■ ORIGINAL ARTICLE ■

Mdm2 and p53 Expression in Radiation–Induced Sarcomas of the Head and Neck: Comparison with *De Novo* Sarcomas

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Background: The pathogenesis of radiation-induced sarcomas (RISs) is not well known. In RIS, *TP53* mutations are frequent, but little is known about Mdm2-p53 interaction, which is a recent therapeutic target of sarcomas. **Methods:** We studied the immunohistochemical expression of Mdm2 and p53 of 8 RISs. The intervals between radiation therapy and diagnosis of secondary sarcomas ranged from 3 to 17 years. **Results:** Mdm2 expression was more common in *de novo* sarcomas than RISs (75% vs 37.5%), and p53 expression was more common in RISs than in *de novo* cases (75% vs 37.5%). While half of the RISs were Mdm2(–)/p53(+), none of *de novo* cases showed such combination; while half of *de novo* sarcomas were Mdm2(+)/p53(–), which are a candidate group of Mdm2 inhibitors, only 1 RIS showed such a combination. Variable immunoprofiles observed in both groups did not correlate with tumor types, except that all of 2 myxofibrosarcomas were Mdm2(+)/p53(+). **Conclusions:** In conclusion, we speculated that both radiation-induced and *de novo* sarcomagenesis are not due to a unique genetic mechanism. Mdm2-expression without p53 overexpression in 1 case of RIS decreases the future possibility of applying Mdm2 inhibitors on a subset of these difficult tumors.

Key Words: Cancer, radiation induced; Head and neck neoplasms; Sarcoma; Mdm2 protein, human; Tumor suppressor protein p53

Development of sarcomas after therapeutic radiotherapy is a rare, but well known complication. The pathogenesis of radiation-induced sarcomas (RISs) has not been elucidated, especially in cases without germline *RB1* mutation. Gonin-Laurent *et al.*[1,2] discovered inactivation of the *TP53* gene in 58% of 36 human RISs with or without *Rb1* mutation and proposed that *TP53* inactivation is an early event caused by irradiation, and not by the *MDM2/MDMX* pathway. In contrast, Roch-Lefevre *et al.*[3] identified recurrent gains/amplifications at many chromosomal regions including the loci of *MDM2* in a series of 16 rat osteosarcomas induced by plutonium-238. In another experiment, moderate to marked Mdm2 immunoreactivity was observed in methylcholanthrene-induced mouse rhabdomyosarcomas (RMSs), and the authors suggested that Mdm2 expression is an important pathogenetic event in this sarcomagenesis.[4] The poor prognosis and therapeutic difficulties of RISs have been described by many researchers.[5-9] Recently, Mdm2 inhibitors

have emerged as novel therapeutic agents for some sarcomas.[10,11] Immunohistochemical examinations of Mdm2 expression in RIS have not been published to date. We compared immunohistochemical expression of Mdm2 and p53 in 8 radiation-induced and 8 *de novo* sarcomas of the head and neck.

MATERIALS AND METHODS

Eight cases of RIS of the head and neck were found from data of Asan Medical Center, Seoul, Korea from 2006 through 2012. The selection was based on the slightly modified criteria applied by Bjerkehagen *et al.*:[12] the sarcoma's location in the field of previous radiotherapy; a latency time of at least 2 years; and histomorphology different from that of the primary tumors. The patients' primary tumors were nasopharyngeal carcinoma in 3, olfactory neuroblastoma in 1, malignant lymphoma in 1, liposarcoma in 1, malignant melanoma in 1, and astrocytoma

Table 1. Radiation-induced sarcomas of head and neck

Tumor type	Site	Age (yr)/Sex	Previous tumor	Treatment	Interval between RT & sarcoma
Osteosarcoma	Mandible	61/F	Malignant lymphoma of palatine tonsil	CT and RT	9 yr 3 mo
Osteosarcoma	Mandible	17/M	Pleomorphic liposarcoma of parotid gland	Wide excision and RT	4 yr
Osteosarcoma	Skull	33/F	Astrocytoma	Tumor resection and RT	3 yr 6 mo
UPS	Nasal cavity	22/M	Olfactory neuroblastoma	Medial maxillectomy, CT and RT	3 yr 3 mo
UPS	Nasopharynx	42/F	Nasopharyngeal carcinoma	RT	6 yr
UPS	Posterior neck	60/M	Nasopharyngeal carcinoma	RT	5 yr 5 mo
Fibrosarcoma	Hard palate	50/F	Malignant melanoma of maxillary sinus	Partial maxillectomy, CT and RT	17 yr
MFS	Submandibular area	51/F	Nasopharyngeal carcinoma	RT	12 yr

