Double-strand breaks and tumorigenesis

Andrew J. Pierce, Jeremy M. Stark, Felipe D. Araujo, Mary Ellen Moynahan, Marianne Berwick and Maria Jasin

The establishment of connections between biochemical defects and clinical disease is a major goal of modern molecular genetics. In this review, we examine the current literature that relates defects in the two major DNA double-strand-break repair pathways – homologous recombination and nonhomologous end-joining – with the development of human tumors. Although definitive proof has yet to be obtained, the current literature is highly suggestive of such a link.

A TRENDS Guide to
Cancer Biology

Andrew J. Pierce
Jeremy M. Stark
Felipe D. Araujo
Maria Jasin*
Cell Biology Program

Mary Ellen Moynahan
Dept of Medicine

Marianne Berwick
Dept of Epidemiology and Biostatistics

Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA.
*e-mail: m-jasin@ski.mskcc.org

Cancer cells often exhibit defects in their response to DNA damage. Whereas normal cells arrest in the cell cycle following exposure to DNA-damaging agents, cancer cells frequently fail to arrest because of defective cell-cycle checkpoints[1]. Cancer cells are also commonly impaired in their ability to repair damaged DNA. Because multiple genetic hits are necessary for tumorigenesis, individuals who carry germline mutations in DNA damage-response genes are particularly cancer prone because of the hypermutability of their cells[1].

One type of DNA damage is a chromosomal double-strand break (DSB), which can be formed by oxygen free-radicals, DNA replication, topoisomerase failure or ionizing radiation (IR). Two major pathways exist in mammalian cells for the repair of DSBs: homology-directed repair (HDR) and nonhomologous end-joining (NHEJ) (Fig. 1)[2]. During HDR, a homologous sequence forms a template for the repair event, with the identical sister chromatid preferred over homology on another chromosome[3]. HDR events between sister chromatids restore the original sequence prior to damage, making this a precise type of repair. During NHEJ, the ends of a break are often modified by the addition and deletion of nucleotides and then ligated to restore covalent continuity to the broken chromosome[4]. Therefore, both NHEJ and HDR preserve global chromosome integrity in the event of a DSB; however, in contrast to HDR, NHEJ does so at the risk of sacrificing local-sequence integrity.

Both HDR and NHEJ have important roles in repairing spontaneously arising lesions, although the nature of these lesions is often not well understood. HDR appears to play a crucial role during the normal cell-division cycle as targeted mutation of some HDR genes leads to cell death[5,6]. By analogy with results obtained in studies in *Escherichia coli*[7], HDR in mammalian cells may be crucial for the repair of strand breaks that arise during DNA replication. In addition, both HDR and NHEJ appear to be crucial for the repair of lesions that arise in certain tissue types, with the consequence that mutation of either of these pathways can lead to developmental defects and embryonic death. In particular, neurogenesis defects have been observed in DSB repair mutants[4,8,9].

Some mouse mutants with disruption of DSB repair genes survive embryogenesis, only to develop tumors of various tissue types and with varying latencies. These tumor studies in the mouse, together with the identification of DSB repair defects in cell lines with mutations in tumor-suppressor genes, suggest a causal relationship between such defects and cancer. This review summarizes recent developments in determining an association between DSB repair defects and tumorigenesis, with an emphasis on the role of components of HDR and NHEJ pathways.

Characterizing DSB repair mutants

Several methods are available to characterize DSB repair mutants, such as direct molecular analysis of repair products following a chromosomal DSB, cellular sensitivity to

 0962-8924/01/$ – see front matter PII: S0962-8924(01)02149-3

radiation or other types of DNA-damaging agents, and cytological associations of factors important for DSB repair. Mechanisms of DSB repair are revealed at the molecular level by introducing a defined DSB into the genome[2]. The DSB is typically generated by expressing the rare-cutting I-SceI endonuclease, whose 18-bp recognition site has been integrated into a chromosomal locus. HDR and NHEJ repair events are then detected by genetic and physical analyses. In addition to assays involving endonuclease-generated DSBs, V(D)J recombination assays are frequently used to examine DSB repair by NHEJ, as this site-specific recombination process requires cellular NHEJ components. Because both HDR and NHEJ are utilized efficiently in mammalian cells, comparing the efficiency of the two pathways in wild-type and mutant cells provides insight into the role of an individual gene product in DSB repair.

In addition to direct molecular analysis, determination of the relative toxicity of DNA-damaging agents or the relative gross physical repair of lesions, as with pulsed-field gel analysis[10], can reveal differences in the efficacy of DSB repair in various cell types or mutants[11,12]. Furthermore, cytological studies have shown that DNA-damaging agents such as IR and crosslinking agents cause the aggregation of specific factors in the nucleus into IR-induced foci (IRIF), often more generally termed 'damage-induced foci', which can also be seen in untreated cells during S phase[13,14]. In several instances, mutation of a gene involved in DSB repair has been found to impair focus formation of another protein involved in DSB repair, an observation that is presumably relevant to the repair process (see below). Not surprisingly, chromosomal aberrations are often observed in DSB repair mutants, whether in the absence or presence of DNA-damaging agents. Although the molecular events leading to chromosomal aberrations are not well understood, several different aberrations are observed in DSB repair mutants, including chromatid or chromosome breaks, exchanges, translocations and deletions. Differences in cellular phenotypes between HDR and NHEJ mutants are summarized in Table 1.

