14 region of the RB tumor suppressor gene and, although survival for this tumor is excellent, a well-known complication is the development of a second nonocular malignant tumor occurring predominately in patients with bilateral retinoblastomas harboring germline RB mutations.³³ Secondary malignant tumors in these patients are believed in large part to be radiation-induced, and the incidence has been

correlated with radiation dose.¹³ In a review of 688 retinoblastoma patients who survived after radiation therapy, 13% developed second tumors, 70% of which occurred in the radiation field.³³ The most common histology was osteosarcoma (39.4%). Radiation-associated craniofacial osteosarcoma in these patients has a poor prognosis.¹⁴

Decreased immunohistochemical pRB staining correlates with decreased expression of the RB gene product. Radiation therapy has been suggested to induce RB mutations as a possible mechanism participating in radiation-associated osteosarcoma.³⁴ In our study, no difference was found in pRB expression between primary and radiation-associated osteosarcoma (56% vs. 50%), providing little evidence for an increased rate of RB gene deletions in patients exposed to radiation.

In summary, our findings demonstrate important biologic and clinicopathologic differences between primary and radiation-associated craniofacial osteosarcoma, indicating that these are distinct clinical entities with different prognoses, which may benefit from different therapeutic approaches. Survival was worse in patients with radiation-associated osteosarcoma, with most deaths resulting from unresectable, locally aggressive tumors. In addition, all were high grade and half were fibroblastic, an otherwise uncommon morphology in craniofacial osteosarcoma. Craniofacial radiation-associated osteosarcomas are more likely to be associated with adverse prognostic markers such as p53 overexpression, TP53 mutation, ezrin expression, and high proliferative activity. These differences parallel those between high- and low-grade extremity osteosarcomas, suggesting histologic grade to be an important variable responsible for the poor outcome in craniofacial radiation-associated osteosarcoma. Although confirmation of these findings with a larger cohort of patients is warranted, these results suggest that craniofacial radiation-associated osteosarcoma has distinctive biologic and clinicopathologic characteristics, knowledge of which may assist in determining prognosis and guiding clinical management.

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