F, female; CT, chemotherapy; RT, radiotherapy; M, male; UPS, undifferentiated pleomorphic sarcoma; MFS, myxofibrosarcoma.

in 1, and the secondary sarcomas comprised 3 undifferentiated pleomorphic sarcomas (UPS), 3 osteosarcomas, 1 fibrosarcoma, and 1 myxofibrosarcoma. The latency period ranged from 4 to 17 years (Table 1). Eight cases of *de novo* sarcomas of the head and neck were retrieved for comparison, and they included 3 osteosarcomas of the maxillary sinus or nasal cavity, 2 UPS of the maxillary sinus or scalp, 2 fibrosarcomas of the nasal cavity, and 1 myxofibrosarcoma of the maxillary sinus.

Tumor tissues from 16 cases were subjected to immunohistochemical staining for Mdm2 (1:100, Zeta, Arcadia, CA, USA) and p53 (1:1,500, Dako, Glostrup, Denmark). The staining was processed on 4-μm sections using a Ventana autostainer and ultraView DAB detection kit (Ventana, Tucson, AZ, USA) according to the manufacturer's instructions. Nuclear staining in more than 10% of tumor cells was rendered positive for both Mdm2 and p53. Statistical analysis of immunohistochemical results between the two groups was performed by chi-square and Fisher's exact test (ver. 18.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Mdm2 expression was less common in RIS (3/8, 37.5%) than in *de novo* cases (6/8, 75%) ($p < .05$), while p53 expression was more common in RIS cases (75% vs 37.5%) ($p < .05$). Variable combination types of expression were observed in both groups (Table 2, Fig. 1); however, while half of RISs were Mdm2(−)/p53(+), none of *de novo* cases showed such combination, and while half of *de novo* sarcomas were Mdm2(+)/p53(−), which can be the candidate group of Mdm2 inhibitors, only 1 RIS showed such a combination. The expression profiles of Mdm2 and p53 did not correlate with tumor types, except that both of the myxofibrosarcomas, 1 RIS case and 1 *de novo* case, were positive for both Mdm2 and p53 (Table 2, Fig. 1).

**Table 2.** Mdm2/p53 expression patterns in two sarcoma groups (RIS and *de novo* sarcoma)

	Mdm2(+)/p53(+)	Mdm2(+)/p53(−)	Mdm2(−)/p53(+)	Mdm2(−)/p53(−)
Radiation-induced				
Osteosarcoma	0	1	2	0
UPS	1	0	2	0
Fibrosarcoma	0	0	0	1
Myxofibrosarcoma	1	0	0	0
De novo				
Osteosarcoma	0	2	0	1
UPS	0	1	1	0

RIS, radiation-induced sarcoma; UPS, undifferentiated pleomorphic sarcoma.

DISCUSSION

RISs develop in a field of prior radiation after a latent period as high-grade sarcoma, most frequently osteosarcoma or undifferentiated pleomorphic sarcoma (former malignant fibrous histiocytoma).[12,13] The pathogenesis of RIS is still unknown even though the initiating events have been identified. Gonin-Laurent et al.[1] detected a high incidence (58%) of inactivating mutation of *TP53* gene in 36 RIS, and the inactivation was subsequently shown to be unrelated to *MDM2* amplification/expression.[2] The same study group recently described a transcriptome signature distinguishing sporadic sarcomas from RISs, and the signature suggested that RISs are characterized by chronic endogenous oxidative stress.[14] On the contrary, Rumenapp et al.[15] showed that 6 of 7 radiation-induced osteosarcomas harbored a high degree of genomic instability similar to that identified earlier in primary osteosarcomas with poor prognosis. They speculated that the poor prognosis is caused by genetic instability, and not by an initiating event.

