RESEARCH COMMUNICATION

Radiation-induced Sarcoma in the Head and Neck Region: a Clinicopathologic and Immunohistochemical Study of 13 Cases

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Abstract

Objectives: To study the clinical and histological features of radiation-induced sarcoma in the head and neck (RISHN). Methods: Medical records of 13 patients with RISHN treated at our institution between 1990 and 2011 were studied, and paraffin-embedded samples were analyzed by haematoxylin and eosin staining and immunohistochemistry to determine mitosis counts and assess expression of Ki-67, bcl-2, and survivin. Results: Positive bcl-2 was observed in 12 (100%) and survivin in 10 (76.9%) patients. The Ki-67 labeling index ranged from 1% to 90%, and it showed significant positive correlation with mitosis count in RISHN tissues, based on Spearman analysis. Percentage of distal metastasis with T2b was significantly higher than T1b stage (P=0.035). Conclusions: Stage T2b may be a useful indicator for predicting distant metastasis of RISHN. The MIB-1 score may be used as a histological grading system for RISHN. In addition, bcl-2 and survivin protein may play an important role in pathogenesis and progression of RISHN.

Keywords: Radiation-induced sarcoma - Ki-67 - Bcl-2 - survivin - head and neck

Asian Pacific J Cancer Prev, **12**, 2995-2999

Introduction

Radiation-induced sarcoma (RIS) is a unique malignant tumor with very low incidence, and previous reports have demonstrated a cumulative incidence of 0.03% to 0.8% (Mark et al., 1994). Despite the very low incidence of RIS, especially RIS of the head and neck (RISHN), its poor prognosis and increasing prevalence (King et al., 2000; Maghami et al., 2005; Wei et al., 2011) demand detailed study of the factors affecting its pathogenesis, progression, and prognosis. Only a few reports to date have examined these processes at the molecular level. Tarkkanen et al. (2001) suggested that a defect of the p53 gene plays an important role in the formation of RIS. Mutated p53 may deregulate apoptosis in cells suffering from radiation-induced genetic defects (Chen et al., 2008), suggesting that resistance to p53-dependent apoptosis participates in RIS RISHN. As important apoptosis regulators that collaborate closely with p53 (Kappler et al., 2004; Hemann et al., 2006), bcl-2 and survivin play an important role in the pathogenesis and development of many tumors (Ghobrial et al., 2005; Guha et al., 2009; Taubert et al., 2010). However, no published study has examined the involvement of these two proteins in RIS. Such work may help us understand the pathogenesis and development of this disease.

Reliable histologic grading of malignant tumors, including soft tissue sarcomas like RIS, is critical for successful diagnosis and treatment. Several grading systems are available for patients with sarcoma; one widely used system is based on Ki-67 immunoreactivity using the MIB-1 antibody, while another is based on the French Federation of Cancer Centers Sarcoma Group (FNCLCC). The Ki-67 system is based on three criteria: tumor differentiation/histologic type, Ki-67 (MIB-1) labeling index and necrosis. The FNCLCC system is based on the same criteria, except that mitotic count is used instead of MIB-1 score. The MIB-1 score has been reported to be the most significant independent prognostic factor for soft tissue sarcoma(Hasegawa et al., 2000; Hasegawa et al., 2001), and the validity and reproducibility of the Ki-67 system was found to be higher than that of FNCLCC(Hasegawa et al., 2002a; 2002b). No published studies have compared the reliability of the two systems for grading RISHN.

Surgery is the standard treatment for RISHN(Xi et al., 2010). However, complete resection of the lesion is very difficult because of its proximity to vital organs in the head and neck and because of the multifocal characteristics of RIS(Holt et al., 2006). The prognosis of patients with incompletely excised tumors is much worse than that of patients with no residual tumor because of tumor insensitivity to chemo- and radiotherapy. The mechanism of this insensitivity is unclear. Some studies have shown

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that anti-apoptosis proteins such as bcl-2 and survivin may protect tumor cells from apoptosis induced by chemo- and radiotherapy(Kirkin et al., 2004; Kim et al., 2007; Gan et al., 2010). Anti-bcl-2 and survivin strategies may reverse this insensitivity and improve treatment effects. However, no study has been published analyzing the expression of bcl-2 or survivin in RISHN tissues.

