SURVEY AND SUMMARY

Overexpression of RAD51 suppresses recombination defects: a possible mechanism to reverse genomic instability

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ABSTRACT

RAD51, a key protein in the homologous recombinational DNA repair (HRR) pathway, is the major strand-transferase required for mitotic recombination. An important early step in HRR is the formation of single-stranded DNA (ss-DNA) coated by RPA (a ss-DNA-binding protein). Displacement of RPA by RAD51 is highly regulated and facilitated by a number of different proteins known as the 'recombination mediators'. To assist these recombination mediators, a second group of proteins also is required and we are defining these proteins here as 'recombination co-mediators'. Defects in either recombination mediators or co-mediators, including *BRCA1* and *BRCA2*, lead to impaired HRR that can genetically be complemented for (i.e. suppressed) by overexpression of RAD51. Defects in HRR have long been known to contribute to genomic instability leading to tumor development. Since genomic instability also slows cell growth, precancerous cells presumably require genomic re-stabilization to gain a growth advantage. RAD51 is overexpressed in many tumors, and therefore, we hypothesize that the complementing ability of elevated levels of RAD51 in tumors with initial HRR defects limits genomic instability during carcinogenic progression. Of particular interest, this model may also help explain the high frequency of *TP53* mutations in human cancers, since wild-type p53 represses RAD51 expression.

INTRODUCTION

Homologous recombinational DNA repair plays an important role in cancer

The central protein involved in the homologous recombinational DNA repair (HRR) pathway is RAD51, a strand transfer protein. Although no mutations in the RAD51 open reading frame have been found in cancers, defects in other HRR genes have been shown to play an important role in carcinogenesis, and particularly in breast cancer. Inherited heteroallelic mutations in *BRCA1* and *BRCA2* each result in a breast cancer predisposition syndrome, and both of these genes are involved in HRR (1). In addition, eight more breast cancer predisposition syndromes have been identified, and all of these also involve defects in HRR-related genes (2). There have been several excellent and comprehensive reviews in recent years that describe the connections between HRR and cancer (particularly breast cancer) (1,3), the role of *TP53* mutations in recombination (4,5) and the observation that RAD51 is overexpressed in many tumors (6). What seems to be missing from these and other reviews is an overall hypothesis explaining the relationship between these three different components of carcinogenesis. Here, we review what we feel is the missing link that helps tie these components together. This missing link is the observation that overexpression of RAD51 partially complements (i.e. suppresses) defects in a number of different HRR genes that encode recombination mediator and co-mediator proteins, including BRCA1 and BRCA2. Recombination mediators and co-mediators are proteins that assist RAD51 in displacing the single-strand DNA (ss-DNA)-binding protein RPA from ss-DNA, thus initiating the formation of the RAD51 filament. Complementation by RAD51 overexpression, which has not been reviewed before, is the central focus of this article.

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RAD51 expression is up-regulated in many cancers

For many years it has been observed that the levels of the RAD51 protein are greatly elevated (~2–7-fold) in many cancer cell lines and in primary tumors [reviewed by (6)]. The wild-type p53 protein plays an important role both in suppressing the transcriptional expression of *RAD51* and in down-regulating RAD51 protein activity (4,5,7,8). The advantage for cancer cells of overexpressing RAD51 has yet to be adequately explained, but it has been suggested that the high levels of RAD51 are involved in tumor progression by destabilizing the genome (9). Another possible explanation is that elevated RAD51 confers a DNA replication advantage during the more rapid cell divisions that follow the activation of oncogenes and inactivation of tumor suppressors. Each of these explanations, at least in their basic form, suffers from the fact that, in general, overexpression of RAD51 is deleterious and slows cell growth *in vitro*, and therefore would also be likely to hinder the growth of cancer cells *in vivo*. Based on published reports about the ability of overexpressed RAD51 to suppress defects in HRR genes, two new alternative hypotheses are presented at the end of this article that may explain why *RAD51* is up-regulated in many human cancers.

RECOMBINATION MEDIATORS AND CO-MEDIATORS

The HRR pathway is composed of a highly orchestrated network of proteins, many of which presumably still remain to be discovered. HRR frequently is initiated by a double-strand break, either due to endogenous lesions at the replication fork, or induced by different DNA damaging agents, including cisplatin, mitomycin C (MMC), camptothecin (CPT) and ionizing radiation. First, endonucleolytic activity produces a long 3′ ss-DNA overhang that is rapidly coated with RPA, the heterotrimeric ss-DNA-binding protein. Displacement of RPA by RAD51 is critical and highly regulated, and initiates the strand transfer process (Figure 1). The proteins directly involved in the displacement of RPA are named 'recombination mediators' (Table 1) (10,11) (here sometimes referred to as just 'mediators'). Recombination mediator activity is ideally assayed by biochemical methods (10,12–15). In place of biochemical evidence, reductions in RAD51 foci formation are frequently utilized as an indirect indicator of a mediator defect (16–18), if the defective/missing protein is not required for the formation of RPA-coated ss-DNA. It is significant that mutations in most of these mediators in mammalian cells result in high levels of genomic instability, as has been demonstrated for cells with deletions of *RAD51D* in both Chinese hamster ovary cells (CHO) and murine embryonic fibroblast cells (MEFs) (19,20). Very high levels of mutagenesis were observed in *Rad51d* deletion CHO cells (19), and increased mutagenesis seems likely in other mediator defective cell lines as well.

