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The Consequences of Rad51 Overexpression for Normal and Tumor Cells

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Abstract

The Rad51 recombinase is an essential factor for homologous recombination and the repair of DNA double strand breaks, binding transiently to both single stranded and double stranded DNA during the recombination reaction. The use of a homologous recombination mechanism to repair DNA damage is controlled at several levels, including the binding of Rad51 to single stranded DNA to form the Rad51 nucleofilament, which is controlled through the action of DNA helicases that can counteract nucleofilament formation. Overexpression of Rad51 in different organisms and cell types has a wide assortment of consequences, ranging from increased homologous recombination and increased resistance to DNA damaging agents to disruption of the cell cycle and apoptotic cell death. Rad51 expression is increased in p53-negative cells, and since p53 is often mutated in tumor cells, there is a tendency for Rad51 to be overexpressed in tumor cells, leading to increased resistance to DNA damage and drugs used in chemotherapies. As cells with increased Rad51 levels are more resistant to DNA damage, there is a selection for tumor cells to have higher Rad51 levels. While increased Rad51 can provide drug resistance, it also leads to increased genomic instability and may contribute to carcinogenesis.

Keywords

Rad51 protein; overexpression of Rad51; genomic instability; tumor cell drug resistance; homologous recombination; gene targeting

1. Introduction

DNA double strand breaks (DSBs) occur through several sources; through the action of exogenous agents such as ionizing radiation, through inhibition of DNA topoisomerases, and from endogenous programmed events such as V(D)J recombination in the immunoglobulin genes and Spo11-promoted meiotic recombination [1]. DSBs can be repaired by end joining pathways that do not utilize significant homology at the broken ends, or through homologous recombination (HR), which uses homology at the broken ends to intact sequences in the genome to repair the breaks. When HR is used for repair, in eukaryotes it is promoted by the recombinase Rad51, which binds to 3'-tailed single strands at the end of DSBs in a helical fashion and promotes pairing with homologous DNA sequences as a prelude to strand invasion and repair of the DSBs [1–4]. Since eukaryotic genomes contain dispersed repeated DNA,

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repair of DSBs through HR can occur not only through an interaction with the sister chromatid or the homolog chromosome, but also with repeats on nonhomolog chromosomes (sometimes called ectopic HR). Ectopic HR can result in genomic rearrangements and instability. It follows then that mitotic HR must be regulated to ensure that DSB repair is not mutagenic.

Although the regulation of mitotic HR is not fully understood, we are now beginning to appreciate the role that Rad51 expression levels play in regulating HR, as Rad51 overexpression is found in human tumors, and its increase is related to p53 function. Rad51 performs a vital function during the normal cell cycle as the *RAD51* knockout in chicken and mouse cells is lethal [5–7]. However, too much Rad51 protein also has deleterious consequences. These findings emphasize that Rad51 expression and activity must be carefully regulated. In fungal systems where Rad51 is not essential, mutations that reduce or destroy Rad51 activity result in increased sensitivity to DNA damaging agents that create DSBs, a reduction in spontaneous mitotic HR rates, an increase in genomic rearrangements and an inability to complete meiotic recombination. Thus HR is an essential feature of a normal cell cycle, but must be held in check to prevent unwanted rearrangements. Increased Rad51 expression in tumor cells also has the unwanted effect of bestowing increased resistance to DNA damage, rendering the cells resistant to drug therapies. Here we review the effects of Rad51 overexpression in yeasts, *Drosophila* and mammalian systems.

2. Fungal Systems

2.1 *S. pombe*

The Rad51 homolog, Rhp51, has been overexpressed in wildtype *S. pombe* cells and results in disruption of the cell cycle [8]. The expression level ranged from 80-fold to 700-fold over the normal level of Rhp51, using an inducible promoter. As the level of overexpression increased, cells became increasingly debilitated, with reduced viability and a defect in chromosome segregation, the “cut” phenotype, which is indicative of a failure to properly segregate the chromosomes at mitosis. The proportion of cells displaying the “cut” phenotype was related to the level of Rhp51 overexpression. The cell morphology suggested that overexpression of Rhp51 induced a DNA damage checkpoint prior to mitosis. This was accompanied by a disruption of microtubule attachments to the chromosomes. A FACS analysis showed that a large fraction of cells had a 1C DNA content, indicative of a delay in entering into S phase. The authors suggest that DNA replication is interfered when Rhp51 is overexpressed. As overexpression of a mutant form of Rhp51 that is most likely defective in binding to DNA based on an identical mutation in the *S. cerevisiae* Rad51 protein [9,10] did not inhibit cell growth, interference with replication and cell growth requires that the overexpressed Rhp51 be bound to DNA rather than causing a defect by binding to other protein factors and depleting these from their normal cellular compartment and function.

