

# **RAD51, genomic stability, and tumorigenesis**

**Christine Richardson**

\*Corresponding author:  
Christine Richardson, Ph.D.  
Department of Pathology,  
Institute for Cancer Genetics,  
College of Physicians and Surgeons, Columbia University,  
1150 St. Nicholas Ave., New York, NY 10032

Phone: 212-851-5277  
Fax: 212-851-5267  
E-mail: [car10@columbia.edu](mailto:car10@columbia.edu)

## **Abstract**

Genomic instability is characteristic of malignant cells, and a strong correlation exists between abnormal karyotype and tumorigenicity. Increased expression of the homologous recombination and DNA repair protein Rad51 has been reported in immortalized cell lines and multiple primary tumor cell types which could alter recombination pathways to contribute to the chromosomal rearrangements found in these cells. In addition, Rad51 participates in a complex network of interactions that includes DNA damage sensors, tumor suppressors, and cell cycle and apoptotic regulators, and mutation of many of these proteins have also been associated with tumor initiation or progression. Insights into the connection between disregulated Rad51 and malignant phenotype indicate that Rad51 is a potential target for new anti-cancer regimens including those that use siRNA technology.

Key words: Rad51, DNA repair, Recombination, Genomic stability, Tumorigenesis

## **1. Introduction**

The faithful repair of DNA damage such as chromosomal double-strand breaks (DSBs) is necessary for the maintenance of genome integrity. DSBs can result from exposure to DNA damaging agents including irradiation, alkylating agents, and topoisomerase II inhibitors, as well as during normal metabolic pathways including DNA replication and antigen receptor rearrangement in developing lymphoid cells. Illegitimate repair of DSBs is expected to result in chromosomal instability and rearrangements including translocations, deletions, duplications, and inversions, that are characteristic of immortalized and malignant cells [1,2]. In support of this, altered function of proteins involved in sensing DNA damage, DSB repair, and HR promotes or stabilizes rearrangements events that lead to malignancy [3-6].

Central to DSB repair by homologous recombination (HR) is Rad51. Rad51 promotes strand invasion and homologous pairing between two DNA duplexes [7-9]. The observation that multiple tumor cell types contain elevated amounts of Rad51 suggests a role in either the initiation or in the progression of tumorigenesis. As a consequence, extensive effort has been devoted to determining the significance of this initial observation. It is now clear that in addition

to its direct role in the HR biochemical reaction, Rad51 participates in an complex network of damage-sensing and cell cycle checkpoint signaling pathways (Figure 1). This cellular cross-talk ensures that appropriate cell cycle checkpoints are initiated to allow for initial pairing and interaction with an appropriate repair template, and the final resolution of repaired products prior to cell division. However, determining the significance of altered Rad51 that results from any spectrum of mutations in malignant cells is complicated by these interactions. Regardless of the direct role of cellular Rad51 protein levels to the etiology of tumorigenesis, multiple assays have demonstrated that dysregulated Rad51 leads to resistance to irradiation or cytotoxic agents, impaired protein interactions, altered fidelity of HR-mediated DSB repair, and gross chromosomal aberrations.

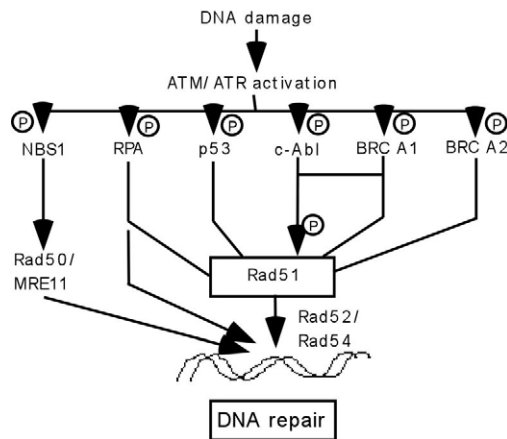


Figure 1. Network of cellular signaling to Rad51 in response to DNA DSBs. Direct interactions and phosphorylation events lead to activation of Rad51 and formation of nucleo-protein filaments on DNA to stimulate HR.

