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Elevated Levels of Rad51 Recombination Protein in Tumor Cells[1]

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ABSTRACT

Rad51 is the key enzyme for homologous recombination, an evolutionarily conserved mechanism for the repair of DNA damage and the generation of genetic diversity. Given the observation that many tumors become resistant to radiation therapy and DNA-damaging chemotherapeutics and also that tumor cell populations can acquire a high number of genetic alterations and then expand clonally, dysfunction of the mammalian Rad51 recombinase could play a major role in the multistep process of tumorigenesis. The data we present provide further strong support for this hypothesis. Using anti-Rad51 immunofluorescence staining, widely different tumor cell lines displayed increased numbers of nuclei with focally concentrated Rad51 protein compared with nonmalignant control cell lines. These nuclear foci are thought to represent a repairosome-type assembly of Rad51 and other proteins required for recombinational DNA repair. By Western blot analyses, the net amount of Rad51 protein was increased 2–7-fold in all tested tumor cell lines. Inhibition of *de novo* protein synthesis by cycloheximide treatment showed a similar half-life of Rad51 protein in normal and tumor cells. Fluorescence *in situ* hybridization experiments did not detect *Rad51* gene amplifications in tumors. Because Northern blot analysis demonstrated highly elevated *Rad51* mRNA levels, we conclude that the increases in Rad51 protein and nuclear foci formation in tumor cells are the result of transcriptional up-regulation.

INTRODUCTION

Rad51, a structural and functional eukaryotic homologue of *Escherichia coli* RecA recombinase, is the key enzyme for DNA DSB[3] repair by homologous recombination. Similar to RecA, both yeast and mammalian Rad51 proteins form nucleoprotein filaments on ssDNA, mediating homologous pairing and strand exchange reactions between ssDNA and homologous double-stranded DNA (1–4). Mammalian Rad51 appears to function as part of a larger recombination complex that includes Rad52 (5) and Rad54 (6), which belong to the same epistasis group as Rad51, and replication protein A (7, 8).

Previously, it was thought that, in contrast to yeast, mammalian cells repaired DSBs primarily through nonhomologous end-joining of the broken ends (9). However, accumulating experimental evidence suggests that the homologous recombination pathway is equally important (10, 11). In particular, in highly replicating mammalian cell types, homologous recombination might play a predominant role for DSB repair (12). *Rad51*-deficient chicken lymphocytes in which a human *Rad51* transgene was inactivated showed increases in chromosome breaks and cell death (13), whereas *Rad51*-overexpressing mammalian cells showed an increased resistance to ionizing radiation (14) and DNA damage-induced apoptosis.[4] Whereas *Escherichia coli* RecA and yeast Rad51 mutants are viable, disruption of both *Rad51* alleles in the mouse led to early embryo lethality, and knockout cells displayed genomic instability, progressive chromosome loss, and cell death (15, 16). One possible explanation is gain of function of Rad51 protein in mammalian cell proliferation and/or genome metabolism.

Observations that mammalian Rad51 protein associates with the tumor suppressors Atm (17), p53 (18), Brca1 (19), and Brca2 (20); the c-Abl oncogene product (21); and the Blm helicase (22) suggest its probable role in tumor development. Aberrant overexpression of Rad51 protein could confer several advantages to tumor cells. First, the DNA repair function of Rad51 may protect cells from DNA damage and apoptosis. Secondly, overstimulation of homologous recombination and chromatid exchange mechanisms by Rad51 protein (23, 24) may contribute to genomic instability and genetic diversity of tumor cells. In this study, we demonstrate elevated levels of Rad51 protein in a variety of tumor cell lines.

MATERIALS AND METHODS

Cell Culture. Primary and SV40-transformed human fibroblasts, as well as most tumor cell lines used in this study (Table 1), were grown as monolayer cultures in DMEM (Life Technologies, Inc.) supplemented with 10% fetal bovine serum, antibiotics, and, when necessary, vitamins and various growth factors. EBV-transformed lymphoblasts and blood tumor cell lines were grown in suspension in supplemented RPMI 1640 (Life Technologies, Inc.). To induce apoptosis, etoposide (4 μg/ml) was added to the culture medium for 24 h. Etoposide interferes with topoisomerase II, an enzyme that binds covalently to double-stranded DNA, cleaves both strands, and reseals the cleaved complex. Etoposide hampers with this breakage and rejoining cycle by trapping the enzyme in the cleaved complex. Irreparable DSBs are then formed, and cell death follows (25). To block *de novo* protein synthesis, cycloheximide was added to the cultures for 8 and 24 h at a final concentration of 10 μg/ml (26).