In this paper we report clinical features and immunohistochemical findings of 13 cases of RISHN. We explore the possible relationships between the clinical and histochemical data in order to understand more about the factors affecting pathogenesis and prognosis of RISHN, and to provide clues for new treatment strategies.

Materials and Methods

Patients

A retrospective review of patients with head and neck tumors who received radiotherapy at our institution was performed. The study was approved by the Ethical Committee of Guangxi Medical University. Data were retrieved from medical records on 164 patients with soft tissue sarcoma in the head and neck treated between January 1990 and March 2011. Thirteen patients were diagnosed as RISHN according to the criteria proposed by Cahan et al. (1948) and Murray et al. (1999): (1) the tumor developed in a field that had been irradiated, (2) the first tumor differed from subsequent one histologically, (3) there was no evidence of the new tumor when recieving radiotherapy, and (4) the new tumor appeared after a certain latent period following radiotherapy. These patients were staged according to the 2002 American Joint Committee on Cancer (AJCC) staging system.

Immunohistochemistry and histologic grading

Histological studies of paraffin wax embedded tissues from all 13 patients were carried out by two experienced pathologists. They were re-sliced and stained with haematoxylin and eosin (H&E) to re-confirm the diagnosis and determine mitosis count. Additional tissue sections(4 mm) were prepared for immunohistochemical study of the expression of Ki-67, survivin and bcl-2. Immunostaining of paraffin sections was performed after dewaxing and rehydrating. Mouse monoclonal primary antibodies specific for survivin (Santa Cruz Biotechnology, CA) (Ki-67 (MIB-1, M7240; DAKO) and bcl-2 (Santa Cruz Biotechnology, CA) were diluted in PBS (1:200 for survivin, 1:500 for Ki-67 and 1:200 for bcl-2). Goat anti-mouse IgG-horseradish peroxidase (HRP; Maxim, Fuzhou, China) was diluted 1:500 in PBS and used as secondary antibody. To calculate the Ki-67 labeling index for each patient, we checked five representative areas containing at least 1000 cells and calculated the percentage of positive cells (Fusco et al., 2008). To score the expression of bcl-2 and survivin, staining intensity and percentage of stained cells were used (Hong et al., 2011). The staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The percentage of stained cells was scored as 0 (negative), 1 (≤10% positive), 2 (11-50% positive), 3 (51-80% positive), or 4 (>80% positive). The final score was obtained by multiplying the intensity score and the score for percentage of positive cells. This final score was used to classify the level of expression as negative (0 point), low (1–4 points), moderate (5–8 points), or high (9–12 points). Histologic grading of RISHN tissues was carried out based on the Ki-67 (MIB-1) score and the FNCLCC system(Guillou et al., 1997; Hasegawa et al., 2002b).

Statistical analysis

Data were statistically analyzed using SPSS 15.0 software (SPSS Inc., Chicago, IL). Rates of overall survival (OS) were calculated using the Kaplan-Meier method. Fisher's exact test was used to compare rates between groups, such as the difference in distal metastasis rate between tumor stage T1b and tumor stage T2b groups, or the difference in lymphatic metastasis rate between patients with a Ki-67 index >60% and patients with an index ≤60%. The relationship between Ki-67 index and mitosis count in RISHN tissues was evaluated by regression analysis using the Spearman rank correlation coefficient. P values were calculated using two-sided statistical tests, with values less than 0.05 considered significant.

Results

From January 1990 to January 2011, 164 cases of soft tissue sarcoma in the head and neck were treated in our hospital, and 13 (7.9%) were cases of RISHN with the mean age at time of diagnosis was 47 years (range, 34 to 58). In 11 of these 13 cases (84.6%) , the first primary tumor was NPC; the remaining 2 cases was breast carcinoma and tongue squamous carcinoma, respectively. Tumor staging of the patients is shown in table 1: eight patients were with stage T1b, and 5 with stage T2b. Only one case with primitive neuroectodermal tumor (PNT) had lymphatic involvement, and no patient showed distal metastasis at the time of diagnosis (Table 1).