## 2. Mechanisms of DSB repair and genome rearrangements

The potentially tumorigenic consequence of illegitimate DNA repair events is inherently linked to the mechanism used for repair. At least two distinct processes contribute to repair of DSBs--non-homologous end-joining (NHEJ) and homologous recombination (HR) (for detailed discussion of DSB repair mechanisms see [1,10,11]). In somatic cells HR-mediated DSB repair is best demonstrated by gene conversion that uses a homologous DNA duplex template to prime DNA synthesis across the damaged region [12-14]. The homologous template preferentially is on a sister chromatid [15], but may be on a homolog [16,17], or non-allelic repeat sequence on a heterologous chromosome [14,18]. As a consequence, chromosome rearrangements resulting from recombination between repetitive elements are associated with several genetic diseases and tumorigenesis [19,20].

### 2.1 Homologous recombination: the RAD52 epistasis group

The RAD52 epistasis group members RAD50, RAD51, RAD52, RAD54, RAD54B, MRE11, and NBS1 participate in HR-mediated DSB repair in mitotic cells [11]. In addition, five human Rad51 paralogs encoded by XRCC2, XRCC3, RAD51B, RAD51C, and RAD51D genes were identified on the basis of sequence identity [10]. Although two independent complexes of Rad51C-XRCC3 and Rad51B-C-D-XRCC2 have been isolated, their precise roles have yet to be defined [21-23]. It has been proposed that these are accessory proteins that assist in the loading

of Rad51 onto single-stranded DNA. These proteins are expressed in multiple tissue types, but with higher levels seen in the testis and proliferating cells. The protein–protein interactions among various members of the group suggest two different complexes involved in DSB-induced recombination, the first involved in presynaptic functions including processing of the DSB ends, and the second involved in synaptic functions for invasion, creation of repair intermediates, and resolution. This second complex has been termed a recombinosome but has not yet been isolated.

## 2.2 *Homologous recombination: Central role of Rad51*

Rad51, the homolog of the *E. coli* RecA protein, is central to HR-mediated DSB repair. Rad51 forms nucleo-protein filaments on single-stranded DNA and mediates homologous pairing and strand exchange between DNA duplexes [7-9]. Rad51 is expressed in proliferating cells with the highest expression in S or S/G2 phase of the cell cycle [24-26]. Not surprisingly, Rad51 is also involved in repair of DNA breaks that arise during replication [27]. Rad51 activity during S phase and activation following exposure to a DNA damaging agent is often characterized by relocalization of diffuse Rad51 protein into discrete foci in the nucleus. The central role of Rad51 and HR in genomic integrity is strongly supported by mouse knockouts of Rad51 which result in early embryonic lethality [28,29], and the inducible loss of Rad51 expression in a DT40 chicken cell line that leads to chromosome breaks, aberrations, and cell lethality [30]. By contrast, moderate increases (2-6 fold) in Rad51 protein levels are tolerated for viability but lead to altered efficacy of HR mechanisms and genomic instability.

## 3. **Rad51 interactions with DNA damage sensors and signaling proteins**

In addition to its centrality to the HR reaction, Rad51 associates with multiple DNA damage sensors and recombination proteins, tumor suppressors, and cell cycle and apoptotic regulators [31-35], likely in multiple dynamic complexes dependent on the type of DNA damage, substrate, and cell cycle phase [36] (Table 1; Figure 1). However, it is still unclear which Rad51-containing complexes form in response to specific damage or cell cycle signals, and the effect of altered Rad51 protein levels on the relative abundance of them. Interactions have been defined by either nuclear co-localization of discrete foci following DNA damage, or by biochemical interactions (Table 1). The exact role of Rad51-containing nuclear foci is yet to be clearly defined, although there is strong evidence that they are markers of broken DNA ends and the process of repair [37,38].

The breadth of interactions so far reported indicates several important points. First, they underscore the importance of Rad51-mediated HR in the normal mitotic cell. Second, they indicate the complex control network to ensure proper activation and regulation of Rad51 to repair multiple types of DNA lesions, and to limit illegitimate repair. In addition to transcriptional regulation of the RAD51 gene, Rad51 protein levels are regulated via multiple post-translational mechanisms including the formation of active monomer structures, cellular localization, phosphorylation, SUMOylation, ubiquitination, cleavage and degradation [39]. Third, the large number of Rad51 interactions provides one clue to why aberrant amounts of Rad51 protein have been observed in a number of transformed cell types that contain mutations in tumor suppressors or oncogenes, thus leading to dysregulation of Rad51.

