Rad51 overexpression contributes to chemoresistance in human soft tissue sarcoma cells: a role for p53/activator protein 2 transcriptional regulation

Jonathan A.F. Hannay,[1] Juehui Liu,[1] Quan-Sheng Zhu,[1] Svetlana V. Bolshakov,[2] Lan Li,[3] Peter W.T. Pisters,[1] Alexander J.F. Lazar,[4] Dihua Yu,[1] Raphael E. Pollock,[1] and Dina Lev[2]

Departments of [1]Surgical Oncology, [2]Cancer Biology, [3]Experimental Therapeutics, and [4]Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas

Abstract

We investigated whether Rad51 overexpression plays a role in soft tissue sarcoma (STS) chemoresistance as well as the regulatory mechanisms underlying its expression. The studies reported here show that Rad51 protein is overexpressed in a large panel of human STS specimens. Human STS cell lines showed increased Rad51 protein expression, as was also observed in nude rat STS xenografts. STS cells treated with doxorubicin exhibited up-regulation of Rad51 protein while arrested in the S-G_2 phase of the cell cycle. Treatment with anti-*Rad51* small interfering RNA decreased Rad51 protein expression and increased chemosensitivity to doxorubicin. Because we previously showed that reintroduction of wild-type p53 (wtp53) into STS cells harboring a p53 mutation led to increased doxorubicin chemosensitivity, we hypothesized that p53 participates in regulating Rad51 expression in STS. Reintroduction of wtp53 into STS cell lines resulted in decreased Rad51 protein and mRNA expression. Using luciferase reporter assays, we showed that reconstitution of wtp53 function decreased *Rad51* promoter activity. Deletion constructs identified a specific Rad51 promoter region containing a p53-responsive element but no p53 consensus binding site. Electrophoretic mobility shift assays verified activator protein 2 (AP2) binding to this region and increased AP2 binding to the promoter in the presence of wtp53. Mutating this AP2 binding site eliminated the wtp53 repressive effect. Furthermore, *AP2* knockdown resulted in increased Rad51 expression. In light of the importance of Rad51 in modulating STS chemoresistance, these findings point to a potential novel strategy for molecular-based treatments that may be of relevance to patients burdened by STS. [Mol Cancer Ther 2007;6(5):1650–60]

Introduction

Soft tissue sarcoma (STS) constitutes a malignancy cluster of putative mesenchymal origin that may occur anywhere in the body. STS is characterized by a high incidence of local recurrence as well as distant metastasis, particularly to the lungs (1). Overall survival is ~50% at 5 years, a rate that has not improved significantly over the past 50 years even with the wide-spread utilization of systemic chemotherapy, including second- and third-generation agents. Even doxorubicin, the single most active STS chemotherapeutic, has a disappointing 30% overall response rate, with breakthrough tumor progression and frequent local and/or distant recurrence after initial sarcoma chemoresponsiveness (2). Chemotherapies damage cellular components, thereby triggering genetically controlled responses, such as apoptosis, that in turn leads to cell death. With tumor progression, cancer cells tend to lose these normal damage responses while acquiring intracellular mechanisms that impair chemoresponse. Abrogating chemoresistance may augment STS patient outcome; however, this mandates improved understanding of STS chemoresistance mechanisms, about which little is currently known.

Previously, we have studied MDR-1/P-glycoprotein expression and activity in STS chemoresistance (3, 4). This transmembrane efflux transporter reduces intratumoral accumulation/retention of cytotoxic drugs and may induce the intracellular redistribution of these agents away from the putative site of action (e.g., outside of the nucleus). In that chemoresistance involves multiple mechanisms, we have examined other possibly relevant intratumoral processes. DNA double-strand breaks (DSB) are a common consequence of chemotherapy (e.g., doxorubicin) and ionizing radiation and are considered the most lethal challenge to genome integrity; a single DSB can trigger DNA damage sensors, arrest cell cycle progression, and activate DNA repair machinery (5, 6). The coordinated repair of DSBs is critical for cell survival. Homologous recombination is integral to DSB repair, and homologous recombination may be important in enabling tumor cells to avoid chemotoxicity. The mammalian Rad51 protein is the structural, biochemical, and genetic homologue of the bacterial RecA and yeast Rad51 proteins

Received 10/18/06; revised 2/23/07; accepted 3/29/07.

Grant support: National Cancer Institute/M. D. Anderson Cancer Center Core grant P30-CA16672 and NIH grants RO1 CA67802 (R.E. Pollock) and CA60488 (D. Yu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Dina Lev, Department of Cancer Biology, The University of Texas M. D. Anderson Cancer Center, Unit 173, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-1637; Fax: 713-792-0722. E-mail: dlev@mdanderson.org

Copyright © 2007 American Association for Cancer Research.

doi:10.1158/1535-7163.MCT-06-0636

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

and plays a central regulatory role in meiotic recombination and homologous recombination DNA repair. *Rad51* is phylogenetically conserved and mice nullizygous for *Rad51* display pre-implantation embryonic lethality (7) with chromosome loss and apoptosis (8). The central role of Rad51 in homologous recombination is further suggested because Rad51 overexpression is sufficient to promote DNA strand exchange without its usual association with the gene conversion complex. However, excessive or uncontrolled homologous recombination poses a threat to genome integrity by inducing chromosome fusion and aberrant karyotype formation and augmenting resistance to DNA damage–induced apoptosis (9). Rad51 overexpression, but not gene amplification or mutation (10), has been observed in a variety of epithelial malignancies (11, 12). High levels of Rad51 are associated with elevated rates of DNA recombination as well as enhanced resistance to DNA-damaging chemotherapies and/or ionizing radiation in several experimental tumor systems (13–15). Antisense strategies have been successfully used to attenuate Rad51-mediated radioresistance in *in vitro* and *in vivo* studies (16, 17). To the best of our knowledge, Rad51 expression, function, or regulation has not been previously studied in STS. Given the chemoresistant nature of STS, it is hoped that investigation of Rad51 yields therapeutically relevant insights.

In this present study, we addressed the following questions: (*a*) is Rad51 overexpressed in human STS; (*b*) does Rad51 overexpression play a role in STS chemoresponsiveness; and (*c*) does *p53*, the most commonly altered mutated gene in STS, regulate the expression of Rad51, and if so, what are the underlying p53 regulatory mechanisms? Our results show that Rad51 protein is overexpressed in primary, recurrent, and metastatic human STS specimens of various histologic subtype and anatomic location. Moreover, inhibiting Rad51 expression in STS cell lines using anti-*Rad51* small interfering RNA (siRNA) markedly increased chemosensitivity to low-dose doxorubicin.

Because we have shown that wild-type *p53* (wtp53) reintroduction into mutant *p53* (mutp53) cells leads to increased STS doxorubicin chemosensitivity (3), we questioned whether p53 also helped regulate STS Rad51 expression. Reintroduction of wt*p53* into a human mut*p53* STS cell line (SKLMS-1 leiomyosarcoma) resulted in decreased Rad51 protein expression, a phenomenon not due to enhanced proteasomal degradation. Moreover, decreased *Rad51* mRNA expression (without evidence of shortened mRNA half-life) was also observed. Examining the *Rad51* gene promoter revealed that activator protein 2 (AP2) binding to its *cis* element binding site mediated the p53-induced Rad51 transcriptional repression.

Taken together, these results suggest that Rad51 has a potential role as a possible therapeutic target in human STS. Novel molecular-based strategies to inhibit Rad51 directly or indirectly via wt*p53* reintroduction may be relevant to patients burdened by STS.