There are also a number of proteins that function to assist these mediators or localize them to the site of the DNA break. These proteins have not previously been
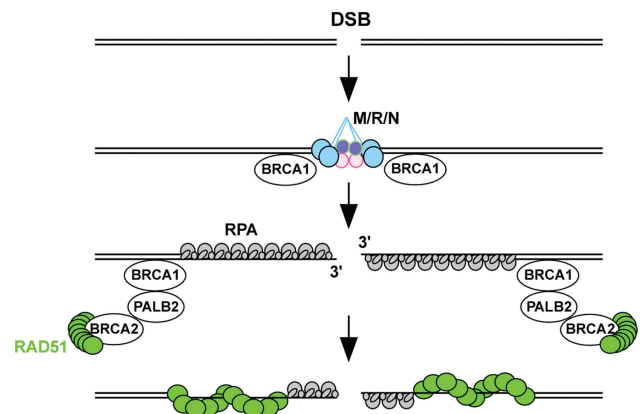
Figure 1. Simplified schematic to depict the mediator step of recombination: displacement of RPA by RAD51. A critical step in homologous re-combinational repair is the displacement of RPA, the trimeric ssDNA-binding protein, by the RAD51 strand transfer protein. This step is highly regulated by cells to ensure that potentially deleterious events are avoided. Many different proteins are involved in assisting RAD51 to displace RPA at this stage, and the proteins that are directly involved are known as the 'recombination mediators' (Table 1). There are also additional proteins that function to assist the mediators or assist in their localization to DNA damage, and in this review, the proteins assisting the mediators are defined as 'recombination co-mediators'. Human BRCA2 is a mediator that interacts directly with approximately eight RAD51 molecules and transports them to the site of ss-DNA bound by RPA, while PALB2 and BRCA1 are co-mediators that directly and indirectly, respectively, interact with BRCA2 and assist in localizing it, with RAD51 bound, to the sites of DNA damage (for references, see Table 1). DSB, double-strand DNA break; M/R/N, MRE11-RAD50-NBS1 complex.

given a general name, but we propose to call them 'recombination co-mediators'. Many of the mediators and co-mediators are known tumor suppressor proteins, and mutant versions frequently can be suppressed by overexpression of RAD51, as discussed below. This makes considerable biological sense, since these proteins function to assist RAD51, but in their absence, RAD51 overexpression can in some cases partially compensate. It is still unknown whether defects in other HRR proteins (i.e. non-mediators such as MRE11 and RAD54) can also be suppressed by RAD51 overexpression. Also, only some of the mediators/co-mediators have been tested for suppression by RAD51 overexpression, and there are additional proteins that may also be mediators/co-mediators, since they interact with BRCA1 or BRCA2 (i.e. BARD1, BCCIPα and β, BRIP1/FANCJ/BACH1 and SHFM1/DSS1). With the use of the RNAi technique, it should be relatively easy to test these other potential human mediators/co-mediators for complementation by RAD51 overexpression. Ideally these experiments should be done in human cell lines that initially express low levels of endogenous RAD51.

FUNCTIONAL SUPPRESSION BY RAD51 OVEREXPRESSION

Functional suppression in model organisms

Overexpression of RAD51 has been shown to partially suppress defects in recombination mediators and

Table 1. Human recombination mediator and co-mediator proteins

	Involved in cancer[a]	Suppressed by RAD51 overexpression in DT40 or human cells	References[b]
Recombination mediators			
BRCA2/FANCD1	Yes	Yes	(13,15)
RAD51 paralogs			
RAD51B/RAD51L1	No evidence yet	Yes	(14,18,56,57)
RAD51C	No evidence yet	Yes	(14,18,56,57)
RAD51D/RAD51L3	No evidence yet	Yes	(18,56,57)
XRCC2	No evidence yet	Yes	(18,56,57)
XRCC3	No evidence yet	Yes	(18,56–58)
RAD52	No evidence yet	nt[c]	(12,59)
Recombination co-mediators			
BRCA1	Yes	Yes	(24,60)
CHK2	Yes	nt	(61)
PALB2/FANCN	Yes	nt	(60,62,63)
SWS1	No evidence yet	nt	(64)

Potential mediators/co-mediators	Main interacting protein partner	References
BARD1	BRCA1	(65)
BCCIPα&β	BRCA2	(66)
BRIP/FANCJ/BACH1	BRCA1	(67)
DSS1/SHFM1	BRCA2	(68)

[a]Direct involvement in cancer, since inherited mutations result in a pre-disposition towards increased cancer, particularly breast and ovarian (1–3), and some mutant forms are also found in sporadic cancers (3).
[b]Top part: recent references to recombination mediator and co-mediator functions; for additional information/references see review articles (1,2,11,63,69,70); bottom part: references to protein interactions with BRCA1 or BRCA2.
[c]nt, not tested yet.

co-mediators in yeast, avian, rodent and human cells, although suppression of the HRR defect by exogenous RAD51 is generally not as complete as the complementation by a wild-type copy of the HRR gene that is mutated. In addition, in these experiments many different end-points were assessed. For each end-point, the relative degree of complementation by exogenous RAD51 differs, for unknown reasons.