Immunoblot Analysis. To compare the net amounts of Rad51 protein between various normal and tumor cell lines, cells were harvested during the logarithmic growth phase. From monolayer cultures, both cells attached to the culture flask and apoptotic cells in suspension were collected. Harvested cells were lysed in PBS [PBS = 136 mM NaCl, 2 mM KCl, 10.6 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.3)] containing 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, and protease inhibitor (Boehringer Mannheim) and disrupted with Qiagen shredder columns. Proteins were precipitated with acetone and resuspended in 25 μl of sample buffer consisting of 50 mM Tris-HCl (pH 6.8), 5% β-mercaptoethanol, 2% SDS, 10% glycerol, and 0.004% bromphenol blue.

Protein extracts were resolved by electrophoresis on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The resulting filters were blocked overnight with 5% nonfat dried milk, incubated with the appropriately diluted rabbit anti-Rad51 antiserum (27) for 1 h, incubated with horseradish peroxidase-conjugated antirabbit IgG (Dianova), and washed. Antibody binding was visualized by chemiluminescence (ECL RPN 2209; Amersham). Although equal amounts of total cellular protein were loaded per lane, all filters were reincubated with anti-β-actin antibody (Sigma Chemical Co.) to compare the protein levels in different cell substrates. The intensity of the Rad51 signals was equilibrated to the intensity of the β-actin signals using PCbas2.0 software.

Northern Blot Analysis. Total RNAs were isolated from tumor and control cell lines with the RNeasy midi kit (Qiagen) and subsequently used for mRNA isolation with Oligotex beads (Qiagen). Three μg of polyadenylated RNA were loaded per lane and separated on a 1% agarose gel containing 2.2 M formaldehyde. Radioactively labeled *Rad51* cDNA (GenBank accession number NM002875) was hybridized to the resulting filters in Quick-Hyb hybridization solution (Stratagene).

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[3] The abbreviations used are: DSB, double-strand break; ssDNA, single-stranded DNA; FISH, fluorescence *in situ* hybridization; YAC, yeast artificial chromosome.

[4] E. Raderschall, A. Bazarov, J. Cao, R. Lurz, A. Smith, W. Mann, H-H. Ropers, J. M. Sedivy, E. I. Golub, E. Fritz, and T. Haaf. Formation of higher-order nuclear Rad51 structures is functionally linked to p21 expression and protection from DNA damage-induced apoptosis, J. Cell Sci., in press.

Table 1 *Percentage of nuclei containing Rad51 foci in normal and tumor cell substrates*

At least 400 nuclei were analyzed for each experiment.

Cell line	Origin	Reference no.	% Nuclei with Rad51 foci		
			Total	Type I	Type II
PF1	Primary fibroblasts		12	11	1
PF2	"		11	10	1
PF3	"		10	10	0
TF1	SV40-transformed fibroblasts		10	9	1
TF2	"		6	6	0
L1	EBV-transformed lymphoblasts		9	7	2
EM2	Chronic myeloid leukemia	54	20	3	17
K562	"	55	52	42	10
HL60	"	56	45	32	13
Kasumi1	Acute myeloid leukemia	57	42	34	8
Jurkat	T-cell leukemia	58	52	52	0
Hut78	T-cell lymphoma	59	n.d.[a]	n.d.	n.d.
U937	Histiocytic lymphoma	60	n.d.	n.d.	n.d.
HeLa	Cervical carcinoma	61	33	31	2
Hey	Ovarian carcinoma	62	6	5	1
SkBr3	Breast carcinoma	63	14	10	4
BT20	"	64	18	13	5
MCF7	"	65	43	38	5
KB	"	66	67	65	2
Tera2	Testis carcinoma	67	24	23	1
LS180	Colon adenocarcinoma	68	7	7	0
SW480	"	64	13	13	0
SW837	Rectal adenocarcinoma	69	23	23	0
Mel2a	Melanoma	70	60	59	1
MeWo	"	71	44	36	8
Bro	"	72	27	20	7
GB	Glioblastoma	Primary tumor	41	39	2

[a] n.d., not determined.