Our cases were treated using surgery, radiotherapy, chemotherapy, or a combination of these (Table 1). Five cases received radiotherapy with a mean dose of 5840 cGY (range, 3000 to 7000). Chemotherapeautic agents included gemcitabine, docetaxel, theprubicin, iphosphamide, dacarbazine, etoposide, methotrexate and vincristine.

The mean follow-up for the 13 cases was 19.2 months (range, 5 to 36), and the OS was 29.5%. Five patients (38.4%) experienced local recurrences within 12 months after treatment, and 3 patients (23.0%) developed distal metastases within 12 months. The organs involved in the distal metastasis included the lungs (2 cases) and the pericardium and bone (1 case). Six cases had died of RISHN by the last follow-up, 5 due to local recurrence and 1 due to distal metastasis. One patient with rhabdomyosarcoma (case 12), who received only radiotherapy, survived more than 36 months. The tumor in her nasopharyngeal cavity disappeared after treatment and showed good sensitivity to radiotherapy.

Histological grading of the patients with RISHN was as follows: four cases were classified as grade I, 6 as grade II, and 2 as grade III based on the FNCLCC system,. Based on the Ki-67 (MIB-1) score, 3 were classified as grade I, 6 as grade II, and 3 as grade III. Histological grade of one

Table 1. Clinical Data of 13 Cases with RISHN

Case	Sex/age (yr)	Tumor site	Tumor size, largest dimension (cm)	T stage	Metastasis		Treatment	Follow-up (month.)	Outcome
					Lymphatic	Distal			
1	F/42	mandible	3	T1b	no	no	S	10	DOD
2	M/49	postnaris	4	T1b	no	no	S	32	DOD
3	M/39	mandible	3	T2b	no	yes	S	13	DOD
4	F/39	cheek	2.5	T2b	no	no	S+RT	13	alive
5	M/50	mandible	3	T1b	no	no	S+CT	36	alive
6	M/34	neck	2.8	T1b	no	no	S+RT	17	alive
7	M/46	hard palate	5	T1b	no	no	S	5	DOD
8	M/55	mandible	6	T2b	no	yes	S	5	alive
9	M/51	hard palate	3	T1b	no	no	S+RT+CT	32	DOD
10	F/58	clavicle	9	T2b	no	yes	CT	5	alive
11	M/ 58	postnaris	6	T2b	no	no	S	32	DOD
12	F/45	NP	3	T1b	no	no	RT	36	alive
13	M/ 52	sinus	2.5	T1b	yes	no	S+RT	15	alive

CT, chemotherapy; DOD, died of disease; F, female; M, male; NP, nasopharynx; RT, radiotherapy; S, surgery; yr, year

Table 2. Pathologic Results of 13 Cases of RISHN

Histological type	Ki-67 labeling index (%)	Mitosis count (per 10 HPs)	Bcl-2 expression	Survivin expression
osteosarcoma	4	2	low	low
osteosarcoma	5	2	moderate	negative
osteosarcoma	16	5	high	moderate
osteosarcoma	40	25	moderate	moderate
MHF	1	1	high	high
MHF	6	1	high	negative
MHF	10	2	high	negative
leiomyosarcoma	9	10	moderate	negative
leiomyosarcoma	17	7	high	moderate
leiomyosarcoma	18	5	moderate	moderate
neuroblastoma	1	1	moderate	high
rhabdomyosarcoma	60	NAa	NAa	high
PNT	90	15	low	low

HP, high power field; MHF, malignant fibrous hystiocytoma; NA, not available; PNT, primitive neuroectodermal tumor; aResults of bcl-2 expression and mitosis count could not be determined because of the limited amount of tissue for H&E study