2.2 *S. cerevisiae*

Several studies have reported on the effects of Rad51 overexpression in *S. cerevisiae*. Since the level of overexpression varies in these studies, in some cases Rad51 overexpression has been more deleterious than in other cases. Rad51 interacts with the mediator proteins Rad52, Rad55 and Rad57, which overcome the inhibitory effect of RPA binding to ssDNA in in vitro reactions for Rad51 nucleofilament formation [11–13]. Overexpression of Rad51 can suppress the in vivo effects of non-null *rad52* mutations [14–17], or *rad55* and *rad57* deletion mutations [18]. The assays for suppression involve DNA damage resistance and an increase in recombination rates. In no case was any deleterious effect of Rad51 overexpression reported, although the level of overexpression ranged from two-fold to what is estimated to be at least a ten-fold increase in protein levels. Rad51 overexpression was also able to partially rescue the meiotic defects of *rad52* mutants, using spore viability as a measure of successful meioses.

S. cerevisiae has a meiotic-specific Rad51 paralog called Dmc1, which acts together with Hop2 to promote meiotic crossing over. *dmc1* and *hop2* mutants are defective in meiotic crossing over and have greatly reduced spore formation and viability. These defects can be counteracted by overexpression of Rad51 [19]. While excess Rad51 could complement meiotic mutants and promote HR under these mutant conditions, it did not enhance HR in wildtype cells.

Rad51 is a dynamic protein that forms nucleofilaments on ssDNA in preparation for HR, then is bound to dsDNA after homologous strand invasion to form a D-loop, the synapsis intermediate, and finally is removed from DNA to allow completion of the HR repair reaction by gap filling strand synthesis. To insure that only appropriate DNA lesions are targeted for HR repair during mitotic growth, cells have devised various mechanisms to prevent inappropriate Rad51 nucleofilament formation. In *S. cerevisiae* this is achieved through the action of the Srs2 DNA helicase, which acts as a translocase to antagonize and disassemble Rad51 nucleofilaments [1]. Thus it might be anticipated that there is a fine balance between Rad51 protein and Srs2 protein in the cell. Indeed, alteration of this balance can have deleterious consequences. Overexpression of Rad51 in an *srs2* null mutant results in increased MMS damage sensitivity, with no consequence in wildtype cells [15,20].

The effects of Rad51 overexpression on the rate and types of DSB-induced HR have been examined in detail by Paffett *et al* [20]. Since strand exchange is promoted by Rad51, one might have expected that increase Rad51 would lead to increased strand exchange tract length. However, this was not found to be the case. In fact, the overall rate of all types of HR events that can repair a DSB were reduced when Rad51 was overexpressed at high levels of at least a ten-fold increase over normal levels. Examination of an *srs2* mutant confirmed previous findings that Rad51 overexpression sensitized these cells to MMS and DSB damage. *yku70* mutants defective in checkpoint signaling and protection of DSB ends were also sensitive to Rad51 overexpression. These effects are attributed to an inability to disassemble Rad51 nucleofilaments. Thus there may be a controlled balance between Rad51 filament assembly and disassembly that is upset when there is excess Rad51 protein. Why this reduces HR is not clear, but it suggests that some HR reactions cannot be finalized into HR products when there is too much Rad51 nucleofilament.

3. Drosophila

The *D. melanogaster* RAD51 homolog is called *spnA* [21–23]. Loss of the *spnA* gene results in a female sterile phenotype and DSB repair deficiency, but otherwise the mutants are viable. Ubiquitous overexpression of Rad51 using two different inducible promoter systems is lethal [24] and the deleterious effects of Rad51 overexpression are due to Rad51 binding to DNA, and not to sequestration of interacting proteins, as a mutant version of Rad51 missing the region required for DNA binding has no effect when overexpressed. Tissue-specific expression using promoters specific for the eye or wing imaginal discs results in cell death due to apoptosis, based on TUNEL assays. When the apoptotic pathway was inhibited by expressing a protein that interferes with caspases, the cell death phenotype from Rad51 overexpression was reduced. The ability of imaginal disc cells to undergo synchronous mitosis was perturbed when Rad51 was overexpressed, showing that Rad51 overexpression can disrupt the normal cell cycle. Whether this is through DNA damage repair or from an interaction with cell cycle progression proteins is not known, but in mammalian cells Rad51 can interact with several cell cycle proteins and its expression is regulated during the cell cycle.

