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Minireview

Homologous recombination as a mechanism of carcinogenesis

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Abstract

Cancer develops when cells no longer follow their normal pattern of controlled growth. In the absence or disregard of such regulation, resulting from changes in their genetic makeup, these errant cells acquire a growth advantage, expanding into pre-cancerous clones. Over the last decade many studies have revealed the relevance of genomic mutation in this process, be it by misreplication, environmental damage or a deficiency in repairing endogenous and exogenous damage. Here we discuss homologous recombination as another mechanism that can result in loss of heterozygosity or genetic rearrangements. Some of these genetic alterations may play a primary role in carcinogenesis, but they are more likely to be involved in secondary and subsequent steps of carcinogenesis by which recessive oncogenic mutations are revealed. Patients whose cells display an increased frequency of recombination also have an elevated frequency of cancer, further supporting the link between recombination and carcinogenesis. In addition, homologous recombination is induced by a wide variety of carcinogens, many of which are classically considered to be efficiently repaired by other repair pathways. Overall, homologous recombination is a process that has been widely overlooked but may be more central to the process of carcinogenesis than previously described.

Keywords: Cancer; Homologous recombination; Loss of heterozygosity; Genomic instability disease; Carcinogen-induced deletion

1. Introduction

Genetic alteration is the fundamental underlying process that allows a normal cell to evolve into a cancerous one. Genetic alterations can take a variety of forms with the essential result being that a gene, or combination of genes, is altered to produce a cell that can bypass normal growth restrictions. The consensus is that the overall alteration is a multi-step process, but the question of how the alteration is produced and what the nature of the alteration is, is still the source of many debates. From examining cancer cells it is obvious that a number of different events can and do take place during carcinogenesis, and that the course of these events is not always the same even within a subclass of a tumor.

The causes of cancer have been determined to be the result of genetic predisposition, environmental exposure, infection by a suitable agent (e.g. human papilloma virus, HTLV1, EBV etc.) or a combination of these. Genetic predisposition is the result of an inherited mutation, often in a gene involved in DNA repair or cell cycle regulation. Such predisposition often results in the early onset of particular cancers in comparison to non-predisposed individuals. These genetically non-predisposed individuals frequently acquire an equivalent mutation, probably

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as the result of a lifetime of environmental exposure, hence the late onset of most cancers without family history, with 80% of cancers being diagnosed in people 55 years and older (for cancer statistics please refer to http://www.cancer.org/statistics/index.html).

Here we will present a body of evidence indicating that one of the important processes of genetic alteration in the generation of cancers is homologous recombination (HR). Evidence from our laboratory, and many others, has demonstrated that certain genetic deficiencies result in higher than normal levels of genomic instability, including a higher frequency of HR. Patients with such genomic instability have a higher probability of developing cancers as the instability allows a higher rate of genetic alteration. These alterations may result in either the direct mutation of a protooncogene or, more likely, these individuals bear an already mutated copy of a cancer predisposing gene. In addition, we will present evidence to demonstrate that environmental exposures to cancer causing agents result in genomic instability, in particular deletion by HR.

2. Models of carcinogenesis

Here we present three commonly accepted models of carcinogenesis (see Fig. 1). There are more complicated possibilities, but, in general, they are most likely to be variations of those presented here. It is not intended that we discuss these models in any detail, as that has been done elsewhere [1–7], but rather we will highlight some of the processes that may involve a HR event.

The simplest model for carcinogenesis is as a one-step event. Most often, a mutation occurs in an oncogene that acts dominantly allowing oncogenesis. An oncogene is a gene that when mutated or expressed at abnormally high levels contributes to converting a normal cell into a cancer cell. Examples of oncogenes include *c-ABL*, *H-RAS*, *c-MYC*, *c-ERBB*, *FOS* and *c-JUN* [8]. Alternatively, the one-step model involves an inherited defect in a tumor suppressing allele that leads to a clinical phenotype by the mutation of its functional counterpart (for a review see [9]). The latter process typically results in loss of heterozygosity (LOH). Two of the most infamous of these inherited disorders are retinoblastoma,

