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Sarcomas and sarcomatoid tumor after radiotherapy of oral squamous cell carcinoma: analysis of 4 cases

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Radiation-induced sarcoma (RIS) or postirradiation sarcoma has been reported rarely as a long-term complication of radiation therapy (RT). We report 4 cases of oral sarcomas or sarcomatoid tumors with a rather short latency period after radiotherapy of the prior OSCC. Histopathological evaluation and immunohistochemical study were performed using a panel of markers including vimentin, cytokeratin, S-100, desmin, myoglobin, HHF-35, p53, and p16. All reported cases were positive for vimentin and negative for cytokeratin. Two cases were positive for myoglobin, desmin, or HHF-35, and were probably myogenic origin. One case was possibly a fibrosarcoma and the subclassification of the other one was not specified. Diverse expression of p53 and p16 was further observed in these 4 cases. Report of the complicated clinical processes and the analysis of genetic markers of these cases provide useful clinical and pathogenetic insights of mesenchymal malignancies associated with a status post OSCC radiation. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;105:65-71)**

Using a high dosage of postoperative radiation therapy (RT) is common in the standard treatment modality of advanced (stages III and IV) oral squamous cell carcinoma (OSCC). However, ionizing radiation has been recognized as a carcinogen ever since Roentgens discovered X rays in 1895. The first recorded case of a radiation-induced malignancy is squamous cell carcinoma, which was observed in the early 20th century. In the following 2 decades, many irradiation therapy–related secondary tumors, mostly sarcomas in the body and extremities, have been reported. Recently, radiation-induced malignancies of the head and neck in the sinuses, pharynx, thyroid, esophagus, and skin have been reported in many studies.[1,2] However, very rare cases of radiation-induced sarcoma (RIS) after OSCC were reported.[3]

The rarity of this tumor in the head and neck makes it difficult to provide a clear definition of the RIS or to clarify between primary and the successive RIS. In addition, because of the rarity of RIS in the head and neck, only clinical cases and review papers were reported initially. In 2000, Mertens et al.[4] first described the chromosome aberrations in cases of RIS. Tarkkanen et al.[5] further analyzed the difference of genetic changes between sporadic sarcoma and RIS by using comparative genomic hybridization. This highlighted a similar scenario in the formation of RIS to any malignancy, in that the defect of critical genes controlling the checkpoints of cell cycles causes the malignancy. The normal functioning of the well-known tumor suppressor gene (TSG), p53, may include the induction of apoptosis in cells suffering from irreversible genetic defects as a result of ionizing radiation. p53 mutation in tumors is associated with a poor response to radiation therapy.[6,7] In addition, inactivation

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Table I. Summary of 4 cases

		Primary OSCC				Sarcoma		
Case	*Age, y/Sex*	*Diagnosis*	*Location*	*Radiation dose, Gy*	*Latency, mo*	*Origin*	*Treatment*	*Outcome*
1	55/F	SCC, T3N0M0	Tongue	80 BRT 103 XRT	40	Muscular	Surgery, RT	DOD
2	63/M	SCC, T3N0M0	Palate	65 XRT	9	Fibroblastic	Surgery, CT	DOD
3	64/M	SCC, T4N0M0	Bucal mucosa	70 XRT	25	ND	Surgery, CT	DOD
4	66/M	SCC, T4N1M0	Buccal mucosa	66 XRT	50	Muscular	Surgery, CT	DOD

BRT, brachytherapy; *CT,* chemotherapy; *DOD,* died of disease; *ND,* not determined; *XRT,* external beam therapy. The age was reported when the patient was diagnosed to have sarcoma or sarcomatoid tumor after radiation therapy.

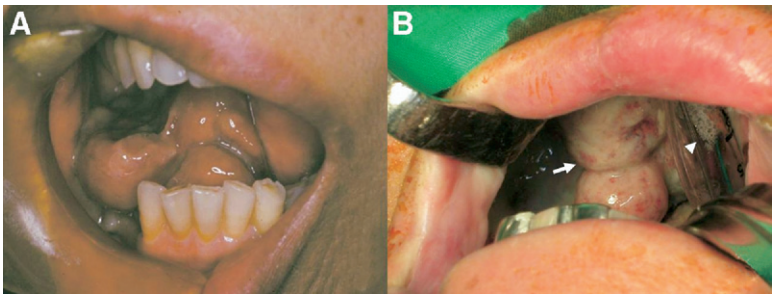

Fig. 1. Clinical manifestations of RIS. (**A**) Case 1: A painless, lobulated, smooth surface soft tissue mass on the right side of the tongue. (**B**) Case 2: A smooth bulging mass on right buccal mucosa (arrow) together with a separated palatal mass with smaller size and surface ulceration (arrow heads).