Table 1. Rad51 associates with multiple DNA damage sensors and recombination proteins, tumor suppressors, and cell cycle and apoptotic regulators, likely in multiple dynamic complexes dependent on the type of DNA damage, substrate, and cell cycle phase. Interactions have been defined by (1) co-localization of discrete nuclear foci following DNA damage or during S phase; or (2) biochemical interaction.

Table 1. Multiple Rad51 interacting proteins.

Protein	Characterization	nuclear foci	biochemical interaction
<b><i>Rad52 Epistasis Group and HR accessory proteins</i></b>			
Rad51B	Rad51 paralog Rad51B-C-D-XRCC2 complex	+	indirectly
Rad51C	Rad51 paralog Rad51C-XRCC3 complex	+	+
Rad51D	Rad51 paralog Rad51B-C-D-XRCC2 complex	+	indirectly
XRCC2	Rad51 paralog Rad51B-C-D-XRCC2 complex	+	indirectly
XRCC3	Rad51 paralog Rad51C-XRCC3 complex	+	+
Mre11	Recombination/Repair Nuclease; Telomere maintenance	+	indirectly
Rad50	Recombination/Repair Enhance Mre11 activity Telomere maintenance	+	indirectly
Nbs1	Recombination/Repair Enhance Mre11 activity Nijmegen breakage syndrome	+	indirectly
Rad52	Recombination/Repair Stimulate Rad51-mediated pairing Single-strand annealing	+	+
Rad54	Recombination/Repair ATP-dependent ATPase Stabilize Rad51-mediated pairing and heteroduplex; SCE	+	+
RPA	ssDNA binding protein	+	+
<b><i>Damage sensors and signaling proteins</i></b>			
p53	Tumor suppressor Li Fraumeni syndrome	+	+
BRCA2	Tumor suppressor Hereditary breast cancer Assist in localization and loading of Rad51 onto DNA	+	+
ATM	Tumor suppressor Ser/Thr kinase; cell cycle checkpoints Telomere maintenance Ataxia telangiectasia		+
c-Abl	Tyrosine kinase		+
FANCD2	Tumor suppressor; Fanconi anemia	+	
<b><i>Other interactions</i></b>			
PLM	Promyelocytic leukemia protein	+	
RNA Pol II	Transcription	+	
Ubc9	SUMOylation		+
Caspase-3	Apoptotic cleavage		+
TGFB1	Signal transduction		indirectly

### 3.1 *Interaction with p53*

The tumor suppressor p53 acts as both caretaker and guardian of the genome [3]. It is mutated in almost 50% of human cancers. In addition to well known functions of p53 in activation of cell cycle checkpoints and modulation of the apoptotic response, recent studies suggest a more direct role of p53 in the process of DNA repair itself. p53 directly binds to both Rad51 and RecA [33,35]. Several genetic assays have demonstrated that p53 can down-regulate Rad51-mediated HR events. How p53 affects HR-mediated repair still remains unclear. Recent biochemical data indicate that p53 inhibits Rad51 strand exchange and replication fork regression reactions [40]. Although several mutant forms of p53 are not capable of this inhibition, it is possible that p53 does not directly inhibit the activity of Rad51 but rather competes for binding to a specific DNA structure. In support of this, p53 can bind to stalled regressed replication forks in the absence of Rad51. Further, ATM and p53 localize to RAG protein-induced DNA breaks, and p53 associates with Mre11-Rad50-Nbs1 complexes, and both of these interactions are associated with the earliest steps of processing of DNA damaged ends prior to their repair [41,42]. Regardless of the exact mechanism, p53 appears to interact at the sites of DNA damage to either enhance cell cycle checkpoint action until appropriate repair is complete, or to block promiscuous HR events in somatic cells that may promote increased genomic instability. This function of p53 provides a rationale for the increased genome instability observed in multiple solid tumors that carry a mutant p53 or in tumors that exhibit increased levels of Rad51 that may abrogate p53-mediated arrest or apoptosis of cells with DNA damage. Conversely, it would explain the lack of multiple genome rearrangements in hematopoietic malignancies that generally maintain wild-type p53 function [43,44].

