

Chapter 2

DNA Repair Dysregulation in Cancer: From Molecular Mechanisms to Synthetic Lethal Opportunities

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Abstract Targeted cancer therapies have excellent potential for increasing long-term patient survival while minimizing short- and long-term side effects of therapy including neurological problems and secondary cancers. DNA repair systems suppress genome instability that drives the acquisition of tumorigenic mutations. Most cancer therapeutics cause DNA damage, yet despite having DNA repair defects, tumors often display resistance to therapy because redundant repair pathways can process DNA damage efficiently. By targeting the redundant repair pathways, tumors can be sensitized to endogenous and/or exogenous DNA damage, described as synthetic lethality or synthetic sensitivity. There have been notable successes in applying synthetic lethal and sensitive approaches to treat cancer, but challenges remain as tumors can acquire resistance due to their rapid evolution driven by ongoing genome instability. It is important to improve our understanding of DNA repair pathways to better exploit tumor weaknesses imparted by DNA repair defects.

Keywords DNA damage • DNA double-strand break repair • Non-homologous end-joining • Homologous recombination • Nucleotide excision repair • Base excision repair • Mismatch repair • Mutagenesis • DNA replication stress • Targeted cancer therapy • Synthetic lethality • Genome instability

2.1 Introduction

DNA repair pathways play critical roles in cancer suppression, etiology, and therapy. DNA damage is ubiquitous, and organisms have evolved sophisticated mechanisms to repair the many types of DNA lesions that arise spontaneously and that are induced by exogenous genotoxins including radiation and reactive chemicals. DNA repair pathways play important roles in the accurate transmission of the

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genome to daughter cells, a central feature of cell division. However, DNA repair is principally designed to restore the *chemical* integrity of DNA without regard for restoring *genetic* integrity, hence repair of DNA damage is sometimes associated with genetic change, i.e., mutation, ranging from single-base substitutions to chromosomal rearrangements. These changes may be detrimental, leading to cancer or other genetic diseases, but they also play important roles in immune system development and evolution.

There are five main classes of DNA repair pathways, each with multiple subpathways. For the most part, specific types of DNA lesions are repaired by a specific pathway/subpathway, but if repair fails, the lesion may be shunted to a secondary (redundant) pathway. In addition, it is often the case that particular steps along a DNA repair pathway create other forms of damage (i.e., single-strand breaks) that require repair. Thus, the constellation of DNA repair pathways function in complex networks. The redundancy inherent in these networks creates robust systems that maintain genome stability and confer resistance to the cytotoxic effects of DNA damaging agents. This is beneficial in normal cells and tissues, but resistance of cancer cells to radio- and chemotherapy presents significant challenges to oncologists. A deep understanding of DNA repair mechanisms and pathway redundancy can reveal weaknesses in cancer cells that can be exploited to improve therapeutic outcomes. Thus, cancer cells with a defect in a primary DNA repair pathway may be dependent on a redundant, secondary pathway for survival. In this case, inhibiting the secondary pathway is lethal to cancer cells, but not normal cells which retain the functional primary pathway (Fig. 2.1a, b). If the damage processed by these pathways arises spontaneously, the lethal combination (“synthesis”) of genetic mutations (and/or inhibited targets) in redundant pathways is termed “synthetic genetic lethality” or simply “synthetic lethality.” If damage is induced by a genotoxin,

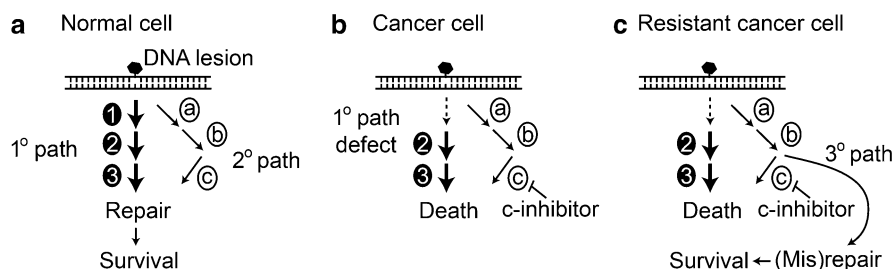


Fig. 2.1 Synthetic lethality. (a) Idealized DNA repair pathways in a normal cell that process a specific DNA lesion, with primary and secondary pathways catalyzed by enzymes 1–3 or a–c, respectively. (b) Cancer cell lacking enzyme 1 shifts repair from the primary to secondary pathway. Inhibition (or mutation) of any step in the secondary pathway is synthetically lethal. (c) Activation of tertiary pathway rescues the cell from synthetic lethality, producing a cancer cell resistant to the inhibitor. The tertiary pathway may be error-prone; the cell may survive the damage but with increased mutation load (genome instability) due to misrepair, which can drive tumor progression and further resistance to subsequent therapy