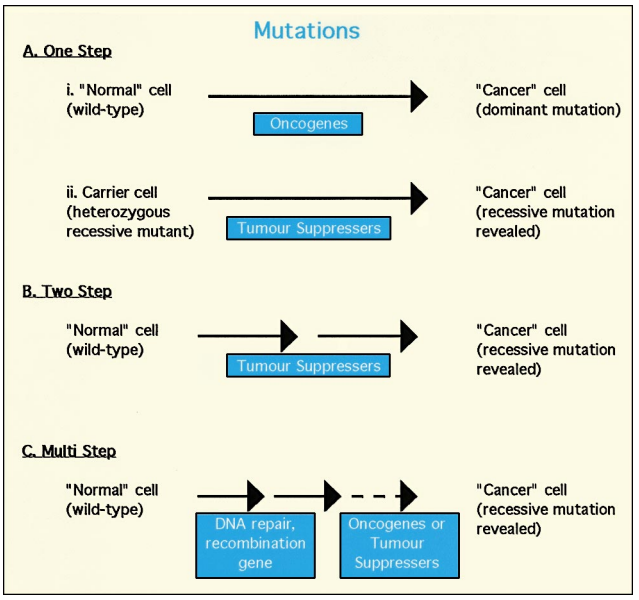

Fig. 1. Model of carcinogenesis. A: The one-step model, where either (i) a normal cell acquires a dominant oncogenic mutation that transforms it to a cancerous one, or (ii) a cell heterozygous for a mutation in a tumor suppressor gene undergoes LOH or mutation of the functional gene copy allowing the cancer phenotype to be revealed. B: The two-step model is an extension of the one-step model, where a normal cell suffers two events, an initial mutation in a tumor suppressor gene and a subsequent LOH to reveal the first mutation and cancer phenotype. C: The multi-step model may simply be a version of the two-step model where more than one tumor suppressor has to be mutated to result in a cancer cell. Alternatively, mutation in a gene responsible for genomic integrity (e.g. genes involved in chromosome segregation, DNA repair, recombination, cell cycle), without genomic fidelity subsequent mutations are more likely to arise, giving a higher probability of producing a cancer cell.

with a mutation in the *RB* gene [10] and Wilm's tumor, with a mutation in *WT1* or *WT2* [11,12].

A simple two-step model allows for the majority of tumor suppressor genes being present as two functional copies, where both copies have to be mutated to incapacitate functionality [13]. In the published literature, LOH is the most commonly reported event, as opposed to mutational heterozygosity. Recombination, be it by deletion of the functional allele or gene conversion of the functional allele into the mutated one, is the most likely mechanism for LOH, as discussed later in this review.

A good example of both the one-step and two-step model is retinoblastoma, a malignant tumor of the retina. There are two forms of retinoblastoma, fam-

ilial and sporadic. The familial form fulfils the one-step hypothesis, with one mutated allele of Rb being inherited from a parent. Random mutation of the second allele in a retinal cell can allow it to grow aberrantly, often resulting in multiple tumors in the retinas of both eyes in the first weeks of infancy. Sporadic retinoblastoma fulfils the two-hit hypothesis, with two normal alleles being inherited and both having to be mutated to result in retinoblastoma formation. In these cases, one typically finds only a single tumor in one eye, usually in early childhood before the retina is fully developed and its mitosis ceases.

The most frequent type of genomic event that results in mutation of Rb is deletion [14–17], usually mediated by HR between *Alu* elements. The susceptibility of proliferating cells to HR-mediated deletion is highlighted later in this review and is probably a determining factor in this mechanism of carcinogenesis.

A multi-step scenario has intriguing implications. Here, the founding, disease-enhancing mutation of an oncogene is not inherited, but rather arises as a result of an intrinsic defect in DNA repair or metabolism. Cells with such a phenotype may accumulate somatic mutations at a higher frequency than normal or may have a higher level of gross genomic instability. Those patients with a predisposition to genomic instability have a difficult prognosis, often experiencing a much earlier onset of cancer than normal individuals.

As yet it has been difficult to determine what gene is initially mutated in most cancers. The reason is two-fold. First, many malignant tumors are composed of a mixture of cells which manifest varying degrees of differentiation and function [5,18–20], often with associated genetic instability [21–24]. Such a complex phenotype may be facilitated by the fact that the initial or founding mutation often affects a DNA repair gene (see below, for reviews see [5,20]). Secondly, not all the genes involved in carcinogenesis have been isolated. Therefore, we may not understand all of the major pathways to carcinogenesis. It seems that defects in several cellular pathways often combine to produce the necessary changes that produce a malignant cell. It is, therefore, very likely that published tumor genotypes are often incomplete (see [25] and http://condor.bcm.tmc.edu/oncogene.html). It is interesting to note that nearly one in 10 normal individuals already harbors oncogenic mutations (p53, Harvey-ras, or N-ras) in their circulating leukocytes [26]. Because overall yearly cancer incidence is nowhere near that high, these observations suggest that such mutations represent only one of several steps in carcinogenesis.

