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Molecular Pathways:

Understanding the Role of Rad52 in Homologous Recombination for Therapeutic Advancement

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Abstract

The Rad52 protein was largely ignored in humans and other mammals when the mouse knockout revealed a largely “no-effect” phenotype. However, using synthetic lethal approaches to investigate context dependent function, new studies have shown that Rad52 plays a key survival role in cells lacking the function of the BRCA1-BRCA2 pathway of homologous recombination. Biochemical studies also showed significant differences between yeast and human Rad52, in which yeast Rad52 can promote strand invasion of RPA-coated single-stranded DNA in the presence of Rad51, but human Rad52 cannot. This results in the paradox of how is human Rad52 providing Rad51 function: presumably there is something missing in the biochemical assays that exists in-vivo, but the nature of this missing factor is currently unknown. Recent studies have suggested that Rad52 provides back-up Rad51 function for all members of the BRCA1-BRCA2 pathway, suggesting that Rad52 may be a target for therapy in BRCA pathway deficient cancers. Screening for ways to inhibit Rad52 would potentially provide a complementary strategy for targeting BRCA-deficient cancers in addition to PARP inhibitors.

Background

Eukaryotic cells are exposed to both endogenous and exogenous insults to their genome. To foster genomic maintenance and protection, an elaborate DNA damage response (DDR) and DNA repair network evolved to encompass multiple repair pathways, each specializing in specific types of DNA lesions. Various sensor, mediator, and effector proteins are required to initiate and complete repair of damaged DNA. Deficiencies in essential components of the DDR or DNA repair pathways results in cell death or accumulation of mutations that can progress to cancer or other disease. For example, ataxia telangiectasia, ataxia-telangectasia-like disorder (AT-LD), Njimegen Breakage syndrome (NBS), and xeroderma pigmentosum are caused by mutations of ATM(1), Mre11(2), NBS1, and XPA respectively. In addition, mutations in the tumor suppressors genes BRCA1 and BRCA2 greatly increase susceptibility to breast, ovarian, and other cancers(3).

One of the most threatening forms of DNA damage is the DNA double-strand break (DSB), as both strands of the DNA duplex are impaired simultaneously. The major repair pathways that cope with DSBs are non-homologous end-joining (NHEJ) and homologous recombination (HR). In many organisms, Rad52 is a key protein involved in the HR

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pathway. This review will focus on Rad52, its role in HR and the translational opportunities available through understanding this protein and its function.

Homologous recombination pathways

Homologous recombination is the exchange of genetic information between allelic sequences and has an essential role in both meiosis and mitosis. In meiosis, HR allows for exchange of genetic material between paternal and maternal alleles within the gamete, and also coordinates proper segregation of homologous chromosome pairs during the first meiotic division.

In the somatic cell, HR maintains genomic integrity by promoting accurate repair of DSBs, damaged replication forks, and DNA interstrand crosslinks. HR repair requires a second, homologous DNA sequence to function as a donor template. In brief, the steps of DSB repair (DSBR) by HR are as follows: (*a*) recognition of a DSB, (*b*) processing of the DSB by nucleases to generate 3' single-stranded DNA (ssDNA) tails, (*c*) formation of a Rad51 recombinase filament on ssDNA ends, (*d*) strand invasion into the homologous sequence with formation of the D-loop intermediate, (*e*) DNA polymerase extension from the 3' end of the invading strand, (*f*) capture of the second end of the DSB by strand annealing after extension of the D-loop, (*g*) formation of two Holliday junctions (HJ), and (*h*) dissolution or resolution of the HJs to form crossover or noncrossover products (Fig 1, *left*). Of note, the Rad51 recombinase is the essential enzyme that mediates strand invasion of a DNA duplex resulting in an exchange of DNA strands during HR.

The synthesis-dependent strand-annealing (SDSA) pathway of HR differs from the DSBR pathway by diverging following step (*e*) and is not dependent on the second HJ formation. Rather, SDSA relies upon displacement of the invading strand and annealing it with the second resected DSB end, ultimately, producing a noncrossover product (Fig 1, *left*).