Immunofluorescence Staining. Harvested cells were washed and resuspended in PBS. Aliquots (10^5 cells in 0.5 ml of PBS) were centrifuged onto clean glass slides using a Shandon Cytospin. Immediately after cytocentrifugation, the preparations were fixed in absolute methanol for 30 min at −20°C and then rinsed in ice-cold acetone for a few seconds. After three washes with PBS, the slides were incubated with blocking solution (3% BSA, 0.1% Tween 20, and 4× SSC) in a Coplin jar for 30 min and then incubated with rabbit anti-Rad51 antiserum (diluted 1:100 with PBS) in a humidified incubator for 30 min. The slides were then washed in PBS another three times for 10 min each and incubated for 30 min with FITC-conjugated antirabbit IgG appropriately diluted with PBS. After three additional washes with PBS, the preparations were counterstained with 1 μg/ml 4′,6-diamidino-2-phenylindole in 2× SSC for 1 min. The slides were mounted in 90% glycerol, 0.1 M Tris-HCl (pH 8.0), and 2.3% 1,4-diazobicyclo-2,2,2-octane.

Images were taken with a Zeiss epifluorescence microscope equipped with a thermoelectronically cooled charge-coupled device camera (Photometrics CH250), which was controlled by an Apple Macintosh computer. Gray scale images were pseudocolored and merged using Vysis and Adobe Photoshop software.

FISH. Chromosomes were prepared from normal and tumor cell lines according to standard procedures. Degenerate oligonucleotide-primed PCR products of flow-sorted chromosomes 15 were used as a chromosome-specific painting probe (28). We have selected several Centre d'Etude du Polymorphisme Humain YACs from chromosome 15q14–15 (29) to find a *Rad51*-containing clone and then determine the copy number of *Rad51* genes in tumor cell lines by FISH. By Southern blot hybridization with *Rad51* cDNA, the 850-kb YAC 844h4 (WI-8897 and WI-9264) was confirmed to contain the *Rad51* gene. DNA probes were labeled with either biotin-16-dUTP or digoxigenin-11-dUTP by standard nick translation.

Before *in situ* hybridization, the slides were treated with 100 μg/ml RNase A in 2× SSC (pH 7.0) at 37°C for 30 min and with 0.01% pepsin in 10 mM HCl at 37°C for 10 min. After refixing the preparations for 10 min in 1× PBS, 50 mM $MgCl_2$, and 1% formaldehyde, they were dehydrated in an ethanol series (70%, 80%, and 100%). Slides were denatured for 1 min at 90°C in 70% formamide and 2× SSC (pH 7.0) and dehydrated again in an alcohol series. For hybridization of one slide, 10–20 ng/μl each of digoxigenated YAC DNA and biotinylated PCR product of the chromosome 15 library were coprecipitated with 100 ng/μl human cot-1 competitor DNA (Life Technologies, Inc.) and 500 ng/μl salmon sperm carrier DNA and redissolved in 50% formamide, 10% dextran sulfate, and 2× SSC. The hybridization mixture was denatured for 10 min at 80°C. Preannealing of repetitive (cot-1) DNA sequences was carried out for 30 min at 37°C, and the hybridization mixture was applied to each slide and sealed under a coverslip. The slides were hybridized overnight in a moist chamber at 37°C and then washed three times for 5 min in 50% formamide and 2× SSC at 42°C and washed once for 5 min in 0.1× SSC (pH 7.0) at 60°C and blocked with 4× SSC, 3% BSA, and 0.1% Tween 20 at 37°C for 30 min. Probes were detected with FITC-avidin (Vector Laboratories) and Cy3-conjugated antidigoxin antibodies (Dianova).

RESULTS

Increased Numbers of Nuclear Rad51 Foci in Tumor Cells. Previously, we have shown that in normal human cell cultures, Rad51 protein is detected in multiple discrete foci in the nucleoplasm of a small number of cells. After DNA damage, the percentage of cells with focally concentrated Rad51 protein increased in a time- and dose-dependent manner (27, 30). Circumstantial evidence suggests that Rad51 recruitment takes place from endogenous Rad protein dispersed throughout the nucleus to damaged chromatin in S-G_2 phase (31–33). In contrast, stably transfected cells that constitutively overexpress Rad51 protein form nuclear foci in the absence of DNA damage,[4] most likely by self-interacting Rad51 molecules (34, 35). The overexpressed Rad51 protein confers resistance to DNA damage-induced apoptosis. In addition, Rad51 foci were observed in meiotic prophase cells (36, 37), which undergo high levels of genetic recombination, and in splenic B cells (38), which are activated for antibody class switch recombination. Collectively, these observations suggest that Rad51 foci are associated with DNA repair and/or stimulation of homologous recombination.