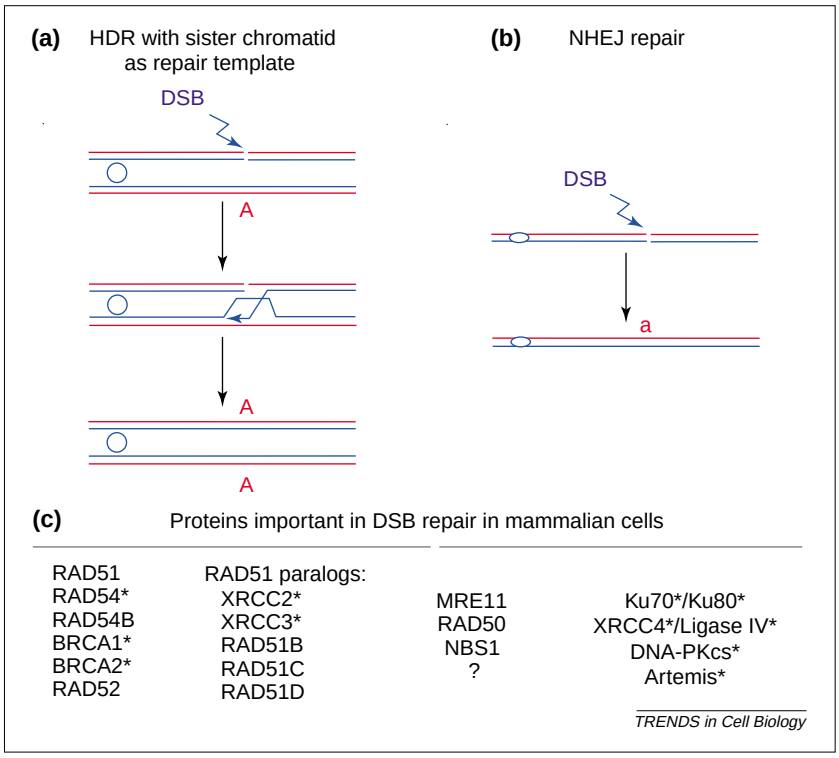

Figure 1. Mammalian cells repair DNA double-strand breaks by homologous recombination or non-homologous end joining

(a) In homology-directed repair (HDR), a homologous sequence templates repair after strand invasion. The invading broken end acts as a primer for DNA synthesis, using the homologous sequence as the template for repair. In yeast, evidence suggests that both leading strand (as shown) and lagging strand (not shown) synthesis occurs during repair. Although the homologous sequences can be on the sister chromatid (as shown), homologous chromosome, or, for sequence repeats, a heterologous chromosome, the sister chromatid is preferred[3]. As they are identical to each other, HDR between sisters will restore the original sequence that was present before the double-strand break (DSB) occurred (and hence the retention of sequence 'A' at the DSB). (b) Non-homologous end-joining (NHEJ) involves processing of DNA ends, finally leading to their ligation. Because nucleotide deletions and insertions can occur during NHEJ repair, the original sequence might not be restored (and hence the alteration of 'A' to 'a'). (c) Proteins expected to be important for each DSB repair pathway are listed, with those verified to be important in mammalian cells by HDR or NHEJ assays indicated by an asterisk. For NHEJ mutants, assays of V(D)J recombination, which generates antigen receptor diversity, are frequently used. The MRE11–RAD50–NBS1 complex might play roles in both pathways.

DSB repair genes as genomic caretakers

Because DSBs are potentially mutagenic, genes involved in both HDR and NHEJ are predicted to have a genomic caretaker role. That is, after either exogenous or endogenous damage, these genes would protect cells from becoming tumorigenic by preventing the accumulation of mutations (for example, in genes controlling cell growth) – similar to the caretaker role of genes involved in mismatch repair or nucleotide excision repair. Each of the aforementioned assays have led to the characterization of genes involved in the DNA-damage response, including genes directly involved in DSB repair, and studies are under way to determine their role in preventing tumorigenesis.

BRCA1 and BRCA2

A striking connection between DSB repair defects and tumorigenesis is found with the hereditary breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2*[15,16]. Families with germline mutations in these genes show an autosomal-dominant inheritance pattern for susceptibility. However, although one allele is inherited in a mutated form, somatic mutation occurs to alter the second allele, such that tumors invariably contain two mutant alleles. Genomic integrity is perturbed by mutation of these genes as *BRCA1* and *BRCA2* mutant cells spontaneously develop a variety of chromosome aberrations[17,18].