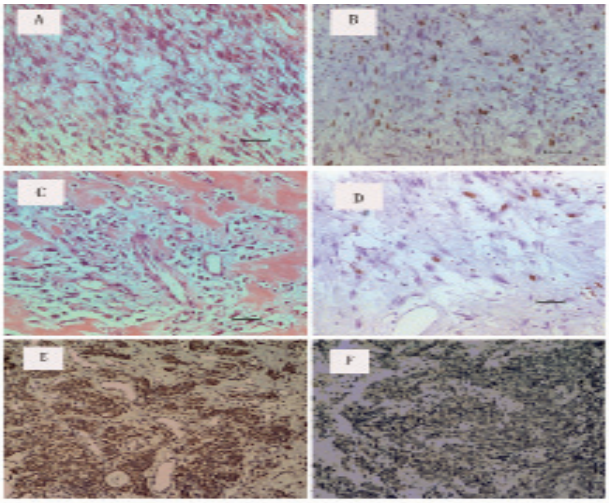

Figure 1. Expression of Ki-67, bcl-2, and Survivin and H&E Staining in RISHN Tissue. A, H&E staining with high mitosis count (case 4); B, high Ki-67 expression in tissue of the same patient (case 4); C, H&E staining with low mitosis count (case 3); D, low Ki-67 expression in tissue of the same patient (case 3); E, high expression of survivin (case 3); F, high expression of bcl-2 (case 3).Original magnifications: 20×(A, ,B,C,D) and 10×(E, F).

Table 3. Search for Factors Associated with Lymphatic Metastasis and Distant Metastasis

Factor	Lymphatic metastasis		P	Distant metastasis		P
	Yes (n)	No (n)		Yes (n)	No (n)	
Ki-67 labeling index (%)						
≤60	0	12	0.077	3	9	1.000
>60	1	0		0	1	
Tumor stage						
T1b	1	7	1.000	0	8	0.035
T2b	0	5		3	2	
Tumor size (largest dimension)						
<5cm	1	7	1.000	1	7	0.510
≥5cm	0	5		2	3	
Bcl-2 expression						
Low	1	1	0.167	0	2	1.000
Moderate-high	0	10		3	7	
Survivin expression						
Negative-low	1	6	1.000	1	3	1.000
Moderate-high	0	6		2	7	

case were not obtained because of the limited amount of tissue available.

Immunohistochemical analysis showed that bcl-2 was present in all 12 of the patients who could be tested; data were not available for one case because of the limited amount of tissue available. Survivin was present in 10 of 13 cases (76.9%) and Ki-67 in 13 (100%) (Table 2 and Figure 1). The median Ki-67 (MB-1) score for all 13 cases was 10.0% (range, 1% to 90%), and the score correlated positively with mitosis count (Spearman correlation coefficient=0.789, P=0.002).

We found a higher rate of lymphatic involvement in the group with a Ki-67 index >60% than in the group with a Ki-67 index ≤60%,but the difference was not significant (P=0.077) (Table 3).The rate of distal metastasis in the group with stage T2b tumors was significantly higher than that of the T1b group (P=0.035) (Table 3).

Discussion

Factors affecting lymphatic metastasis in RISHN are unclear. In our study population, only 1 of 13 cases (7.7%) showed lymphatic involvement at the time of diagnosis, similar to other reports (Bjerkehagen et al., 2008; Xi et al., 2010). This indicates the rarity of regional

lymphatic involvement in RISHN. We found a weak positive relationship between an index of Ki-67 >60% and lymphatic metastasis in the neck, suggesting that extremely high expression of Ki-67 may be associated with lymphatic involvement. This possibility requires further investigation based on a larger number of cases.