### 3.2 *Interaction with BRCA proteins*

The BRCA1 and BRCA2 genes are commonly mutated in hereditary breast cancer. Both BRCA1 and BRCA2 interact with Rad51 [32,45]. Expression of BRCA1, BRCA2, and Rad51 are cell cycle regulated, with highest levels in S phase, and have similar RNA and protein expression patterns [24-26]. Mutation of BRCA1 or BRCA2 leads to a cellular defect in HR, but the physiological relevance of this defect to breast cancer is not yet understood [46-48]. BRCA1 has been implicated in DNA repair and HR, possibly coordinating the DNA damage response via protein modifications of multiple proteins. BRCA2 appears to play a direct role in HR since it directly interacts with, and appears in discrete nuclear foci with, Rad51 [45,49]. The BRC repeats located in exon 11 of BRCA2 are necessary and sufficient for binding to Rad51 [50-52]. This interaction may promote relocalization of Rad51 from the cytoplasm to the nucleus, dissociation of inactive Rad51 heptamer rings into active Rad51 monomers, and assist in the loading of Rad51 monomers onto DNA as part of the DNA damage response [51,53-55]. The link between the BRCA proteins and DNA repair provides one explanation for the high degree of chromosomal instability observed in BRCA mutant cell lines [49]. Presumably, mutations of the BRCA proteins in breast epithelial tissue lead to chromosomal instability and further mutation in oncogenes to promote tumorigenesis.

### 3.3 *Upstream Kinase Effectors: ATM and c-Abl*

The ataxia-telangiectasia mutated (ATM) kinase belongs to the phosphatidylinositol-3 kinase family that surveys genomic integrity, cell cycle progression, and processing of DSBs that occur during mitosis or as a result of exposure to DNA damaging agents [56]. ATM<sup>-/-</sup> cells have defects similar to other mutant cell types deficient in HR including a high frequency of

spontaneous chromosomal aberrations, high rates of intrachromosomal and error-prone HR [56,57]. Such cells have increased sensitivity to irradiation likely due to the DNA repair defect, as supported by the increased numbers of chromosomal aberrations after irradiation [57,58]. Mice mutated in the homologue of the ATM gene have similar defects and are predisposed to tumors [59-61].

ATM is a "hierarchical kinase" capable of initiating many pathways simultaneously [56]. Molecular targets include ATM itself, ATR, c-Abl, BRCA1, BRCA2, chk-1, chk-2, RPA, and NfκappaB/IκappaBα. In turn, several of these proteins interact with or activate Rad51. c-Abl may participate in responding to the extent of DNA lesions to maintain proper checkpoint control. ATM and DNA-PK contribute to the induction of c-Abl activity in the response to DNA damage [62,63]. In turn, c-Abl may regulate activation of Rad51 through phosphorylation at Tyr315 [64,65] which enhances the interaction between Rad51 and Rad52 and presumably stimulates HR [66]. However, although formation of radiation-induced Rad51 foci requires ATM, it does not require c-Abl, at least in a chicken DT40 cell line [67]. Consistent with this, the yeast homolog of ATM (MEC1) is required for phosphorylation of RPA as a response to radiation-induced DNA damage [68], and in turn RPA has been shown to interact with Rad51 at the site of DNA damage [69] bypassing the requirement for c-Abl signaling. Since ATM and c-Abl activate multiple pathways, it would be simplistic to suggest that activation or regulation of Rad51 occurs in a stepwise linear progression [70,71]. The complexity of these interactions is exemplified by the impact of the BCR-ABL fusion protein resulting from the t(9;22) characteristic of chronic myelogenous leukemia (CML), as discussed below.

#### **4. Rad51 Polymorphisms in Cancer Susceptibility**

The presence of polymorphisms of RAD51 and related HR genes have been associated with several tumor types, further suggesting that fidelity of DSB repair is an important step in their pathogenesis. Because of its interaction with BRCA1 and BRCA2, it has been proposed that polymorphism or mutation of the RAD51 gene may predispose to breast cancer. One study of Japanese hereditary breast cancer patients found two out of 45 patients who contained a single G to A transition in exon 6 of the Rad51 coding region (Arg150Gln). This Rad51 mutation was not present among 200 sporadic breast cancer or 100 colon cancer cases suggesting it is a disease-associated mutation, but a large population study is needed to determine if it is a rare polymorphism [72]. Two single base polymorphisms have been identified in the 5' untranslated region of RAD51: 5'UTRg135c and 5'UTRg172t [73]. Rad51-135c itself has not been demonstrated to lead to elevated risk of breast cancer or be predictive of survival of breast cancer patients [74,75]. However, Rad51-135c has been associated with an elevated risk of breast cancer in BRCA2 mutation carriers [76,77]. One study identified the Rad51-135c variant in slightly more BRCA1 mutation carriers affected with breast cancer as compared to healthy carriers [73], but a matched case study of Polish women shows instead that Rad51-135c is associated with decreased risk of breast cancer in women who also carry the BRCA1 mutation 5382insC [78]. The conflicting findings of these studies suggest the presence of additional modifiers within individual populations. The impact of these polymorphisms on protein activity is not yet clear, although it is presumed that they result in diminished repair function.