When a DSB is flanked closely by sequence repeats, repair of the DSB may occur through a process called single-strand annealing (SSA). In SSA the DSB ends are resected, but rather than identifying a homologous DNA template for strand invasion, the resected strands anneal to one another using the repeat sequences for annealing. The result is a deletion of the sequence between the direct repeats (Fig 1, *right*). Since SSA is independent of strand invasion, the components involved in this process, such as Rad51 filament formation, and the downstream events, including HJ resolution, are not required(4).

Single-ended DSBs can occur at telomeres or at broken replication forks. These can be repaired by HR through a single-ended invasion process known as break-induced replication (BIR)(5). Most BIR events are dependent on Rad51 and other HR factors used in DSBR and SDSA. This process can also occur without Rad51 protein although the Rad51-independent process is thought to be only a minor component of this reaction.

A number of excellent reviews have been published with more in-depth discussion of HR and its sub-pathways (SSA, BIR)(4; 6; 7).

Saccharomyces cerevisiae Rad52 protein and its function in mediating recombination

The *S. cerevisiae* Rad52 protein (ScRad52) was identified in a genetic screen for mutants that are sensitive to ionizing radiation (IR)(8) and is the most studied recombination mediator. Indeed, many other HR genes are labeled under the heading of *RAD52* epistasis group, which includes *RAD50*, *RAD51*, *RAD54*, *RAD55*, *RAD57*, *RAD59*, *MRE11*, and *XRS2*. Of all members of the *RAD52* epistasis group, the absence of *RAD52* confers the most severe defects due to its involvement in all known pathways of HR, both *RAD51*-dependent (DSBR, SDSA) and the *RAD51*-independent pathway of SSA(6).

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Rad52 forms an oligomeric ring, and the oligomerization is mediated by the N-terminal portion of the protein(9). This N-terminal portion specifically binds ssDNA(10). ScRad52 mediates Rad51 strand invasion by physically associating with the Rad51 protein(11) and allowing for highly efficient reversal of replication protein A (RPA)-imposed inhibition of the ssDNA-dependent ATPase and recombinase activity of Rad51(12–14).

Rad52 also directly associates with RPA(15), a hetero-trimeric ssDNA binding protein that coats the resected ends of the DSB (Fig 1). Both the largest (RPA70) and intermediate (RPA32) subunits demonstrate direct binding with ScRad52(16; 17). The specific interaction of RPA and ScRad52 appears important for the recombination mediator function of ScRad52, as demonstrated when RPA is substituted with the *Escherichia coli* single-stranded DNA-binding protein (SSB), ScRad52 is not able to overcome SSB-inhibition and subsequently fails to promote Rad51-mediated homology search and strand exchange(13).

Additional functions of *S.cerevisiae* Rad52

ScRad52 is required for the Rad51-independent SSA and BIR reactions(4; 5). In concordance with its role in SSA, ScRad52 is able to anneal DNA strands that are either bare or coated with RPA(16; 18). There is some evidence to suggest that Rad52-mediated annealing of RPA-coated ssDNA strands is important for second ssDNA end capture in the DSBR pathway of HR(19).

Human recombination mediators: Rad52 and BRCA2

Human Rad52 (hRad52) is similar to ScRad52 structurally and biochemically. hRad52 exists in an oligomeric form, binds ssDNA, promotes ssDNA annealing and, under certain specialized conditions, simulates Rad51-mediated homologous DNA pairing(20). Like ScRad52, hRad52 has conserved the ability to directly interact with RPA(17).

Subsequent studies have examined the N-terminal portion of hRad52 to determine in detail its interaction with ssDNA. Investigation by X-ray crystallography revealed an undecameric (11-subunit) ring structure with a deep groove on the surface that is lined by a vast number of positively charged basic and aromatic residues(21; 22). These hRad52 residues have been shown to be important for ssDNA binding by mutational analysis(22; 23) and deletions of equivalent residues in ScRad52 demonstrate deficiencies in DNA repair and HR(24).

