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Citation

Kacker, C., Marx, A., Mossinger, K., Svehla, F., Schneider, U., Hogendoorn, P. C. W., ... Strobel, P. (2013). High frequency of MYC gene amplification is a common feature of radiation-induced sarcomas: further results from EORTC STBSG TL 01/01. *Genes, Chromosomes & Cancer*, 52(1), 93-98. doi:10.1002/gcc.22009

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Note: To cite this publication please use the final published version (if applicable).

High Frequency of MYC Gene Amplification Is a Common Feature of Radiation-Induced Sarcomas. Further Results from EORTC STBSG TL 01/01

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Irradiation is a major causative factor among the small subgroup of sarcomas with a known etiology. The prognosis of radiation-induced sarcomas (RIS) is significantly worse than that of their spontaneous counterparts. The most frequent histological subtypes include undifferentiated pleomorphic sarcomas, angiosarcomas, and leiomyosarcomas. A high frequency of MYC amplifications in radiation-induced angiosarcomas, but not in primary angiosarcomas, has recently been described. To investigate whether MYC amplifications are also frequent in RIS other than angiosarcomas, we analyzed the MYC amplification status of 83 RIS and 192 sporadic sarcomas by fluorescence in situ hybridization. We found significantly higher numbers of MYC amplifications in RIS than in sporadic sarcomas ($P < 0.0001$), especially in angiosarcomas, undifferentiated pleomorphic sarcomas, and leiomyosarcomas. Angiosarcomas were special in that MYC amplifications were particularly frequent and always high level, while other RIS showed low-level amplifications. We conclude that MYC amplifications are a frequent feature of RIS as a group and may contribute to the biology of these tumors.  2012 Wiley Periodicals, Inc.

INTRODUCTION

Radiation-induced sarcomas (RIS) are uncommon neoplasms, accounting for less than 5% of all sarcomas, and are generally associated with a poor prognosis (Brady et al., 1992; Neuhaus et al., 2009; Gladdy et al., 2010). The most common reasons for previous irradiation are breast cancer (Karlsson et al., 1998; Huang and Mackillop, 2001), lymphomas, and cervical cancers (Neuhaus et al., 2009). RIS develop with a latency of 1.3–74 years (median 10 years; Kirova et al., 2005; Neuhaus et al., 2009; Gladdy et al., 2010). The cumulative risk to develop RIS increases from 0.07% at 5 years to 0.48% at 15 years (Kirova et al., 2005), and risk has been shown to be related to radiation dosage (Karlsson et al., 1998). RIS appear to be frequently of high-histological grade, deep seated, and truncal with a poor prognosis (Laskin et al., 1988; Gladdy et al., 2010).

The most frequent single entities among RIS are pleomorphic undifferentiated sarcomas (previously so-called malignant fibrous histiocytomas), angiosarcomas (Karlsson et al., 1998; Huang and Mackillop, 2001; Kirova et al., 2005), fibrosarcomas (Karlsson et al., 1998; Gladdy et al., 2010), leiomyosarcomas (Gladdy et al., 2010), osteosarcomas (Brady et al., 1992; Kirova et al., 2005), and various others.

Supported by: EORTC Soft Tissue and Bone Sarcoma Group, Grant number: TLR grant 01/01; Deutsche Krebshilfe (KO.SAR network), Grant number: 109742.

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Received 25 June 2012; Accepted 16 August 2012

DOI 10.1002/gcc.22009

Published online 25 September 2012 in Wiley Online Library (wileyonlinelibrary.com).

TABLE 1. Clinicopathologic Characteristics of Radiation-Induced Sarcomas

Entity	Grade	Primary disorder	Radiation dosage	Latency (median)	n
UPS	24 × G3	9 × BCA 12 × other 3 × n.a.	43–60 Gy	4–40 years (13)	24
LMS	6 × G2 8 × G3	4 × BCA 4 × other 6 × n.a.	36–60 Gy	3–19 years (6)	14
AS	10 × G1 8 × G2 18 × G3	22 × BCA 14 n.a.	50–66.5 Gy	4–17 years (10)	7 (29) ^a
MPNST	1 × G2 5 × G3	2 × BCA 3 × other 1 × n.a.	50 Gy	6–23 years (12)	6
SS	2 × G3	1 × BCA 1 × other	n.a.	0.8–1.1 years (1)	2
RMS	1 × G3	1 × other	55 Gy	8	1

AS, angiosarcomas; UPS, undifferentiated pleomorphic sarcomas, type "malignant fibrous histiocytoma"; LMS, leiomyosarcomas; MPNST, malignant peripheral nerve sheath tumors; SS, synovial sarcomas; RMS, rhabdomyosarcomas; BCA, breast carcinoma; Other, various other neoplasias including (in descending frequency) lymphomas, carcinomas of different sites, and histological subtypes, germ-cell tumors, other sarcomas; n.a., not available.