4. Regulation of RAD51 expression in mammalian systems

Since many tumors exhibit resistance to therapeutic drugs that damage DNA, it is important to understand the molecular mechanisms causing this DNA damage resistance. In several cases

it appears that the resistance is from enhanced *RAD51* expression, due to oncogene-induced expression of *RAD51* and inhibition of pathways that limit Rad51 protein levels. Cells that express the oncogene BCR/ABL have increased levels of Rad51 protein [25]. This occurs through STAT5-dependent transcription of *RAD51* and inhibition of Rad51 protein cleavage by caspase-3. These cells have increased resistance to cisplatin and mitomycin C, drugs whose damage requires Rad51 for repair. Additionally, double strand break-induced homologous recombination is elevated in these cells. Increased drug resistance was not universal, as MNNG resistance was not increased. MNNG damage can be repaired by the base excision repair pathway and as this pathway is not enhanced by BCR/ABL expression, cells remain sensitive to MNNG. Another level of regulation of Rad51 activity occurs through protein modification. Rad51 is a target of the BCR/ABL kinase and is phosphorylated on tyrosine 315. Mutation of this tyrosine residue to phenylalanine resulted in increased sensitivity to cisplatin and mitomycin C, suggesting that Rad51 recombinational repair of DNA crosslink damage is controlled through Rad51 tyrosine 315.

In another study, overexpression of Bcl-2 or Bcl-x(L) were found to inhibit DNA damaged induced HR requiring Rad51 protein [26]. Bcl-2 inhibited double strand break-induced HR that was enhanced by Rad51 overexpression, and increased error-prone repair of DNA damage. The mechanism of inhibition of Rad51-dependent HR was not fully defined, but seems to involve inhibition of Rad51 post-translation modification.

RAD51 expression is also regulated through p53. Overexpression of the c-myc, β -catenin or human papilloma virus E7 oncogenes results in induction of *RAD51* and increased protein levels [27]. *RAD51* induction is dependent on the ATM and ATR kinases acting on p53 and promoting transcriptional activation of downstream targets. Thus there is a correlation between p53 phosphorylation and downregulation of Rad51 levels. Increased Rad51 levels are correlated with an induction of the DNA damage response when oncogenes are overexpressed, using formation of γ -H2AX foci as a marker of DNA damage. Further studies suggested that the ARF tumor suppressor pathway also regulated Rad51 levels through p53 activation.

The *RAD51* promoter of mammalian cells contains a p53 response element that acts through AP2 and p53 binding, resulting in decreased *RAD51* mRNA and Rad51 protein levels [28, 29]. p53 also regulates Rad51 function as it inhibits the formation of Rad51 nuclear foci after the induction of DSBs. The p53 contact mutant R280K does not down regulate *RAD51* mRNA and protein levels, nor does it inhibit the formation of Rad51 foci after DNA damage induced by treatment with the etoposide VP16 [28]. Since p53 negatively regulates Rad51 levels and increased Rad51 is correlated with increased drug resistance, many tumors harboring p53 mutations may be resistant to chemotherapeutic treatments due to increased Rad51 levels. The role of increased Rad51 in malignant transformation is not as clear, but increased Rad51 levels in p53 mutant cells may contribute to increased genomic instability through inappropriate HR.

Increased Rad51 levels have been associated with p53-dependent expression of p21^{Waf-1}, topoisomerase IIa and GADD45 [30]. These can impact the expression of BRCA1, a protein mutated in heritable breast cancer and required for genomic stability through checkpoint signaling of DNA damage, transcriptional regulation and homologous recombination. Cell cycle arrest that is seen when Rad51 is overexpressed is thought to occur through p21. This could increase HR and genomic instability by keeping cells in G2, the cell cycle phase where HR is most active, and promoting inappropriate recombination, including translocations and other rearrangements.