Materials and Methods

Cells, Culture Conditions, and Treatments

Human SKLMS1 leiomyosarcoma (harboring a double G245S and M237K *p53* point mutation), SW684 (fibrosarcoma), HT1080 (fibrosarcoma), SW872 (liposarcoma), A204 (rhabdomyosarcoma), RD (rhabdomyosarcoma), MRC-5 (normal diploid human lung fibroblasts), and HUVE (human umbilical vein endothelial) cells were obtained from the American Type Culture Collection. Cells were cultured in DMEM (A204 in McCoy's 5A) supplemented with 10% FCS (Life Technologies, Inc). SK-Ala-10, SK-Ala-14, SK-Ala-21, and SK-neo cell lines were generated from SKLMS1 as previously described (18) and cultured as per parental cell culture conditions. SK-Ala-10, SK-Ala-14, and SK-Ala-21 translate p53 transcripts from the transfected temperature sensitive V143A *p53* gene as mutant p53-mimicking conformation at 37°C but as a DNA binding and transcription activating wild-type conformation at 32°C (19).

Viral Incubation. AdEV (empty vector), AdLacZ (β-galactosidase transgene), AdFLAGp53 (FLAG-tagged wtp53), or Adp53 (non-tagged wtp53) were incubated at 37°C for 48 h and at 1:2,000 cell to viral particles ratios unless indicated otherwise.

Proteasome Inhibition. SKLMS1 cells were pretreated with AdFLAGp53 or AdLacZ for 48 h before addition of MG132 (or DMSO) to a final concentration of 10 μmol/L for 0, 2, 4, and 6 h before cell lysis and Western blotting.

siRNA Western Blotting. SKLMS1 and RD cells (50,000) were plated per well of six-well plate and incubated overnight at 37°C. The following morning siGENOME Rad51 siRNA constructs (Dharmacon, Inc.) were transfected using LipofectAMINE PLUS (Invitrogen) reagents according to manufacturer's instructions. Forty-eight hours later, monolayers were harvested in modified radioimmunoprecipitation assay buffer and clarified, and equimolar amounts of protein are separated by 10% SDS-PAGE. Proteins immobilized on nitrocellulose membranes were blocked with 5% bovine serum albumin-TBST and probed with anti-Rad51 (Lab Vision clone 3C01) 1:400 overnight and detected with IRdye680-conjugated secondary antibody. Similar experiments were done using human *AP2*–specific siRNA si*GENOME SMART* pool (Dharmacon). Proteins harvested were used for AP2 and Rad51 Western blot.

siRNA Chemosensitivity Assay. Proliferating SKLMS1 and RD cells (2,000) were plated per well of 96-well plates and incubated overnight. Medium was then aspirated and replaced with LipofectAMINE PLUS–prepared 100 nmol/L siRNA (anti-Rad51 construct #4, scrambled or mock) in 70 μL Opti-MEM 1 (Invitrogen) per well for 6 h then supplemented with 70 μL DMEM/20% FCS per well overnight. After 48 h, fresh medium containing increasing concentrations of doxorubicin was added for a further 48 h before 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium assay (Promega) according to manufacturer's instructions.

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

Antibodies

Anti-Rad51. Monoclonal antibody clone 51RAD01 (same as 3C10) as Ab-1 from Lab Vision Corp. Anti Rad51 (Ab-1) polyclonal antibody and (Ab-2) monoclonal antibody (Calbiochem) were used additionally for Rad51 immunohistochemical control.

Anti-FLAG. Monoclonal M2 and M5 (Sigma-Aldrich). Anti-p21$^{CIP1/WAF1}$: C-19 polyclonal (Santa Cruz Biotechnology, Inc.). Anti-P-glycoprotein antibody C219 (Centocor).

Anti-β-actin. Monoclonal (Sigma-Aldrich). Anti-AP2: 3B5 monoclonal and C-18 polyclonal (Santa Cruz Biotechnology).

Anti-p53. DO1 monoclonal (Santa Cruz Biotechnology). Horseradish peroxidase–conjugated secondary antibodies were detected by enhanced chemiluminescence (Amersham Biosciences Plc.). Alexa Fluor 680 (Molecular Probes) and IRdye800 (Rockland Immunohistochemicals, Inc.) second-conjugated secondary antibodies were detected using Odyssey Imaging (LI-COR Biosciences).

Immunohistochemistry

Paraffin sections (5 μm) of human STS samples were heated, dewaxed, and hydrated in xylene and ethanol/H_2O. Rehydrated sections were boiled in 10 mmol/L citrate (pH 6.0) antigen retrieval buffer for 20 min before quenching endogenous peroxidase activity with 1% H_2O_2 for 30 min. Endogenous protein binding activity was blocked using DAKO blocking agent (DAKO Corp.) overnight at 4°C before incubation at 4°C with anti-Rad51 primary antibody diluted 1:100 in 1% bovine serum albumin-PBS. DAKO Envision system was used for secondary antibody conjugation, and color was developed with Zymed DAB system (Zymed Laboratories, Inc.). Slides were counterstained with hematoxylin. Rad51 expression scoring was done by two independent observers; % positive staining cells and expression intensity (1, low; 2, moderate; and 3, strong) were determined.

Western and Northern Blot Analysis

Protein extraction from either cultured cells or frozen human STS specimens was done as previously described. For some experiments, nuclear and cytoplasmic protein fractions were isolated; 50 μg proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes for Western blotting. Northern blots were done using 20 μg of total RNA. Full-length cDNAs for *Rad51*, glyceraldehyde-3-phosphate dehydrogenase, and *p21* were labeled using a random labeling kit (Roche Diagnostics GmbH) and used as probes for Northern blot.

Flow Cytometry

Cell monolayers were trypsinized and collected by centrifugation and washed twice with ice-cold PBS. Resuspended cells and debris were aliquoted for 70% ice-cold ethanol/PBS fixation or lysis in radioimmunoprecipitation assay buffer for Western blot. Fixed cells were treated with 50 μg/mL RNase and stained with 50 μg/mL propidium iodide for 30 min. Cells were analyzed in a FACSCalibur, and data were analyzed with Cell Quest and ModFitLT v3.1 software (Verity Software House).

Luciferase Reporter Assay

Transfections were done in six-well cluster plates using Fugene 6 transfection reagent (Roche Diagnostics) according to manufacturer's instructions, with 100 ng Rad51-Luc luciferase reporter per well and 20 ng pRL/CMV control per well for normalization. SKLMS1 cells were pretreated with wt*p53* or lacZ expressing adenoviruses (5,000 viral particles/cell ratios) or mock infected for 48 h before transfection. The total amount of DNA transfected per well was 120 ng. Lysates were prepared 48 h after transfection, and luciferase activity was measured using Promega dual luciferase assay kit according to manufacturer's instructions.

Rad51 Promoter Constructs

The *Rad51* promoter sequence (AF203691) from −403 to +63 of the transcription start site was amplified by PCR and cloned into pGL3 using primers: forward, 5′-CATTACCTCTTGGGAGTCGTGGTCT-3′ and reverse, 5′-ACTCTCCTTAGGGCTCGGTCTCTGG-3′. PCR-based deletion strategies generated truncated wild-type sequence Rad51 promoters using the following forward primers: pRad-295Luc, 5′-GGGTAGGAGTAGGGGCGTTG-3′; pRad-185Luc, 5′-GGCATAAAGTTTGAATTAGT-3′; pRad-50Luc, 5′-GGGGATACGTTACGTCGACG-3′. Substitution mutations (lowercase) were incorporated into the −193 to −183 bp AP2 consensus site (underlined) using the primer 5′-CGTGAGCCA*ttGCCttt*GGCATAA AGTTTG-3′, and 10 bp were deleted using the primer 5′-GGACTACACGCGTGAGCCATTTATAAAGTTTGAATTAGTCC-3′.

Electrophoretic Mobility Shift Assay

Nuclear extracts were prepared from SKLMS1 cells in cold nuclear extraction buffer. Binding reaction was initiated by adding 5 μg nuclear extract to binding buffer and 1 μg poly(deoxyinosinic-deoxycytidylic acid; Amersham Biosciences), 3×10^5 counts per minute ^{32}P-labeled AP2α double-stranded oligonucleotide, and 1% NP40 (total volume 20 μL) and incubated for 30 min at 37°C. The reaction was terminated by adding 4 μL 6× DNA loading dye and placing samples on ice prior 5% native PAGE. The dried gel was autoradiographed. For supershifts, 1 μg rabbit polyclonal anti-AP2α antibody (Santa Cruz Biotechnology) was added to the nuclear extract and incubated at room temperature for 30 min before being added to the binding reaction. Probe sequence was 5′-CGTGAGCCACCGCCCCCGGCATAAAGTTTG-3′.