Table 1. Phenotypes of cells deficient in components of the two major pathways for DSB repair[a]

Phenotype	HDR mutants	NHEJ mutants	Refs[b]
IR sensitivity	Mild	Strong	11,12,15,47
Crosslinking-agent sensitivity	Strong	Mild	11,12,17,18,21
Spontaneous chromosomal abnormalities[c]	+	+,–	11,12,15,17,18,62,63
IR-induced chromosomal abnormalities	+	+	11,12
Centrosome abnormalities	+	–	18
Altered kinetics of DSB rejoining by PFGE	–	+	10

[a]Abbreviations: DSB, double-strand break; IR, ionizing radiation; PFGE, pulsed-field gel electrophoresis.
[b]See also references within these reviews or primary research papers.
[c]Although not exhaustively analyzed, some differences are noted for different cell types. For NHEJ mutants, abnormalities have been reported for mouse embryonic fibroblasts but not hamster cell mutants.

Cellular phenotypes of BRCA1 and BRCA2 mutants

A role for *BRCA1* and *BRCA2* in DSB repair was suggested by the discovery that both proteins interact with RAD51[15,16]. As the mammalian homolog of the *E. coli* RecA protein, RAD51 catalyzes strand exchange, an early step in homologous recombination that results in the formation of heteroduplex DNA molecules[19]. The three proteins colocalize in IRIF[15] and, notably, cells deficient in *BRCA1* and *BRCA2* are defective in RAD51 IRIF formation[17,20–22].

Direct evidence for a role for *BRCA1* and *BRCA2* in promoting HDR was obtained using the I-SceI system. Cells containing hypomorphic (i.e. partial loss of function) alleles for either of these proteins exhibit HDR defects[18,23,24]. NHEJ does not appear to be impaired[17,23,25]. As with other HDR mutants, *BRCA1*- and *BRCA2*-deficient cells exhibit a mild sensitivity to IR but a more profound sensitivity to crosslinking agents[17,18,21]. Although the nature of their role in HDR is unclear, biochemical functions of *BRCA1* and *BRCA2* are beginning to be ascertained. In recent reports, *BRCA1* has been shown to bind DNA with a preference for branched DNA structures[26], and peptides from *BRCA2* have been demonstrated to modulate the binding of RAD51 to DNA[27]. The subcellular localization of RAD51 is also abnormal in a tumor cell line containing a common *BRCA2* mutation[27].

Compounding the repair defects, a *BRCA1* mutant has recently been shown to have a defective S-phase checkpoint response to IR, and evidence in some studies also supports a G2/M checkpoint defect, suggesting that *BRCA1* mutant cells will continue to progress through the cell cycle with unrepaired damage arising during replication[28]. Several of the chromosome-instability syndromes (see below) exhibit defects in both repair and checkpoint pathways, raising a fundamental question regarding the importance of checkpoint defects for allowing a repair phenotype to manifest itself. If cell-cycle checkpoints are intact, cells with unrepaired or misrepaired damage should be effectively eliminated from the population. Thus, disruption of cell-cycle checkpoints could be an important step for the accumulation of mutations in cells with defective DSB repair. Nevertheless, checkpoint defects are not universally observed in cancer syndromes involving DNA repair defects.

BRCA1 and BRCA2 mutants and tumor development

BRCA1 and *BRCA2* are essential for normal development. Patients in which both alleles of *BRCA1* or *BRCA2* are mutated have not been identified[29]. In mice, null mutations of *BRCA1* or *BRCA2* result in embryonic lethality at approximately day 6.5; hypomorphic alleles result in survival to later embryonic stages, or in some cases even to adulthood[16,30,31]. Recently, viable mice were obtained with a *BRCA1* truncation allele, although, in this mouse model, embryonic lethality occurs on certain strain backgrounds, indicating the existence of strain-specific modifiers[31].

Tumor development has been examined in viable mice obtained with the *BRCA1* or *BRCA2* hypomorphic alleles. As is common in mouse tumor models, lymphomas are frequently observed, although in the longer-lived mice with the *BRCA1* truncation allele, mammary tumors, sarcomas and other carcinomas have been found after long latency (i.e. 18 months)[31]. In addition, mice in which *BRCA1*[32] or *BRCA2*[33] is conditionally disrupted in the mammary gland develop mammary tumors between 10–17 months of age. Tumor latency is significantly decreased on a $p53^{+/-}$ background in mice with either the *BRCA1* truncation allele or the mammary-specific *BRCA1*

Table 2. SNPs and mutations identified in genes involved in homologous recombination[a]