Rad51 protein forms nuclear foci after DNA damage that causes double strand breaks. When Rad51 is overexpressed, foci and higher order structures are seen even in the absence of induced DNA damage and even when cells are not in S phase [31]. This is correlated with an increase

in p21, the Cdk inhibitor. When Rad51 expression is reduced by antisense inhibition, the level of p21 decreases and cells become sensitive to etoposide drug treatments that cause double strand breaks. While the relationship between the higher order structures and DNA repair centers is not clear, cells that expressed higher levels of Rad51 showed increased resistance to etoposide treatment with fewer DNA breaks and chromatid aberrations. The nature of the higher order structures remains unknown. They could represent Rad51 bound to single stranded DNA that accumulates at replication forks, however, these would be expected to occur in S phase cells. Rad51 could be bound nonspecifically to chromatin, as has been postulated to occur on yeast meiotic chromosomes when Rad51 is not properly regulated [32]. The increase in p21 when Rad51 is overexpressed could reflect an arrest of the cell cycle in G1 in those cells that have Rad51 bound promiscuously to chromatin.

5. Rad51 overexpression in human tumors and cell lines

Rad51 has been found to be increased in expression in a wide range of human tumors, most likely contributing to drug resistance of these tumors. Increased Rad51 expression does not come from amplification, but rather is due to increased transcription of the *RAD51* gene and possibly post-translational modifications.

Since there is a correlation between tumor resistance to radiation and chemotherapy and the ability to repair DNA damage by homologous recombination, tumor cell lines were screened for increased Rad51 protein by immunofluorescence staining [33]. Increased numbers of Rad51 nuclear foci were found in cell lines derived from chronic myeloid leukemia, acute myeloid leukemia, T-cell leukemia, T-cell lymphoma, histiocytic lymphoma, cervical carcinoma, ovarian carcinoma, breast carcinoma, testis carcinoma, colon adenocarcinoma, rectal adenocarcinoma, melanoma and glioblastoma. Increased Rad51 expression was not the result of gene amplification. Rad51 protein levels were increased 2–7 fold as measured by Western blot analysis. Increased Rad51 expression in malignant prostate cancer cells has also been reported [34]. In this report several gene products involved in homologous recombination were increased 2–5 fold over normal cells. In spite of the increase in homologous recombination factors, the cells had an abnormal response to DNA damage and showed defective repair of DNA breaks, oxidative base damage and alkali-labile sites.

The FLT3 kinase is activated in about 30% of patients with acute myeloid leukemia [35]. Among these are individuals with two mutations in FLT3, a length mutation and a mutation in the tyrosine kinase domain. This FLT3 double mutant is associated with drug resistance towards PTK inhibitors and the topoisomerase II inhibitor daunorubicin, which occurs through hyperactivation of STAT5, with an ensuing increase in Rad51 and Bcl-x(L). Cell lines expressing the FLT3 double mutant arrest in G2/M following daunorubicin treatment instead of undergoing apoptosis or arresting in G0/G1 phase of the cell cycle. Arrest of cells in G2/M might provide the opportunity for Rad51 to repair double strand break damage by homologous recombination and increased drug resistance.

Since fusion tyrosine kinases are associated with oncogenes arising from chromosome translocations and the BCR/ABL fusion kinase is expressed in chronic myelogenous leukemia patients and some acute lymphocytic leukemia patients can increase Rad51 expression through STAT5 activation, the effect of different fusion tyrosine kinases on drug resistance, HR, extension of the G2/M phase of the cell cycle and reduced apoptosis was examined [36]. Fusion tyrosine kinases arising from translocations found in a variety of leukemias and lymphomas were transformed into the murine BaF3 pro-B lymphoid cell line. BCR/ABL, TEL/ABL, TEL/PDGFR, TEL/JAK2 and NPM/ALK transformed cells displayed increased resistance to cisplatin and mitomycin C and were increased in Rad51 expression by a factor of five to eight fold. Antisense targeting of *RAD51* reduced Rad51 levels and concomitantly, drug resistance.

Further studies showed that Rad51 expression was activated through the STAT5 pathway and that Bcl-xL expression was also increased by the fusion tyrosine kinases, which could increase tolerance of DNA damage by preventing the apoptotic response to excessive DNA damage.