Results

Rad51 Is Overexpressed in Human STS Specimens

Increased Rad51 overexpression has been observed in several epithelial malignancies (11); therefore, we investigated whether similar overexpression could also be identified in mesenchymal origin tumors. Immunohistochemical staining (anti-Rad51 DO1 Ab, Labvision) to detect Rad51 overexpression was done on a panel of 62 human primary, recurrent, and metastatic STS samples of different histologic subtype and location randomly selected from our sarcoma tissue bank, showing diffuse nuclear, perinuclear,

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

and robust cytoplasmic Rad51 staining patterns of varying intensity in 59 of 62 specimens. Only three tumors exhibited no Rad51 expression (Fig. 1A–G). In specimens exhibiting Rad51, level of expression varied both in distribution and intensity. Rad51 expression was observed in <20% of tumor cells in 16 samples, 20% to <80% in 39, and 80% to 100% in 24. Intensity was scored as 1 in 26, 2 in 18, and 3 in 15. Prevalent nuclear staining was observed in 5 samples, cytoplasmic in 10 samples, and both cytoplasmic and nuclear in 44 samples. In contrast, Rad51 expression was low to absent in paired normal tissues retrievable in either tumor sections, resection margin normal tissues, or temperospatially distant autologous normal tissues (Fig. 1H). A small cohort of five human malignant fibrous histiocytoma immunostained with a different set of Rad51 antibodies (anti-Rad51 Ab-1, Ab-2, Calbiochem) confirmed the increased Rad51 expression and subcellular localization observed above. Increased Rad51 expression in STS in comparison with autologous normal tissues was further confirmed using Rad51 Western blotting (Fig. 1I). The diversity of STS specimens we examined suggests that Rad51 overexpression is commonly observed in STS regardless of histology, location, or stage.

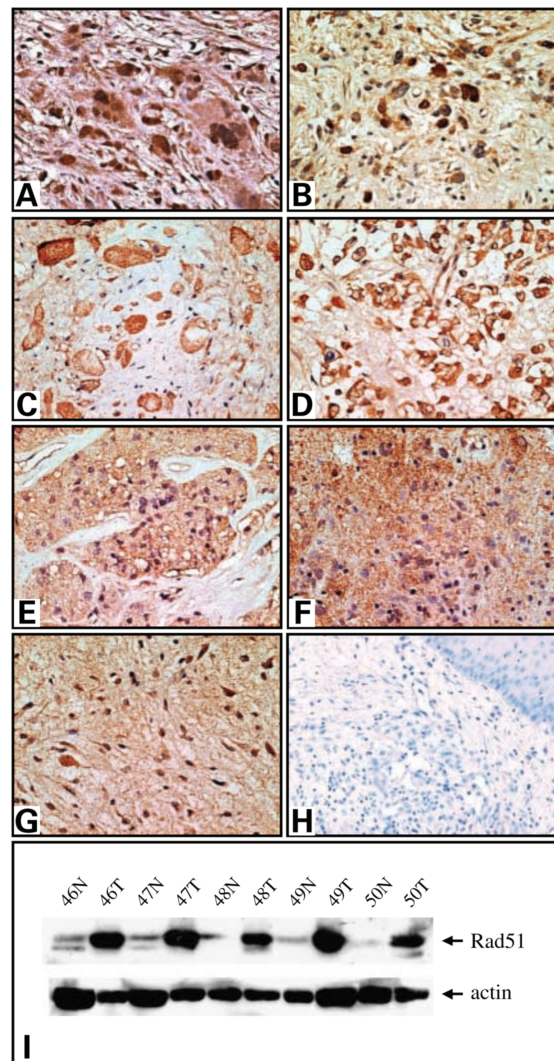

Figure 1. Rad51 protein is aberrantly expressed in human STS samples of different histologic subtypes and stage. Representative cases: **A** and **B,** primary malignant fibrous histiocytoma; **C,** primary rhabdomyosarcoma; **D,** metastatic rhabdomyosarcoma; **E,** recurrent alveolar soft part sarcoma (*ASPS*); **F,** metastatic fibrosarcoma; **G,** primary liposarcoma; **H,** normal tissue adjacent to an malignant fibrous histiocytoma exhibiting no Rad51 expression (no tumor is seen in this slide). Immunohistochemistry done as per Materials and Methods. All original images were captured at ×200 magnification. **I,** Rad51 Western blot exhibiting increased Rad51 expression in protein extracted from selected human STS specimens when compared with expression in matched normal tissue.

Rad51 Is Overexpressed in STS Cell Lines *In vitro* and *In vivo*

To further evaluate the role of Rad51 in STS, we first asked whether STS cells exhibit Rad51 overexpression when grown in cell culture and as xenografts in nude rats. Rad51 protein expression was detected to varying degree in all tested STS cells lines; no Rad51 expression was detected in NHF cells; and only a minimal level was expressed in human umbilical vascular endothelial cell (Western blot; Fig. 2A). Immunocytochemistry clearly revealed Rad51 in the nucleus, perinuclear cytoplasm, and cytoplasm of the SKLMS1 cells (Fig. 2B). SKLMS-1 leiomyosarcoma xenografts excised from nude rats were immunohistochemically stained for Rad51 using the same method as the patient analysis above and showed the same diffuse nuclear, perinuclear, and robust cytoplasmic staining patterns as seen above (Fig. 2C). In contrast, positive-staining control normal rat testis tissue showed focal staining confined to the nucleus (Fig. 2D). Rad51 Western blot of nuclear and cytoplasmic protein fractions of the SKLMS1 and RD cell lines further confirmed the protein localization observed by immunohistochemistry (Fig. 2E). Our results show that Rad51 overexpression is observable *in vitro* and *in vivo* in a manner comparable with Rad51 overexpression in human STS, suggesting the utility of these models to further investigate STS chemoresistance and Rad51 regulation.

Rad51 Protein Levels Increase in Response to Doxorubicin-Induced S-G_2-M Phase Cell Cycle Arrest

Because the principal basis of doxorubicin cytotoxicity is induction of DSBs during DNA synthesis, we questioned whether doxorubicin exposure might affect Rad51 STS protein levels. Proliferating SKLMS1 cells were treated with 0.5 μmol/L doxorubicin for 24 and 48 h and collected (with treatment media) for Western blotting and fluorescence-activated cell sorting analysis. Rad51 protein levels were consistently higher in cells treated with doxorubicin than in paired untreated cells (Fig. 3A). Propidium iodide sorting by fluorescence-activated cell sorting indicated that doxorubicin-treated SKLMS1 cells arrested in the S-G_2-M cell cycle phase without concomitant increase in the apoptotic sub-G_1 fraction. Consistent with other studies showing a cell cycle phase–related expression of Rad51 (20), our findings indicate that Rad51 levels are highest in that phase of the cell cycle where doxorubicin exerts its principal effects. Because Rad51 overexpression per se stimulates

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

homologous recombination and gene conversion (21), our findings suggest that Rad51 could contribute to STS doxorubicin chemoresistance.