Given the hypothesis that increased recombinational DNA repair is advantageous for tumor cells, formation of repairosome-type Rad51 foci might also occur during tumorigenesis. Here we have determined the number of Rad51 foci in widely different tumor cell lines, including leukemias, carcinomas, melanomas, and glioblastoma, by anti-Rad51 immunofluorescence. Compared with nonmalignant cell sub-

strates (lymphoblasts and primary and transformed fibroblasts), most tumor cell lines exhibited an increased percentage of nuclei with Rad51 foci (Table 1). Type I nuclei showed many (>10) discrete foci scattered throughout the entire nuclear volume (Fig. 1*A*), whereas type II nuclei contained clusters of brightly fluorescing foci that were highly enriched with Rad51 protein (Fig. 1*B*). In particular, the type II foci were very rare in nonmalignant cell substrates.

Elevated Rad51 Protein Levels in Tumor Cells. The observed accumulation of Rad51 protein in nuclear foci could be due to a net increase in Rad51 protein in tumor cells or to a relocalization of dispersed Rad51 protein to nuclear foci. To determine the Rad51 protein levels in tumor *versus* normal cells, we have performed quantitative Western blot experiments. In primary fibroblasts, anti-Rad51 antibodies detected one predominant band at 39 kDa, which corresponds to the full-length Rad51,[4] and another much weaker band at approximately 37 kDa (Fig. 2). After induction of DNA damage by etoposide treatment (4 μg/ml for 24 h), the intensity of the 39-kDa band and, consequently, the net amount of total Rad51 protein drastically decreased. This demonstrates that formation of DNA damage-induced Rad51 foci is due to redistribution of the protein (27, 33), but not to *de novo* protein synthesis. In contrast, the intensity of the faster-migrating 37-kDa band remained unchanged or slightly increased after etoposide treatment of primary cells. It has been speculated that the lower band is a product of proteolytic cleavage during apoptosis (39). Cleavage of Rad51 by caspase 3 after DNA damage results in loss of its recombinase activity (40).

By Western blotting, there was a 2–7-fold increase in full-length Rad51 protein in all tumor cell lines compared with primary (PF1 and PF2) cells (Fig. 3). Because of the very prominent 39-kDa band, the lower 37-kDa band was hardly visible. The observation that the amount of the proteolytic Rad51 cleavage product did not increase argues against the notion that the elevated Rad51 protein levels are the result of high levels of DNA damage in tumor cells. After Rad51 protein staining, the same filters were reincubated with anti-β-actin antibody. The β-actin signals were used to equilibrate slightly different amounts of cell extract loaded per lane. To determine the half-lives of Rad51 protein in primary (PF1) fibroblasts and representative tumor (SkBr3 and Mel2a) cells, cultures were treated with cycloheximide for 8 and 24 h (Fig. 4). In the absence of *de novo* protein synthesis, the Rad51 protein level decreased approximately 30% in 1 day. There was no detectable difference in protein stability between primary and tumor cells.

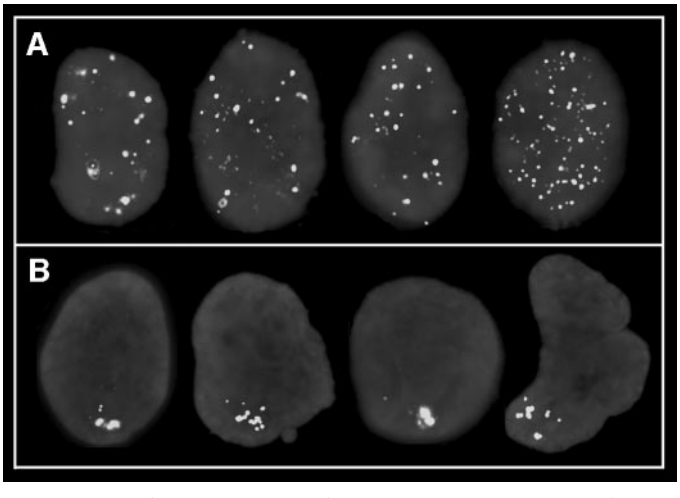

Fig. 1. Immunofluorescence staining of Rad51 protein in Mel2a (*A*; type I foci) and EM2 (*B*; type II foci) tumor cells, representing the two different Rad51 foci types.