or down-regulation of p16 (CDKN2a/INK4a) is required to immortalize the cells,[8] and abnormal function of p16 is associated with local treatment failure in radiotherapy.[9] Thus, the lack of normal functioning of TSGs, such as p53, p16, and others, have been correlated significantly with the transformation or progression of many malignancies, especially those having received high efficient megavoltage radiation therapy.

In the present case analysis, 4 patients having received a high dosage of radiotherapy developed sarcomas or sarcomatoid tumors at anatomical locations near primary cancers. The differential diagnosis was made in the primary cancer and successive malignancies by a series of conventional histological and immunohistochemistry (IHC) examinations including p53 and p16.

CASE REPORTS

Four patients (1 female, 3 male) with features of sarcomas or sarcomatoid change of tumors were treated at Taipei Veterans General Hospital (Taipei-VGH) from 1992 to 2003. Patients' ages ranged from 52 to 62 years. All of the patients were irradiated for primary OSCC. After a variable period of latency, sarcomas or sarcomatoid tumors occurred in the previously irradiated field (Table I).

Case 1

A 52-year-old female first presented with a smaller than 2-cm ulcerative lesion in the right ventral tongue and mouth floor in 1996 at another medical center and the lesion was pathologically shown to be squamous cell carcinoma (SCC). She refused surgical treatment and took herbal medicine. Because the lesion had no palliation, she came to our hospital to seek help. She had a history of smoking without alcohol consumption or betel quid chewing. Physical examination and magnetic resonance image revealed an ulcerative mass about 3 × 3 cm in size in the right side tongue base area without invasion into pharynx area. She did not have surgery and opted to do interstitial brachytherapy as the treatment modality with therapeutic dose of 8000 cGy in 40 fractions for the primary lesion and an additional dose of 5587 cGy in the right neck and 4687 cGy in the left submandibular area. After radiotherapy, the lesion totally subsided and the patient was regularly followed in our hospital.

Three years later, in 1999, a 2 × 2-cm bulging mass occurred in the mucosa of the right cheek. The mass was excised with a pathological diagnosis of "granulation tissue." Unfortunately, in July 2000, the lesion recurred in a similar location and grew rapidly into a 4 × 3-cm mass within 1 month (Fig. 1, *A*). A computed tomographic (CT) scan revealed a 4-cm heterogeneously enhanced soft tissue mass protruding from the right buccal mucosa, extending to the right side of the tongue, mouth floor, and submandibular region without evidence of neck lymphadenopathy. She underwent wide excision, partial mandibulectomy, and suprahyoid neck dissection with free fibula flap reconstruction. Final pathologic report confirmed the lesion as a rhabdomyosarcoma. She subsequently received adjunctive external beam radiotherapy for 6200 cGy and chemotherapy. Unfortunately, she expired as a result of tumor recurrence and massive bleeding 10 months after this treatment.

Case 2

In 1994, a 57-year-old male presented with a 2.5 × 2-cm indurated, ulcerative exophytic mass over the right side of the palate for 2 months. He had a history of smoking and betel quid chewing for more than 20 years. A CT scan showed no evidence of neck lymphadenopathy. The lesion was proven to be SCC by incision biopsy with a final staging of pT3N0M0, stage III. After wide excision for the tumor, he was regularly followed in the outpatient clinic until 6 years later, in December 2000, a 2 × 2-cm new mass was found over the right palate again. The mass was excised with a 1-cm safe margin and pathologically diagnosed as a well-differentiated SCC. However, the tumor recurred in 7 months and extended to the underlying bone, right upper gingival, and soft palate. Another wide excision operation plus right side partial maxillectomy was performed. Postoperative external beam radiation therapy with a total dosage of 6480 cGy was delivered for local control of the tumor.

Nine months after radiotherapy, 2 distinct masses were noted. One was a 3.5-cm exophytic mass with a smooth surface on the right buccal mucosa, and the other was a 2.5-cm exophytic and ulcerative mass over the median border of the palate adjacent to the surgical margin of previous partial maxillectomy (Fig. 1, *B*). After the operation, the pathological report revealed 2 distinct diagnoses of lesions with a sarcoma in the buccal mucosa and a recurrent SCC in the palate. He soon received adjunctive chemotherapy but unfortunately died of sepsis and cachexia at 2 months after chemotherapy.