Rad51 expression is cell cycle-regulated, being highest in S/G2 and lowest in nongrowing cells. Since increased Rad51 expression is correlated with increased HR, but also increased genomic instability, the consequences of increased Rad51 expression were studied in human cells. A human fibrosarcoma cell line was transfected with a tet-repressible *HsRAD51* transgene, to generate cell lines that expressed high levels of HsRad51 [37]. High HsRad51 resulted in a decrease in plating efficiency and growth rate, an increase in apoptosis and cells in G2. Comet assays suggested that there was not increased spontaneous DNA damage in the cells with increased HsRad51 levels. The deleterious effects on cells growth appeared to be similar to those seen in *Drosophila* cells with increased Rad51 expression [24]. Whether these are the consequence of unbalanced HR components or uncontrolled HR and genomic rearrangements is not known. However, the authors did observe that after extended HsRad51 high expression, some cells became adapted to the HsRad51 levels and returned to normal growth, perhaps reflecting selection in tumors for cells that are resistant to the deleterious growth consequences of increased Rad51 expression while at the same time having increased HR rates and drug resistance.

In a study examining differentially expressed genes in pancreatic cancer cells by cDNA microarray analysis, among the thirty identified genes was *RAD51* [38]. The overexpression was confirmed by RT-PCR in nine pancreatic cancer cell lines. Rad51 expression by RT-PCR using frozen pancreatic tumor tissue from patients showed that three out of eight were significantly increased. Overexpression was confirmed at the protein level by immunostaining of a pancreatic adenocarcinoma tissue array. Of 35 samples stained, 74% showed significant increase in staining and 13% stained very strongly. Increased Rad51 expression has also been seen in soft tissue sarcomas (STS). Using a panel of 62 human STS samples of differing histologic subtype, strong Rad51 cytoplasmic staining was seen in 59 samples. Nuclear staining was observed in 49 of the samples. Rad51 expression was also increased in STS cell lines. These cell lines showed increased Rad51 expression after doxorubicin treatment, which arrested cells in S-G2-M. *RAD51*siRNA reduced the resistance of these cells to doxorubicin treatment, providing further support for the link between drug resistance and Rad51 levels. Maacke et al [39] also found Rad51 to be overexpressed in human pancreatic adenocarcinoma specimens. Using three-dimensional cell culture and orthotopic xeno-transplants, the level of Rad51 overexpression was found in 66% of the tissue specimens examined. The increased Rad51 expression was correlated with increased survival following DNA damage by double strand breaks. Rad51 expression was significantly increased when tumor cells were grown in 3D culture as compared to monolayer growth.

Increased Rad51 expression by immunostaining has also been found in invasive ductal breast cancer [40]. The level of overexpression correlated with the histological grading of the tumors. No correlation between Rad51 overexpression and loss of BRCA1 was found, nor was there a direct correlation between Rad51 staining in tissue samples and p53 staining. These findings implicate Rad51 in sporadic breast cancer and suggest that multiple factors can lead to an increase in Rad51 expression.

The breast cancer susceptibility gene *BRCA1* is involved in the cellular response to DNA damage, in repair through HR. Chicken DT40 cells deleted for *BRCA1* are viable, but exhibit growth defects, DNA damage sensitivity and reduced HR [41]. These defects can be suppressed by overexpression of hRad51 to about 15-fold increase. Resistance to ionizing radiation and cisplatin could be achieved by hRad51 overexpression. Since these are features of some breast tumors that are defective in BRCA1, BRCA1-deficient tumors were screened by microarray

for expression levels of Rad51 and Rad51-related proteins. Both Rad51 and Rad51 cofactor proteins were found significantly elevated in BRCA1-deficient tumors. These results show that an increase in Rad51 and HR can rescue growth defects in BRCA1-deficient cells and suggest that there may be an intrinsic selection for increased Rad51 and HR in BRCA1-deficient human tumors. Since these cells are also associated with chromosome abnormalities and rearrangements, this suggests that excess or uncontrolled HR can be aberrant and deleterious.

6. Effects of Rad51 overexpression on recombination in mammalian cells

Overexpression of Rad51 in mammalian cells has been used to try to improve homologous recombination rates. Overexpression of *CgRAD51* in CHO cells stimulated homologous recombination as measured by recombination between a direct repeat of LacZ genes to give a LacZ⁺ recombinant [42]. Cells also were more resistant to ionizing radiation, particularly in G2 of the cell cycle. Overexpression of *CgRAD51* in the Chinese hamster cell line SPD8 led to a 2–3 fold increase in spontaneous homologous recombination using a recombination reporter that had a partial duplication [43]. The SPD8 cell line was mutated in p53, which could affect endogenous Rad51 levels and also regulate the use of homologous recombination pathways through increased expression of other HR factors. A separate study by this same group found that overexpression of *CgRAD51* could increase spontaneous nonhomologous recombination in Chinese hamster cells, but was ineffective in stimulating nonhomologous recombination promoted by camptothecin and etoposide [44]. Overexpression of *CgRAD51* did increase resistance of Chinese hamster cells to the etoposide VP16 and other DNA damaging agents through double strand break repair [45].