Polymorphisms in multiple pathways may act synergistically with environmental carcinogen damage to increase cancer susceptibility. Mutations in mismatch repair and metabolite clearance genes increase the risk of development of therapy-related leukemias [79-81]. Both *de novo* and therapy-related acute myeloid leukemia (AML and t-AML) have been

associated with the Rad51-135c polymorphism [82]. The associated Rad51 paralog XRCC3-Thr241Met polymorphism alone is not associated with AML or t-AML [82] but has been associated with bladder cancer [83], melanoma [84], and higher levels of DNA adducts [83,85]. Interestingly, the presence of both Rad51-135c and XRCC3-241Met further increases AML and t-AML risk [82], and the triple combination of Rad51-135c, XRCC3-241Met, and glutathione S-transferase M1 (GSTM1) detoxification gene deletion variant leads to a significant increase in AML risk [82].

Inactivating mutations of Rad51 in tumors have not been identified. A high frequency of tumors exhibit loss of heterozygosity (LOH) of the chromosomal band 15q14-15 where the Rad51 locus has been mapped [86]. Analysis of 21 metastatic brain tumors or 42 breast carcinomas with LOH of this region did not reveal mutation or loss of the Rad51 coding region [87]. Since Rad51<sup>-/-</sup> mice are inviable, and induced Rad51 loss in a chicken DT40 cell line leads to chromosome fragmentation and cell death, loss of Rad51 would be expected to lead to cell lethality. However, the impact of Rad51 loss in all somatic tissues has not been examined. The t(12;14) observed in some uterine leiomyomas results in the allelic loss of a uterine-specific alternatively spliced form of RAD51B [88], and provides evidence for tissue-specific variation and tolerance for mutation of Rad51 paralogs in certain tumor types.

## **5. Impact of Elevated Rad51**

### *5.1 Increased Rad51 detected in multiple tumor types*

Multiple immortalized and tumor cell lines over-express Rad51, and screening of primary tumor samples has also identified elevated levels of wild-type Rad51 protein [26,89,90]. There has been much speculation about the significance of this finding and whether dysregulation of Rad51 protein can be an early step in transformation or secondary to mutations in regulators of Rad51. Similarly, elevated levels of Rad51 could reflect the disruption of its normal cleavage by caspase-3 during apoptosis, since malignant cells tend to bypass apoptotic signals normally induced following DNA damage or express one of the anti-apoptotic BCL-2 family members [91-94]. Alternatively, elevated levels of Rad51 could simply reflect the high proliferative capacity and normal activity of Rad51 during S phase of malignant cells.

Elevated levels of wild-type Rad51 protein were detected in 5-30% of pancreatic cell lines, and in 66% of primary pancreatic adenocarcinoma samples [95]. Significantly, Rad51 appeared to be a tumor-specific antigen in these samples, and therefore a good target for immunotherapy, at least in pancreatic cancer [95]. Histological grading on sporadic invasive ductal breast cancer correlates with the concomitant down-regulation of BRCA1 and the increase of Rad51 [96]. However, decreased levels of Rad51 in tumors have also been detected. Rad51, as well as BRCA1, expression and protein levels are reduced in almost one third of breast tumor cell lines and primary sporadic breast cancer cells [97-100], suggesting that disruption of normal HR-mediated DSB repair may be an early step in sporadic breast tumorigenesis. Taken together, these studies indicate that the maintenance of genome stability can be sensitive to alterations both up and down in the balance of repair proteins in the cell.