Yeast. In *Saccharomyces cerevisiae*, two different groups observed that cells with deletion mutations in *RAD55* and *RAD57*, the two yeast RAD51 paralogs, are partially suppressed by overexpression of RAD51 (21,22). In addition, overexpression of RAD52 resulted in partial suppression of both X-ray sensitivity and gene conversion defects, and simultaneous overexpression of both RAD51 and RAD52 gave near complete suppression for both phenotypes (21). Furthermore, although most mutations in *RAD52* are not suppressed by RAD51 overexpression, there is one mutation in *RAD52* that is suppressed (23). Since *S. cerevisiae* lacks both *BRCA1* and *BRCA2* genes, it is not possible to test defects in these genes for complementation by RAD51 overexpression.

Chicken DT40 cells. The chicken DT40 cell line (p53 deficient) has been extensively used to characterize DNA repair defects. Each of the five RAD51 paralog genes has been separately knocked out in DT40 cells, and each knockout is partially complemented by overexpression of human RAD51 (16,17). The complementation of $RAD51B^{-/-}$ by hRAD51 overexpression was the most extensively studied, and very interestingly, the authors observed significant complementation for MMC, cisplatin

and X-ray sensitivity and for genomic instability, but not for homologous integration events (16). This result argues that in DT40 cells and under conditions where a RAD51 paralog is lost, overexpression of human RAD51 largely, but not fully, ameliorates the HRR-deficiency. Interestingly, this complementation occurred in $RAD51B^{-/-}$ cells in which the level of the hRAD51 was approximately equal to the level of the endogenous avian RAD51, suggesting that simply doubling the level of RAD51 was sufficient for such suppression in these cells. In addition, RAD51 overexpression partially complemented a *BRCA1* deletion, since overexpression of human RAD51 (~15-fold increase relative to the endogenous protein) in $BRCA1^{\Delta/\Delta}$ DT40 cells almost completely rescued defects in cell proliferation (i.e. slow growth) and in DNA damage survival (Figure 2), and also partially rescued the defect in gene targeting frequency (24). These investigators also showed that human tumors with *BRCA1* mutations frequently exhibit elevated *RAD51* transcripts, as well as elevated transcripts of *RAD51AP1* and *RAD54*, encoding two relatively late acting HRR proteins (24). This is indirect evidence that these *BRCA1*-deficient tumors had undergone selection for overexpression of *RAD51*, *RAD51AP1* and *RAD54*, and the authors suggested a somewhat similar hypothesis to the one presented here: 'The growth and viability defects of BRCA1-deficient cells are difficult to reconcile with the deregulated growth of BRCA1-deficient tumors. The findings we present here suggest that one mechanism by which this apparent paradox is resolved is via genetic suppression of BRCA1 phenotypes by up-regulation of
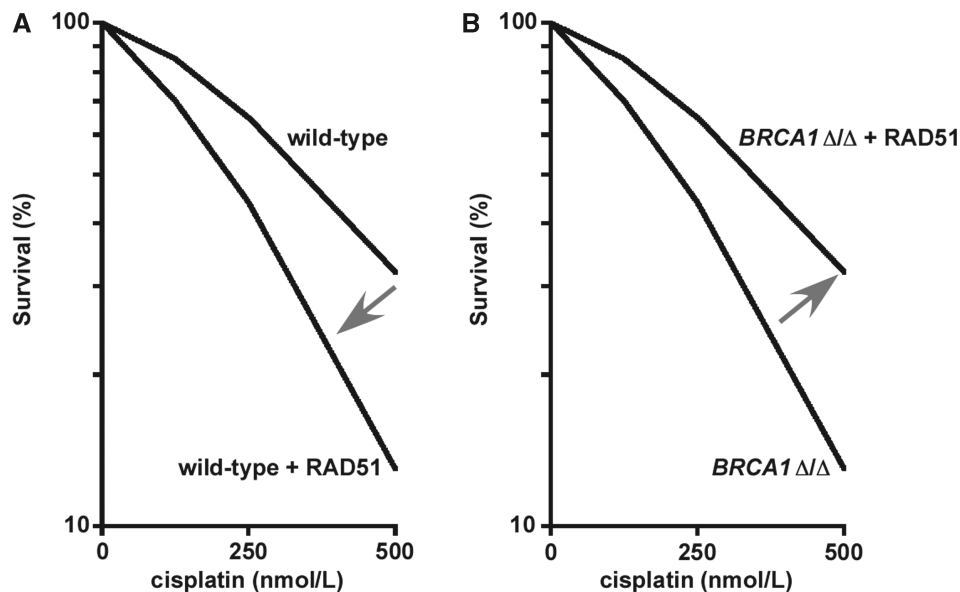
Figure 2. Effects of RAD51 overexpression in isogenic wild-type and *BRCA1*-deleted DT40 cells. (**A**) In cells with wild-type HRR, overexpression of RAD51 results in a reduced ability to repair cisplatin-induced DNA damage. Such increased sensitivity to a DNA cross-linking agent frequently coincides with genomic instability in untreated HRR-defective cells, presumably due to unrepaired spontaneous damage (16,17,44). (**B**) Alternatively, in cells with a *BRCA1* homozygous deletion, the HRR defect is complemented by RAD51 overexpression. The complemented cells also presumably have increased genomic stability, a concept incorporated into Model I. Importantly, comparing just the cells overexpressing RAD51 (in both panels), the *BRCA1* deletions actually result in increased resistance to cisplatin, and presumably also increased genomic stability, a concept incorporated into Model II. This figure is adapted directly from the data in Figure 2D of (24), with permission from Douglas K. Bishop and AACR.