More recently, a second DNA binding domain has been discovered in hRad52, which appears to bind dsDNA(25). Mutations of these amino acid residues are defective in promoting Rad51-mediated D-loop formation in ScRad52(26). Further study of this second DNA binding domain is necessary to understand how it contributes to the known functions of Rad52.

Interesting, although hRad52 did not demonstrate recombination mediator activity in reconstituted biochemical assays(27), it appears to mediate Rad51 function in human cancer cells deficient in *BRCA1, PALB2* (28)or *BRCA2*(29). We postulate that hRad52 may perform its mediator function in combination with partner proteins. It has been suggested that the Rad51 paralogs, Rad51B/C/D-XRCC2, may fulfill this role. However, the Rad51 paralogs appear to function primarily in the BRCA2 pathway and operate independently of hRad52 in mediating the Rad51 recombinase (Chun J and Powell SN, unpublished observations). There remains other Rad51 paralogs that may be candidate co-factors, including hSWS1 (homolog of yeast Shu2) and SWSAP1(30). Alternatively, hRad52 recombination mediator activity may be dependent on specific posttranslational modifications (e.g. phosphorylation, SUMOylation, etc.) that have yet to be examined biochemically.

Besides its role in Rad51 mediation, it has recently been suggested that BRCA2 participates in the stabilization of RAD51 filaments from degradation by MRE11 through interaction of BRCA2's conserved C-terminal domain with Rad51(31). It is unknown whether Rad52 has any role to play in this proposed mechanism of replication fork protection.

Species spectrum of Rad52 and BRCA2

BRCA2 and its homologs appear to have appropriated subsets of Rad52 function, especially in mediating recombination by Rad51, and are present in a variety of multi-cellular organisms(See Table 1)(32–39).

A speculative assumption is that BRCA2 has evolved for the greater level of insults to the genome in multi-cellular organisms in comparison to unicellular organisms. Seen from another perspective, increased -- but regulated -- mutagenesis is evolutionarily preferential in unicellular life; however in multi-cellular organisms more stringent control of DNA repair, especially in stem cells, via BRCA2 is essential for vitality. *U. maydis* exists as a unique unicellular exception to possessing a BRCA2 homolog, possibly, to enable it to tolerate the greater levels of exogenous DNA damage (e.g. high UV exposure, etc.) it encounters in its native environment budding on crops of corn or in support of its multi-cellular filamentous form.

Rad52 and its synthetically lethal interactions across various species

The dual role of ScRad52 in both mediating Rad51 function and performing the annealing step in second end capture and SDSA explains why *Sc*R*ad52* mutants display more severe phenotypes than defects in Rad51 protein. Unexpectedly, in organisms containing a BRCA2 homolog, including *U. maydis*, chicken, and mice, inactivation of Rad52 causes minimal or no HR and DNA repair defects(39–41). However, in human cancer cell-lines deficient in *BRCA1, PALB2*(28), *or BRCA2*(29), *RAD52* depletion increases damage-induced chromosomal abnormalities, decreases clonogenic survival, and further reduces the rates or frequency of HR(28, 29). Thus, *RAD52* appears to exist in a relationship with *BRCA1*, *PALB2*, or *BRCA2*, known as synthetic lethality, where simultaneous inactivation of two genes leads to cell death, whereas inactivation of only one of these genes does not affect viability. This observation is in accordance with other *RAD52* synthetically lethal phenotypes seen in chicken DT40 cells, where inactivation of *rad52* is lethal with a defect in *XRCC3*, a RAD51 paralog(42), and, in *U. maydis*, where a Rad51 paralog mutant, *rec2*, demonstrates synthetic lethality with loss of *rad52*(39).

Interestingly, in DT40 cells, *rad52* deletion in a *BRCA2* mutant background did not enhance cytotoxicity, instead epistasis was observed between BRCA2 and Rad52, as well as other HR proteins(43). Also, in *U. maydis, Brh2* mutants in combination with *Rad52* defects demonstrate a more subtle synthetically lethal phenotype, which the study's authors interpret as a compensatory interaction(39). These observed divergences from human and other studied organisms will require further investigation to delineate the hierarchy and functional nuances of these various HR proteins across the evolutionary spectrum.