^aPlus 29 previously published cases (Manner et al., 2010).

MYC is among the most frequently altered genes in human tumors. It has been estimated that 70% of all human malignancies show deregulation of *MYC* due to genetic and epigenetic alterations (Albihn et al., 2010; Klapproth and Wirth, 2010). MYC proteins (c-MYC, MYCN, and MYCL) regulate processes involved in many if not all aspects of cell fate [reviewed in Albihn et al. (2010)] with hundreds to thousands of potential transcriptional targets (Fernandez et al., 2003; Lawlor et al., 2006). MYC is a helix–loop–helix leucine zipper transcription factor that dimerizes with its partner protein Max to bind specific DNA sequences and transactivates genes (Blackwood and Eisenman, 1991), although Max-independent MYC activity is also emerging (Galant and Steiger, 2009). A conserved core set of MYC target genes seems to be involved in ribosomal and mitochondrial biogenesis, energy metabolism, and regulation of cell cycle (Dang et al., 2009). In addition to its role in cancer, MYC is one of four transcription factors that collectively can reprogram differentiated adult cells back to a pluripotent stem-cell state (Takahashi and Yamanaka, 2006). In embryonal development, MYC has been shown to sustain stem-cell features and to antagonize terminal differentiation (Kim et al., 2008). Genetic alterations of *MYC*, particularly gene amplifications, have also been reported in primary sarcomas (Schmidt et al., 1999; Stock et al., 2000; Tarkkanen et al., 2001; Zielenska et al., 2001; Ozaki et al., 2002; Squire et al., 2003; Morrison et al., 2005; Tarkka-

nen et al., 2006) and RIS (Tarkkanen et al., 2001). We and others have recently described a high frequency of *MYC* high-level gene amplifications as a distinctive feature of radiation-induced angiosarcomas as opposed to primary angiosarcomas (Manner et al., 2010; Guo et al., 2011), where *MYC* amplifications were not detectable. To investigate whether this high frequency of *MYC* amplifications is a unique feature of radiation-induced angiosarcomas or whether *MYC* amplifications are also more frequent in other RIS when compared with sporadic sarcomas, we analyzed a large series of RIS and primary sarcomas by fluorescence in situ hybridization (FISH).

MATERIALS AND METHODS

Archival paraffin-embedded tissues of 83 radiation-induced soft tissue sarcomas were collected in the setting of the RIS-EORTC-STBSG translational study 01/01. This study aimed at the collection and molecular characterization of radiation-induced soft-tissue sarcomas from international institutions. Collected cases were reviewed and reclassified by reference sarcoma pathologists applying up-to-date criteria (Fletcher et al., 2002; Hogendoorn et al., 2004) and subsequently analyzed for *MYC* amplifications (clinicopathologic characteristics shown in Table 1). This series was compared to 192 sporadic sarcomas [33 angiosarcomas (Manner et al., 2010), 36 undifferentiated pleomorphic sarcomas, 34 leiomyosarcomas, 36 malignant peripheral nerve sheath tumors

TABLE 2. Frequency of MYC Amplifications in Primary (PRS) and Radiation-induced (RIS) Sarcomas

Entity	MYC HLA		MYC LLA		MYC NA		P value
	PRS (%)	RIS (%)	PRS (%)	RIS (%)	PRS	RIS (%)	
AS	0/33	21/36 (58)	0/33	0	33/33 (100)	15/36 (42)	0.001
UPS	2/36 (6)	7/24 (29)	4/36 (11)	8/24 (33)	30/36 (83)	9/24 (38)	0.004
LMS	2/34 (6)	5/14 (36)	6/34 (18)	6/14 (43)	26/34 (76)	3/14 (21)	0.032
MPNST	5/36 (14)	1/6 (17)	3/36 (8)	2/6 (33)	28/36 (78)	3/6 (50)	n.s.
SS	1/36 (3)	0/2	0/36	2/2 (100)	35/36 (97)	0/2	n.s.
RMS	1/17 (6)	0/1	5/17 (29)	0/1	11/17 (65)	1/1 (100)	n.s.
Total	11/192 (6)	34/83 (41)	18/192 (9)	18/83 (22)	163/192 (85)	31/83 (37)	$P < 0.0001$

AS, angiosarcomas; UPS, undifferentiated pleomorphic sarcomas; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; SS, synovial sarcoma; RMS, rhabdomyosarcoma; HLA, high-level amplification; LLA, low-level amplification; NA, not amplified; n.s., not significant.