Rad51 Suppression by siRNA Attenuates RD and SKLMS-1 Doxorubicin Chemoresistance

Next, we employed a Rad51 siRNA knockdown strategy to examine whether Rad51 participates in STS chemoresistance. RD and SKLMS-1 cells were incubated with increasing doses of anti-Rad51 specific siRNA. Efficient dose-dependent Rad51 knockdown was shown (Western blot; Fig. 3B) with Rad51siRNA, whereas scrambled siRNA did not affect Rad51 expression levels. Western blot for P-glycoprotein showed no change in P-glycoprotein levels after Rad51 inhibition (data not shown). Using 100 nmol/L concentrations of siRNA against Rad51, we next did 96-well plate 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium cell viability assays for increasing concentrations of doxorubicin. Rad51 siRNA alone did not affect the growth rate of the

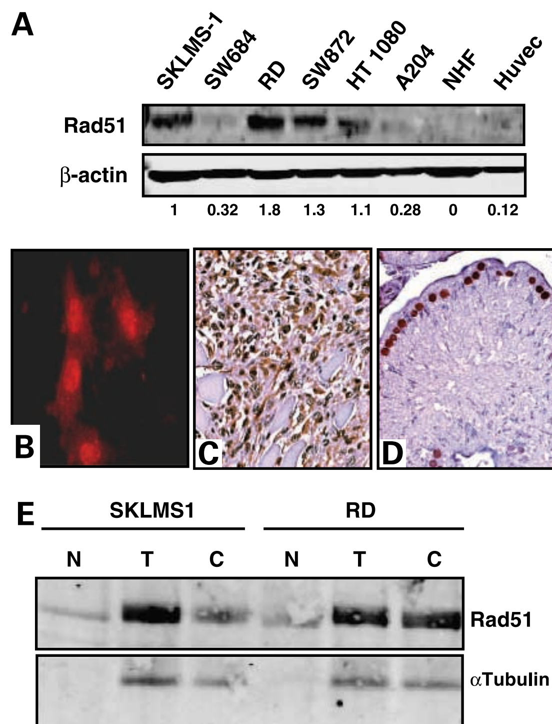

Figure 2. Rad51 protein is aberrantly expressed in human STS cell lines *in vitro* and *in vivo*. **A,** Western blot comparison of Rad51 levels in STS cell lines. Relative Rad51/actin expression values (SKLMS1 Rad51/actin ratio determined as 1) measured by densitometry (*bottom*). NHF, normal human fibroblasts; HUVEC, human umbilical vein endothelial cells. **B,** florescent immunocytochemistry for Rad51 in cultured SKLMS1 cells showing Rad51 in the cytoplasmic and nuclear compartments. **C,** immunohistochemistry shows abundant and deregulated Rad51 staining throughout nuclear and cytoplasmic cellular compartments of SKLMS-1 xenografts invading local normal rat musculature in comparison to (**D**) highly discrete nuclear Rad51 staining in rat testis (positive control). **E,** Rad51 Western blot showing Rad51 expression in both the cytoplasm (C, cytoplasmic protein extract) and in the nucleus (N, nuclear protein extract) of SKLMS1 and RD cells (T, total cell lyset).

tumor cells compared with control scrambled siRNA and/or mock transfection. Furthermore, we found that Rad51-siRNA enhanced doxorubicin chemosensitivity in both cell lines to varying degree (Fig. 3C and D). Rad51 inhibition led to 1.75-fold reduction in doxorubicin IC_{50} (from 0.7 to 0.4 μmol/L) in SKLMS1 cells and 3.2-fold in RD cells (from 3.2 to 1 μmol/L).

Induction of wtp53 Leads to Suppression of Rad51 Protein Levels in STS Cells

Previously, we have shown that restoration of wtp53 in mutp53 STS cells leads to loss of chemoresistance in these cells. We explained this observation as due to p53 induced down-regulation of MDR1/Pgp expression with concordantly reduced activity (3, 4). However, it is likely that the wtp53-induced chemosensitivity is a multifactorial process. Taking into account our finding that Rad51 inhibition leads to enhanced chemoresponse as well as extensive studies by others showing that wtp53 inhibits Rad51 function (22), we wanted to further explore the mechanism underlying wtp53 regulation of Rad51 in STS. Previous reports have focused on non-transcriptional protein-protein interacting domains of p53 and Rad51 whereby p53 inhibits Rad51 function (22); however, studies examining p53 transcriptional regulation of Rad51 production have been very limited.

To address the potential affect of wtp53 induction on Rad51 status in STS, we used our panel of SK-Ala mutants previously generated in our laboratory (18). As shown in Fig. 4A, sustained induction of wtp53 function (shown by $p21^{CIP1/WAF1}$ protein up-regulation) leads to down-regulation of Rad51 protein levels. To acutely induce wtp53 function, we also infected SKLMS1, RD, and A204 STS cells with log doses of adenoviruses expressing FLAG-tagged wtp53 or empty vector controls. Rad51 levels fell in each cell type within 48 to 72 h after transfection when wtp53 was expressed at high levels (Fig. 4B). These data indicate that wtp53 leads to suppression of Rad51, and that this phenomenon occurs in multiple STS cell lines.

wtp53 induction leads to transcriptional up-regulation of many target genes, including its own negative regulator Mdm-2. Mdm-2 acts as an E3-ligase, polyubiquitinating p53 and targeting it for proteasomal degradation. To assess possible wtp53 suppression of Rad51 protein via proteasomal degradation, we incubated AdFLAGp53- and AdLacZ-infected SKLMS1 cells with the proteosome inhibitor MG132 for incremental time periods and harvested cellular proteins for Western blotting. As expected, FLAGp53 accumulated in cells treated with MG132, confirming proteosome inhibition. Rad51 protein marginally accumulated in those AdLacZ-treated cells where the proteosome was inhibited, indicating that Rad51 protein degradation takes place, at least partly, via a proteosome-dependent mechanism. However, no such accumulation was seen in those cells treated with AdFLAGp53 in which the proteosome was inhibited, and proteosome inhibition failed to rescue Rad51 attenuation (data not shown). This result indicates that wtp53 does not principally use a proteosome-dependent mechanism to suppress Rad51 levels.

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

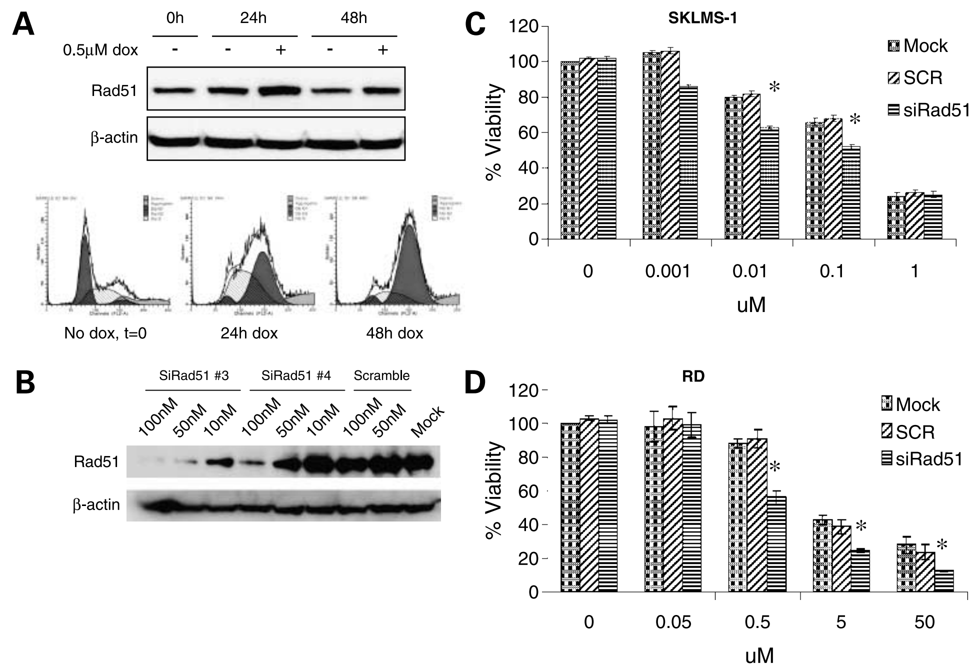

Figure 3. SKLMS-1 cells incubated with doxorubicin exhibit increased Rad51 and arrest in S-G_2-M without induction of apoptosis; however, abolishing Rad51 with siRNA enhances doxorubicin chemosensitivity. **A,** incubation of SKLMS-1 cells with 0.5 μmol/L doxorubicin (*dox*) for the indicated periods of time leads to increased Rad51 protein levels, as observed in Western blotting, and arrest of cells in the S-G_2-M phase of the cell cycle, by FACS analysis. No concomitant increase in sub-G1 fraction of total sorted cells was seen. Representative of three experiments (Western blot and fluorescence-activated cell sorting graphs). **B,** Western blot for Rad51 levels in RD cells incubated with indicated doses of two different anti-Rad51 siRNA constructs (siRNA #3 corresponds to NM-002875.2 sequence 802 – 821, GCAGTGATGTCCTGGATAAT and #4 to 361 – 379, CCAACGATGTGAAGAAATT). Mock, LipofectAMINE PLUS transfection agent alone; SCR, scrambled Rad51 siRNA sequence. **C,** before 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium cell viability assay shows that 100 nmol/L siRNA#3 pretreatment of SKLMS-1 and RD cells enhances chemosensitivity after a 48-h exposure to varying concentrations of doxorubicin. *Columns,* viability; *bars,* SD. *, $P < 0.05$, statistically significant decreases (Student's *t* test).