HR. The fact that the majority of BRCA1-deficient tumors examined exhibit significantly up-regulated expression of HR genes suggests either that there is a selection for such up-regulation during tumor development or that a high level of expression is a precondition for tumorigenesis of BRCA1-deficient cells.' (24)

Functional suppression in mammalian cells

In the first direct studies in mammalian cells, two recent independent publications report that *BRCA2* defects are suppressed by RAD51 overexpression. In one study, the *BRCA2* mutant Capan-1 human pancreatic carcinoma cell line (with mutant p53) was shown to be partially complemented by overexpression of RAD51, assaying for cell survival after exposure to X-rays or cisplatin (25). In addition, complementation was better, and nearly complete, when a mutant form of RAD51 that is resistant to caspase-3 cleavage was expressed. In the same study, similar results on complementation by overexpression of wild-type RAD51 were also observed for *Brca2* mutant MEFs. In the second study, mouse hybridoma cell lines depleted for *Brca2*, using stable expression of siRNA, were studied for the effect of overexpression of the mouse Rad51, Rad52 and Rad54 proteins (26). In mouse hybridoma cells with low levels of Brca2, the reduced frequency of targeted integration and DNA damage-induced Rad51 foci was partially complemented by overexpression of Rad51, but not by overexpression of either Rad52 or Rad54.

We have found only one relevant report that demonstrated a lack of complementation by human RAD51, and these experiments were carried out in

Xrcc3-deficient CHO cells (27). In this study, complementation by RAD51 was assessed using an integrated recombinational reporter plasmid. It is possible that, if these *Xrcc3*-deficient CHO cells had been tested for the cellular sensitivity to MMC or cisplatin, a different result with respect to RAD51 complementation would have been observed, as discussed earlier for the DT40 *RAD51B*$^{-/-}$ cell line (16). It is worth noting that RAD51 is one of the most highly conserved proteins known. Consequently, the fact that the heterologous human RAD51 protein was expressed in *Xrcc3*-deficient CHO cells is unlikely to have biased the results reported by Pierce and collaborators (27). There is a *Brca2* mutant hamster V79 cell line (with mutant *Trp53*) (28), which would provide a unique tool to study whether or not wild-type human RAD51 can complement *Brca2*-deficiency in a hamster background.

EFFECTS OF ECTOPIC OVEREXPRESSION OF RAD51

Since the effects of RAD51 overexpression in many different cellular systems have recently been extensively reviewed (6), the results of only some studies will be discussed here. In chicken DT40 cells with wild-type HRR, overexpression of human RAD51 resulted in increased cellular sensitivity to cisplatin, and this enhanced sensitivity of wild-type DT40 cells was equal to the cellular sensitivity of *BRCA1*$^{\Delta/\Delta}$ DT40 cells that did not express ectopic RAD51 (24). On the other hand, when RAD51 was overexpressed in the *BRCA1*$^{\Delta/\Delta}$ DT40 cells, near

complete complementation was observed for cell survival after both cisplatin and X-rays. These experiments suggest that overexpression of RAD51 in cells with normal HRR can be deleterious, while overexpression in cells with an HRR defect can be beneficial (Figure 2). (Note that the authors did not see X-ray sensitization of wild-type DT40 cells following RAD51 overexpression, but this test is not very sensitive, since non-homologous end-joining is the major repair pathway for X-ray damage.)

In wild-type (i.e. HRR-proficient) mammalian cells, the effects of ectopic RAD51 overexpression are somewhat contradictory, but this may relate to the end-points assessed, the levels of RAD51 overexpression achieved, the fraction of RAD51 that is cytoplasmic versus nuclear and the p53 status of the cell lines utilized. In CHO cells, overexpression of hamster RAD51 resulted in increased HRR, while a second study reported that overexpression of human RAD51 in CHO cells reduced homologous recombination at a double-strand break (29,30). In mouse ES cells, RAD51 overexpression led to aneuploidy and chromosomal rearrangements (9). In the human fibrosarcoma HT1080 cell line, ectopic overexpression of human RAD51 was very deleterious to cells, resulting in a slow growth phenotype and increased levels of apoptosis (31).