Gene	SNP or mutation	Frequency[b]		Patient tissue genotype[c]		Tumor type	Refs
		Normal	Patient	Germline	Tumor		
RAD51	5′UTR g135c	6/73	12/121 (BRCA1)	+/v	n.d.	Breast and/or ovarian (BRCA1)	39
			8/46 (BRCA2)	+/v	n.d.	Breast and/or ovarian (BRCA2)	
	Arg150Gln	n.d.	2/45	+/v	+/v or v/v	High-risk breast[d]	37
			0/200			Sporadic breast	
			0/100			Colon	
RAD52	Ser346ter	5/102	3/99	+/v	n.d.	Early-onset breast	36
	Tyr415ter	3/102	2/99	+/v	n.d.	Early-onset breast	36
RAD54	Pro63His	0/100	1/13[e]	+/+	+/v	Colon	49
	Gly325Arg	0/100	1/95[e]	+/v	v/v	Breast	49
	Val444Glu	0/100	1/24[e]	n.d.	+/v	Lymphoma	49
	Ser657Cys	0/100	1/100	+/v	n.d.	Early-onset breast	36
RAD54B	Asp418Tyr	0/80	1/19[f]	n.d.	v/v	Colon	70
	Asn593Ser	0/80	1/26	n.d.	v/v	Lymphoma	70
XRCC3	Thr241Met	23/211	21/125	v/v	n.d.	Malignant melanoma	51[g]
		78/211	65/125	+/v	n.d.	Malignant melanoma	
		16/85	27/124	v/v	n.d.	Bladder	52[h]
		27/85	64/124	+/v	n.d.	Bladder	

[a]Abbreviations: n.d., not determined; SNP, single nucleotide polymorphism.
[b]Number of individuals with SNP or mutation, divided by the total number of individuals analyzed.
[c]+, common allele; v, variant (i.e. SNP or mutation).
[d]Includes breast cancer families (20 patients) or other factors, such as early-onset, bilateral or tumor of another organ (25 patients).
[e]A total of 132 unselected primary tumors (i.e. colon, breast and lymphoma) were analyzed for each of these *RAD54* mutations.
[f]A total of 45 unselected primary tumors (i.e. colon and lymphoma) were analyzed for each of these *RAD54B* mutations.
[g]Met allele frequency: 0.30 (control) and 0.43 (melanoma). In the study by Butkiewicz *et al.*[53], the Met allele frequency was 0.33 (control) and 0.32 (lung). In the study that identified this variant the Met allele frequency was 0.38 (Ref. 50).
[h]Met allele frequency: 0.35 (control) and 0.48 (bladder).

disruption allele. Comparing these studies, *BRCA2* mammary tumors are morphologically quite uniform, whereas the *BRCA1* tumors are heterogeneous. Differences between *BRCA1* and *BRCA2* mouse tumors are perhaps not surprising considering that *BRCA1*- and *BRCA2*-associated human breast tumors have different morphological and immunohistochemical characteristics[34], including different gene-expression profiles[35]. The observed differences between *BRCA1*- and *BRCA2*-associated human or mouse tumors probably underscore a fundamental difference in the cellular activity of these two proteins.

Although the tumor-suppressor function of *BRCA1* and *BRCA2* is expected to involve the DNA damage response, it is not certain that the HDR defects are causal for tumorigenesis as both proteins interact with a diverse set of proteins involved in other cellular functions[16]. Supporting a causal relationship are studies using similar *BRCA1* hypormorphic alleles, in which HDR is impaired and mammary tumors are observed[23,32]. Definitive proof, however, might require clear separation-of-function alleles in which HDR is impaired while other cellular functions of *BRCA1* or *BRCA2* remain intact.

Despite these reservations, the interaction of *BRCA1* and *BRCA2* with RAD51 has prompted searches for alterations of *RAD51* in patients with breast tumors. In one study of 27 patients with early-onset breast cancer, no germline alterations were identified[36]. However, a single-base-pair transition was identified in another study in the germline of two patients with bilateral breast cancer (Table 2)[37]. This base-pair change creates an amino acid substitution that makes the protein identical to *RAD51* homologs in other organisms, making the significance of the substitution uncertain. In other studies to identify genetic modifiers of *BRCA1* and *BRCA2*, a single-nucleotide polymorphism (SNP) found in the 5′ untranslated region (5′UTR) of *RAD51* was associated with an increased breast cancer risk in *BRCA2* carriers[38,39]. The functional consequence of this SNP on *RAD51* expression remains to be determined.

Other HDR genes

Several other proteins have been identified that are involved in HDR in mammalian cells, some of which promote RAD51 strand exchange[4,12]. These proteins are the RAD51-interacting proteins RAD52, RAD54 and RAD54B, which form IRIF with RAD51, and the RAD51 paralogs (paralog: a gene that probably arose by duplication of an ancestral gene, but which evolved distinct functions) RAD51B (a.k.a. RAD51L1), RAD51C (a.k.a. RAD51L2), RAD51D (a.k.a. RAD51L3), XRCC2 and XRCC3[40]. RAD52 binds to DNA ends and has strand-annealing activity, whereas RAD54 and RAD54B are members of a superfamily of DNA-dependent ATPases[4,41]. The RAD51 paralogs, which share approximately 25–30% identity with RAD51, physically interact with each other and with RAD51 in various pairwise combinations. RAD54[42], XRCC2[43], and XRCC3[44] have been shown to be important for HDR of an I-SceI-induced DSB in mammalian cells. In addition, disruption of each of the RAD51 paralogs in vertebrate cells results in decreased sister-chromatid exchange, a further indicator of HDR[40].

Mice with disruption of some of the paralogs (i.e. *Xrcc2*, *Rad51b* and *Rad51d*) have been reported, but studies of tumor development have been precluded because these mice die during embryogenesis[8,45,46]. In contrast, mouse disruptions of *Rad52* and *Rad54* are viable and fertile[47,48], although tumors have not been observed.