Dependency of BRCA1-PALB2-BRCA2 deficient human tumor cells on Rad52-Rad51-mediated HR

BRCA1, PALB2 and BRCA2 appear to be linked in a sequential manner. BRCA2 recruitment to foci requires interaction with PALB2, and abolishment of the physical interface between BRCA1 and PALB2 impairs BRCA2 function and subsequently Rad51-mediated HR(44–46). Evidence suggests Rad52 provides an alternative mediator pathway to the BRCA1-PALB2-BRCA2 pathway and allows tumor cells to proliferate in the absence of

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the BRCA pathway (Fig 1). The loss of Rad52 in a BRCA1, PALB2 (28), or BRCA2(29) mutant leads to cell death.

These observations set the potential foundation for any BRCA1-PALB2-BRCA2 pathway deficient tumor to be therapeutically targeted by Rad52 inactivation. This approach for targeting BRCA pathway deficient cancers is distinct from other strategies that take advantage of synthetic lethality in *BRCA* mutant tumors, namely poly-(ADP-ribose) polymerase (PARP) inhibition. These translational approaches and discoveries that exploit HR defective cancers will be highlighted in the subsequent section.

Clinical–Translational Advances

Exploiting synthetic lethal interactions in HR deficient tumors

Synthetic lethality can be exploited therapeutically by identifying cells with a cancer-related mutation or loss of a single gene that can then be evaluated for a second gene or protein target that would render the cancer cells non-viable. Since the loss of the second gene alone is not lethal to normal cells, tumor-specific kill can be achieved. This approach has been employed to great promise and effect in HR deficient *BRCA1* and *BRCA2* mutant cancers, and is being expanded to many cancers with tumor-specific alterations that are potentially exploitable, such as oncogene addiction.

PARP inhibition in BRCA1-BRCA2 deficient tumors

PARP inhibitors (PARPi) are synthetically lethal with *BRCA* defective tumor cells(47; 48). The presumed mechanistic model for this lethal interaction is the inhibition of PARP prevents repair of single-strand DNA breaks which accumulate and are then converted into DSBs during replication. The DSB repair HR pathway is impaired in *BRCA1-BRCA2* mutant tumors, leading to a lethal amount of DSBs after PARP inhibition. However, new evidence has suggested other models that build upon or modify this assumed mechanism. One model proposes that PARPi may cause PARP-1 to be trapped onto DNA repair intermediates that then stall replication forks, or another suggests that PARP is directly involved in catalyzing the restart of stalled replication forks. There are excellent reviews for further discussion of this topic(49; 50).

Regardless of the mechanism, the use of PARPi to target *BRCA* mutant cancers has moved beyond the laboratory and into clinical trials with promising results(51–54). However, even in these studies, some *BRCA* mutant carriers respond poorly(51) and resistance to PARPi therapy via *BRCA2* reversion mutations has been documented(55). In addition, expression of the multi-drug resistance transporter also results in resistance to PARPi. These observations highlight the need to identify biomarkers that will predict patient response to PARP inhibition and the development of additional strategies for exploiting BRCA pathway deficiency.

Targeting Rad52 in BRCA1-BRCA2 deficient tumors

The rationale for targeting Rad52 in *BRCA*-deficient tumors was established by the pre-clinical evidence covered in the preceding sections. In addressing the inactivation of Rad52 in the clinical setting, a variety of approaches can be examined. One approach would be to target Rad52 directly. Currently, there are no known enzymatic or kinase functions of Rad52, which preclude the more well-studied pharmacologic approaches. However, as advances are made by molecular pharmacologists and medicinal chemists, creating or identifying a compound that disrupts the oligomer ring structure or binds in the vicinity of the DNA binding groove of Rad52 to prevent access by the DNA substrate may be potential molecular mechanisms. These approaches will require high-throughput screening with

libraries of compounds as a top-down approach coupled with other investigations from a bottom-up mechanistically driven strategy.