rather than arising spontaneously, these types of genetic interactions are properly described as conferring “synthetic sensitivity” to the genotoxin. While therapeutic approaches based on synthetic lethality and sensitivity clearly have merit, it is important to note that tertiary repair pathways may exist, or they may arise or be activated through other mutations, allowing cancer cells to evade synthetic lethality or sensitivity (gain resistance) (Fig. 2.1c). The promise of synthetic lethality and synthetic sensitivity hinges on detailed knowledge of DNA repair pathways for the design of effective targeted cancer therapies.

2.2 DNA Repair Mechanisms

2.2.1 *Base Excision Repair, Nucleotide Excision Repair, and Mismatch Repair*

There are five classes of DNA repair pathways, each with two or more subtypes (Fig. 2.2). Three sets of pathways act on damage present on single strands. Base excision repair (BER) comprises several subpathways that repair non-bulky DNA lesions such as ring-opened bases and small adducts like oxidized bases (Fig. 2.2a). The first step in BER is lesion recognition by one of several glycosylases that remove the damaged base, producing an abasic site. PARP1, APE1 endonuclease, and deoxyribosephosphodiesterase activities create a single-strand break and DNA repair is completed by DNA polymerase and DNA ligase activities (Krokan and Bjoras 2013). Nucleotide excision repair (NER) processes bulky lesions that distort the double helix, including pyrimidine dimers and large DNA adducts Kamileri et al. 2012) (Fig. 2.2b). NER requires lesion recognition proteins and endonucleases that create single-strand breaks ~15 nt from the lesion, a helicase removes the ~30 nt oligonucleotide carrying the lesion, DNA polymerase then fills the single-strand gap and ligase completes the repair. Mismatch repair (MMR) differs from all other DNA repair pathways in that there is no “damage” per se, but instead chemically intact mismatched bases occur on otherwise complementary strands (Hsieh and Yamane 2008). Mismatches arise via replication errors, strand exchange during homologous recombination (HR), and deamination of 5'-methyl cytosine which produces thymidine and a G-T mismatch. MMR also processes single- and multi-base loops that arise when bases are inserted or deleted – insertion/deletion loop mismatches can arise by replication errors (especially in sequences with mononucleotide repeats, or certain trinucleotide repeats that are prone to self-annealing as these form relatively stable hairpin structures), or during homologous recombination (see below). MMR involves mismatch recognition, single-strand nicking 5' and/or 3' of the mismatch from which long-tract single-strand excision removes the mismatched base(s), re-synthesis to fill the single-strand gap, and ligation (Fig. 2.2c). Long-tract MMR operates on mismatches that arise during replication and HR; G-T mismatches can be repaired by long-tract MMR or by a specialized G-T MMR