3. HR in mammalian cells

In mammalian cells, HR has, in the past, been viewed as less prevalent than an alternative recombination pathway, namely non-homologous end-joining (NHEJ) [27]. Thus, as a process of DNA repair, HR had been underemphasized [28]. This idea was widely accepted, since it is well known that a large proportion of the mammalian genome contains repetitive DNA sequences [29], with dispersed SINES, LINES, microsatellite and minisatellites. There are also structural elements of chromosomes, centromeres, telomeres and origins of replication. Ribosomal DNA sequences for the 18S and 28S rRNA are present in many copies. Further, there are duplicated genes such as the globin genes that are very closely related at the sequence level, categorized into families or superfamilies (e.g. the immunoglobulin (Ig) superfamily) and even pseudogenes. If HR were a prevalent event after DNA damage, for example, one might expect that recombination between these different repetitive elements would produce adverse gross genomic rearrangements.

In contrast with previous convictions about HR, a recent study has shown that mammalian cells are, in fact, quite proficient in HR [30]. Moreover, Liang et al. have demonstrated that this process is not always perfect, since a site-specific break between two copies of a gene results in homologous deletion at a relatively high frequency (30–50%). Furthermore, in the same study they determined that sister chromatid exchange is highly prevalent, with HR between homologues being next most common. In the last decade we, among several other researchers, have demonstrated that spontaneous deletion can be mediated by HR between repeated DNA fragments [31] and that the frequency of such events is elevated following exposure to cancer causing agents [33–35].

4. HR in carcinogenesis

HR may play a fundamental role in carcinogenesis. In the following sections, we outline six situations in which HR may play a significant role in cancer progression. First, we believe that HR can be a major mechanism in LOH, fulfilling the second step of the two-step model or a later event in a multi-step model of carcinogenesis. Secondly, there are some cancer-prone diseases in which genetic instability is a major phenotype. Some of these diseases also display an elevated level of HR. An increased frequency of HR makes it more likely that LOH can occur at an accelerated rate, but also raises the possibility that HR will beget aberrant genomic rearrangements that may act as the primary step towards carcinogenesis. We also present recent evidence showing that HR is more prevalent in proliferating cells and that a variety of carcinogens induce HR, including oxidative damage, an endogenous by-product of normal metabolism. Finally, we highlight very recent reports of HR acting as an alternative mechanism of telomere maintenance. Together, these arguments provide strong evidence that HR is an important factor in the multiple steps required for carcinogenesis (see Table 1).

Table 1
Possible HR events that may result in phenotypes observed in tumor cells

HR[a] event	LOH[b]	GGI[c]	Heterogeneity[d]	Telomere maintenance
Gene conversion	v			
Deletion	v	v	v	
Unequal crossover	v	v	v	
Gene amplification		v	v	
Translocation	v	v	v	v

[a]HR, homologous recombination.
[b]LOH, loss of heterozygosity.
[c]GGI, gross genomic instability.
[d]Heterogeneity, the continuing evolution of new genotypes in tumor cells.

4.1. Mechanisms of LOH

There are various mechanisms that can result in LOH (see Fig. 2). Basically, LOH results from one allele being lost from a cell that is then either homozygous or hemizygous for the remaining allele. Homozygosity can be attained when a gene conversion event occurs. Hemizygosity can occur when one allele is simply lost. The latter event may occur by deletion of the region containing the gene or by whole or partial chromosome loss.

Gene conversion [29,36,37] is a unidirectional transfer of information. In such an event, DNA is copied [38,39] from one chromosome or chromatid to another without necessarily altering the arrangement of flanking markers. The frequency by which this HR mechanism occurs is difficult to determine as most gene conversions probably go undetected. Much of our understanding of this and other recombination mechanisms comes from analogous comparison to works performed in *Saccharomyces cerevisiae*.

Chromosome loss is a major mechanism of LOH. This type of event results in an abnormality in chromosome number resulting in a cell that is aneuploid. It is interesting to note that almost most invasive epithelial cancer cells are aneuploid (for review see [40]). Aneuploidy represents a form of genetic instability, as highlighted in cases of congenital aneuploidy. Patients with this congenital abnormality often display a high incidence of neoplasia (for reviews see [41,42]).

A translocation is the transfer of a part of one chromosome to a non-homologous chromosome. Translocations are often reciprocal, exchanging two different DNA segments. The break point of a translocation event may occur within a gene, thus destroying/perturbing its function or altering its expression pattern, e.g. Burkitt's lymphoma. One such translocation, that in the Philadelphia chromosome (chromosome 9/22 translocation), produces a *bcr-abl* 'compound gene' and gives rise to chronic myelogenous leukemia. Two studies mapped the break point of the Philadelphia chromosome and found that the 9/22 translocation is likely mediated by a region of shared homology [43,44], implicating HR as a participant in the translocation process.