Induction of wtp53 Leads to Suppression of Rad51 mRNA Levels in STS Cells

To assess the effect of wtp53 induction on Rad51 mRNA transcript levels, Northern blot was done on mRNA isolated from SK-Ala temperature-sensitive mutants, parental, and neo-control cell lines following differential temperature incubation to induce wtp53 or mutp53. Figure 4C shows that induction of wtp53 is associated with abolition of Rad51 mRNA levels in the temperature-sensitive cells but not in the parental or control cells. To further confirm this finding, Northern blot was done on mRNA extracted from SKLMS1 cells that had been infected with AdFLAGp53 or AdLacZ at increasing viral doses. Similarly above, induction of exogenous wtp53 was also associated with suppressed Rad51 transcript levels (data not shown). Next, we investigated whether the decreased Rad51 mRNA could be attributed to wtp53-induced shortened Rad51 mRNA half-life. SKLMS1 cells pre-infected with control or wtp53-expressing adenoviruses were treated with actinomycin D to arrest generation of nascent mRNA, and mRNA was harvested at subsequent incremental time points. Comparable Rad51 mRNA half-lives were found independent of p53 status, indicating that wtp53 does not enhance Rad51 mRNA transcript degradation as a mechanism of Rad51 suppression (data not shown). Similar results were found using SK-Ala temperature-sensitive cells, thereby controlling for the possibility that adenoviral proteins could be interfering with host cell mRNA transcript processing, transportation, and translation machinery. Taken together, these data suggest that wtp53 regulates Rad51 STS expression via a transcriptional mechanism.

wtp53 Suppresses *Rad51* Promoter Activity via an AP2 Response Element

The *rad51* gene possesses a GC-rich TATA-less promoter that indicates a possible Rad51 housekeeping function. To examine wtp53 involvement in transcriptional control of Rad51, the −403 to +63 bp region of the Rad51 promoter (relative to the transcription start site) was subcloned from SKLMS-1 genomic DNA into the pGL3 luciferase reporter plasmid to generate pRad-403Luc for promoter activity studies. pRad-403Luc was transfected into SK-Ala-10, SK-Ala-14, SK-Ala-21, parental, and neo-control cell lines under mutp53- or wtp53-inducing conditions. As shown in Fig. 5A, we confirmed that induction of wtp53 in cells harboring mutp53 leads to suppression of transcription by the −403-bp rad51 promoter segment.

To locate a potential genetic element that wtp53 may be acting through, we analyzed the proximal promoter fragment sequence using DNAsys software and generated

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

serially truncated (−403, −295, −185, and −50 bp) promoter constructs flanking identified consensus binding sites for AP-2, Sp-1, and a previously reported p53 response element (23). No classic p53-binding site was identified in this analysis. The effect of wtp53 on transactivation from the truncation mutants was investigated in SKLMS1 cells infected with mock or wtp53-expressing adenoviruses. Induction of wtp53 in AdFLAGp53-infected SKLMS1 cells consistently suppressed transcriptional activity by the −403 and −295 constructs. However, the transcription attenuating effect of wtp53 induction was lost when the −185 promoter was studied (Fig. 5B).

Because AP2 is known to directly interact with p53, and because a putative AP2 binding site was suggested in the −193 to −183 region of the Rad51 promoter, we next investigated the relevance of this AP2 site to the p53 effect on Rad51 promoter activity. We first confirmed AP2 binding to the identified site with an electrophoretic mobility shift assay using a known AP2 binding sequence and a 30-bp radiolabeled DNA probe corresponding to the identified AP2 site and short flanking sequences (Fig. 5C). Autoradiography of the PAGE-separated mixtures confirmed AP2 binding to the sequence from the Rad51 promoter and also showed ''supershift'' migration following anti-AP2α antibody pre-incubation. Incomplete ''shifting'' of the entire band implied that AP2 isoforms other than the α subtype may also be binding this sequence. Moreover, increased AP2 binding was observed in the nuclear extracts from Adp53-transfected SKLMS1 cells compared with parental or AdLacZ transfectants. Western blotting showed no difference in AP2 protein level in the presence of wtp53 (data not shown), suggesting that wtp53 affects the activity but not the expression of AP2. Specific deletion of 10 bp internal to the AP2 binding site was done on the −403 promoter construct, and luciferase-based reporter assays were repeated in virally infected SKLMS1 cells. Mutating this site alone led to loss of wtp53 suppression of *Rad51* promoter activity in our luciferase reporter gene assays (Fig. 5D). Similar results were achieved after specific substitution mutation of the AP2