As with *RAD51*, studies of human populations and tumor samples have been undertaken to identify alterations in HDR genes, with the eventual goal of determining whether these alterations are associated with tumorigenesis. Several alterations have been identified, although as yet the functional consequence of the various gene alterations on HDR activity has not been determined. Normal tissue DNA from either healthy or cancer-stricken individuals is generally used to determine the genotype of the HDR gene of interest in the germline. However, in some studies, tumor samples from patients have been genotyped to ascertain whether mutations arose during development of the disease (Table 2). Several of these alterations will be discussed, to emphasize the variety of outcomes that can be obtained from such genetic screens – although, as yet, little definitive evidence exists for a role of these alterations in promoting tumorigenesis.

For *RAD52*, two SNPs giving rise to truncations were identified in the germline of several early-onset breast cancer patients, although the SNPs were as prevalent in healthy individuals[36]. For *RAD54* (a.k.a. *RAD54L*), four alterations giving rise to amino acid substitutions were found in cancer patients but not in healthy individuals[36,49]. At least one substitution (Pro63His) found in a tumor arose somatically, as it was not found in normal tissue from the individual, but, because tumors are often genetically unstable, it is not certain whether this mutation had any consequence for tumor development. Another substitution, which occurs in a conserved domain of RAD54 (Gly325Arg), was heterozygous in normal tissue from the individual, but was homozygous (or hemizygous) in the tumor. Such a loss of heterozygosity (LOH) can support the significance of genetic alterations; however, regions of LOH tend to be extensive, involving large chromosomal segments, such that the significance of a single event is uncertain. Another substitution (Ser657Cys) was found in the normal tissue of a patient with early-onset breast cancer; however, as this substitution was found in only one of 100 patients and none of 100 controls, it is not clear whether this is a rare, benign polymorphism in the population or relevant to the disease status of the individual.

The RAD51 paralogs provide other examples of genetic alterations that can be observed in DNA repair genes. For XRCC3, a common allelic variant (Thr241Met) has been identified in the population[50]. This variant has been associated with the development of malignant melanoma[51], whether individuals carry one or two copies of the variant allele, and bladder cancer[52], but not lung cancer[53]. The frequency of the variant allele was found to differ in different studies by 0.08 (allele frequency range was 0.30–0.38), and a 0.05-greater frequency above this range (allele frequency 0.43) was found in patients with melanoma, emphasizing the need for well-matched control groups to rule out population variations as a cause for increased risk. For *RAD51B*, gross chromosomal rearrangements of the gene have been reported in tumors (i.e. uterine leiomyomas) that involve translocations with the high-mobility group protein gene *HMG1C*, creating in-frame fusion proteins in some translocations[54]. As gross chromosomal rearrangements are common in solid tumors, it is not clear whether these translocations were important in the etiology of these tumors.

Genes involved in chromosome-instability disorders

The chromosome-instability disorders include a diverse set of autosomal-recessive diseases, which are characterized by cancer predisposition. The inherent chromosome instability in cells from patients with these disorders, as well as sensitivity to DNA-damaging agents, suggests defects in DNA replication and/or repair, although the specific repair pathways that are disrupted in the various disorders are not well understood. Several recent reviews have covered these disorders[55–57], and so they will be briefly summarized.

Three disorders, Nijmegen breakage syndrome (NBS), ataxia-telangiectasia (A-T), and the rare A-T-like disorder (A-TLD), are characterized at the cellular level by IR sensitivity[56]. A-T and NBS patients are highly cancer prone, primarily developing lymphoid malignancies; the rarity of A-TLD patients has not allowed a determination of their cancer predisposition. A-T arises from mutation of the *ATM* gene, which encodes a Ser/Thr kinase, whereas NBS and A-TLD arise from mutations in the *NBS1* and *MRE11* genes, respectively, which, together with *RAD50*, encode members of the MRE11 complex. The MRE11 complex apparently has diverse functions, including recombination and telomere maintenance, and, like the ATM kinase, has a role in the S-phase checkpoint response to DSBs, during which ATM phosphorylates NBS1[56,58]. The functional complexity of the MRE11 complex precludes a definitive conclusion as to which activity is responsible for cancer predisposition; however, the similarity among the three disorders suggests a common pathway, which might involve the S-phase checkpoint.

Other chromosome-instability disorders include those syndromes caused by mutations in *RECQ* helicase genes [i.e. Bloom (*BLM*), Werner (*WRN*) and Rothmund-Thomson (*RECQL4*) syndromes[57]], as well as Fanconi anemia, which is caused by mutation in one of seven genes (i.e. the *FANC* genes)[55]. The tumor spectrum for Bloom syndrome patients is extremely diverse; Werner and Rothmund-Thomson patients are prone to sarcomas. Patients with Fanconi anemia are at greatly increased risk for leukaemias, squamous cell carcinomas and other tumors. Unlike the RECQ helicases, the biochemical function of proteins encoded by the *FANC* genes is unknown. A recent report has associated them with *BRCA1* – in part because of colocalization of the FANCD2 protein with *BRCA1* in IRIF[59]. Interestingly, other proteins disrupted in chromosome-instability disorders are found in IRIF with *BRCA1*, including ATM, BLM and the MRE11 complex[60].