UP-REGULATION OF RAD51 IN TUMORS AND CANCER-DERIVED CELL LINES

Many different cancer-derived cell lines, as well as many tumors have been shown to have higher than normal levels of RAD51, as recently reviewed (6). The exact cause of this overexpression is not known, but there are important clues. For example, wild-type p53 has been reported to suppress the transcriptional regulation of *RAD51*, and *TP53* deletions and some *TP53* point mutations have been shown to up-regulate the expression of RAD51 (7). In addition to its role in transcriptional repression of *RAD51*, the wild-type p53 protein directly interacts with the RAD51 protein, inhibiting its activity (8).

There are also a number of additional factors that appear to play a role in *RAD51* regulation. There is evidence that transcriptional activator protein 2 (AP2), in combination with p53, functions to down-regulate *RAD51* transcription (32). In addition, there is evidence that the oncogenic fusion tyrosine kinase BCR/Abl, the result of translocations, increases RAD51 expression (33) and indirect evidence that c-Abl is also involved in up-regulating *RAD51* transcription, since imatinib (Gleevec), a relatively specific inhibitor of c-Abl, reduced the elevated levels of RAD51 in some cancer cell lines (34,35). Recently, it has also been reported that unlike p53, wild-type p63 and p73 act as inducers of both basal and DNA-damage-induced levels of RAD51 in MEFs (36). Although, many cancer cell lines and primary tumors overexpress RAD51 (6), chronic hypoxia has been shown to decrease the level of RAD51, leading to reduced levels of HRR in hypoxic tumors that also show increased sensitivity to DNA cross-linking agents (37,38). In general, a more complete understanding of how

RAD51 is regulated may lead to better treatments for cancer patients.

TP53 MUTATIONS ARE UNDER REPRESENTED IN MISMATCH REPAIR-INITIATED CANCERS

Approximately 50% of all cancers have mutations in *TP53*. In particular, cancers initiated by general genomic instability frequently have mutations in *TP53*. Alternatively, cancers initiated by mutations in genes of the mismatch repair (MMR) pathway, and the subsequent microsatellite instability (MSI), are less frequently associated with mutations in *TP53* (39,40). This disparate occurrence of *TP53* mutations has never been satisfactorily explained. The hypothesis presented here, that one of the main functions of mutant p53 is to up-regulate RAD51, takes this disparity into account and provides an explanation for it, as discussed below. It is important to note that *TP53* mutations are very heterogeneous (4,41), and that only a few mutant versions, other than the *TP53* homozygous deletion, have been tested for their effects on up-regulating *RAD51* expression.

Model I: UP-REGULATION OF RAD51 SUPPRESSES HRR DEFECTS TO RESTABILIZE THE GENOME

We proposed that carcinogenesis is frequently initiated by defects in HRR genes (particularly in genes encoding recombination mediators or co-mediators) that result in genomic instability (Figures 3A and 4A). There are likely to be many ways for a normal cell to develop an HRR defect, since HRR is a complex DNA repair pathway with many known genes involved and presumably some genes yet to be discovered. In addition, there is evidence for haploinsufficiency for some HRR defects, and epigenetic silencing events also may account for compromised HRR ability. Interestingly, there is evidence that point mutations in known HRR genes are not common in either breast or colorectal cancers: in a study of these two cancers, most of the transcripts from 11 cancers of each type were sequenced and only a small number of mutations in a few HRR-related genes were identified in cancers of the breast (i.e. *BCCIP*, *BRCA1*, *BRCA2*, *MRE11*, *FANCA* and *FANCM*) and of the colon (*FANCG*) (42). Of these, only mutations in *BRCA1* and *BRCA2* are known to be suppressible by RAD51 overexpression.