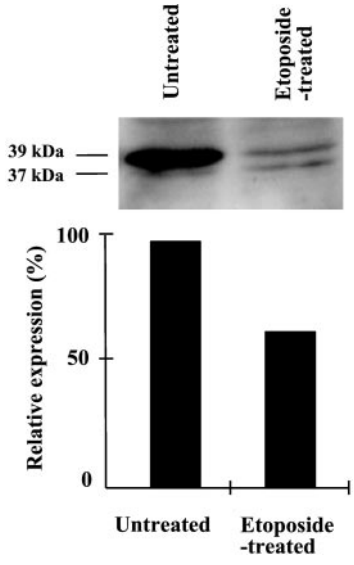

Fig. 2. Immunoblot analysis of Rad51 protein in total cell extracts from untreated and etoposide-treated primary (PF1) fibroblasts. The full-length (39-kDa) Rad51 protein band predominates in cells without DNA damage.

The *Rad51* Gene Is Not Amplified in Tumor Cells. To test whether *Rad51* gene amplification causes up-regulation of Rad51 protein, we have visualized the *Rad51* locus in eight representative tumor cell lines (Table 2) by metaphase FISH. YAC 884h4 containing the *Rad51* gene produced a discrete and specific hybridization signal on chromosome 15q14–15 (Fig. 5, *red signals*), but not on any other chromosomal region. To facilitate the detection of numerical and structural aberrations involving chromosome 15, we have simultaneously painted the target chromosome 15 with a chromosome-specific DNA library (Fig. 5, *green*). Although some of the eight tumor cell lines analyzed showed chromosome 15 rearrangements, the critical region 15q14–15 was not disrupted by translocation or inversion (data not shown). In addition, none of the tumors showed a cytologically detectable amplification of the 15q14–15 region. The copy number of YAC signals/haploid tumor genome ranged from 0.6–1.5 (mean = 0.94; SD = 0.26). In fact, only one cell line, SkBr3, had more than one copy of the YAC/haploid genome. The intensity of YAC signals was comparable in normal and tumor metaphase spreads. Thus, gene amplification does not seem to contribute to the elevated Rad51 protein levels in tumor cells.

Increased *Rad51* mRNA Expression in Tumor Cells. Northern blot hybridization was used to determine the *Rad51* mRNA levels in three tumor cell lines (SkBr3, Mel2a, and HL60) of different tissue origin and primary (PF1) fibroblasts. Only one mRNA species (of approximately 1.3 kb) hybridized with *Rad51* cDNA in all tested cell substrates (Fig. 6). Compared with the control, all three tested tumors showed a dramatically (6–11-fold) increased *Rad51* expression. Hybridization with a control *glyceraldehyde-3-phosphate dehydrogenase* cDNA produced a band of equal intensity in both normal and tumor cells. Thus, up-regulation of the *Rad51* gene occurs at the transcriptional level in tumors.

DISCUSSION

Insights into the mechanisms underlying the development of radiation/chemotherapy resistance and the increased chromosome mutation rate could help to improve diagnosis and treatment of tumors. Because the Rad51 recombinase is critical to DSB repair and the generation of genetic diversity in higher eukaryotes, it might also contribute to the development of cancer. The potential functional