Disruption of c-Abl and formation of the BCR-ABL fusion protein by the CML-associated t(9;22) leads to dysregulation of Rad51 through multiple pathways. BCR-ABL stimulates transcription of Rad51, inhibits degradation of Rad51 through suppression of caspase-3, leads to constitutive Rad51 Tyr315 phosphorylation, and altered efficiency of HR-mediated DNA repair [101]. As a result, increased levels of Rad51 protein are observed in primary CML samples with higher expression in cells in blast crisis, a phase of the disease marked by increased

genome instability and multiple chromosome rearrangements [101]. It is tantalizing to interpret this observation to mean that increases in Rad51 expression found in tumor cells may contribute to genomic instability by stimulating illegitimate recombination between short repetitive elements or homeologous sequences on different chromosomes [26,89,102]. However, since c-Abl activates multiple pathways, the genomic instability associated with progression of CML could be reflective of other interactions and cell cycle checkpoint defects [70,71].

Altered amounts of Rad51 observed in tumor cells correlate with differences in radioresistance and chemoresistance. Over-expression of Rad51 correlated with pancreatic tumor cell survival following exposure to calicheamicin  $\gamma$ - induced DSBs in a dose dependent manner [103]. Expression levels of Rad51 may be predictive of cell sensitivity and B-cell chronic lymphoid leukemia (B-CLL) patient response to nitrogen mustards [104,105]. Small cell lung cancer cell lines that have a high level of Rad51 protein are more resistant to both irradiation and etoposide/VP-16 [106]. BCR-ABL in CML cells leads to resistance to irradiation, cisplatin, and mitomycin C, consistent with increases in Rad51 protein levels and altered activation, and this resistance can be abrogated by the direct inhibition of BCR-ABL activity with Gleevec [101]. These observations may have the most impact on cancer therapy since they suggest that anti-Rad51 approaches will be effective adjunct therapy modalities.

## 5.2 *Experimental Approaches to Examine Rad51 Over-expression*

Multiple experimental systems have been aimed at determining the direct impact of increased amounts of Rad51 in cells. Cells that over-express Rad51 are resistant to multiple cytotoxic agents including irradiation, etoposide, cisplatin, mitomycin C, hydroxyurea, and thymidine. Resistance to a particularly broad spectrum of agents underscores the fact that Rad51 is central to multiple repair pathways. Moderate 2-4 fold increases in Rad51 protein levels have been associated with increased gene targeting [107,108], intrachromosomal HR and sister chromatid exchange [101,109-111], as well as interchromosomal HR-mediated repair leading to translocations and aneuploidy [112]. Rad51 quantity or activation may normally be a limiting factor to the establishment of HR intermediates in mitotic cells. By contrast, elevated Rad51 may be sufficient to overcome disruption of Rad51 pre-synaptic filaments by DNA helicases, as shown with Srs2 [113,114], thus favoring the formation of illegitimate intermediate structures. Consistent with this, the chromosomal rearrangements induced by elevated Rad51 in experimental systems is similar to the phenotypes of abrogating mutations in BLM and WRN helicases (responsible for Bloom's and Werners Syndromes) that have increased numbers of Rad51-containing foci in response to DNA damage, elevated HR, genome instability, and a predisposition to cancer [34,115]. However, reports on the effects of Rad51 over-expression are conflicting [116] and may reflect different cell types and assay systems. It remains unclear what threshold amount of active Rad51 protein *in vivo* is sufficient to stimulate strand invasion for levels of HR necessary for cell survival, what level is necessary to stimulate illegitimate HR events leading to genome rearrangements, or how large increases in Rad51 protein levels (4-10 fold and greater) may lead to suppression of such events or promote apoptosis [102,116].

Elevated Rad51 also may disrupt additional steps in cell cycle progression [102] not directly related to DNA strand invasion and HR. Elevated levels of Rad51 may indirectly interfere with DNA metabolism that results in recombinogenic DNA lesions [92,117,118], alter its normal interactions with p53, p21, or bcl-2 and allow aberrantly repaired cells to escape apoptosis [90,93,101], or disturb the G2/tetraploid checkpoints resulting in aneuploidy [119,120]. Chromosomal instability and aneuploidy in cells with elevated levels of Rad51 could

be the result of defective centrosome function [112]. Interestingly, similar results can be obtained in cells with decreased activity of Rad51. Chinese hamster ovary (CHO) cells that express a dominant negative form of Rad51 also exhibit centrosome defects and aneuploidy [121], as well as cells that carry mutations in other DNA repair genes [122-126].