Unlike spontaneous soft tissue sarcoma(Behranwala et al., 2004), the incidence of distal metastasis in RISHN is low(Patel et al., 2002; Xi et al., 2010). Consistent with these previous reports, none of our 13 cases showed involvement of distal organs at the time of RISHN diagnosis. Of the three cases who suffered distal metastases after treatment, none showed evidence of local recurrence, suggesting that micrometastasis to distal organs had occurred before treatment. Factors that influence distal metastasis in RISHN are unclear. In the present study, the rate of subsequent distal metastasis in patients with a primary tumor stage of T2b (60%) was significantly higher than in those with a primary tumor stage of T1b (0%), indicating that T stage may be a useful indicator to predict distal metastasis.

Ki-67 is expressed in all phases of the cell cycle except G0, indicating that this protein may reflect the proliferative capability of cells(Khoury et al., 2009). In RISHN, little is known about the expression of Ki-67(Junior et al., 2003; McHugh et al., 2006). A previous study(McHugh et al., 2006) reported that 4 of 6 patients with radiation-associated craniofacial osteosarcoma showed >50% Ki-67 nuclear staining, which was higher than that observed in patients with primary osteosarcoma. In the present study, however, we found >50% Ki-67 nuclear expression in only 2 of 13 (15.4%) RISHN cases and in 0 of 4 cases with osteosarcoma. This discrepancy may indicate that not all cases of RISHN are highly proliferative or that Ki-67 is not a useful marker for evaluating the proliferative capability of RISHN cells, perhaps because not all tumor cells expressing Ki-67 enter mitosis (Scholzen et al., 2000; Ladstein et al., 2010). Since our results show a significant positive correlation between Ki-67 labeling index and mitosis count, the more likely explanation seems to be that not all cases of RISHN are highly proliferative.

Although the MIB-1 system has been used to grade some malignant tumors, including soft tissue sarcomas, its validity in RISHN has not been reported. In the present study, we graded RISHN tissue based on both the MIB-1 score and the FNCLCC score. Although the Spearman rank correlation coefficient in our results is not high, we believe that it will increase when more cases are included. Thus, our results provide preliminary evidence that the MIB-1 score is applicable to RISHN.

Bcl-2 is the founding member of a family of antiapoptotic proteins, and its overexpression is believed to contribute to tumor initiation and progression(Del Bufalo et al., 1997; Miyake et al., 1999). To our knowledge, no studies have examined possible associations between bcl-2 expression and RISHN. In our study, all 12 of the RISHN cases that we could examine showed bcl-2 staining, including 5 cases with strong staining, indicating the participation of bcl-2 in the pathogenesis and progression of disease. We also found that survivin was expressed in 9 of 13 (69.2%) of our RISHN cases. As the smallest member of the Inhibitor of Apoptosis gene family, survivin is overexpressed in human tumors but is undetectable or expressed at very low levels in most normal adult tissues(Kappler et al., 2004). The strong expression of survivin in our patients suggests that this protein plays a critical role in the pathogenesis or development of RISHN.

Consistent with previous studies showing the difficulty of completely excising RISHN tumors(Holt et al., 2006), 9 of our 12 cases who underwent surgery could not have their tumor completely removed and experienced recurrence within 1 year. Of 4 patients who underwent only radiotherapy without receiving surgery, 3 died of RIS within 1 year. The effectiveness of other treatments, including chemo- and radiotherapy, is limited because of the insensitivity of RISHN to these methods. The mechanism of this insensitivity is not fully explained, though some authors have suggested that bcl-2 and survivin may be involved. In fact, the results of experimental and clinical studies suggest that inhibiting bcl-2 or survivin expression renders tumor cells chemo- and radio-sensitive(Kim et al., 2007; Altieri, 2008; Mita et al., 2008). Given the strong expression of both proteins in our RISHN patients, treatments that target one or both of these proteins may be effective.

Based on our clinical and immunohistochemical data, we suggest that RISHN is a malignant disease with infrequent lymph node involvement and distal metastasis, and patients of RISHN with T2b stage may have a higher risk distal metastasis compared with those with T1b. The anti-apoptotic effect of bcl-2 and survivin may play an important role in the pathogenesis and progression of RISHN, and treatments that target one or both of these proteins may help make RISHN tissues more sensitive to chemo- and radiotherapy. The Ki-67 (MIB-1) score correlates with mitosis count, which may mean that it can be used to grade RISHN tissue, but this should be confirmed in studies with a larger number of cases.