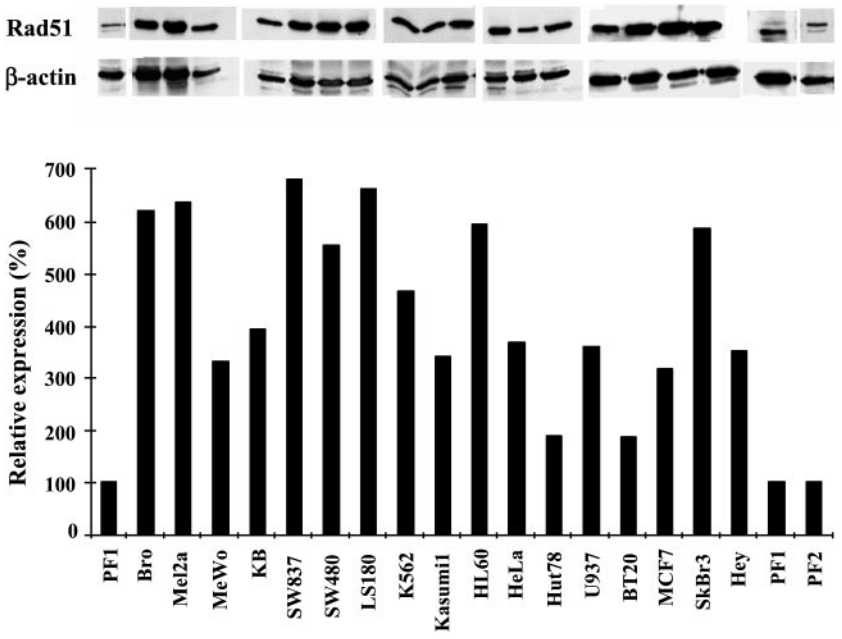

Fig. 3. Up-regulation of Rad51 protein in tumor cells. Equal amounts of total cellular protein from exponentially growing tumor cell lines and controls (PF1 and PF2) were separated by electrophoresis and subjected sequentially to immunoblot analysis with antibodies to Rad51 and β-actin. Antibody binding was quantified by densitometric analysis. The β-actin signal was used to equilibrate slightly different amounts of cell extract loaded per lane. The amount of protein in fibroblast cells was chosen as a reference (100%).

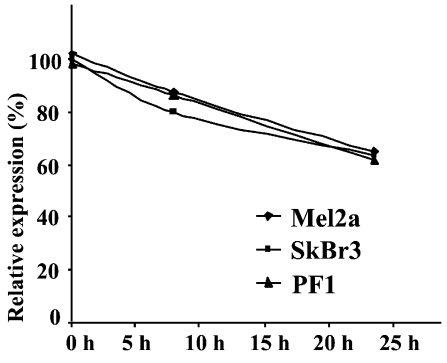

Fig. 4. Relative expression of full-length Rad51 protein in primary (PF1) fibroblasts and tumor (Mel2a and SkBr3) cells after treatment with 10 μg/ml cycloheximide for 8 and 24 h. For each cell line, the amount of protein in untreated cells was chosen as a reference (100%). The absolute amount of protein differed between primary and tumor cells (see Fig. 3).

Table 2 *Number of Rad51 loci/haploid genome*

At least 20 metaphases were analyzed per cell line.

Cell line	Chromosomes/ metaphase	*Rad51*-YAC signals/ metaphase	*Rad51* genes/haploid genome[a]
K562	65	1.8	0.6
Hey	46	1.5	0.8
SkBr3	76	5.1	1.5
KB	70	2.9	1.0
LS180	50	2.2	1.0
Mel2a	60	2.4	0.9
MeWo	50	2.0	0.9
Bro	55	1.8	0.8

[a] Number of YAC signals/23 chromosomes.

association of mammalian Rad51 protein with tumor suppressors, including Atm (17), p53 (18), Brca1 (19), and Brca2 (20), has led to the proposal that Rad51 also may be a tumor suppressor. Interestingly, in addition to their colocalization with Rad51 to nuclear foci, the breast cancer predisposition gene products Brca1 and Brca2 may themselves participate in the repair of DNA damage. Cells with defective *Brca1* or *Brca2* alleles displayed radiation hypersensitivity and an increased number of chromosome breaks (20, 41–43). However, the fact that the *Rad51* gene is not targeted by mutations in tumors (44, 45) does not support a role for Rad51 as a tumor suppressor. Interaction of the c-Abl oncogene product with Rad51 may be required for the correct posttranslational modification of Rad51 and the assembly of DNA repair protein complexes (17, 21).