Apart from the genetic instability, prognosis of RIS is expected to be poor, considering the high histologic grade, limitations in further radiotherapy, and the presence of pre-existing malignancy. Bjerkehagen et al.[5] compared 98 patients with RIS
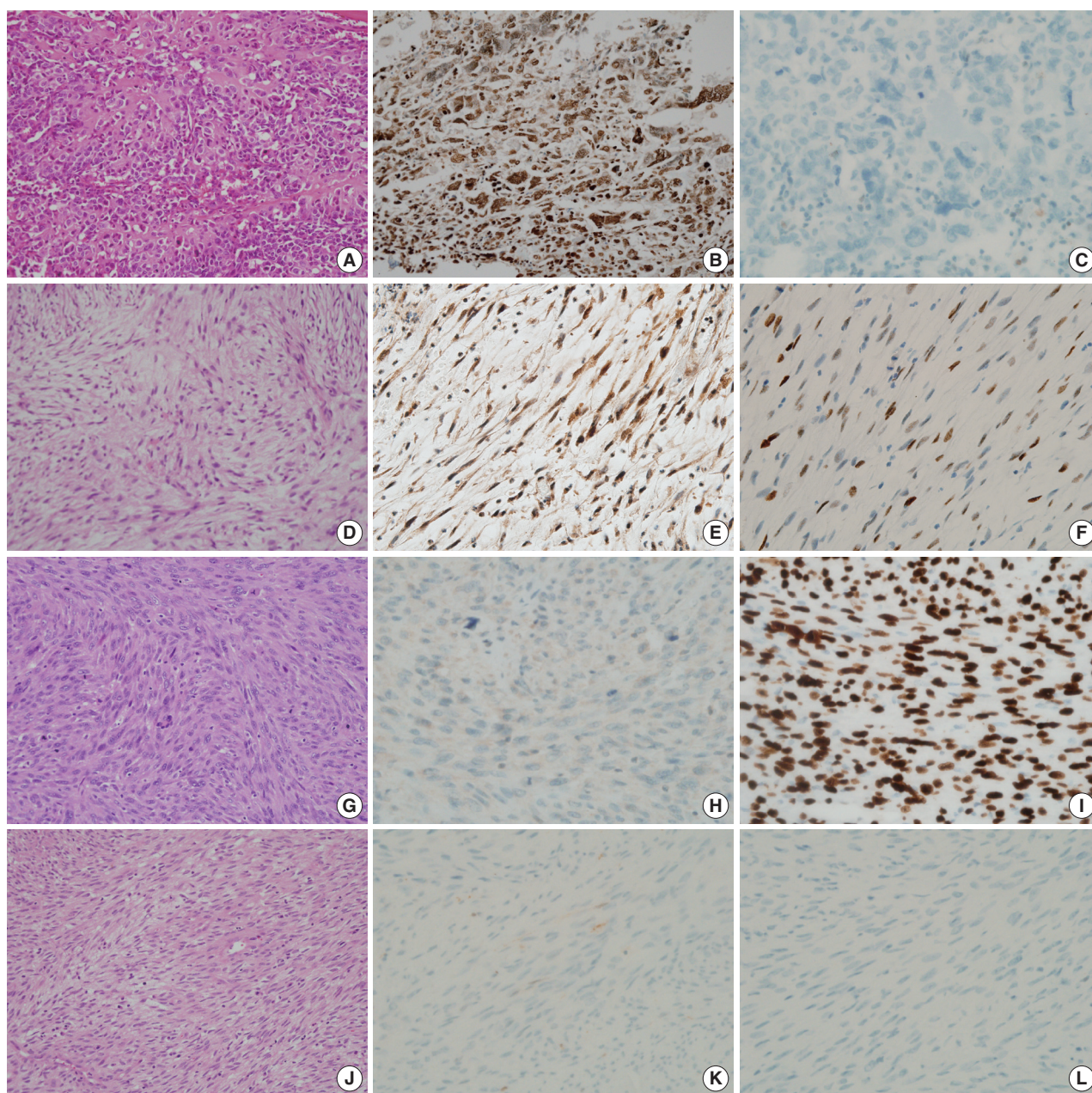
Fig. 1. Mdm2 (B, E, H, K) and p53 (C, F, I, L) expression in sarcomas. (A–C) Mdm2-positive, p53-negative *de novo* osteosarcoma. (D–F) Mdm2-positive, p53-positive *de novo* myxofibrosarcoma. (G–I) Mdm2-negative, p53-positive radiation-induced osteosarcoma. (J–L) Mdm2-negative, p53-negative radiation-induced fibrosarcoma.