There are three basic mechanisms that may produce a DNA deletion event (see Fig. 3), replication slippage, intrachromosomal recombination, and interchromosomal recombination. Deletions associated with replication slippage tend to be small [45–49] and occur, most often, in special regions where short tandemly reiterated sequences exist. The most common

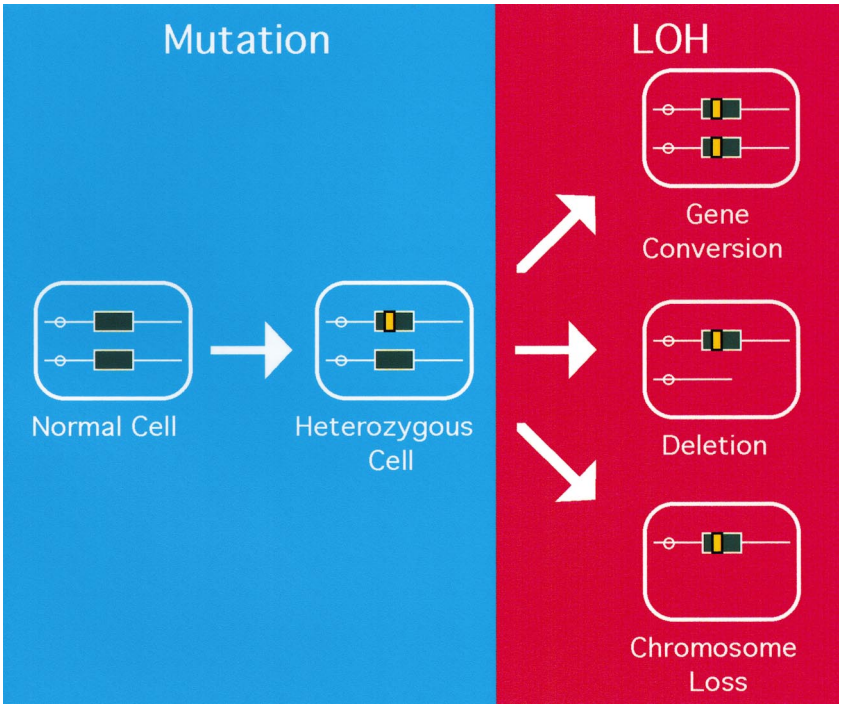

Fig. 2. There are three mechanisms by which LOH may occur. LOH is an important step in carcinogenesis revealing deleterious mutations in genes that restrict cancer phenotypes. The mechanisms shown are gene conversion, where the mutation is copied from the mutated gene to the functional counterpart, deletion of the functional counterpart and chromosomal loss, where the chromosome containing the functional counterpart is lost.

example of this is microsatellite instability, a phenomenon most prominent in hereditary non-polyposis colon cancer. The underlying mutations in this disease map to certain mismatch repair genes and result in a lack of replication proofreading [50–53] and, therefore, an increased frequency of replication errors.

Intrachromosomal deletions arising from aberrant recombination are often large and are typically mediated by regions of homology. Such deletions have been identified as the source of several diseases which include X-linked ichthyosis, where 1.9 Mb of DNA are deleted mediated by flanking homologous S232 elements [54,55], hereditary neuropathy with liability to pressure palsies where 1.5 Mb are deleted mediated by CMT1A-REP [56–58], as well as Prader–Willi syndrome [59], DiGeorge syndrome [60] and familial hypercholesterolemia [61]. There are several mechanisms that may produce an intrachromosomal deletion, two of the most likely being an intrachromosomal crossover event or single strand annealing (reviewed in [62]). Single strand annealing is initiated by a double strand break (DSB) in a non-homologous region between repeats or within a single repeat element. DNA degradation of single strands from exposed 5′ ends of DSBs leads to single-stranded regions which anneal with each other once the degradation has exposed the repeated sequences. The 3′ tails are processed, and nicks are ligated, producing the deletion.

The final class of deletion is an interchromosomal event, such as unequal crossing over between misaligned homologous regions on homologous chromosomes or sister chromatids producing a deletion on one chromosome or chromatid and a duplication of the same region on the other.