NHEJ genes

Defects in genes involved in NHEJ have also been analyzed for an effect on tumorigenesis. These genes were first identified in the context of repair of the RAG1/RAG2 recombinase-induced DSBs used to generate antigen receptor diversity in B- and T-cell lineages[4,61]. Thus far, six protein factors have been identified: the DNA end-binding KU70–KU80 heterodimer (a.k.a. G22P1/XRCC5), which is a component of the DNA-dependent protein kinase (DNA-PK) when paired with the catalytic subunit DNA-PKcs (a.k.a. PRKDC), the XRCC4–DNA ligase IV (LIG4) complex and more recently, the Artemis protein. NHEJ mutant cells are typically extremely sensitive to IR, but, unlike HDR mutants, they are not very sensitive to crosslinking agents. In NHEJ mutant cell lines, chromosome aberrations are highly induced by treatment with DNA-damaging agents such as IR; however, chromosome aberrations arise even in the absence of exogenous damage in some cell types[62,63].

Mice lacking *Ku70*, *Ku80* and *DNA-PKcs* are viable, and, as expected by their deficiency in V(D)J recombination, show arrested B- and T-cell development. However, *Xrcc4*- and *Lig4*-null mouse mutants die during embryogenesis, apparently because of massive neuronal apoptosis. An *Artemis* mouse mutant has not been reported; patients with null mutations are short-lived and succumb during the first year of life from infections[61]. *Ku70* mutant mice have been reported to have accelerated tumor development, in particular of thymic lymphomas. These lymphomas, which are common in mice, might have arisen from aberrations in the few cells that did manage to complete V(D)J rearrangements in the *Ku70* mutant.

Evidence for an effect of NHEJ mutations on tumorigenesis is primarily based upon crosses of mutant mice with p53 mutant mice. Providing a $p53^{-/-}$ (or even $p53^{+/-}$) background rescues the lethality of both $Xrcc4^{-/-}$ and $Lig4^{-/-}$ mutations, possibly by preventing the neuronal apoptosis[64,65]. Double-mutant animals (i.e. $Xrcc4^{-/-}/p53^{-/-}$, $Lig4^{-/-}p53^{-/-}$, $Ku80^{-/-}/p53^{-/-}$ and $DNA\text{-}PKcs^{scid/scid}/p53^{-/-}$) develop pro-B-cell lymphomas with an early onset, in contrast to $p53^{-/-}$ mutant animals, which develop thymic lymphomas at approximately five months[62,64–67]. The pro-B-cell lymphomas have a characteristic t(12;15) translocation between the *IgH* locus and *c-myc*, frequently involving amplification of these loci. In contrast with the thymic lymphomas arising on a $p53^{-/-}$ background, tumors in $DNA\text{-}PKcs^{scid/scid}/p53^{-/-}$ mutant mice are suppressed by *RAG* gene mutation, consistent with tumorigenesis initiating with misrepair of a DSB during V(D)J recombination[66]. Tumors have also been examined in $Ku80^{-/-}/p53^{+/-}$ mutant mice[67]. As with $p53^{+/-}$ mice, $Ku80^{-/-}/p53^{+/-}$ mice develop a broader spectrum of tumors than just lymphomas, including several types of sarcoma, with *Ku80* mutation accelerating the process. In addition to lymphomas, a recent report has also implicated the *DNA-PKcs* gene in IR-induced mammary tumor suppression[68]. BALB/c mice have two amino acid substitutions in the *DNA-PKcs* gene compared with C57BL/6 mice and have a 10-fold greater breast cancer risk, although thus far the increased tumor risk has not been conclusively determined to be caused by the substitutions in the *DNA-PKcs* gene.

To date, only one cancer patient with NHEJ deficiency has been reported. This developmentally normal person was found to be hypersensitive to radiotherapy during treatment for leukemia, with the cause for the sensitivity determined to be a homozygous missense mutation in *LIG4*, which created a hypomorphic allele[69].

Concluding remarks

Because cancer cells are often impaired in their DNA damage response, it follows that identification and understanding the role of genes involved in DNA repair will lead to insights into the etiology of cancer. Mutations in *BRCA1*, *BRCA2* and some genes involved in maintaining chromosomal stability are clearly linked to cancer predisposition and lead to defects in DSB repair. Identifying the causal relationship of these phenotypes is the next challenge and will likely require separation-of-function mutations in which repair defects are disentangled from other cellular phenotypes. Recent work has identified SNPs and mutations in genes involved in HDR, although, as yet, the functional significance of these alterations is unclear. Nevertheless, this approach warrants further investigation as it could lead to the identification of modifier genes for tumor risk in the population. In general, these studies underline the importance of a comprehensive understanding of the mechanisms of DSB repair and the role of mutations in repair genes for promoting tumorigenesis.