Case 3

A 62-year-old male was diagnosed with gingival SCC of the right mandible in March 2001. He refused surgery, but received external beam radiation therapy with a total dosage of 7020 cGy in another hospital. One year later, the tumor recurred and he came to our hospital for surgical treatment. Wide excision with radical neck dissection was performed. The pathology revealed a diagnosis of a stage IV pT4N0M0 SCC. No other adjunctive treatment was given. One year later, in March 2003, a 1 × 1-cm ulcerative lesion was noted over the right lower gingiva adjacent to the previous surgical site. The lesion was excised and pathologically proved as a recurrent SCC. After surgery, a 3 × 3-cm bulging-out soft tissue mass occurred in the right submental area perforating through the skin. He received another ablative excision with partial mandibulectomy. Unfortunately, the pathological report showed the malignant tumor in the upper neck to be consistent with a sarcoma. After surgery, only chemotherapy was administered because previous full-dosage radiotherapy had been given. During the period of administering chemotherapy, his condition worsened with signs of sepsis and massive bleeding. He expired as a result of sepsis and respiratory failure in July 2003.

Case 4

The 62-year-old male presented with a 4 × 5-cm exophytic ulcerative, indurated mass over the left buccal mucosa in January 1999. He had a history of smoking, alcohol drinking, and betel quid chewing for more than 30 years. Physical examination showed a firm, but movable 2-cm neck mass with a central necrosis pattern in CT scan. Wide excision combined with radical neck dissection was performed, and the final pathological diagnosis of moderately differentiated SCC was made (pT4N1M0, stage IV). Postoperative concurrent chemoradiotherapy was given with a total radiation dosage of 6600 cGy in 33 fractions. In April 2003, a 3 × 2-cm new hemorrhagic soft mass lesion was found over the right buccal mucosa and a wide excision was performed. The pathological report revealed the diagnosis of a sarcoma. Five months later, sarcoma recurred again in the previously dissected region with neck metastasis. He refused to have any ablative surgery, but only palliative chemotherapy. The patient's systemic condition worsened and he expired with sepsis and respiratory failure within 2 months.

IMMUNOHISTOCHEMISTRY

Surgical specimens from these 4 cases were processed routinely by tissue sectioning and evaluated by experienced oral pathologists. Hematoxylin and eosin (H&E) stain was used for evaluating the morphology of tumor cells. IHC was carried out using protocols previously established.[10] The primary antibodies for IHC included those against cytokeratin (Signet, Dadhem, MA), vimentin, S-100, desmin, myoglobin, HHF-35 and p53 (Dako, Carpinteria, CA), and p16 (BioCare, Concord, CA). After rinsing with phosphate-buffered saline (PBS), standard IHC staining was carried out using a LSAB2 streptavidin-biotin complex system (Dako) with 3, 3′-diaminobenzidine as the chromogen. The slides were counterstained with hematoxylin and mounted with Clearmount (Zymed, South San Francisco, CA). An identical section without the addition of the primary antibody was processed in the same manner as the negative control. The extent of immunoreactivity was independently scored based on the percentage of positive cells found in each tissue section. Cell content ≤10% was considered positive (+) in cases with strong staining, while positive cell content less than 10% was considered negative (−). Case of ambiguous immunoreactivity was recorded as (+/−).

RESULTS

Histopathology of tumors after radiotherapy

Case 1. Histological observation revealed spindle and pleomorphic malignant cells with eosinophilic cytoplasm. IHC for cytokeratin was negative, but vimentin was positive. IHC for myoglobin, a striated muscle specific oxygen-binding protein, was positive. Although the IHC for HHF-35 was negative, the overall IHC is suggestive a rhabdomyosarcoma (Fig. 2, *A*).