Inter- and intrachromatid recombination events are only distinguishable by the presence or absence of a reciprocal duplication product. In this respect, it is interesting to note that Charcot–Marie–Tooth syndrome type 1A occurs from a duplication of the same region as is deleted in hereditary neuropathy with liability to pressure palsies [58,63,64]. Similarly, a tandem duplication within the *ALL-1* gene is medi-

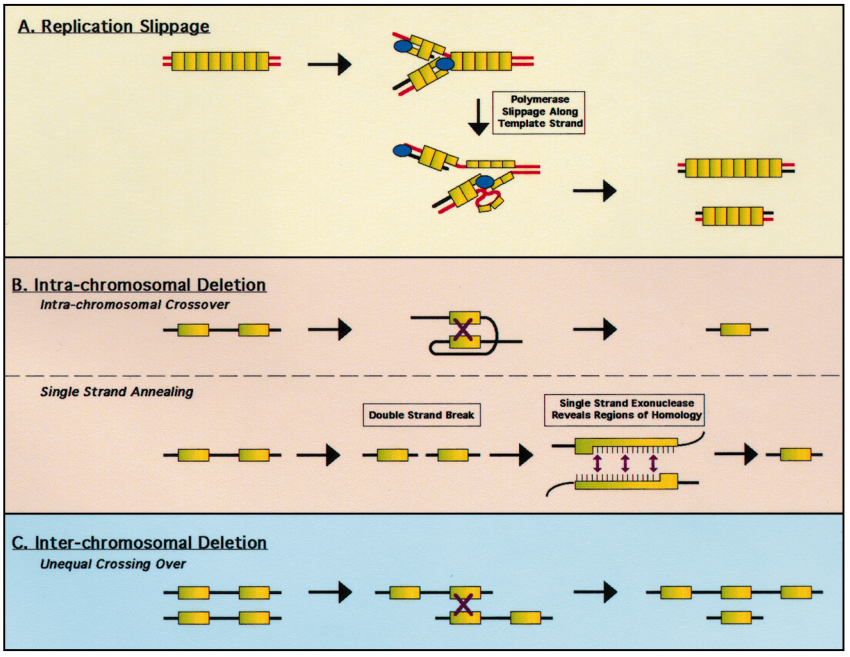

Fig. 3. Mechanisms of deletion. A: Replication slippage, where DNA polymerase dissociates from its template and reanneals to homologous sequences nearby resulting in either a deletion (shown) or insertion (not shown) of sequences. These tend to be relatively small deletions or insertions and are usually in regions of repetitive DNA. B: Intrachromosomal or intrachromatid deletion may be mediated by a number of different mechanisms, two of the most likely being a crossover event and single strand annealing. A crossover event is mediated by aligning homologous sequences, strand invasion, possibly following a single-stranded break, allows strand exchange and recombination between the two homologous sequences. The result is a deletion of the intervening sequences. Single strand annealing is another likely mechanism that requires a DSB between the homologous sequences. A single strand exonuclease can degrade one strand at the DNA ends until homology is revealed allowing the broken ends to anneal and the intervening sequences to be clipped off. C: Interchromosomal or interchromatid deletion is most likely to result from an unequal crossover event. Again, the event is mediated by a repeated region of homology, but in these events two products are formed, a deletion and a triplication on the two resultant recombinant chromosomes.

ated by *Alu* recombination and is associated with acute myelogenous leukemia [65]. These duplications suggest that an interchromatid crossing over mechanism is responsible for these events.

Gene conversion, deletion, and, perhaps, translocation may be mediated by HR. In the past decade, we have used homologous deletion to detect genomic instability in a yeast model systems [66,67], in human cells [34] as well as in vivo in mice [33,35,68,69]. Some of the most interesting results from these studies are discussed later in this review.

4.2. Genetic instability syndromes

If genome rearrangements and deletion events contribute to the development of a significant proportion of cancers, there should be a positive correlation between those mutations that result in a higher recombination frequency and result in cancer predisposition. In fact, there are several genetic diseases that have a genetic instability phenotype and, indeed, have a high frequency of carcinogenesis. These include Ataxia telangiectasia (AT) [70], Li-Fraumeni syndrome [71], Blooms syndrome [72], Werner's syndrome [73], Cockayne's syndrome, Fanconi's anemia (FA), Lynch syndromes I and II, Wiscott–Aldrich syndrome and xeroderma pigmentosum [74].

Li-Fraumeni syndrome is a genetically dominantly disorder characterized by early cancer onset. The most prominent of these cancers are carcinoma of the breast followed by sarcomas, brain tumors, leukemia, lymphoma, lung carcinoma, and adrenocortical carcinoma, usually in children and young adults. The overall risk of cancer in these patients is generally high, with penetrance values sometimes approaching 100% and with over 50% of patients de-

veloping breast cancer by age 50 (reviewed [75]). Li-Fraumeni syndrome patients who carry a recessive mutation in *TP53* have an exceptionally high risk of developing multiple primary cancers [76]. p53 has been proposed to be involved in maintaining the stability of the genome [71,77–84]. At early passages, fibroblasts from p53−/− mice develop several chromosomal abnormalities [85]. Tumors from p53−/− mice are often aneuploid [86,87]. In addition, p53 is suspected of contributing to the regulation of HR via a previously demonstrated interaction with RAD51, again highlighting its role in the maintenance of genomic stability [81,88]. The details of how p53 involvement is manifest are still not clearly understood, though many studies have undertaken to examine the relationship, most showing that cells lacking p53 have a higher than normal frequency of HR [81–83,89–91].