Our results demonstrate increased levels of Rad51 protein and increased numbers of cells with nuclear Rad51 foci in a wide variety of tumor cell lines. Two conceptually related studies (46, 47) found that wild-type Rad51 protein is up-regulated in pancreatic adenocar-

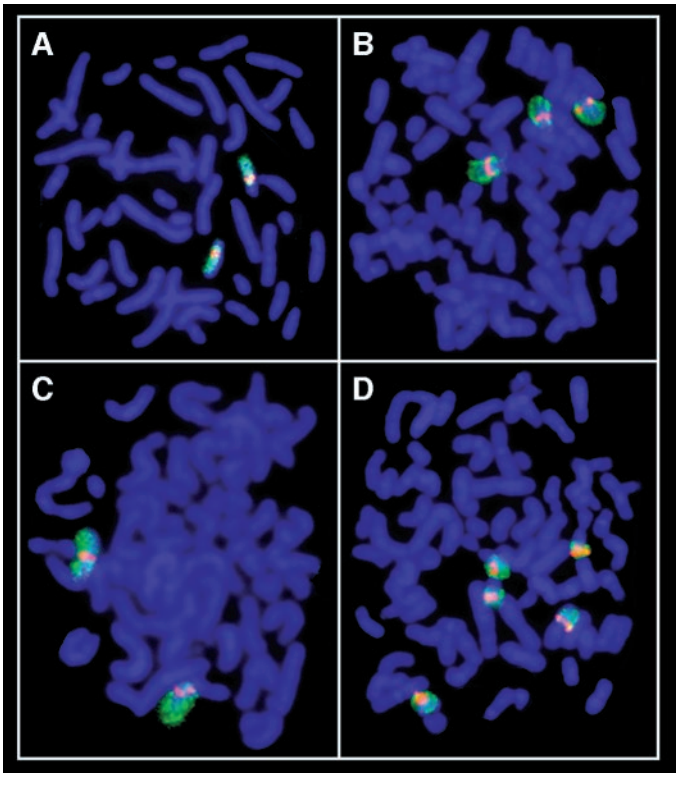

Fig. 5. FISH with a *Rad51*-containing YAC (*red Cy3 signals*) and a chromosome 15-specific DNA library (*green FITC fluorescence*) on normal PF1 (*A*) and representative LS180 (*B*), MeWo (*C*), and SkBr3 (*D*) tumor metaphase spreads. Chromosomes are counterstained with 4′,6-diamidino-2-phenylindole (*blue*).

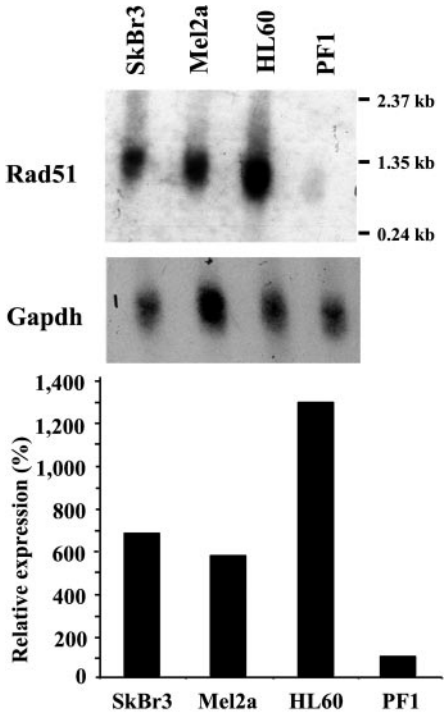

Fig. 6. Northern blot with RNAs from primary (PF1) fibroblasts and tumor (SkBr3, Mel2a, and HL60) cells hybridized with *Rad51* cDNA. As a control, the same blot was rehybridized with *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*). The *Rad51* mRNA levels are increased by 6–11-fold in tumor cells compared with the control.

cinoma and breast cancer and that overexpression correlates with histological grading. Although both Rad51 expression (48) and foci formation (19, 31, 32) appear to be higher in S and G_2 than in G_1 phase, cell cycle differences between cell lines, *i.e.,* the somewhat prolonged G_1 phase of primary cells compared with that of rapidly cycling tumor cells, cannot explain the up to 11-fold increase in *Rad51* mRNA and the up to 7-fold elevated Rad51 protein levels in tumors. Both primary and transformed cell substrates were used as controls and consistently showed low percentages of nuclei with Rad51 foci ($9.7 \pm 2.1\%$) compared with tumor lines ($33.2 \pm 18.1\%$). Secondly, all experiments were performed on exponentially growing cell cultures with a maximum number of S and G_2 phase cells.