Case 2. Histological evaluation of the buccal mucosa lesion revealed a diffuse infiltration of spindle-shaped tumor cells arranged in a fascicle pattern, with pale

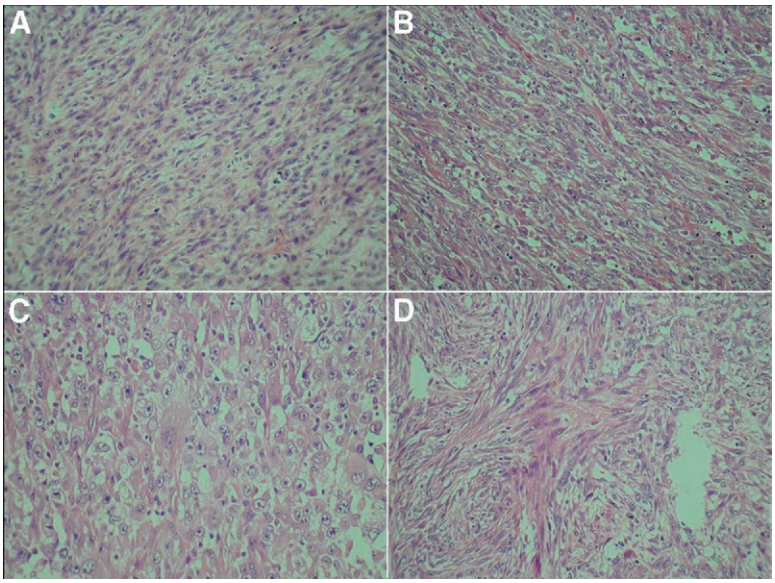

Fig. 2. Histopathology of RIS. (**A–D**) Case 1–case 4, respectively. Cases 1, 2, and 4 are composed of spindle cells with cigar-shaped nuclei and eosinophilic cytoplasm. Case 3 consists mainly of rather large polygonal or irregular cells with vesicular nuclei. Malignant features including pleomorphism, nuclear hyperchromatism, and frequent mitoses are present in tumors (hematoxylin and eosin, ×200).

Table II. Expression of tissue markers in sarcomas

	Sarcoma					
Case	*Cytokeratin*	*Vimentin*	*S-100*	*Desmin*	*Myoglobin*	*HHF35*
1	−	+	−	−	+	−
2	−	+	−	−	−	−
3	−	+	−	−	−	−
4	−	+	−	+/−	−	+/−

+/− represents heterogeneous weak positive.

Table III. p53 and p16 immunoreactivity in primary OSCC and sarcoma

	OSCC		*Sarcoma*	
Case	*p53*	*p16*	*p53*	*p16*
1	NA	NA	+	−
2	+	−	+	+/−
3	−	−	−	−
4	+	−	−	+

+/− represents heterogeneous weak positive; *NA,* not available.

eosinophilic cytoplasm and plump cigar-shaped nuclei. Prominent nucleoli and frequent mitotic figures were present (Fig. 2, *B*). Tumor cells were diffusely positive for vimentin and negative for cytokeratin, HHF-35, desmin, or myoglobin.

However, in the other lesion localized to the palate, nests of well-differentiated SCC with stromal invasion were observed. In the invasion front, spindle-shaped cells that were positive for both cytokeratin and vimentin were noted. All malignant cellular components in this tumor were negative for HHF-35, myoglobin, and S-100. Moreover, CD117 highlighting c-kit expression specific for gastrointestinal stromal tumor was also negative.

Case 3. Histological evaluation showed stromal tissue and skeletal muscle infiltrated with mainly plump polyhedral cell clusters and some short spindle-shaped tumor cells. These tumor cells appeared pale and plump with eosinophilic cytoplasm, vesicular nuclei, prominent nucleoli, pleomorphism, and frequent mitotic figures (Fig. 2, *C*). The tumor cells were positive for IHC staining of vimentin, but were negative for cytokeratin, HHF-35, desmin, or S-100 protein.

Case 4. Sections of the excised mass showed a picture of sarcoma composed of spindle cells in a fascicle, whorl, or occasional herringbone arrangement. Eosinophilic cytoplasm and plumped hyperchromatic nuclei with frequent mitoses were seen in tumor cells (Fig. 2, *D*). Masson trichrome stained the tumor cells red. Tumor cells also exhibited strong immunoreactivity for vimentin and were focally positive for desmin and HHF-35. The CD117 stain was negative.

IHC is summarized in Table II. Case 1 and case 4 exhibited positive staining for myoglobin, desmin, or HHF-35, and were considered of myogenic origin. It is likely that case 1 is a rhabdomyosarcoma and case 4 is a leiomyosarcoma. Case 2 is possibly a fibrosarcoma. The differentiation of case 3 could not be defined with these markers.