Overexpression of *MmRAD51* in p53 defective Chinese hamster cells stimulated homologous recombination but did not stimulate tumorigenesis when cells expressing high levels of Rad51 were injected into nude mice [46]. In contrast, overexpression of a dominant negative version of *MmRAD51* that inhibits homologous recombination resulted in a higher frequency of tumors when those cells were injected into nude mice. This led the authors to conclude that inhibition of HR through defective Rad51 activity can lead to tumorigenesis.

In another study using mouse hybridoma cell lines, overexpression of *MmRAD51* enhanced the cell growth rate [47]. Cells also exhibited increased resistance to ionizing radiation and gene targeting frequencies. This was dependent on a functional Rad51 protein, as overexpression of mutant Rad51 proteins did not show an increase in gene targeting frequency.

Using a HR reporter system that is based on induced double strand breaks at I-SceI sites, Richardson et al [48] found that increased HsRad51 protein in mouse ES cells led to a novel spectrum of recombination events, including crossovers and chromosome translocations, multiple chromosome rearrangements and aneuploidy. A further study examining double strand break repair in multipotent hematopoietic cells found that reciprocal translocations were a common result of double strand break repair but were not seen in actively proliferating myeloid cells [49]. Rad51 overexpression through a transgene did not affect the frequency of recovered translocations, but did alter the type of recombination repair event associated with the translocation such that increased loss of heterozygosity and tandem duplications were recovered. These results suggest that hematopoietic cells are particularly prone to chromosome rearrangements during double strand break repair.

As some tumor cells have increased Rad51 levels and are treated with agents such as etoposides that cause double strand breaks, this combination could promote genomic instability in those cells. The above results highlight the fact that there are multiple pathways for HR repair, and that excess Rad51 can provoke use of alternative pathways that can repair a double strand break, but are associated with gross karyotype abnormalities. It suggests that use of HR pathways and Rad51 must be carefully controlled to prevent such instabilities from arising

during the normal cell cycle response to DNA damage. In fact, crossovers are rarely associated with mitotic HR events and are suppressed by the Sgs1/BLM and other RecQ-family helicases [50–56].

Increased hRad51 expression has been reported to enhance gene targeting in human cells [57]. In this study Rad51 expression was increased four-fold. Gene targeting of HPRT and the interferon gene 6–16 were increased about two-fold.

In a situation in reverse of those cited above, Wang et al [58] found that prostate cancer cell lines were sensitive to cisplatin when grown in anchorage-independent conditions. This was associated with a decrease in Rad51 expression, a lack of colocalization of Rad51 with double strand breaks, a reduction in HR repair of an induced double strand break, and increased DNA damage sensitivity. When cells were grown under conditions that allowed reattachment, or in the presence of proteasome inhibitors, Rad51 levels increased and Rad51 protein colocalized with induced double strand breaks. The reduction in Rad51, which appears to be at the level of degradation and not transcriptional, could lead to increased aberrant DNA damage repair and when this is associated with metastatic growth, could lead to selection for cells with increased DNA damage resistance.

7. Conclusions

Although increased Rad51 can result in increased drug resistance and recombination in mammalian cells, there clearly must be a balanced relationship between Rad51 and other repair factors. Excess Rad51 in *Drosophila* or yeast does not increase HR and in fact has deleterious consequences on cell cycle and cell survival. Rad51 activity can be regulated at the level of expression and action. In mammalian cells p53 and the downstream STAT5 pathway act to keep Rad51 expression in check. BRCA2 interaction with Rad51 keeps Rad51 in a monomeric state [59–61], which controls the ability of Rad51 to form a nucleofilament and initiate HR strand exchange. Excess Rad51 could disrupt the balance with BRCA2 such that the BRCA2-bound Rad51 can form multimeric higher order structures. Whether these are pathological or can engage in a HR reaction is unknown.

The correlation between Rad51 levels and resistance to chemotherapeutic drugs suggests that targeted inhibition of Rad51 through siRNA or small molecule strategies may improve the response to drug treatments by reducing HR. Since excessively reduced Rad51 levels can also result in genomic rearrangements, any protocol to reduce Rad51 levels should also ensure that damaged cells are destroyed through an active apoptotic pathway.

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