Acknowledgements

This study was supported by the Guangxi Natural Science Foundation of China for Youth (No. 2010GXNSFB013067), the Open Foundation of the Experimental Center of Medical Science of Guangxi Medical University (KFJJ2010-44) and Guangxi Medical University Research Foundation for Ph.D. (304193). The author(s) declare that they have no competing interests.

References

Altieri, DC (2008). Survivin, cancer networks and pathway-directed drug discovery. *Nat Rev Cancer*, **8**, 61-70.

Behranwala KA, A'Hern R, Omar, AM, Thomas JM (2004). Prognosis of lymph node metastasis in soft tissue sarcoma. *Ann Surg Oncol*, **11**, 714-9.

Bjerkehagen B, Smeland S, Walberg L, et al (2008). Radiation-induced sarcoma: 25-year experience from the Norwegian Radium Hospital. *Acta Oncol*, **47**, 1475-82.

Cahan WG, Woodard HQ, et al (1948). Sarcoma arising in irradiated bone; report of 11 cases. *Cancer*, **1**, 3-29.

Chen YW, Tu HF, Wu TH, et al (2008). Sarcomas and sarcomatoid tumor after radiotherapy of oral squamous cell carcinoma: analysis of 4 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod,* **105**, 65-71.

Del Bufalo D, Biroccio A, Leonetti C, Zupi G (1997). Bcl-2 overexpression enhances the metastatic potential of a human breast cancer line. *FASEB J*, **11**, 947-53.

Fusco A, Zatelli MC, Bianchi A, et al (2008). Prognostic significance of the Ki-67 labeling index in growth hormone-secreting pituitary adenomas. *J Clin Endocrinol Metab*, **93**, 2746-50.

Gan XL, Li JK, Yu TH, et al (2010). High expressions of bcl-2 and survivin, and decreased apoptosis in uterine cervical carcinosarcoma compared to cervical squamous cell carcinoma. *Arch Gynecol Obstet*.

Ghobrial IM, Witzig TE, Adjei AA (2005). Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin*, **55**, 178-94.

Guha M, Altieri DC (2009). Survivin as a global target of intrinsic tumor suppression networks. *Cell Cycle*, **8**, 2708-10.

Guillou L, Coindre JM, Bonichon F, et al (1997). Comparative study of the national cancer institute and french federation of cancer centers sarcoma group grading systems in a population of 410 adult patients with soft tissue sarcoma. *J Clin Oncol*, **15**, 350-62.

Hasegawa T, Seki K, Hasegawa F, et al (2000). Dedifferentiated liposarcoma of retroperitoneum and mesentery: varied growth patterns and histological grades--a clinicopathologic study of 32 cases. *Hum Pathol*, **31**, 717-27.

Hasegawa T, Yokoyama R, Matsuno Y, et al (2001). Prognostic significance of histologic grade and nuclear expression of beta-catenin in synovial sarcoma. *Hum Pathol*, **32**, 257-63.

Hasegawa T, Yamamoto S, Nojima T, et al (2002a). Validity and reproducibility of histologic diagnosis and grading for adult soft-tissue sarcomas. *Hum Pathol*, **33**, 111-5.

Hasegawa T, Yamamoto S, Yokoyama R, et al (2002b). Prognostic significance of grading and staging systems using MIB-1 score in adult patients with soft tissue sarcoma of the extremities and trunk. *Cancer*, **95**, 843-51.

Hemann MT, Lowe SW (2006). The p53-Bcl-2 connection. *Cell Death Differ*, **13**, 1256-9.

Holt GE, Thomson AB, Griffin AM, et al (2006). Multifocality and multifocal postradiation sarcomas. *Clin Orthop Relat Res*, **450**, 67-75.

Hong B, Krusche, CA, Schwabe K, et al (2011). Cyclooxygenase-2 supports tumor proliferation in vestibular schwannomas. *Neurosurgery*.