and 239 sporadic high-grade sarcomas and concluded that the poorer prognosis of RIS is related to the central tumor site, incomplete surgical remission, microscopic tumor necrosis, and the presence of metastases. McHugh *et al.*[6] studied primary versus radiation-associated craniofacial osteosarcomas and showed radiation-associated cases were more aggressive, because of their unresectability, high grade, and expression of adverse markers including p53, Ki-67, and ezrin.

The Mdm2-p53 interaction in sarcomas has recently received attention on account of a new therapeutic approach targeting this interaction.[10,11,16] *MDM2* amplification/overexpression is characteristic of well-differentiated/dedifferentiated liposarcomas (DDLS), but is also identified in a small percentage of other sarcomas.[17,18] Studies on Mdm2 in RIS are very rare. Gonin-Laurent *et al.*[2] identified *MDM2* mRNA expression in only 5 of 36 RIS cases (13.9%). In contrast, Roch-Lefevre *et al.*[3] detected

recurrent gains/amplifications at many chromosomal regions including the loci of *MDM2* in a series of 16 rat osteosarcomas induced by plutonium-238. In a chemical sarcomagenesis experiment, a high percentage of Mdm2 immunoreactivity was observed in mouse RMS, and the authors suggested that Mdm2 expression is an important pathogenetic event in this sarcomagenesis.[4]

Our study is the first immunohistochemical study on Mdm2 expression of RIS. Mdm2 expression was more common in *de novo* than RISs (p < .05), and p53 expression was more common in RIS than *de novo* cases (p < .05). Frequent Mdm2 expression of *de novo* nonlipogenic sarcomas in this series might be due to the high percentage of included osteosarcoma cases. Frequent p53 overexpression in RISs suggests mutations of *TP53* are pathogenetic events in radiation-induced sarcomagenesis, as proposed earlier.[1] Mutations in *TP53* are basically not affected by Mdm2, but concurrent overexpression of Mdm2 was observed in 2 cases. While half of the RISs were Mdm2(–)/p53(+), none of *de novo* cases showed such combination, and while half of *de novo* sarcomas were Mdm2(+)/p53(–), which can be the candidate group for Mdm2 inhibitors, only 1 RIS showed such combination. Mdm2-p53 interaction, that is p53 inactivation by Mdm2 activation, appears to not be a major pathogenetic step in radiation-induced sarcomagenesis.

RMSs are aggressive pediatric tumors, and there have been recent preclinical trials of Mdm2 inhibitors in RMS.[10,11] Radiation-induced RMS has rarely been described, mostly among Chinese patients treated for nasopharyngeal cancer.[8,9,19,20] Since our series included no RMS cases, Mdm2-p53 status in radiation-induced RMS is not known. Among osteosarcomas, UPS and fibrosarcomas, Mdm2-p53 status showed no correlation with tumor type, but 2 myxofibrosarcomas (1 radiation-induced and the 1 *de novo*) were both positive for both Mdm2 and p53, but the meaning of this is uncertain for now.

UPSs with *MDM2* amplification/expression, especially of the retroperitoneum, were considered DDLS by one research group,[21] and the same group recently published a similar result on peripheral UPS.[22] In addition, it has been documented that immunostaining results for Mdm2 are well correlated with *MDM2* gene amplification.[23] Our study resulted in 2 cases of Mdm2-positive UPS, 1 radiation-induced and 1 *de novo*. Nevertheless, it appears to be hasty to regard these nonlipogenic sarcomas as DDLS, since no example of radiation-induced DDLS has been reported.

In summary, in the comparison of Mdm2 and p53 expression of RIS with that of *de novo* sarcomas of the head and neck, we speculated that radiation-induced and *de novo* sarcomagenesis are not due to a unique genetic mechanism. Mdm2 expression without p53 overexpression in 1 case of RIS decreases the future possibility of applying Mdm2 inhibitors on a subset of these difficult tumors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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