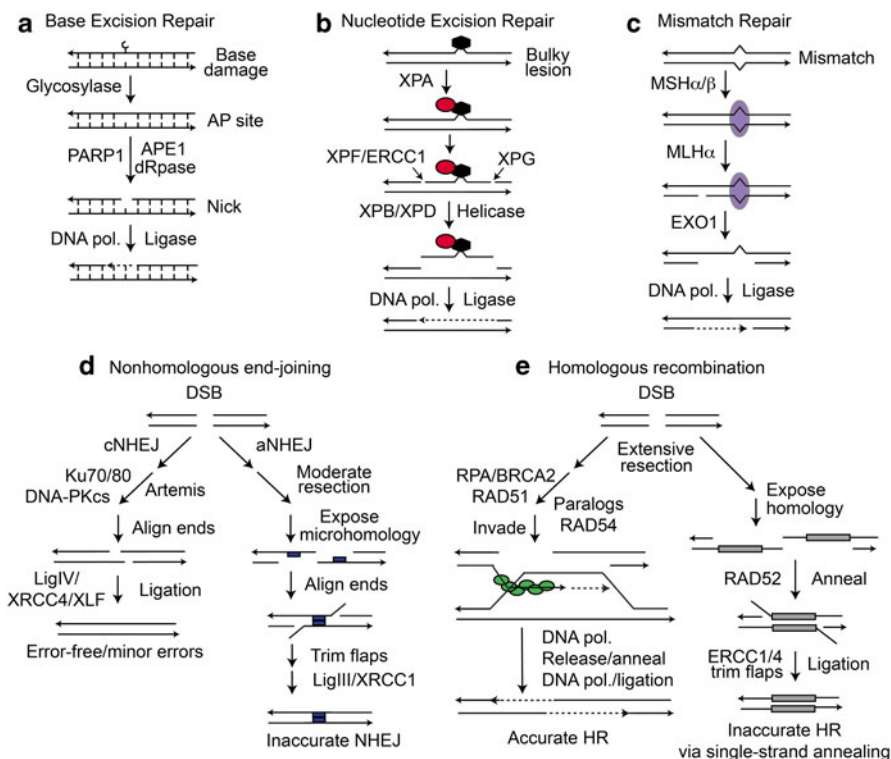


Fig. 2.2 DNA repair pathways. (a) Repair of base damage by BER results in short patch repair and is promoted by PARP1. (b) Bulky lesion repair by NER removes a ~30 nt single strand oligonucleotide carrying the lesion. (c) MMR involves long-patch excision and resynthesis initiated at nicks distant from the mismatch. (d) NHEJ includes relatively accurate cNHEJ, and inaccurate aNHEJ pathways distinguished by the extent of end resection, requirement for microhomology (blue boxes). (e) HR catalyzed by RAD51 (green ovals) is generally accurate. BRCA1 and FANC proteins (not shown) also function in RAD51-dependent HR. SSA between linked repeats (grey boxes) deletes one repeat and intervening sequences

system that is more akin to BER as it is initiated by a G-T specific glycosylase (Bill et al. 1998; Wiebauer and Jiricny 1990). BER, NER, and MMR are relatively accurate repair mechanisms because each uses an intact complementary strand opposite the lesion to direct repair. However, repair polymerases tend to be less accurate than replicative polymerases, and this can result in localized mutagenesis. In addition, BER proceeds through abasic intermediates that can be subject to translesion DNA synthesis by Y-family polymerases, which are low processivity, error-prone DNA polymerases, providing another path to localized mutagenesis (Simonelli et al. 2005). There are also examples where the accuracy of DNA repair systems are downregulated to specifically enhance mutagenesis, e.g., MMR induced trinucleotide repeat expansion and antibody maturation (Pena-Diaz and Jiricny 2012).

2.2.2 *Features and Roles of DSBs and DSB Repair Pathways*

DSBs are the most important DNA lesion because they can trigger genome rearrangements and unrepaired DSBs are usually lethal. DSBs are induced by ionizing radiation, and by endogenous nucleases during meiosis (SPO11) and V(D)J recombination (RAG1/2) (Keeney and Neale 2006; Nishana and Raghavan 2012). AID deaminates cytosine to uracil which can be processed to staggered single-strand breaks to create DSBs that trigger immunoglobulin class switch recombination or gene conversion (Daniel and Nussenzweig 2013), which along with V(D)J recombination are important mechanisms for generating antibody diversity. DSBs also arise during DNA replication when forks encounter blocking lesions (single-strand breaks, many types of base damage, most DNA adducts, pyrimidine dimers, and intra- and inter-strand crosslinks) (Allen et al. 2011; Budzowska and Kanaar 2009). DSBs are marked by phosphorylated histone H2AX (γ -H2AX) (Ward and Chen 2001) which plays important roles in DNA damage checkpoint signaling and DSB repair (Chanoux et al. 2008; Downey and Durocher 2006).

DSBs are repaired by nonhomologous end-joining (NHEJ) and HR (Fig. 2.2d, e). DSB repair by NHEJ is frequently inaccurate, yielding short (1–20 nt) deletion or insertion mutations (Deriano and Roth 2013). When two DSBs occur simultaneously on different chromosomes, NHEJ can mediate translocations (Lieber et al. 2006; Nickoloff et al. 2008; Weinstock et al. 2006). HR is generally accurate, but since HR can occur between any two homologous sequences (sister chromatids, homologous chromosomes, linked repeats in inverted or direct orientation, and repeats on non-homologous chromosomes), HR poses significant risks of medium- to large-scale genome rearrangements including deletions, inversions, amplifications, small- to large-scale loss of heterozygosity (LOH), and translocations (Nickoloff 2002).