AT is an autosomal recessive syndrome. Among the phenotypes that patients display are chromosomal instability, radiosensitivity, and a predisposition to lymphoid cancer in childhood. Though AT is a relatively rare disorder, it has been estimated that about 1% of the general population is heterozygous for *ATM* mutations [92]. These heterozygous carriers may have a predisposition to sporadic breast cancer though this correlation is still under discussion (for a review see [93]).

Cells from AT patients display chromosomal instability, both spontaneously and following induction by ionizing radiation or radiomimetic agents (reviewed in [94,95]). Cytogenetic analysis of AT patients' cells revealed a higher spontaneous incidence of chromosome breaks, chromosome gaps, acentric fragments, dicentric chromosomes, and aneuploidy. In addition, T-lymphocytes have an elevated frequency of translocations with break points mapping to the T cell antigen receptor genes and the Ig heavy chain genes (reviewed in [95]). Following exposure to ionizing radiation or radiomimetic agents, cells from AT patients have an increased frequency of chromosomal aberrations compared to normal cells [94,95]. In vivo, we have reported that ATM-deficient mice have an increased frequency of spontaneous HR [126]. In comparison, Turker et al. demonstrated that ATM-deficient mice do not display a mutator phenotype [96], and more recently, Cappelli et al. demonstrated that base excision repair works efficiently in AT patient cells [97]. These results suggest that an increased frequency of HR in AT patients may be a molecular basis for the etiology of the disease.

ATM is generally thought to be important in activating a p53 in response to DNA damage [98,99]. Recent reports have highlighted the multifunctional aspect of ATM, including that it phosphorylates BRCA1 [100,101] and NBS-1 [102–105] following irradiation.

Germ line mutations in the BRCA1 or 2 gene are associated with early onset breast and/or ovarian cancer. Selective inactivation of BRCA1 in mouse mammary epithelial cells results in genetic instability such as aneuploidy and chromosomal rearrangements, as well as breast cancer, especially when the relevant animals bear at least a heterozygous loss of function mutation in p53 [106]. In addition, BRCA1 plays a role in HR, in its absence HR repair of double-stranded breaks is defective [107]. BRCA1 and 2 interact, physically, with RAD51, and it has been reported that BRCA1, BRCA2 and RAD51 normally from S-phase form foci in the nucleus, and these foci are enhanced following DNA damage in an ATM-dependent manner [100,101,108–110].

RAD51 is a component of the RAD52 epistasis group, a series of genes defined in yeast to be necessary for a HR reaction. Homologues of this epistasis group that have been identified in mammals to date include RAD51, RAD54 and RAD52. Their involvement in HR is complex, a subject that has been reviewed by others ([111–113]). How the observed foci relate to the induction and/or performance of HR is still unclear.

The genes mutated in Blooms syndromes and Werner's syndrome, *BLM* and *WRN*, respectively, are highly homologous to RecQ helicases [114,115], and have been postulated to be involved in recombination. Cells from Blooms syndrome patients show a high frequency of sister chromatid exchanges, hyper recombination and chromosomal breakage. Patients with Blooms syndrome also show a greatly elevated predisposition to cancer of the sites and types that affect the general population [116]. Similarly, cells from Werner's syndrome patients show a 50-fold elevation in mutation rate, with the predominant form of mutations being gross DNA deletions [73]. Werner's syndrome patients age prematurely and show

features like early onset of cataracts, generalized hair loss, loss of skin elasticity, osteoporosis, atherosclerosis and short stature [117]. They also develop non-epithelial solid tumors rather frequently and, to a lesser extent, leukemia and carcinomas. These cancer-prone diseases have, in common, a defect in genomic stability.

FA is an autosomal recessive genetic disorder characterized clinically by progressive bone marrow failure, skeletal deformities, and a predisposition to tumor development [118,119]. Patient cells manifest extreme chromosomal instability and hypersensitivity to polyfunctional alkylating agents. Most interestingly, cells from FA patients, as well as cell extracts, show a much elevated frequency of HR measured with model plasmids [120].