We have previously shown (33) that after induction of DNA damage and during meiotic prophase, nuclear Rad51 foci are formed at sites of ssDNA. Because most models of recombination and repair involve a ssDNA intermediate, DNA damage-induced and meiotic Rad51 foci are thought to represent active sites of recombinational DNA repair. Consistent with this hypothesis, Rad52 (5), Rad54 (6), replication protein A (7, 8), Xrcc2 (49), and Xrcc3 (50) all of which facilitate Rad51-mediated homologous recombination, are also highly enriched in these nuclear Rad51 foci types. However, in tumors, mechanisms other than induction of the DNA damage response pathway by elevated DSB levels are very likely to be at work. In normal cells, the formation of Rad51 foci after γ-irradiation or etoposide treatment is not associated with increased Rad51 protein levels (27, 33), suggesting posttranslational modification. In contrast to DNA damage-induced redistribution of Rad51 to ssDNA (32, 33), fibroblasts stably infected with the human *Rad51* gene exhibited a high number of nuclei with Rad51 foci even in the absence of DNA damage.[4] In fact, a 2-fold increased Rad51 protein level was already sufficient to induce foci formation by self-interaction of overexpressed Rad51 molecules. Similar to the situation in stably transfected cell lines, the constitutive overproduction of Rad51 in tumors can be expected to destroy the balance between different components of the DNA repair system. Tumor suppressor proteins such as p53 (18) and Brca2 (20), which interact with Rad51 directly, are thought to keep Rad51 in an inactive monomeric state. When Rad51 molecules are overexpressed and/or tumor suppressors become functionally inactivated in tumor cells, Rad51 may form multimeric complexes because of potentially limiting concentrations of interacting tumor suppressor proteins. Although the Rad51 protein steady-state level and Rad51 foci formation are interrelated, they may be regulated in different ways and may not exhibit a simple linear relationship. Foci formation after DNA damage occurs despite decreased amounts of full-length Rad51 protein. On the other hand, the dramatically increased Rad51 protein levels in some tumor cell lines (*i.e.,* Hey and LS180) were not associated with increases in the number of Rad51 foci-positive cells. Defects in *Rad54* (6), *Xrcc2* (49), *Xrcc3* (50), or other genes required for assembly and stabilization of multimeric Rad51 protein complexes may account for this defect in Rad51 foci formation.

Cycloheximide treatment for 24 h led to a comparable (relative) decrease in full-length Rad51 protein in normal and tumor cells. Thus, elevated protein levels are not likely to be due to a prolonged half-life of Rad51 in tumor cells. In contrast, the highly elevated *Rad51* mRNA levels in Northern blots suggest that the *Rad51* gene is transcriptionally up-regulated in tumor cells. By FISH we have excluded the possibility that *Rad51* gene amplifications and gross chromosomal changes involving the *Rad51*-containing region 15q14–15 account for the increased *Rad51* expression. Interestingly, the *Rad51* mRNA levels appeared to be even more increased than the Rad51 protein levels, which is consistent with a tight regulation of Rad51 not only on the transcriptional level but also on the protein level.

The increased amounts of full-length Rad51 protein in tumor cells and possible interactions with other proteins and/or ssDNA result in nuclear Rad51 foci. Similar to the overexpressed Rad51 protein in stably infected cell lines,[4] the increased amount of Rad51 protein may protect the tumor cells from undergoing apoptosis in response to DNA damage. In addition, increased Rad51 protein levels may be associated with enhanced recombination and genomic instability. The generality of Rad51 up-regulation and foci formation in widely different tumors suggests that this is not only a secondary phenomenon but is implicated in malignant transformation and/or tumor progression. Interestingly, and in contrast to constitutive overexpression of Rad51 in stably transfected cell lines and tumors, abrupt Rad51 overexpression under the control of a repressible promoter resulted in a decreased growth rate and an increased apoptotic rate (51). These cytotoxic and cytokinetic effects of acute Rad51 overexpression are thought to protect multicellular organisms against hyperrecombination and genomic instability. Evidently, tumor cells become adapted to increased Rad51 protein levels during clonal selection and tumor evolution. However, additional studies will be required to determine at which point during the multistage process of tumorigenesis Rad51 up-regulation occurs and to understand its clinical significance. Our findings have possible diagnostic and therapeutic applications. Firstly, Rad51 could serve as a diagnostic/prognostic marker to improve tumor classification. More importantly, down-regulation of Rad51 protein by *Rad51* antisense oligonucleotides (52, 53) or Rad51-inhibitory drugs could be used to sensitize tumors to radiation or chemotherapy.

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