2.2.3 *DSB Repair by Nonhomologous End-Joining*

NHEJ comprises two pathways, classical and alternative NHEJ (cNHEJ, aNHEJ) (Fig. 2.2d). Although both pathways are inaccurate, cNHEJ is more accurate and is the dominant DSB repair pathway in mammalian cells; aNHEJ appears to serve as a backup pathway to cNHEJ as it is typically observed when cells have a defect in a cNHEJ factor (Deriano and Roth 2013; Weinstock et al. 2007; Wray et al. 2010, 2013). cNHEJ involves little to no end-resection, whereas moderate end-resection is key to exposing microhomologies on complementary strands central to the aNHEJ pathway (aNHEJ is sometimes called “microhomology-mediated end-joining”). 53BP1 and BRCA1 are implicated in regulation of end-resection, which in turn regulates cNHEJ – aNHEJ pathway choice (and NHEJ – HR choice; see below) (Deriano and Roth 2013; Panier and Boulton 2014; Symington and Gautier 2011).

cNHEJ initiates with Ku70/Ku80 binding to DSB ends to which DNA-PKcs is recruited, activating its kinase. Artemis is a nuclease required for processing certain

types of broken ends (Jacobs et al. 2010). Metnase is a recently evolved nuclease and protein methylase that promotes cNHEJ by enhancing recruitment and retention of other NHEJ factors by methylating histone H3 (Fnu et al. 2011), and possibly through nucleolytic end processing (Hromas et al. 2008). Together these factors promote association of broken ends with little to no base-pairing (Lieber 2010). Prior to ligation by Ligase IV and accessory factors XRCC4 and XLF, DNA-PKcs phosphorylation by itself or ATM stimulates its dissociation from ends (Dahm 2008; Lieber 2010). Genetic defects in, or inhibition of, cNHEJ factors shunts DSBs to aNHEJ, which depends on moderate end-resection by MRE11-RAD50-NBS1 (MRN) and CtIP to expose microhomologies, and requires PARP1 and Ligase III-XRCC1 (Deriano and Roth 2013; Rupnik et al. 2010; Wray et al. 2013; You et al. 2009). By suppressing resection by MRN and CtIP, 53BP1 prevents aNHEJ and thus reduces the risk of aNHEJ-mediated translocations (Bothmer et al. 2010). PARP1 inhibitors also prevent aNHEJ-mediated translocations, and may be useful in reducing the risk of oncogenic translocations associated with cancer chemotherapy (Wray et al. 2013).

2.2.4 DSB Repair by Homologous Recombination

HR comprises two pathways, a conservative, accurate pathway that involves strand invasion mediated by RAD51, and a non-conservative, error-prone, RAD51-independent pathway termed single-strand annealing (SSA) (Fig. 2.2e). Both HR pathways require extensive end-resection (100–1,000s of bases). Cells with defects in RAD51-dependent HR shunt DSBs to SSA, destabilizing the genome (Tutt et al. 2001). DSB repair pathway choice is apparently regulated by proteins that control resection, including 53BP1 and BRCA1 (Panier and Boulton 2014), with increasing resection along a cNHEJ – aNHEJ – HR continuum. The extensive resection required for HR begins with end-processing by MRN-CtIP followed by BLM helicase and two nucleases, DNA2 and EXO1 (Symington and Gautier 2011). RAD51-dependent HR can lead to localized LOH termed gene conversion, a mechanism that nearly always results in unidirectional (non-reciprocal) transfer of information from an unbroken donor molecule to a broken homologous molecule during DSB repair – the LOH region is termed a gene conversion tract and these can range from just a few bp to many kbp. Gene conversions are sometimes associated with reciprocal exchange of sequences flanking the conversion tract, and during HR between homologous chromosomes, 50 % of crossovers can cause LOH of an entire chromosome arm, extending from the point of the crossover to the telomere (Nickoloff 2002). In cells with defects in HR proteins, RAD51-mediated strand exchange may abort after the strand invasion and repair synthesis is initiated but before second end capture. In this case, synthesis continues to the end of the donor chromosome, which also results in LOH from the DSB to the telomere in a process termed “break-induced replication” (Llorente et al. 2008). As noted above, crossovers also pose significant risk

of large-scale genome rearrangement, and are suppressed by proteins like BLM (Cheok et al. 2005). RAD51-dependent HR is generally restricted to S and G2 phases when sister chromatids serve as closely associated, essentially 100 % accurate repair templates with low risk of large-scale genome rearrangement.