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

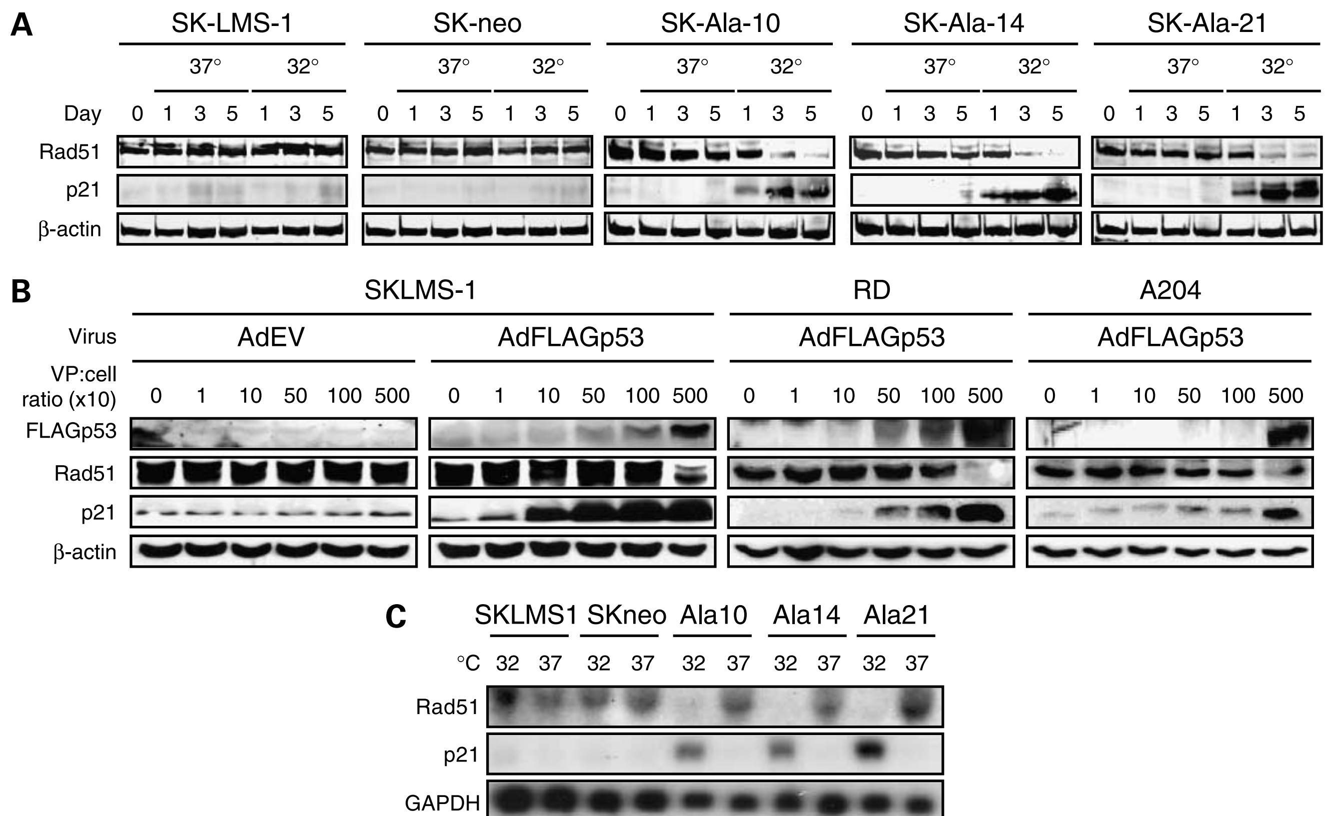

Figure 4. Induction of endogenous or exogenous wtp53 leads to suppression of Rad51 in STS. **A,** Western blot. SK-Ala-10, SK-Ala-14, SK-Ala-21 p53 temperature-sensitive, parental SKLMS-1, and SK-neo control cell lines were incubated at 37°C and 32°C to differentially induce mutant or functional wtp53, respectively. Protein lysates were harvested at indicated time points. Rad51 protein levels were decreased following induction of wtp53 function, confirmed by p21 induction, in temperature-sensitive p53 cells at 32°C (wtp53 function) but not at 37°C (mutp53 function). **B,** Western blot. SKLMS-1, RD, and A204 cells were incubated with empty vector (AdEV) or wtp53-expressing (AdFLAGp53) adenoviruses at the indicated cell to virus particle ratios for 72 h. Induction of exogenously delivered wtp53 leads to suppression of Rad51 protein levels. **C,** Northern blot. Parental and temperature-sensitive transfectants were cultured as in (**A**). Northern blotting of RNA extracts showed suppression of rad51 mRNA levels following induction of wtp53 function, confirmed by increased levels of p21 mRNA.

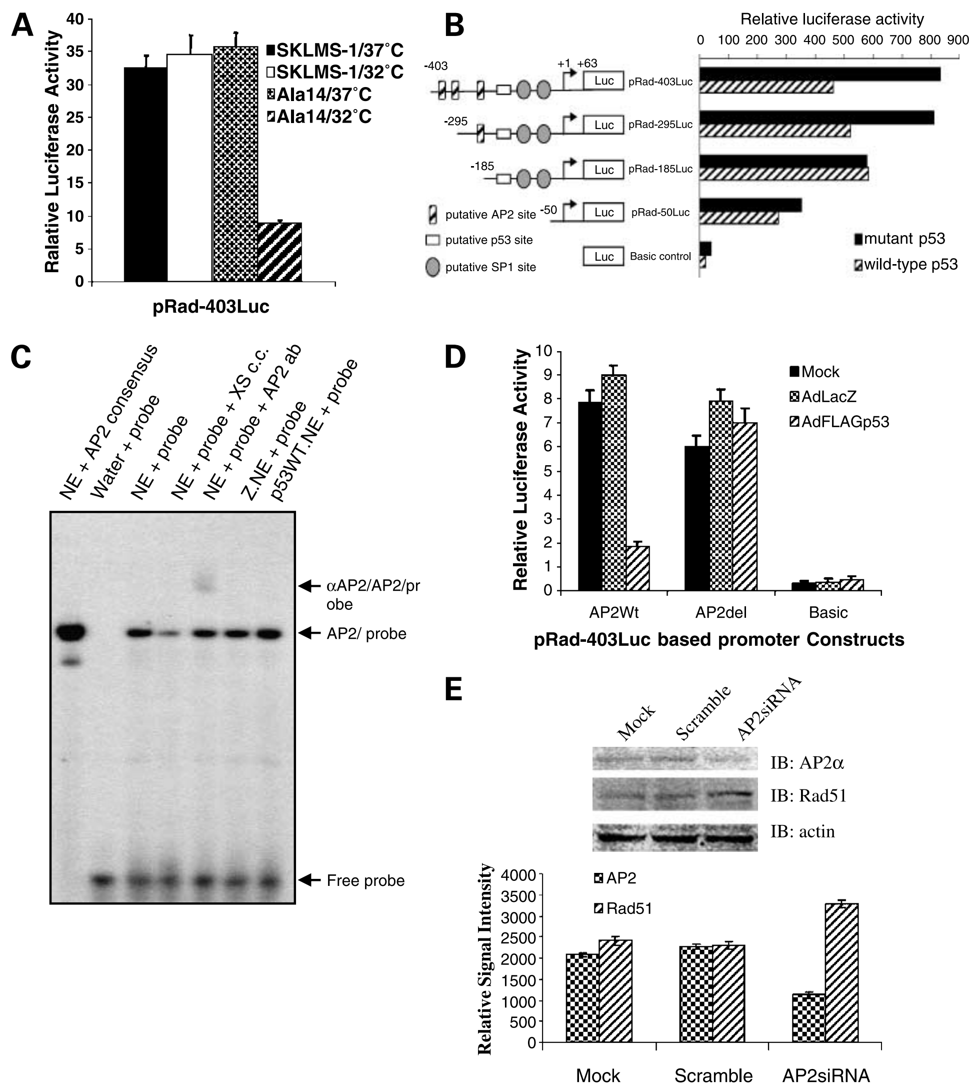

Figure 5. wtp53 induction leads to rad51 transcriptional repression through the −193- to −183-bp AP2 consensus binding site. **A,** induction of wtp53 function leads to suppression of rad51 promoter activity. SK-Ala-14 temperature-sensitive and SKLMS-1 parental cells were incubated at 37°C and 32°C for 72 h before cotransfection with pRad-403Luc and control plasmids. Luciferase activity was assayed 48 h later and normalized to internal control activity. Representative of three separate experiments. **B,** wtp53 induction exerts repression of rad51 transcriptional activity via a genetic element between −295 and −185 bp. SKLMS-1 cells pretreated for 48 h with mock or wtp53 expressing adenovirus were cotransfected with control and reporter plasmids for 48 h before assay for luciferase activity. Cartoon schematics of putative response elements present on each deletion construct (*left*). Representative of three separate experiments. **C,** electrophoretic mobility shift assay shows the ability of SKLMS-1 nuclear extracts (*NE*) to retard the migration of a radiolabeled AP2 commercial consensus site and a 30-bp probe of identical sequence to that spanning the intact −193 to −183 bp AP2 consensus binding site. Prior incubation of nuclear extracts with anti-AP2 antibodies results in supershift of the migrating protein-DNA probe. Result representative of three electrophoretic mobility shift assays. Furthermore, increased AP2 binding can be observed in the nuclear extracts containing wtp53 compared with those with mutp53. **D,** an intact −193- to −183-bp AP2 consensus binding site is required for wtp53 suppression of rad51 promoter activity. SKLMS-1 cells pretreated with indicated adenoviruses for 48 h were transfected with pRad-403Luc harboring an intact −193 to −183 AP2 consensus binding site (*AP2wt*) or harboring a 10-bp deletion of the binding site (*AP2del*). Luciferase activity was assayed 48 h later and normalized to internal control activity. Representative of three separate experiments. **E,** AP2α knockdown with AP2 siRNA results in increased Rad51 expression in SKLMS-1 cells; relative expression levels measured via densitometry (*bottom*).

binding site (data not shown). Furthermore, to validate that AP2 binding to the Rad51 promoter leads to transcriptional repression, we transiently transfected SKLMS1 cells with a specific *AP2* siRNA, leading to decrease in AP2 expression in these cells and a simultaneous increase in Rad51 expression (Fig. 5E). The increase in Rad51 after AP2 inhibition in SKLMS1 cells harboring mutp53 suggests that AP2 can also repress the *Rad51* promoter in SKLMS1 cells in a wtp53-independent manner. Taken together, these data suggest that induction of wtp53 transcriptional suppression of Rad51 requires an intact AP2 binding site at −193 to −183 bp in the *Rad51* promoter.