References

1 Vogelstein, B. and Kinzler, K.W. (1998) *The Genetic Basis of Human Cancer*, McGraw-Hill

2 Jasin, M. (2001) Double-strand break repair and homologous recombination in mammalian cells. In *DNA Damage and Repair* (Vol. III) (Nickoloff, J.A. and Hoekstra, M.F., eds), pp. 207–235, Humana Press

3 Johnson, R.D. and Jasin, M. (2001) Double-strand-break-induced homologous recombination in mammalian cells. *Biochem. Soc. Trans.* 29, 196–201

4 van Gent, D.C. *et al.* (2001) Chromosomal stability and the DNA double-stranded break connection. *Nat. Rev. Genet.* 2, 196–206

5 Lim, D-S. and Hasty, P. (1996) A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol. Cell. Biol.* 16, 7133–7143

6 Tsuzuki, T. *et al.* (1996) Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. U. S. A.* 93, 6236–6340

7 Cox, M.M. *et al.* (2000) The importance of repairing stalled replication forks. *Nature* 404, 37–41

8 Deans, B. *et al.* (2000) Xrcc2 is required for genetic stability, embryonic neurogenesis and viability in mice. *EMBO J.* 19, 6675–6685

9 Gu, Y. *et al.* (2000) Defective embryonic neurogenesis in Ku-deficient but not DNA-dependent protein kinase catalytic subunit-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 97, 2668–2673

10 Wang, H. *et al.* (2001) Efficient rejoining of radiation-induced DNA double-strand breaks in vertebrate cells deficient in genes of the RAD52 epistasis group. *Oncogene* 20, 2212–2224

11 Jeggo, P.A. (1998) DNA breakage and repair. *Adv. Genet.* 38, 185–218

12 Thompson, L.H. and Schild, D. (1999) The contribution of homologous recombination in preserving genome integrity in mammalian cells. *Biochimie* 81, 87–105

13 Paull, T.T. *et al.* (2000) A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol.* 10, 886–895

14 Mirzoeva, O.K. and Petrini, J.H. (2001) DNA damage-dependent nuclear dynamics of the Mre11 complex. *Mol. Cell. Biol.* 21, 281–288

15 Scully, R. and Livingston, D.M. (2000) In search of the tumour-suppressor functions of BRCA1 and BRCA2. *Nature* 408, 429–432

16 Welcsh, P.L. *et al.* (2000) Insights into the functions of BRCA1 and BRCA2. *Trends Genet.* 16, 69–74

17 Yu, V.P. *et al.* (2000) Gross chromosomal rearrangements and genetic exchange between nonhomologous chromosomes following BRCA2 inactivation. *Genes Dev.* 14, 1400–1406

18 Moynahan, M.E. *et al.* (2001) Homology-directed DNA repair, mitomycin-C resistance, and chromosome stability is restored with correction of a *Brca1* mutation. *Cancer Res.* 61, 4842–4850

19 Cox, M.M. (1999) Recombinational DNA repair in bacteria and the RecA protein. *Prog. Nucleic Acids Res. Mol. Biol.* 63, 311–366

20 Yuan, S.S. *et al.* (1999) BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex *in vivo*. *Cancer Res.* 59, 3547–3551

21 Bhattacharyya, A. *et al.* (2000) The breast cancer-susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA crosslinking agent cisplatin. *J. Biol. Chem.* 275, 23899–23903

22 Huber, L.J. *et al.* (2001) Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. *Mol. Cell. Biol.* 21, 4005–4015

23 Moynahan, M.E. *et al.* (1999) Brca1 controls homology-directed repair. *Mol. Cell* 4, 511–518

24 Moynahan, M.E. *et al.* (2001) BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol. Cell. Biol.* 7, 263–272

25 Mak, T.W. *et al.* (2000) Brcal required for T cell lineage development but not TCR loci rearrangement. *Nat. Immun.* 1, 77–82

26 Paull, T.T. *et al.* (2001) Direct DNA binding by Brca1. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6086–6091

27 Davies, A.A. *et al.* (2001) Role of BRCA2 in control of the RAD51 recombination and DNA repair protein. *Mol. Cell* 7, 273–282

28 Xu, B. *et al.* (2001) Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol. Cell. Biol.* 21, 3445–3450

29 Kuschel, B. *et al.* (2001) Apparent human BRCA1 knockout caused by mispriming during polymerase chain reaction: implications for genetic testing. *Genes Chromosomes Cancer* 31, 96–98

30 Hohenstein, P. *et al.* (2001) A targeted mouse Brca1 mutation removing the last BRCT repeat results in apoptosis and embryonic lethality at the headfold stage. *Oncogene* 20, 2544–2550

31 Ludwig, T. *et al.* (2001) Tumorigenesis in mice carrying a truncating Brca1 mutation. *Genes Dev.* 15, 1188–1193

32 Xu, X. *et al.* (1999) Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat. Genet.* 22, 37–43

33 Ludwig, T. *et al.* (2001) Development of mammary adenocarcinomas by tissue-specific knockout of Brca2 in mice. *Oncogene* 20, 3937–3948