Targeting post-translational modifications of Rad52 that are essential for function is another promising avenue of approach. Rad52 is phosphorylated by the c-Abl kinase which affects its ability to form sub-nuclear foci(56) and enhances its annealing functions(57). Disrupting the c-Abl kinase through genetic and pharmacological approaches in preliminary studies appeared to disrupt Rad52 function (Lok BH and Powell SN, unpublished observations). Another post-translational modification that contributes to Rad52 function is SUMOylation, which may be another potential target for impeding Rad52 function(58–62). The interaction of Rad52 with RPA may also be a potentially targetable site, but developing drugs to inhibit protein-protein interactions has always been a challenging problem. Whether these strategies will result in clinically applicable therapeutics remains to be seen.

Identification of BRCA pathway deficient cancers susceptible to synthetically lethal therapeutic approaches

Even with the mechanistic understanding of these realized and potential therapeutic approaches, proper identification of tumors susceptible to these interventions is equally important. A potent intervention applied to an improper scenario/disease-state will result in disappointingly ineffectual or even harmful outcomes.

Indeed, one of the primary target proteins of PARPi, PARP-1, displays a spectrum of protein expression levels even within genetically-similar tumor types. In this regard, not all *BRCA1*-associated breast cancers are created equal, with up to 18% of these tumors expressing none or low levels of nuclear PARP-1 protein(63). This is suggestive that investigating target protein expression levels in tumors may be one approach to predict patient response to PARPi therapy.

The HR proficiency of a tumor can be measured by observing sub-nuclear focus formation of HR proteins induced by *ex-vivo* irradiation, including BRCA1 and Rad51 amongst others(64; 65), and these HR foci formation assays correlate with the PARPi sensitivity of these tumors(65). This functionally-driven approach allows for more precise patient identification, in addition to expanding the potential pool of targetable tumors, through establishing a predictive biomarker for HR-deficient tumors that are not identified by the traditional genetic test for the *BRCA1* or *BRCA2* mutation.

Conclusions

We are entering an exciting era of precision-targeted and personalized cancer therapy. With the greater understanding and continual investigation of the complex genetic interactions of oncogenesis and tumor proliferation, rational design of and molecular target identification by various therapeutic methods can be accomplished. Success will also depend on the development of biomarkers of DNA repair function to identify appropriate patients for targeted therapy. Continual and further elucidation of the mechanisms of DNA repair will be paramount to devising therapeutics, identifying appropriate patients, evaluating responsiveness, and preventing resistance to targeted therapeutic strategies.