One key question that remains is whether aberrant levels of Rad51 are sufficient to initiate tumorigenesis. Separation of function studies could potentially determine which of the Rad51 activities directly impacts either tumor initiation or progression and additional genome instability. Recent evidence has demonstrated that even transient increases in Rad51 protein levels during the repair of DSBs is sufficient to promote crossovers, a type of HR-mediated repair that by definition leads to translocations, as well as aneuploidy and genome instability [112]. However, another study examined the potential for Rad51 to promote tumorigenesis at the earliest steps following injection of Rad51-transfected cells into athymic nude mice. Over-expression of wild-type Rad51 was not sufficient to increase the rate of tumor formation or size of tumor recovered 20 days post-injection suggesting that the defects observed in cultured cells are not sufficient to promote tumorigenesis *in vivo* [121]. Increased levels of Rad51 protein alone may not be sufficient for tumorigenesis, but require a second co-operating mutation. Long-term *in vivo* transgenic mouse models could address this question.

## **6. Rad51 as a Therapeutic Target**

Although irradiation produces multiple types of DNA damage, cell lethality is most associated with the DSBs that are substrates for Rad51-mediated repair. Elevated amounts of Rad51 have been linked to radioresistance of multiple tumor types [89], and suggest that abrogation of Rad51 in tumors could inhibit cell growth, and increase efficacy of current therapeutic approaches. Several approaches have targeted Rad51 expression in tumor cells to increase radiosensitivity. Exposure of murine m5S skin cells and 203G glioma cells to Rad51 anti-sense oligonucleotides leads to a 70% decrease in Rad51 mRNA and protein levels that is accompanied by decreased cell growth and a dose-dependent increase in radiosensitivity [127,128]. In a similar approach, transfection of LNCaP prostate tumor cells with an anti-Rad51 ribozyme oligonucleotide moderately reduces Rad51 protein levels by 20-50% with a concomitant 2-fold increase in radiosensitivity [129]. Exposure of U251 or SF539 glioma cells, immortalized fibroblasts, or CML cells to the kinase inhibitor Gleevec reduced Rad51 expression and increased radiosensitivity [101,130].

Alkylating agents lead to DNA cross-links that are repaired by the nucleotide excision pathway. The removal of damaged DNA adducts produces DNA strand breaks that can act as substrates for Rad51-mediated HR repair [10]. Therapeutic targeting of Rad51, or its paralogs, to increase sensitivity to this class of chemotherapeutic agents has provided conflicting results. Anti-sense Rad51 oligomers do not appear to enhance sensitivity of cells in the Lewis lung tumor model to either cisplatin or taxol [131]. This is consistent with the observations that cisplatin/melphalan resistance was not dependent on Rad51 protein levels alone in epithelial cell lines [104], and that over-expression of Rad51 in CHO cells only produced a mild elevation in resistance [110]. However, peptide-based inhibition of RAD51C has provided promising results. Direct transduction of CHO cells with a synthetic peptide corresponding to the residues of RAD51C important for multiple protein-protein interactions reduced the appearance of DNA damage-induced nuclear foci and also increased sensitivity to cisplatin [132]. Although Rad51C is thought to be an accessory protein for Rad51 activity, it may have a separate or larger function than previously appreciated. Screening of random peptide phage display libraries may become a

valuable tool in the search for other therapeutic peptides capable of blocking Rad51 protein-protein interactions.

These studies provide promising preliminary evidence that suppression of Rad51 may be a new strategy for the treatment of tumors. Studies will need to examine the effect of moderate down-regulation of Rad51 on illegitimate HR or gross chromosomal stability in the surviving fraction of cells that may have further implications for tumor progression. The use of new and more effective siRNA should enhance these effects.

## 7. Future Directions

Much progress has been made in the field of HR-mediated repair of DSBs, and their impact on genomic stability and tumorigenesis. A role for Rad51 in the genomic alterations observed in tumor cells is now beginning to be appreciated, although it still remains to be determined if Rad51 directly impacts tumor initiation, progression, or both. An understanding of how specific damage recognition and DNA repair pathways are altered in human cancers should ultimately facilitate the rational design of agents that selectively sensitize cells. New technologies such as synthetic peptides and siRNA may be combined with individual proteomic profiling and provide significant progress toward the future of cancer therapy.

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