Though AT has been identified to be the result of a mutation in the *ATM* gene, two other mutations result in syndromes that were originally mistaken to be AT. These variants of AT are caused by mutations in NBS (the syndrome is called Nijmegen Break Syndrome) [121] and in MRE11 [122], and present similar phenotypes, including genetic instability. NBS, MRE11 and RAD50 form a complex that is functionally influenced by NBS, once it is phosphorylated by ATM in response to DNA damage [102–104]. In yeast, it has been shown that RAD50 and MRE11 are involved in NHEJ [123–125], a mechanism that can repair DSBs and competes with HR. Assuming that the mammalian homologues of these genes are also involved, at least in part, in NHEJ, it seems plausible that a deficiency in ATM also results in a slight deficiency in NHEJ. Therefore, damage would be channelled into HR via an alternative pathway, possibly contributing to the hyper recombination phenotype that we found in ATM-deficient mice [126]. Most recently, it has been demonstrated that WRN interacts with the Ku heterodimer [127], the complex thought to bind DSB ends at the initiation of NHEJ [27,128–130]. Thus, in a fashion similar to AT, WRN deficiency might lead to an increased frequency of HR by the absence of its ability to contribute to NHEJ, leaving HR as the remaining avenue to repair of a DSB.

4.3. Carcinogen induction of HR

If genomic rearrangements and deletion events contribute to the development of some tumors, one might predict that certain carcinogens would increase the frequency of genome rearrangements. This has, in fact, been demonstrated in yeast [67,131,132], in human cells [34], as well as in vivo in mice [33,35,68,69].

We have constructed and/or used several different intrachromosomal recombination assays to score for deletion events between repeated DNA elements. These include a repeated mutation allele of *his3* in the yeast *S. cerevisiae* [66], an internal duplication of exons 2 and 3 of the *hprt* gene in a human lymphoblastoma cell line [34], and two partially deleted alleles of the *p* gene in the mouse (the p^{un} (for pink-eyed unstable) mutation) [33]. In yeast, intrachromosomal recombination events resulting in deletions (DEL events) occur more frequently after treatment with many carcinogens, including carcinogens whose activity in this regard is not detected in other assays [67,68,133–135]. In the same way, DEL recombination events in human cells [34] and in mice [33,35,68,69] are inducible by both Ames positive and Ames negative carcinogens (see Table 2).

4.4. Oxidative damage induction of HR

Reactive oxygen species (ROS) are by-products of the normal respiration of a cell [136]. Every living cell metabolizes an energy source to release energy that it harnessed to drive other reactions within the cell. Aerobic respiration is a good source of energy, basically using a carbon source and oxygen to form water and carbon dioxide. Though efficient, this process is not absolutely contained, some energy is lost and some of the intermediate molecules escape metabolism, appearing as ROS. The most potent of these ROS are oxygen radicals such as super oxide and hydrogen peroxide. These are highly reactive agents that can indiscriminately attack a wide variety of cellular macromolecules, including DNA. Several forms of DNA damage are thought to result from these attacks, including thymine glycols, single strand breaks, DSBs, intrastrand crosslinks, and even DNA–protein crosslinks [136].

Considering that oxidative damage is a normal by-product of respiration, it is interesting that oxidative mutagens are powerful inducers of DNA deletions [134]. In addition, several DEL assay positive carci-

Table 2
Inducibility of DEL recombination by carcinogens in the yeast, human cells and mouse assays

Carcinogen	DEL assay			Carcinogen	DEL assay		
	Yeast	Human cells	Mouse		Yeast	Human cells	Mouse
2,4-Diaminotoluene [139]	+			Epichlorohydrin [131]	+		
3-Amino-1,2,4-triazole [131]	+			Ethionine [131]	+		
4-NQO [131]	+			Ethylene dibromide [131]	+		
Acetamide [131]	+			Ethylene oxide [170]	+		
Acrylonitrile [132]	+			Ethylenethiourea [131]	+		
Aflatoxin B1 [131]	+			Formaldehyde [131]	+		
Aniline [131]	+			Hexamethyl phosphoramide [132]	+		
Arochlor 1260 (PCB) [68]			+	Ionizing radiation [33,34,69,131]	+	+	+
Aroclor 1221 (PCB) [68]	+	+	+	Methyl eugenol [133]	+		
Arsenate [35]			+	Methylene chloride [131]	+		
Auramine O [135]	+			MMS [34,35,131]	+	+	+
Benzene [34,35,131]	+	+	+	Nitrogen mustard [131]	+		
Benzo(a)pyrene [35,69]			+	*o*-Anisidine [171]	+		
Cadmium chloride [131]	+			*o*-Toluidine [171]	+		
Cadmium sulfate [131]	+			Propylene oxide [170]	+		
Carbon tetrachloride [131]	+			Safrole [133]	+		
Cyclophosphamide [132]	+			Tobacco smoke [172]			+
DDE [131]	+			TCDD [68]			+
Dimethylhydrazine [131]	+			Thioacetamide [131]	+		
EMS [35,131]	+		+	Thiourea [34,131]	+	+	
ENU [35]			+	Urethane [131]	+		
				UV irradiation [34,131]	+	+	