34 Phillips, K.A. *et al.* (1999) Breast carcinomas arising in carriers of mutations in BRCA1 or BRCA2: are they prognostically different? *J. Clin. Oncol.* 17, 3653–3663

35 Hedenfalk, I. *et al.* (2001) Gene-expression profiles in hereditary breast cancer. *New Engl. J. Med.* 344, 539–548

36 Bell, D.W. *et al.* (1999) Common nonsense mutations in RAD52. *Cancer Res.* 59, 3883–3888

37 Kato, M. *et al.* (2000) Identification of Rad51 alteration in patients with bilateral breast cancer. *J. Hum. Genet.* 45, 133–137

38 Wang, W. *et al.* (2001) A single nucleotide polymorphism in the 5′ untranslated region of RAD51 and risk of cancer among BRCA1/2 mutation carriers. *Cancer Epidemiol. Biomarkers Prev.* 10, 955–960

39 Levy-Lahad, E. *et al.* (2001) A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. *Proc. Natl. Acad. Sci. U. S. A.* 98, 3232–3236

40 Takata, M. *et al.* (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. *Mol. Cell. Biol.* 21, 2858–2866

41 Tanaka, K. *et al.* (2000) A novel human rad54 homologue, Rad54B, associates with Rad51. *J. Biol. Chem.* 275, 26316–26321

42 Dronkert, M.L. *et al.* (2000) Mouse RAD54 affects DNA double-strand break repair and sister chromatid exchange. *Mol. Cell. Biol.* 20, 3147–3156

43 Johnson, R.D. *et al.* (1999) Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. *Nature* 401, 397–399

44 Pierce, A.J. *et al.* (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev.* 13, 2633–2638

45 Shu, Z. *et al.* (1999) Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(−/−) background. *Mol. Cell. Biol.* 19, 8686–8693

46 Pittman, D.L. and Schimenti, J.C. (2000) Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* 26, 167–173

47 Essers, J. *et al.* (1997) Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* 89, 195–204

48 Rijkers, T. *et al.* (1998) Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol. Cell. Biol.* 18, 6423–6429

49 Matsuda, M. *et al.* (1999) Mutations in the RAD54 recombination gene in primary cancers. *Oncogene* 18, 3427–3430

50 Shen, M.R. *et al.* (1998) Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.* 58, 604–608

51 Winsey, S.L. *et al.* (2000) A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Res.* 60, 5612–5616

52 Matullo, G. *et al.* (2001) DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. *Int. J. Cancer* 92, 562–567

53 Butkiewicz, D. *et al.* (2001) Genetic polymorphisms in DNA repair genes and risk of lung cancer. *Carcinogenesis* 22, 593–597

54 Schoenmakers, E.F. *et al.* (1999) Allelic knockout of novel splice variants of human recombination repair gene RAD51B in t(12;14) uterine leiomyomas. *Cancer Res.* 59, 19–23

55 Vessey, C.J. *et al.* (1999) Genetic disorders associated with cancer predisposition and genomic instability. *Prog. Nucleic Acids Res. Mol. Biol.* 63, 189–221

56 Petrini, J.H. (2000) The Mre11 complex and ATM: collaborating to navigate S phase. *Curr. Opin. Cell Biol.* 12, 293–296

57 Mohaghegh, P. and Hickson, I.D. (2001) DNA helicase deficiencies associated with cancer predisposition and premature ageing disorders. *Hum. Mol. Genet.* 10, 741–746

58 Wu, X. *et al.* (2000) ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response. *Nature* 405, 477–482

59 Garcia-Higuera, I. *et al.* (2001) Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Mol. Cell* 7, 249–262

60 Wang, Y. *et al.* (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* 14, 927–939

61 Moshous, D. *et al.* (2001) Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 105, 177–186

62 Difilippantonio, M.J. *et al.* (2000) DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* 404, 510–514

63 Ferguson, D.O. *et al.* (2000) The nonhomologous end-joining pathway of DNA repair is required for genomic stability and the suppression of translocations. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6630–6633

64 Frank, K.M. *et al.* (2000) DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. *Mol. Cell* 5, 993–1002

65 Gao, Y. *et al.* (2000) Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature* 404, 897–900

66 Vanasse, G.J. *et al.* (1999) Genetic pathway to recurrent chromosome translocations in murine lymphoma involves V(D)J recombinase. *J. Clin. Invest.* 103, 1669–1675

67 Lim, D.S. *et al.* (2000) Analysis of ku80-mutant mice and cells with deficient levels of p53. *Mol. Cell. Biol.* 20, 3772–3780

68 Yu, Y. *et al.* (2001) Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-dependent protein kinase catalytic subunit) gene. *Cancer Res.* 61, 1820–1824

69 Riballo, E. *et al.* (2001) Cellular and biochemical impact of a mutation in DNA ligase IV conferring clinical radiosensitivity. *J. Biol. Chem.* 276, 31124–31132

70 Hiramoto, T. *et al.* (1999) Mutations of a novel human RAD54 homologue, RAD54B, in primary cancer. *Oncogene* 18, 3422–3426