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

Discussion

This report is the first to suggest a role for the key DNA homologous repair protein Rad51 in STS. We show that Rad51 is overexpressed in a wide range of STS and possibly plays a role in doxorubicin chemoresistance. We have also shown that wtp53 can transcriptionally repress the *Rad51* promoter, resulting in decreased Rad51 mRNA and protein expression. Moreover, *Rad51* transcriptional regulation by wtp53 involves enhanced promoter repression induced by AP2.

Elevated Rad51 expression protects tumor cells from apoptosis and improves their survival by decreasing DNA damage (24). Additionally, there is evidence that tumor cell Rad51 overexpression may contribute to genomic instability by stimulating aberrant recombination between short repetitive elements and homologous sequences, leading to increased rates of chromosomal translocations as well as both abnormal gain and loss of chromosomal material (25). This finding may be specifically relevant in STS in that non-random chromosome translocations are found in more than a third of these tumors (26), a process we are currently studying.

Integrity of the genome mandates tight regulation of Rad51 (27). Several tumor suppressor gene products, such as ATM (28), BRCA2 (29), c-Abl (28), and p53 (22, 30), as well as oncogene products, such as Bcl-2 (31), and BCR/ABL (32), interact with Rad-51. We focused on p53 regulation of Rad51 because the p53 tumor suppressor pathway is commonly altered in STS and contributes to a spectrum of STS-promoting behaviors, such as loss of cell cycle control (18), enhanced angiogenesis (33), and STS invasion and metastasis (34), as we have shown. Even more pertinent to this study, we have shown that p53 mutation contributes to STS chemoresistance (3, 4), whereas reintroduction of wt*p53* into mutp53 STS cells results in significant doxorubicin chemosensitization via decreased P-glycoprotein drug efflux pump expression and phosphorylation. Here, we suggest that Rad51 might also play a role in p53-regulated STS chemoresistance.

p53 regulation of Rad51 has been previously studied; until recently, this was attributed to p53 physical association with Rad51 in a manner not requiring p53 transactivating capacities (22, 35). This protein/protein interaction leads to inhibition of the Rad51-mediated rate of homologous recombination as well as the homologous recombination machinery per se, such as Rad51 ATPase activity and polymerization into high-order nuclear filamentous structures (27, 36). In contrast, mutp53 seems to exacerbate Rad51 promotion of homologous recombination (37, 38) perhaps because the most common *p53* mutations affect the DNA binding domain, the region of wtp53 that associates with Rad51 (39).

Although these reports all show direct protein/protein binding non-transcriptional regulation of Rad51 homologous recombination function by p53, the possibility of additional regulatory mechanisms are not precluded by these findings. Recently, it has been shown by Arias-Lopez et al. that transcriptional regulation of *Rad51* by p53 also occurs (23). Working with Tet-Off-wtp53 inducible cell lines in a p53-null cell background, it was shown that p53 transcriptionally represses the *Rad51* promoter. wtp53 was found to specifically bind to a non-canonical p53-responsive element ~160 bp upstream of the transcription starting point of the Rad51 promoter. wtp53-mediated repression was abolished by deletion of the 42-bp promoter region containing the p53 response element. Moreover, repression was partially reduced by introducing specific point mutations into the p53 binding-site while preserving its palindromic structure. Collectively, this suggests that transcriptional repression of the *Rad51* gene by p53 may work cooperatively with non-transcriptional Rad51 regulatory mechanisms to increase genome fidelity. Our study reinforces and materially expands the above findings that wtp53 induces transcriptional repression of Rad51 in STS, resulting in decreased Rad51 mRNA and protein expression. However, we have additionally identified a novel mechanism of wtp53-induced Rad51 transcriptional repression that entails p53 and AP2 crosstalk. Our reporter deletion assay identified a region in the Rad51 promoter (−295 to −185) that lacks a p53 binding site but does have a functional AP2 binding site (−193 to −183) as shown by electrophoretic mobility shift assay. The importance of this AP2 binding site is suggested by our findings that wtp53 enhances the binding of AP2 to this site, and that selective deletion or specific mutations in this site lead to abrogation of the wtp53-mediated repression. Possible interactions between AP2 and wtp53 has been previously investigated, focusing on p53/AP2 protein/protein interactions in AP2 augmentation of p53-mediated transcriptional activation (40–42). The interaction between AP2 and p53 has been shown *in vitro* by yeast two-hybrid and glutathione *S*-transferase pull down and confirmed *in vivo* by coimmunoprecipitation and colocalization assays (43). Using a p53-responsive reporter assay, AP2 was shown to coactivate p53-mediated transcription and seemed to act through the p53 regulatory element (41). Our present study extends these previous observations by showing that in the context of the *Rad51* promoter, functional wtp53 can coactivate AP2. Thus, our findings support the possibility that the activity of AP2 at certain promoters is dependent upon the presence of functional p53. Although we show that AP2 binding to the *Rad51* promoter seems to be necessary for wtp53 repression of *Rad51*, our results do not contradict those of Arias-Lopez et al. (23) because it is entirely plausible that AP2 and p53 are cooperatively involved in promoter regulation through tandem regulatory elements. A similar transcriptional partnership has been recently reported for the regulation of the *KAI1* gene promoter (44).

Loss of AP2 expression is a common denominator in a wide variety of solid malignancies, correlating with tumor stage and patient outcome (45–47). Interestingly, two recent reports have suggested a role for AP2 loss in tumor chemoresistance. The reintroduction of AP2 into a variety of naturally low expressing AP2 cancer cells resulted in many-fold chemosensitivity increases in these cells to chemotherapeutic agents such as doxorubicin and

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

cisplatinum (42, 48). An association between the loss of AP2 and Rad51 expression should be further studied.

In conclusion, our results suggest a role for Rad51 in STS and further extend the previous findings of the complex p53/Rad51 regulatory process. This regulation not only involves non-transcriptional protein/protein interaction but also requires transcriptional repression of the *Rad51* promoter involving both wtp53 as well as AP2. An important clinical objective in STS management is to ameliorate chemoresistance; this might possibly be achieved by down-modulating Rad51-mediated homologous recombination and thus warrants further study. Therapeutic molecularly targeted strategies incorporating wtp53 restoration, AP2 activation, and possibly also direct Rad51 blockade, perhaps via siRNA, should be further studied in preclinical animal models.

References

1. Clark MA, Fisher C, Judson I, Thomas JM. Soft-tissue sarcomas in adults. N Engl J Med 2005;353:701 – 11.

2. Bramwell VH, Anderson D, Charette ML. Doxorubicin-based chemotherapy for the palliative treatment of adult patients with locally advanced or metastatic soft tissue sarcoma. Cochrane Database Syst Rev 2003:CD003293.

3. Zhan M, Yu D, Lang A, Li L, Pollock RE. Wild type p53 sensitizes soft tissue sarcoma cells to doxorubicin by down-regulating multidrug resistance-1 expression. Cancer 2001;92:1556 – 66.

4. Zhan M, Yu D, Liu J, Glazer RI, Hannay J, Pollock RE. Transcriptional repression of protein kinase Calpha via Sp1 by wild type p53 is involved in inhibition of multidrug resistance 1 P-glycoprotein phosphorylation. J Biol Chem 2005;280:4825 – 33.

5. Bennett CB, Lewis AL, Baldwin KK, Resnick MA. Lethality induced by a single site-specific double-strand break in a dispensable yeast plasmid. Proc Natl Acad Sci U S A 1993;90:5613 – 7.

6. Allen C, Miller CA, Nickoloff JA. The mutagenic potential of a single DNA double-strand break in a mammalian chromosome is not influenced by transcription. DNA Repair (Amst) 2003;2:1147 – 56.

7. Tsuzuki T, Fujii Y, Sakumi K, et al. Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. Proc Natl Acad Sci U S A 1996; 93:6236 – 40.

8. Lim DS, Hasty P. A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. Mol Cell Biol 1996;16:7133 – 43.

9. Raderschall E, Bazarov A, Cao J, et al. Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis. J Cell Sci 2002;115: 153 – 64.