Chemical abbreviations: DDE: 2,2-bis[4-chlorophenyl]-1,1-dichloroethylene, 4-NQO: 4-nitroquinoline *N*-oxide, ENU: 1-ethyl-1-nitrosourea, MMS: methyl methanesulfonate, EMS: ethyl methanesulfonate, TPA: 12-*O*-tetradecanoylphorbol-13-acetate, PCB: polychlorinated biphenyl, TCDD: 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin. Data for carcinogenicity can be found in the Carcinogenesis Potency Database (http://potency.berkeley.edu/chemicalsummary.html).

nogens, that are negative in other short-term tests, cause oxidative stress [137–140]. The ability of the DEL assay to differentiate between carcinogen–non-carcinogen structural analogs correlates with the more potent ability of carcinogens (compared to non-carcinogens) to induce oxidative stress [138–140]. The presence of an antioxidant, *N*-acetyl cysteine, during the exposure to such a carcinogen reduced both its toxicity, as well as its induction of DEL recombination frequency. These studies indicate that measuring the frequency of deletions is an extremely sensitive method of detecting an oxidative mutagen. These observations support the notion that deletions accumulate over a lifetime, probably because of the constant onslaught of ROS produced by endogenous as well as exogenous factors. This proposed accumulation of deletions over the lifetime correlates with the strong age dependence of the occurrence of most non-familial cancers.

4.5. Susceptibility of proliferating cells to HR

Actively dividing cells are thought to be the most prone to developing cancer. Mitogenesis (induced cell division) has been proposed to be an important contributor to carcinogenesis [141,142] as evidenced by a higher risk for cancer after tissue damage and regeneration [143–145] and has been suggested to occur in a similar fashion following wound healing [142]. Furthermore, chemical carcinogenesis and transformation are most efficient if the target cells are treated just prior to or during S-phase [4,146]. Studies on yeast conducted in our laboratory have probed the effect of cell cycle arrest on carcinogen-mediated induction of deletions mediated by HR. It was found that only DSBs induce DEL recombination in arrested cells. For induction of DEL by other forms of DNA damage, such as DNA single strand breaks, UV lesions, as well as exposure to

alkylating agents, there must be DNA replication [147,148].

As mentioned earlier, HR events are, in part, mediated by the RAD52 epistasis group. It is interesting to note that the protein and mRNA levels of this group tend to correlate with cell proliferation. For example, RAD51 expression is highest in intestinal and uterine epithelia [149] which are highly proliferative. Consistent with the correlation with cell proliferation, both RAD51 and RAD54 are maximally transcribed at late G1/early S, i.e. at the beginning of DNA synthesis [149,150]. Recently it has been proposed that HR performs a special function during replication, namely in resolving stalled replication forks [112,151]. In a recent study from our laboratory, we have correlated cells that are proliferating during development with a susceptibility to HR induced by carcinogen exposure [152], thus affirming that HR is a normal repair process most prevalent in dividing cells. Altogether, it appears that HR is a relatively common event in normal cells and may be more active in highly proliferative cancer cells facilitating their evolution.

4.6. Telomere maintenance by HR

In the generation of cancer, immortalization is sometimes a determining event. It is well known that a cell is capable of a finite number of cellular divisions in tissue culture. At each round of cellular division, the chromosomal termini, or telomeres, are shorter than in the previous generation [153–156]. Eventually, telomeres become too short, and cell crisis develops. Few cells manage to pass this crisis barrier to become immortal, but those that do are broadly classified as having reactivated their telomerase activity [157,158] or found another mechanism to maintain functional telomere length [159–161]. This alternative mechanism appears to be HR-dependent, as has been shown elegantly in yeast [162–164] and now in mammalian cells [165–168]. Here is another way in which HR plays a vital role in the emergence of a viable neoplastic cell.

5. Conclusions

In conclusion, we have presented a body of evidence showing that HR can participate in various stages of carcinogenesis. We believe that HR functions mostly as a secondary step in cancer progression and have presented evidence that a wide variety of carcinogenic agents induce HR. The latter suggests that normal, day-to-day exposure to a variety of environmental stimuli increases the frequency of HR. As can be observed in those patients who have an intrinsically upregulated level of HR, an abnormal tendency to perform HR events can be deleterious. In addition, the extraordinary sensitivity of proliferating cells to HR correlates closely with such cells being more prone to carcinogenesis and fits with current models of replication/recombination. Finally, HR is likely to play a major role in producing the observed biological and genetic heterogeneity in many tumors. All in all, it seems likely that HR is a more prevalent contributor to carcinogenesis than previously considered.

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