(MPNST), 36 synovial sarcomas, and 17 rhabdomyosarcomas]. Following the review, all sarcomas were arranged on a multitissue array as previously described (Zettl et al., 2003). The procedures of this study were approved by the local ethics committee (ref No. 2012-289N-MA).

FISH Analyses

Fluorescence in situ hybridization (FISH) was performed as previously described (Manner et al., 2010). Low-level amplification was assumed if more than two times as many gene signals compared to centromere signals were detected in >25% of tumor cells. Detection of >9 gene signals or tight clusters of at least 10 gene signals were considered high-level amplification. Osteosarcomas were excluded from FISH studies as decalcification procedures applied hampered reliable hybridization.

Statistical Analyses

Statistical differences were determined using Cochran Armitage trend test, Chi square test, and Mann–Whitney *U* test, where appropriate, using the SPSS software (Version 2000, SPSS, Chicago, IL). For all analyses, a *P* value < 0.05 was considered significant.

RESULTS

Clinicopathologic Features of RIS

The most frequent sarcoma types observed in the EORTC–STBSG study were undifferentiated pleomorphic sarcomas (39%), followed by leiomyosarcomas (19%), osteosarcomas (14%), angiosarcomas (11%), and MPNST (10%). Seventy percent of the cases were grade 3 sarcomas. The median radiation dosage was 60 Gy (range, 36–66.5 Gy) with a median latency period of 10 years (0.8–40 years). The main indications for irradiation

had been breast carcinoma and non-Hodgkin's or Hodgkin's lymphoma.

MYC High-Level Amplification Is a Prominent Feature of Radiation-Induced Angiosarcomas, But Are Also Prevalent in Other RIS

Table 2 gives a detailed overview of the results obtained by FISH analysis and primary sarcomas and radiation-induced sarcomas (RIS). We have previously reported on a high frequency of *MYC* high-level amplification in radiation induced, but not in primary angiosarcomas (Manner et al., 2010). In comparison with other RIS, *MYC* high-level amplification was almost twice as frequent in angiosarcomas (58%) as in any other type (17–36%). Low-level amplification of *MYC* was not observed in radiation-induced angiosarcomas. However, *MYC* high-level amplifications were also significantly more frequent in the other RIS types compared to their sporadic counterparts (Cochran Armitage trend test $P < 0.0001$), particularly in undifferentiated pleomorphic sarcomas and leiomyosarcomas. The other histological subtypes in this series were too rare to allow for a meaningful direct statistical comparison. In addition, RIS other than angiosarcomas also showed *MYC* low-level amplification in a high percentage of cases (Table 2 and Fig. 1). *MYC* amplification status was not correlated with tumor grade ($P = 0.97$, Chi-square test), but there was a nonsignificant statistical trend toward a shorter latency between primary tumor and sarcoma in cases with amplification [median latency 96 months (48–408 months) in cases with vs. 114 months (72–360) in cases without *MYC* amplification; $P = 0.2$, Mann–Whitney *U* test].

DISCUSSION

In this study, we compared the frequency of *MYC* gene copy number alterations in RIS and

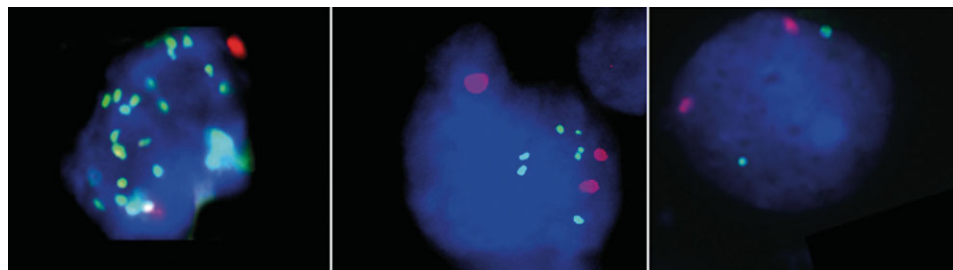


Figure 1. Representative images showing interphase fluorescence in situ (FISH) results for *c-MYC*. Left panel, high-level amplification; middle panel, low-level amplification; right panel, normal cell (blue, DAPI; green, *MYC* probe; red, chromosome 8 centromeric probe).