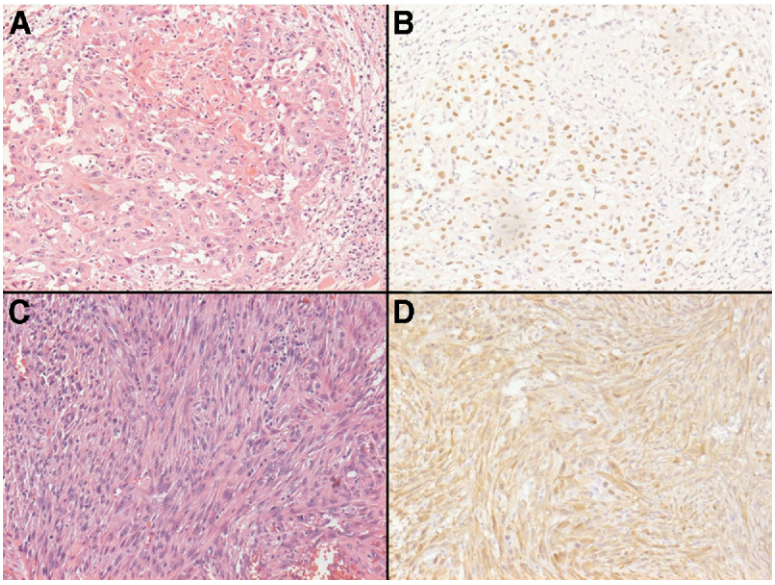

Fig. 3. Representative p53 and p16 immunoreactivity. (**A**) Hematoxylin and eosin stain of primary OSCC of case 3. (**B**) Positive p53 nuclear immunoreactivity in primary OSCC for case 3. (**C**) Hematoxylin and eosin stain of RIS in Case 4. (**D**) Cytoplasmic p16 immunoreactivity for case 4. (Magnification, ×200.)

IHC for p53 and p16 of the primary OSCC and sarcoma

We also investigated the expression of p53 and p16 using IHC. Differential p53 and p16 expression between primary OSCC and sarcoma in the same patient were noticed (Table III). Two of the 3 primary OSCCs showed positive p53 immunoreactivity. In the sarcoma counterparts, 2 were positive and 2 were negative for p53. All 3 available primary OSCCs were negative for p16 immunoreactivity. As for sarcomas, case 1 and case 3 were negative and case 4 was positive for p16 immunoreactivity (Fig. 3).

DISCUSSION

The occurrence of sarcomas in the head and neck region is rare. According to the sarcoma database recorded by Patel et al.,[11] only 4.2% of 3796 sarcoma patients had a lesion involving the head and neck. Among all malignant tumors in this area, sarcomas account for less than 1%. The etiology of sarcomas includes tumorigenic effects of prior radiation therapy and the spontaneous development of second malignancies in the form of sarcomas. In 1948, Cahan et al.[12] described the criteria used for the diagnosis of RIS in irradiated bone. The guideline was modified by Arlen et al.[13] in 1971 as follows: (1) history of radiation therapy, (2) the development of neoplasm within the field of the radiotherapeutic beam, (3) a relatively long period of latency of at least 5 years, and (4) histological proof of a sarcoma. Although the above criteria are still used in the diagnosis of a RIS, controversies remain in regard to the definition of the RIS and, especially, the latency period. In fact, many cases of RIS reported in recent literature showed a latency period of less than 5 years.[1,3,14-16] Thus, Murray et al.[17] in 1999 revised the original criteria by removing the limitations on the latency period and emphasized that radiation-induced malignancies were not merely sarcomas. When a new lesion was histologically different from the irradiated original lesion, it may be radiation-induced.

The incidence of sarcoma after radiation therapy ranges from 0.03% to 0.80%. In the head and neck region, the incidence of radiation-induced secondary cancers (including carcinoma and sarcoma) was reported to be 0.70%.[18] They most commonly involve skin and thyroid tissue. Regarding the types of malignancies, most of the radiation-induced malignancies were found to be carcinoma.[2,18,19] However, in a recent survey of a series of 13 patients with radiation-induced malignancy (RIM) of the head and neck, RIS accounted for the most frequent RIM.[20] The 4 cases presented here had received large dosages of radiation therapy ranging from 65 to 80 Gy. All of the new neoplasms were located within the field of prior radiation and were proven to be sarcomas that were histologically different from the primary tumors. IHC evaluation demonstrated that all cases had negative cytokeratin expression and positive vimentin expression highlighting the sarcomatous features.