10. Rapakko K, Heikkinen K, Karppinen SM, Winqvist R. Screening for RAD51 and BRCA2 BRC repeat mutations in breast and ovarian cancer families. Cancer Lett 2006;236:142 – 7.

11. Maacke H, Jost K, Opitz S, et al. DNA repair and recombination factor Rad51 is over-expressed in human pancreatic adenocarcinoma. Oncogene 2000;19:2791 – 5.

12. Thacker J. The RAD51 gene family, genetic instability and cancer. Cancer Lett 2005;219:125 – 35.

13. Raderschall E, Stout K, Freier S, Suckow V, Schweiger S, Haaf T. Elevated levels of Rad51 recombination protein in tumor cells. Cancer Res 2002;62:219 – 25.

14. Vispe S, Cazaux C, Lesca C, Defais M. Overexpression of Rad51 protein stimulates homologous recombination and increases resistance of mammalian cells to ionizing radiation. Nucleic Acids Res 1998;26: 2859 – 64.

15. Xu ZY, Loignon M, Han FY, Panasci L, Aloyz R. Xrcc3 induces cisplatin resistance by stimulation of Rad51-related recombinational repair, S-phase checkpoint activation, and reduced apoptosis. J Pharmacol Exp Ther 2005;314:495 – 505.

16. Taki T, Ohnishi T, Yamamoto A, et al. Antisense inhibition of the RAD51 enhances radiosensitivity. Biochem Biophys Res Commun 1996; 223:434 – 8.

17. Ohnishi T, Taki T, Hiraga S, Arita N, Morita T. *In vitro* and *in vivo* potentiation of radiosensitivity of malignant gliomas by antisense inhibition of the RAD51 gene. Biochem Biophys Res Commun 1998; 245:319 – 24.

18. Pollock R, Lang A, Ge T, Sun D, Tan M, Yu D. Wild-type p53 and a p53 temperature-sensitive mutant suppress human soft tissue sarcoma by enhancing cell cycle control. Clin Cancer Res 1998;4:1985 – 94.

19. Zhang W, Guo X, Hu G, Liu W, Shay J, Deisseroth A. A temperature-sensitive mutant of human p53. EMBO J 1994;13:2535 – 44.

20. Chen F, Nastasi A, Shen Z, Brenneman M, Crissman H, Chen DJ. Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. Mutat Res 1997;384: 205 – 11.

21. Lambert S, Lopez BS. Role of RAD51 in sister-chromatid exchanges in mammalian cells. Oncogene 2001;20:6627 – 31.

22. Linke SP, Sengupta S, Khabie N, et al. p53 interacts with hRAD51 and hRAD54, and directly modulates homologous recombination. Cancer Res 2003;63:2596 – 605.

23. Arias-Lopez C, Lazaro-Trueba I, Kerr P, et al. p53 modulates homologous recombination by transcriptional regulation of the RAD51 gene. EMBO Rep 2006;7:219 – 24.

24. Henning W, Sturzbecher HW. Homologous recombination and cell cycle checkpoints: Rad51 in tumour progression and therapy resistance. Toxicology 2003;193:91 – 109.

25. Richardson C, Stark JM, Ommundsen M, Jasin M. Rad51 over-expression promotes alternative double-strand break repair pathways and genome instability. Oncogene 2004;23:546 – 53.

26. Borden EC, Baker LH, Bell RS, et al. Soft tissue sarcomas of adults: state of the translational science. Clin Cancer Res 2003;9:1941 – 56.

27. Bertrand P, Saintigny Y, Lopez BS. p53's double life: transactivation-independent repression of homologous recombination. Trends Genet 2004;20:235 – 43.

28. Chen G, Yuan SS, Liu W, et al. Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. J Biol Chem 1999;274:12748 – 52.

29. Yang H, Li Q, Fan J, Holloman WK, Pavletich NP. The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction. Nature 2005;433:653 – 7.

30. Janz C, Wiesmuller L. Wild-type p53 inhibits replication-associated homologous recombination. Oncogene 2002;21:5929 – 33.

31. Saintigny Y, Dumay A, Lambert S, Lopez BS. A novel role for the Bcl-2 protein family: specific suppression of the RAD51 recombination pathway. EMBO J 2001;20:2596 – 607.

32. Slupianek A, Schmutte C, Tombline G, et al. BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. Mol Cell 2001;8: 795 – 806.

33. Zhang L, Yu D, Hu M, et al. Wild-type p53 suppresses angiogenesis in human leiomyosarcoma and synovial sarcoma by transcriptional suppression of vascular endothelial growth factor expression. Cancer Res 2000; 60:3655 – 61.

34. Pollock RE, Lang A, Luo J, El-Naggar AK, Yu D. Soft tissue sarcoma metastasis from clonal expansion of p53 mutated tumor cells. Oncogene 1996;12:2035 – 9.

35. Boehden GS, Akyuz N, Roemer K, Wiesmuller L. p53 mutated in the transactivation domain retains regulatory functions in homology-directed double-strand break repair. Oncogene 2003;22:4111 – 7.

36. Yun S, Lie ACC, Porter AC. Discriminatory suppression of homologous recombination by p53. Nucleic Acids Res 2004;32:6479 – 89.

37. Saintigny Y, Rouillard D, Chaput B, Soussi T, Lopez BS. Mutant p53 proteins stimulate spontaneous and radiation-induced intrachromosomal homologous recombination independently of the alteration of the transactivation activity and of the G1 checkpoint. Oncogene 1999;18: 3553 – 63.

38. Saintigny Y, Lopez BS. Homologous recombination induced by replication inhibition, is stimulated by expression of mutant p53. Oncogene 2002;21:488 – 92.

39. Friedler A, Veprintsev DB, Rutherford T, von Glos KI, Fersht AR.

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024

Binding of Rad51 and other peptide sequences to a promiscuous, highly electrostatic binding site in p53. J Biol Chem 2005;280:8051 – 9.

40. Stabach PR, Thiyagarajan MM, Woodfield GW, Weigel RJ. AP2alpha alters the transcriptional activity and stability of p53. Oncogene 2006;25: 2148 – 59.

41. McPherson LA, Loktev AV, Weigel RJ. Tumor suppressor activity of AP2alpha mediated through a direct interaction with p53. J Biol Chem 2002;277:45028 – 33.

42. Wajapeyee N, Somasundaram K. Cell cycle arrest and apoptosis induction by activator protein 2alpha (AP-2alpha) and the role of p53 and p21WAF1/CIP1 in AP-2alpha-mediated growth inhibition. J Biol Chem 2003;278:52093 – 101.

43. Modugno M, Tagliabue E, Ardini E, et al. p53-dependent downregulation of metastasis-associated laminin receptor. Oncogene 2002;21:7478 – 87.

44. Marreiros A, Dudgeon K, Dao V, et al. KAI1 promoter activity is dependent on p53, junB and AP2: evidence for a possible mechanism underlying loss of KAI1 expression in cancer cells. Oncogene 2005;24: 637 – 49.

45. Gershenwald JE, Sumner W, Calderone T, Wang Z, Huang S, Bar-Eli M. Dominant-negative transcription factor AP-2 augments SB-2 melanoma tumor growth *in vivo*. Oncogene 2001;20:3363 – 75.

46. Ruiz M, Pettaway C, Song R, Stoeltzing O, Ellis L, Bar-Eli M. Activator protein 2alpha inhibits tumorigenicity and represses vascular endothelial growth factor transcription in prostate cancer cells. Cancer Res 2004;64: 631 – 8.

47. Ropponen KM, Kellokoski JK, Pirinen RT, et al. Expression of transcription factor AP-2 in colorectal adenomas and adenocarcinomas; comparison of immunohistochemistry and *in situ* hybridisation. J Clin Pathol 2001;54:533 – 8.

48. Wajapeyee N, Raut CG, Somasundaram K. Activator protein 2alpha status determines the chemosensitivity of cancer cells: implications in cancer chemotherapy. Cancer Res 2005;65:8628 – 34.

Downloaded from http://aacrjournals.org/mct/article-pdf/6/5/1650/1877330/1650.pdf by guest on 09 October 2024