Genomic instability presumably facilitates both the activation of oncogenes and the inactivation of tumor suppressor genes (with the exception of *TP53*, which in the current model occurs as a later event). However, genomic instability initiated by defects in HRR is also deleterious due to the inhibitory effects on cell growth. Therefore, there is pressure to suppress genomic instability initiated by HRR deficiencies, and *TP53* mutations and/or mutations in other genes are selected for partially because they lead to the up-regulation of RAD51, which then at least partially suppresses the initial HRR defect. It also seems likely that due to synergistic effects, some cancers may
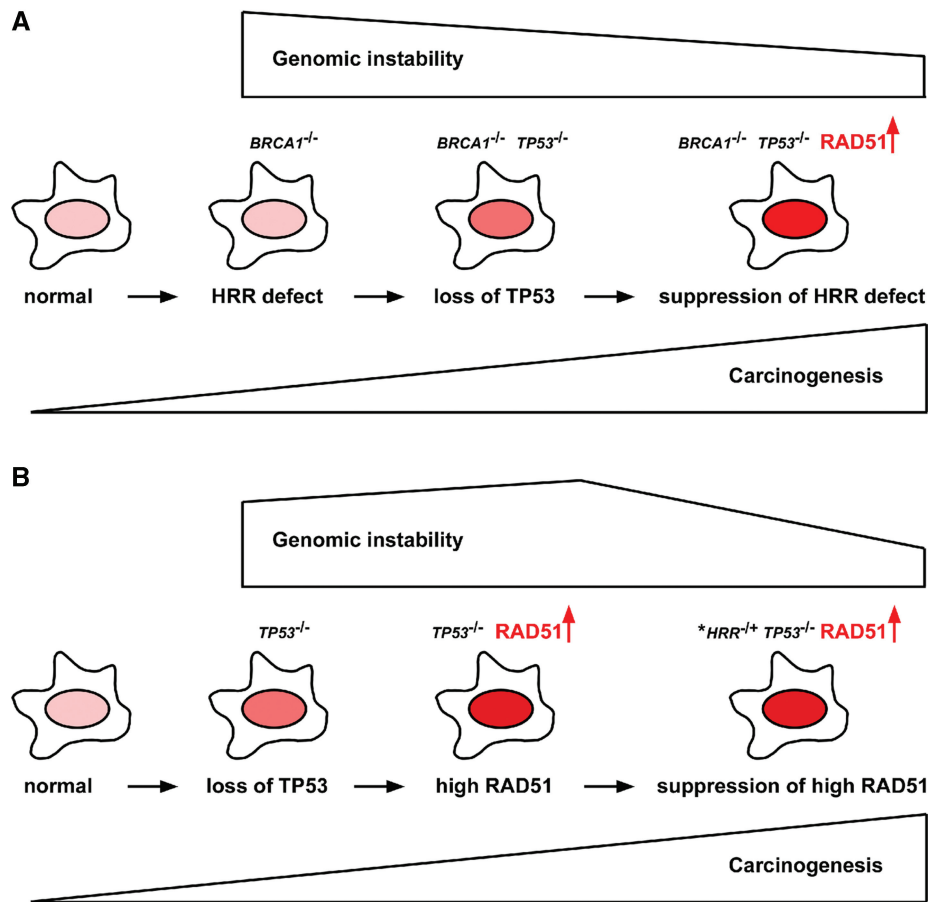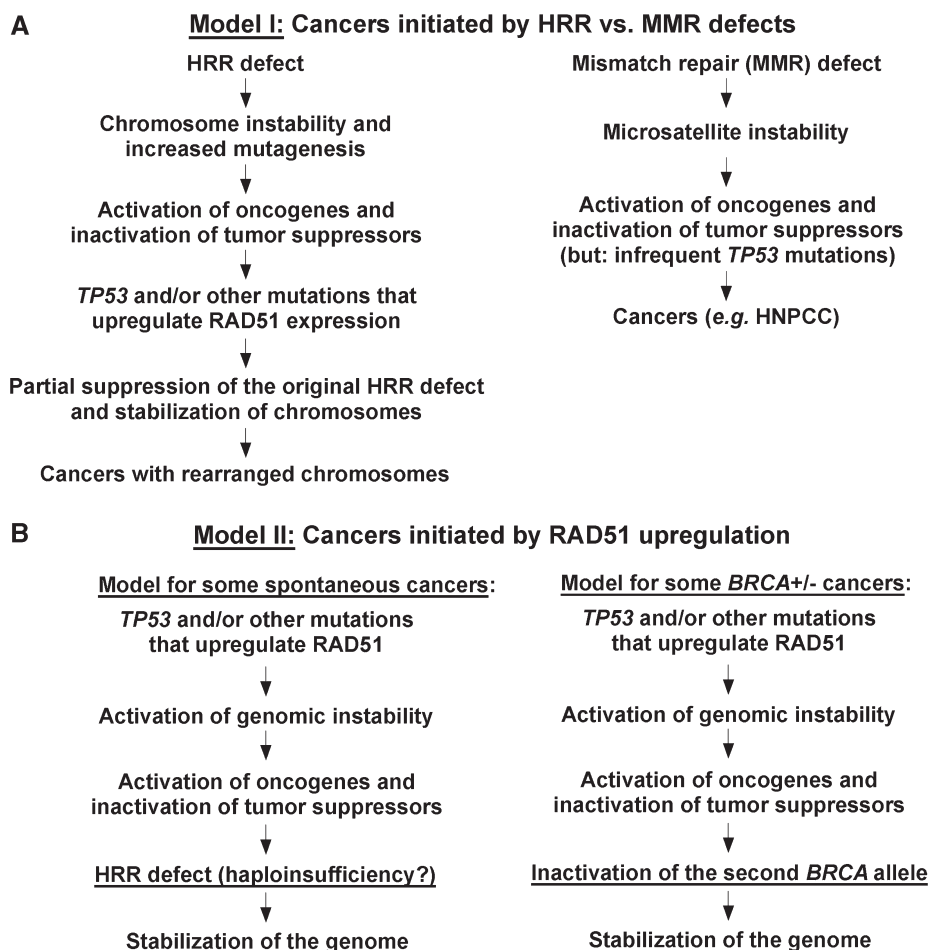
Figure 3. Schemes to explain the order of events in the two proposed models. (**A**) Model I: normal cells that develop an HRR defect (e.g. $BRCA1^{-/-}$) select for loss of *TP53*, which then acts to up-regulate RAD51 expression (darker nuclei represent higher levels of RAD51). Overexpression of RAD51 can in some cases lead to partial suppression of the original HRR defect, helping to stabilize the genome. (**B**) Model II: in some cancers, *TP53* mutations are early events and these mutations may up-regulate RAD51 expression, resulting in increased genomic instability. Subsequent mutations/silencing in an HRR function partially suppresses the phenotype of RAD51 overexpression, reducing genomic instability. The asterisk indicates that for sporadic cancers many of these HRR mutations are likely to be in genes that exhibit haploinsufficiency, or the mutations themselves may be dominant-negative alleles.