In reviewing the histopathology of different types of RIS, the 3 most commonly seen RIS types were osteo-

sarcoma, malignant fibrous histiocytomas, and fibrosarcoma.[3,17,21,22] Because of the heterogeneity and the lack of expression of tissue markers, one fourth of the reported RIS cases could not be subclassified. In this study, except for case 3, the cases exhibited typical features of malignant spindle cells, which could be categorized as muscle, nerve, bone, or fibroblast in origin. Using selected markers, we showed clearly that cases 1 and 4 were rhabdomyosarcoma and leiomyosarcoma, respectively. The morphological, cytological, and IHC features of case 2 suggested a fibrosarcoma or sarcomatoid tumor. The classification of case 3 was not definitive. However, since it occurred in submental space and had intact mucosal surface, a radiation-induced soft tissue–originated malignancy is highly suspected. In case 2, in addition to sarcoma over the buccal mucosa, there were mixed epithelial and focal mesenchymal elements that were positive for both cytokeratin and vimentin in the invasion front of the palatal lesion. This histological characteristic is compatible with "sarcomatoid carcinoma" or "spindle cell carcinoma." However, such features only occurred in an invasion front; therefore, it is considered an epithelial-mesenchymal transition.[23,24] The true mechanism underlying such changes is unknown, and recurrence or radiation might contribute to such de-differentiation.[25,26] For this case, either the frequent recurrence of tumors or the effect of radiation may be the reason for cells to become more anaplastic. Alternatively, multiclonal origin may also explain a synchronous genesis of hybrid tumors.[27,28]

Ionizing radiation is known universally as having a mutagenic potential. The p53 gene is a well-known TSG that has a pivotal role in regulating cell cycle, genomic stability, differentiation, and apoptosis. Overexpression of mutant p53 represents the most common genetic change in OSCC and the aberrant p53 immunoreactivity is correlated with oral tumorigenesis.[10,29] p53 mutations in the process of radiation-induced malignant transformation have also been reported.[8,30] A high correlation between the effect of radiation therapy and p53 expression has been shown. Animal studies supported the idea that an absence or reduction of normal p53 function enhances radiation-induced tumorigenesis by increasing genetic instability at various loci.[31,32] The p16 gene is another TSG that acts as a negative cell-cycle regulator. p16 has been found to be altered in most OSCC.[33-37] p16 expression also enhances radiation-induced cell killing.[38] Thereby, p53 and p16 alterations have been recognized in association with the treatment response to radiation therapy and the consequent occurrence of radiation-induced malignancies. In this study, we observed diverse staining results in the 4 cases. Only p16 immunoreactivity was similarly all negative in the primary OSCC. Nuclear accumulation of p53 was found in 2 of 3 cases. These preliminary findings suggest that p16 down-regulation and p53 alteration might be involved in oral carcinogenesis. This pilot analysis revealed diverse expression profiles in p53 and p16 in the primary and successive tumor pairs in cases 2, 3, and 4 except that the specimen of primary OSCC in case 1 was unavailable. The discordant staining pattern suggests that these short-latency-period sarcoma cases may originate from cells other than carcinoma cells, and possibly be induced by radiation.

Successful treatment of RIS is difficult, especially for high-grade lesions. Surgical excision remains the only definitive treatment for RIS.[22] The extent and adequacy of excision determine the incidence of local recurrence, distal metastasis, and survival. In RIS of the head and neck, an adequate surgical margin is difficult to achieve because of the difficulty in determining their surgical safe margins. Therefore, regional metastasis of sarcomas in the head and neck after surgery occurred frequently. Other treatment options, such as adjunctive postoperative radiation therapy and chemotherapy, should be considered, although their ability to achieve overall survival has yet to be evaluated. Although the incidence of RIS is rare, its biological behavior is more aggressive than spontaneous tumors. All of the 4 patients reported had received aggressive surgical intervention after successive tumors were found. All cases showed tumors that recurred several months after surgery, and they died within 2 years after treatment. This characteristic warrants even more special attention to the detection of secondary malignancies after the radiation therapy. Extreme care should be taken in the follow-up or biopsy obtained from any suspected lesions that occurred in or adjacent to the postirradiated area after a latency time from months to years.

CONCLUSIONS

To implicate radiation therapy in the causation of tumors is difficult. When tumors occur that are histologically or phenotypically different from the primary cancer and having radiation history, the possibility of radiation-induced malignancy should be taken into consideration.

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