sporadic sarcomas and found a strong association between irradiation and *MYC* gene amplification. Among RIS, *MYC* high-level amplifications were found in 17–58% compared to 0–14% in sporadic cases. *MYC* amplifications were particularly frequent in angiosarcomas (Manner et al., 2010; Guo et al., 2011), followed by leiomyosarcomas (36%) and undifferentiated pleomorphic sarcomas (29%). Interestingly, the 58% frequency of *MYC* high-level amplifications in our series of radiation-induced angiosarcomas, which included mainly sarcomas of the breast but also other organs, was considerably lower than the 84–100% reported for other series that included only angiosarcomas of the breast (Guo et al., 2011; Mentzel et al., 2012). When only angiosarcomas of the breast were counted, the frequency of *MYC* high-level amplifications in our series was also 86%. One could hypothesize from these observations that angiosarcomas of the breast might be biologically different from angiosarcomas in other organs, as exemplified, for example, by the unusually high percentage of tumors with lymphatic differentiation among angiosarcomas of the breast (Mentzel et al., 2012).

When high-level and low-level amplifications were considered, leiomyosarcomas showed *MYC* alterations in 79% and undifferentiated sarcomas in 62% of cases. Frequent genetic gains of the chromosomal region 8q24 with putative alterations of *MYC* have also been observed in a study on RIS using comparative genomic hybridization (CGH; Tarkkanen et al., 2001), and *MYC* amplification has also been described in radiation-induced tumors generated in animals (Sawey et al., 1987). This observation may indicate a prominent role of *MYC* in the pathogenesis of RIS in general and angiosarcomas in particular. Another locus frequently affected in RIS appears to be located on chromosome arm 3p (Mertens et al., 2000).

Recurrent chromosomal gains of the *MYC* locus 8q24 have also been reported in many sporadic sarcomas, although most of these data came from CGH studies and did not directly demonstrate amplification of *MYC*. Sarcoma entities with bona fide involvement of *MYC* either in their pathogenesis or their progression include osteosarcoma (Tarkkanen et al., 1999a; Stock et al., 2000; Ozaki et al., 2002; Squire et al., 2003), and so-called malignant fibrous histiocytoma of bone (Tarkkanen et al., 2006), high-grade chondrosarcoma (Morrison et al., 2005), proximal type epithelioid sarcoma (Lualdi et al., 2004), Ewing sarcoma (Tarkkanen et al., 1999b), and myxoid liposarcoma (Schneider-Stock et al., 2003). Involvement of the 8q24 region has repeatedly been reported to be associated with clinical high-risk profiles (Gladdy et al., 2010) e.g. in leiomyosarcomas (El-Rifai et al., 1998) and synovial sarcomas (Skytting et al., 1999). Together, these data indicate that *MYC* may be an important oncogene in the development and progression of a variety of different sarcoma types. *MYC* has been shown to play a role in the spontaneous transformation of murine bone marrow-derived mesenchymal stem cells (BMMSCs; Miura et al., 2006; Rosland et al., 2009). After numerous passages, BMMSCs may obtain unrestricted cell division and undergo malignant transformation with in vivo formation of osteosarcomas (Mohseny et al., 2009). Interestingly, during immortalization and transformation, the BMMSCs were shown to accumulate chromosomal abnormalities including loss of the p16 locus and to specifically amplify *MYC* in double-minute chromosomes (Miura et al., 2006). One oncogenic mechanism of *MYC* amplification in these cells may involve increased telomerase activity. The functional consequences of altered *MYC* levels in sarcomas are currently not known. *MYC* overexpression has been reported to increase genomic instability (Felsher and Bishop,

1999). DNA damage-induced checkpoints are important safeguard mechanisms for genomic stability. MYC overexpression in irradiated cells alters cell-cycle arrest to DNA damage at the G₁/S boundary (Sheen and Dickson, 2002), for example, through induction of cyclinD1 (Sheen et al., 2003). At the same time, MYC also sensitizes the cell for apoptosis (Askew et al., 1991). These two seemingly contradictory mechanisms, amplification of DNA damage on one hand and “apoptosis-assisted” selective pressure on the other, could favor the selection of genomic alterations in irradiated tumors (Sheen and Dickson, 2002). These mechanisms may contribute to the highly aggressive behavior of postirradiation sarcomas (Robinson et al., 1988; Gladdy et al., 2010).

Our finding that *MYC* gene amplifications were so prevalent in RIS, but not in sporadic sarcomas, points to a specific role of MYC in the pathogenesis of RIS. However, because MYC drives the transcription of most active genes, even small changes in MYC levels may have profound impact on cellular functions (Levens, 2010; Rahl et al., 2010). Absence of high-level gene amplifications does thus not necessarily exclude a profound role of MYC also in the pathogenesis of primary sarcomas. Given the emerging perspective that MYC has finally become a druggable cellular target (Delmore et al., 2011), it will be important to determine the specific role of MYC in different subsets of sarcomas.

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