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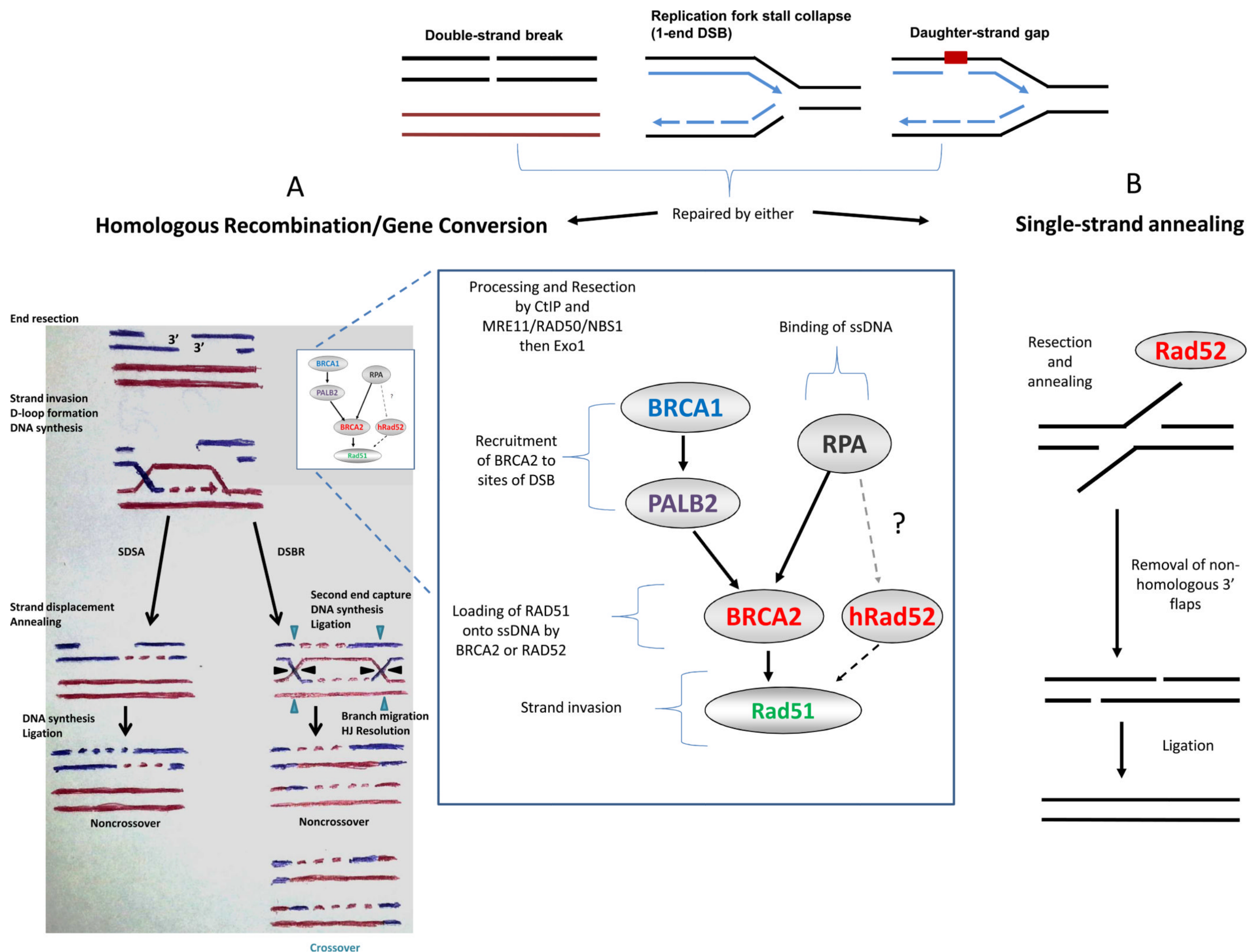

Fig 1.

The BRCA and RAD52 pathways of DNA double-strand break (DSB) repair. Various DNA lesions lead to DSBs that can be repaired by homologous recombination (HR) or single-strand annealing (SSA). A) In the HR pathway, after activation of the DNA damage response pathway, DNA ends are resected to expose 3' single-stranded DNA (ssDNA) which become substrates for binding by RPA. BRCA2 or, in its absence, Rad52 can recruit Rad51 for loading onto ssDNA, displacing RPA, allowing for homology searching and strand invasion by the Rad51 nucleofilament. Subsequently, noncrossover or crossover ligation products are generated in the double-strand break repair (DSBR) pathway or only noncrossover products when synthesis-dependent strand-annealing (SDSA) is utilized. B) SSA requires repetitive sequences around the DSB. After resection, Rad52 mediates annealing of the exposed complementary sequences. After removal of the 3'-flaps, ligation leads to repair, with loss of the intervening sequence. *There is currently no clear evidence that 1-end DSBs or daughter-strand gaps are repaired by single-strand annealing.
The left half of panel A is adapted by permission from Macmillan Publishers Ltd: *Nature Reviews Molecular Cell Biology* (66), copyright 2006.

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Table 1

The spectrum of BRCA2 and RAD52 across various species.

		Biochemical activity		
Species		**Mediator**	**Annealer**	**HR Phenotype**
S. cerevisiae	No BRCA2			
	Rad52	Yes	Yes	++
U. maydis	Brh2	Yes	Yes	++
	Rad52	Low	Yes	−
A. thaliana	BRCA2	?	?	+
	Rad52	?	?	+
D. melanogaster	BRCA2	?	?	+
	No Rad52			
C. elegans	BRC-2	Yes	Yes	++
	No Rad52			
H. sapiens *M. musculus*	BRCA2	Yes	No	++
	RAD52	No	Yes	(+)

++ = strong phenotype, + = intermediate phenotype, (+) = weak phenotype; ? = unknown/no published reports identified.