select for the overexpression of both RAD51 and HRR proteins that function later than the recombination mediators/co-mediators. [Note that there is evidence that RAD51AP1, which is not a RAD51 paralog, functions later in HRR than the recombination mediators (43,44), and RAD51AP1 has been shown to be up-regulated in a number of cancers (24,45,46)]. With regard to genomic rearrangements, there is evidence from cell lines derived from breast cancers, that genomic stability appears to be partially restored during tumor development (47), consistent with the proposed model. The concept that genomic instability might be restored by RAD51 overexpression during tumor progression, as previously suggested by the Bishop laboratory (24,48), has been expanded here.

We also propose that cancers initiated by MMR defects do not select for *TP53* mutations. Such mutations might up-regulate RAD51, which would be deleterious in HRR-proficient cells. Besides, in such cancers there is no need to up-regulate RAD51 for suppression purposes, and the precancerous cells achieve the additional benefits of *TP53* inactivation, such as down-regulation of both

apoptosis and checkpoint functions, by selecting for mutations in other genes that do not affect RAD51 expression.

The model presented here, if correct, has profound implications for how cancers might be treated more effectively in the future. Our proposed hypothesis argues that many more cancers may arise from HRR defects than has previously been suggested. If so, restoration of RAD51 to normal levels (i.e. no longer suppressing the original HRR defect) may sensitize these tumor cells to genotoxic agents that kill HRR defective cells. Imatinib has been reported to suppress the overexpression of RAD51, while not strongly affecting the levels of RAD51 in non-cancerous cells (35). Imatinib sensitizes tumors to radiation and to chemotherapy (34,35), and the model presented here provides a new perspective on such cancer treatment, that is based on the pro-oncogenic activity of RAD51 overexpression. Recently, PARP inhibitors have been used to target tumors with HRR defects such as *BRCA1* and *BRCA2* (49). Since PARP inhibitors preferentially target HRR-defective tumor cells, we propose that a combination therapy using both imatinib-type compounds and

A   <u>Model I: Cancers initiated by HRR vs. MMR defects</u>

HRR defect

↓

Chromosome instability and
increased mutagenesis

↓

Activation of oncogenes and
inactivation of tumor suppressors

↓

TP53 and/or other mutations that
upregulate RAD51 expression

↓

Partial suppression of the original HRR defect
and stabilization of chromosomes

↓

Cancers with rearranged chromosomes

Mismatch repair (MMR) defect

↓

Microsatellite instability

↓

Activation of oncogenes and
inactivation of tumor suppressors
(but: infrequent *TP53* mutations)

↓

Cancers (*e.g.* HNPCC)

B   <u>Model II: Cancers initiated by RAD51 upregulation</u>

<u>Model for some spontaneous cancers</u>:

TP53 and/or other mutations
that upregulate RAD51

↓

Activation of genomic instability

↓

Activation of oncogenes and
inactivation of tumor suppressors

↓

<u>HRR defect (haploinsufficiency?)</u>

↓

Stabilization of the genome

<u>Model for some *BRCA*+/- cancers</u>:

TP53 and/or other mutations
that upregulate RAD51

↓

Activation of genomic instability

↓

Activation of oncogenes and
inactivation of tumor suppressors

↓

<u>Inactivation of the second *BRCA* allele</u>

↓

Stabilization of the genome

Figure 4. (**A**) Summary of Model I explaining cancer development initiated by HRR defects. The model suggests that many cancers with genomic instability are initiated by HRR defects, just as cancers with MSI are initiated by MMR defects. In cancers with HRR defects, selection for up-regulation of RAD51 acts to suppress the original HRR defect, partially restoring genomic stability and enhancing cell proliferation. *TP53* mutations frequently result in the up-regulation of RAD51, and are selected for in cancers with a pre-existing HRR defect. HNPCC, hereditary non-polyposis colorectal cancer. (**B**) Summary of Model II explaining cancer development initiated by RAD51 up-regulation. In this model, inactivation of *TP53* and up-regulation of RAD51 occur early in sporadic oncogenesis (i.e. cancers initiated by spontaneous non-inherited mutations) (left side). In some *BRCA* heterozygous cells (*BRCA1*$^{+/-}$ or *BRCA2*$^{+/-}$), these events occur prior to functional loss of HRR resulting from a mutation or silencing of the second *BRCA* allele (right side). These are identical models, with the exception of the underlined phrases.