Junior AT, de Abreu Alves F, Pinto CA, et al (2003). Clinicopathological and immunohistochemical analysis of twenty-five head and neck osteosarcomas. *Oral Oncol*, **39**, 521-30.

Kappler M, Bache M, Bartel F, et al (2004). Knockdown of survivin expression by small interfering RNA reduces the clonogenic survival of human sarcoma cell lines independently of p53. *Cancer Gene Ther*, **11**, 186-93.

Khoury T, Arshad A, Bogner P, et al (2009). Apoptosis-related (survivin, Bcl-2), tumor suppressor gene (p53), proliferation (Ki-67), and non-receptor tyrosine kinase (Src) markers expression and correlation with clinicopathologic variables in 60 thymic neoplasms. *Chest*, **136**, 220-8.

Kim DW, Seo SW, Cho SK, et al (2007). Targeting of cell survival genes using small interfering RNAs (siRNAs) enhances radiosensitivity of Grade II chondrosarcoma cells. *J Orthop Res*, **25**, 820-8.

King AD, Ahuja AT, Teo P, et al (2000). Radiation induced sarcomas of the head and neck following radiotherapy for nasopharyngeal carcinoma. *Clin Radiol*, **55**, 684-9.

Kirkin V, Joos S, Zornig M (2004). The role of Bcl-2 family members in tumorigenesis. *Biochim Biophys Acta*, **1644**, 229-49.

Ladstein RG, Bachmann IM, Straume O, Akslen LA (2010). Ki-67 expression is superior to mitotic count and novel proliferation markers PHH3, MCM4 and mitosin as a prognostic factor in thick cutaneous melanoma. *BMC Cancer*, **10**, 140.

Maghami EG, St-John M, Bhuta S, Abemayor E (2005). Postirradiation sarcoma: a case report and current review. *Am J Otolaryngol*, **26**, 71-4.

Mark RJ, Poen J, Tran LM, et al (1994). Postirradiation sarcomas. A single-institution study and review of the literature. *Cancer*, **73**, 2653-62.

McHugh JB, Thomas DG, Herman JM, et al (2006). Primary versus radiation-associated craniofacial osteosarcoma: Biologic and clinicopathologic comparisons. *Cancer*, **107**, 554-62.

Mita AC, Mita MM, Nawrocki ST, Giles FJ (2008). Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics. *Clin Cancer Res*, **14**, 5000-5.

Miyake H, Hara I, Yamanaka K, et al (1999). Overexpression of Bcl-2 enhances metastatic potential of human bladder cancer cells. *Br J Cancer*, **79**, 1651-6.

Murray EM, Werner D, Greeff EA, Taylor DA (1999). Postradiation sarcomas: 20 cases and a literature review. *Int J Radiat Oncol Biol Phys*, **45**, 951-61.

Patel SG, Meyers P, Huvos AG, et al (2002). Improved outcomes in patients with osteogenic sarcoma of the head and neck. *Cancer*, **95**, 1495-503.

Scholzen T, Gerdes J (2000). The Ki-67 protein: from the known and the unknown. *J Cell Physiol*, **182**: 311-22.

Tarkkanen M, Wiklund TA, Virolainen M J, et al (2001). Comparative genomic hybridization of postirradiation sarcomas. *Cancer*, **92**, 1992-8.

Taubert, H, Heidenreich, C, Holzhausen, H J, et al (2010). Expression of survivin detected by immunohistochemistry in the cytoplasm and in the nucleus is associated with prognosis of leiomyosarcoma and synovial sarcoma patients. *BMC Cancer*, **10**, 65.

Wei Z, Xie Y, Xu J, et al (2011). Radiation-induced sarcoma of head and neck: 50 years of experience at a single institution in an endemic area of nasopharyngeal carcinoma in China. *Med Oncol*, (in press).

Xi M, Liu MZ, Wang HX, et al (2010). Radiation-induced sarcoma in patients with nasopharyngeal carcinoma: a single-institution study. *Cancer*, **116**, 5479-86.