PARP inhibitors may be highly potent in the treatment of RAD51 overexpressing cancers.

MODEL II: RAD51 UP-REGULATION LEADS TO GENOMIC INSTABILITY FIRST, FOLLOWED BY SELECTION FOR INACTIVATION OF AN HRR GENE TO RESTABILIZE THE GENOME

Overexpression of RAD51, like mutations in HRR, can destabilize the genome (9), as previously discussed. An alternative to Model I is that in some cancers the up-regulation of RAD51 is an early event (Figures 3B and 4B). Subsequently, as carcinogenesis proceeds, there is selection for an HRR defect to specifically counteract the effects of RAD51 overexpression. The consequence of each of these two proposed cancer developmental processes (i.e. Models I and II) is the same (i.e. cells with both an HRR defect and higher than normal levels of RAD51 protein), but the order of events is reversed.

The alternative Model II helps to explain why in some *BRCA*$^{+/-}$ carriers, inactivation of the second *BRCA* allele is a late event (50). This model suggests that in cancers arising by this order of events, the *BRCA* heterozygosity has little to do with the early events of carcinogenesis, but is required to rescue highly unstable cancer cells from the RAD51 up-regulation. It is important to note that, although both models are mutually exclusive to explain the origin of a single tumor, each hypothesis may correctly explain the origin of some cancers. For both sporadic and inherited predisposition-related cancers, some may have evolved with an HRR defect first, while others may have evolved first with a *TP53* mutation and/or other mutation that up-regulates RAD51.

Although there appears to be no cancer-related mutations in the *RAD51* coding region, there is a single-nucleotide polymorphism (SNP) in the 5′-untranslated region of *RAD51* that increases the likelihood of breast cancer in *BRCA2*$^{+/-}$ carriers (51). This SNP has been

shown to significantly increase the relative levels of the SNP-containing *RAD51* transcripts ($\sim$2–3-fold) for a reporter construct expressed in the U2-OS osteosarcoma cell line (52). The increased cancer predisposition of *BRCA2* carriers with this *RAD51* SNP can be explained by either of the two different models presented. For each model, the up-regulation of RAD51 is a necessary event for carcinogenesis, and the reported *RAD51* SNP may help to facilitate this up-regulation.

FUTURE RESEARCH FOR EXAMINING RAD51 OVEREXPRESSION AND SUPPRESSION

There are a number of questions that should be experimentally addressed to further examine RAD51 overexpression and complementation. (i) Which HRR genes/defects are suppressed by RAD51 overexpression, are all of these suppressible HRR defects in either recombination mediator or co-mediator genes, and is there suppression of mediators that also function at an additional stage of HRR? (ii) Are elevated levels of RAD51AP1 and/or RAD54 synergistic to RAD51 overexpression in this suppression? (iii) In addition to *TP53, TP63, TP73, c-Abl* and *AP2*, what other genes are involved in regulating the expression of *RAD51*? (iv) Is RAD51 expression and/or activity regulated by translational and/or post-translational mechanisms (presumably fruitful areas for further study)? (v) Do most tumors that overexpress RAD51 have suppressed HRR defects and also *TP53* mutations? (vi) If epigenetic silencing is involved in inactivating HRR functions, can these silencing events be reversed by drugs, and if so, is this reversal lethal or semi-lethal to cancer cells with high levels of RAD51 protein, even in the absence of genotoxic agents?

There is also a great need for additional mechanisms to assay for the levels of *RAD51* transcript, protein and foci in primary tumors. A recent study successfully analyzed RAD51 foci, as well as BRCA1 and FANCD2 foci, in sporadic breast cancer biopsies (treated with X-rays *ex vivo*), and the absence of such foci was closely correlated with likely defects in the BRCA1 pathway (53). Improved mechanisms for analyzing RAD51 levels and re-localization/DNA-damage foci should be useful in the analyses of primary tumors and help to determine potential treatment modalities.

CONCLUDING REMARKS

In summary, overexpression of RAD51 in a number of different organisms has been shown to partially suppress defects in recombination mediator and co-mediator functions. In addition, *RAD51* is frequently up-regulated in cancer cell lines and in primary tumors, although *RAD51* overexpression in the absence of any underlying HRR defect is frequently detrimental to growth of cells, at least in tissue culture.

Largely based on the published findings reviewed here, two closely related models are proposed that may help to explain the role of *RAD51* up-regulation in cancers.

It is worth remembering that cancer is an extremely complex set of diseases, and that cancer cells develop many different mechanisms to achieve the same phenotype of independent and uncontrolled growth (54,55). The models presented here strive to explain certain aspects of oncogenic progression, and if these models prove correct, they will point the way to how some cancers arise and to novel roles for HRR functions during oncogenesis.

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