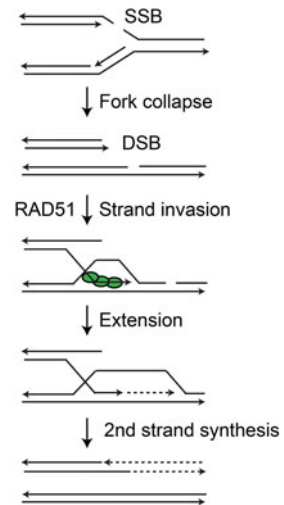
End resection produces long, 3' ssDNA tails bound by RPA that is exchanged for RAD51 in a reaction promoted by “mediator” proteins BRCA2, RAD52, and RAD51 paralogs (XRCC2, XRCC3, RAD51B, RAD51C, and RAD51D). The RAD51-ssDNA nucleoprotein filament searches for and invades homologous sequences, the invading strand is extended by DNA polymerase, and then released and “captured” by the resected end on the opposite side of the DSB. This one-ended invasion mechanism, termed synthesis-dependent strand annealing (SDSA), poses low risk of crossovers (Fig. 2.2e). In some cases, both ends invade, creating a double Holiday junction intermediate that is resolved by BLM-TOP3 α -RMI1-2 without crossing over (Heyer et al. 2010). In the absence of BLM, crossovers are much more frequent and this confers a genome-instability and cancer-prone phenotype (Cheok et al. 2005). Some RAD51 mediator proteins also function later in HR, stabilizing the strand invasion intermediate and removing RAD51 from ssDNA after the RAD51-ssDNA filament invades the homologous sequence to prepare for repair synthesis (Brenneman et al. 2002; Fortin and Symington 2002). RAD54 (and possibly RAD54B) also act late, altering chromatin in donor sequences to promote strand invasion (Heyer et al. 2010).

The SSA pathway (Fig. 2.2e) is RAD51-independent and at least in yeast, depends on the strand annealing activity of RAD52 (Heyer et al. 2010). SSA involves extensive resection to expose complementary sequences in linked, direct repeats which anneal to produce a deletion product lacking one of the repeats and the intervening sequences. SSA can also mediate translocations (Weinstock et al. 2006). SSA and RAD51-mediated gene conversion are competing HR pathways, and the balance shifts toward SSA for closely spaced repeats (Schildkraut et al. 2005).

2.2.5 Role of HR in Replication Stress Responses

Nearly all DNA lesions, whether induced by chemotherapeutics or ionizing radiation, block replicative DNA polymerases, causing “replication stress.” RAD51-mediated HR plays a critical role in restarting stalled and collapsed replication forks, and it is this role that accounts for the fact that RAD51 is an essential protein in mammalian cells (Allen et al. 2011). When replication forks encounter blocking lesions, the fork stalls and the replisome is stabilized by DNA repair and checkpoint proteins including RPA, ATR-ATRIP, ATM, BLM, and INO80 (Budzowska and Kanaar 2009; Davies et al. 2007; Shimada et al. 2008; Zou et al. 2006). If a stalled fork is not restarted in timely manner, it collapses to a DSB. Unlike DSBs induced by nucleases or ionizing radiation, fork collapse yields single-ended DSBs that cannot be readily repaired by NHEJ. Like two-ended DSBs, one-ended DSBs are

Fig. 2.3 Restart of collapsed replication fork. Fork encounter with lesion (single-strand break in this example) causes collapse to one-ended DSB. RAD51 loads onto resected 3' end that invades the sister chromatid, is extended, and then serves as template for second strand synthesis to complete DNA replication



marked by γ -H2AX, and the induction and resolution of this signal are measures of fork collapse and repair (De Haro et al. 2010; Kim et al. 2014). Stalled and collapsed replication forks can be restarted by several HR-related mechanisms (Allen et al. 2011; Budzowska and Kanaar 2009); one mechanism is shown in Fig. 2.3. A fork stalled by a blocking lesion can “regress”, and this allows the lesion to be bypassed via an HR mechanism (Budzowska and Kanaar 2009). Lesion bypass via fork regression is not a repair mechanism, but a damage tolerance mechanism. Another type of damage tolerance mechanism is mediated by error-prone, translesion synthesis (TLS) polymerases (Sale 2013). Understanding tumor cell responses to replication stress, and in particular, the role of checkpoint and HR pathways in these responses, has emerged as a critical topic in cancer biology.

2.3 DNA Repair Pathway Regulation and Networks

DNA repair pathways are highly regulated and exist in complex, interacting networks. DNA repair can be regulated in many ways. For example, repair proteins may be absent (gene knockout) or exist as hypomorphs with reduced or altered function; mRNA levels can be altered by transcription factors, microRNAs, and other factors that regulate mRNA stability; and protein stability and function can be altered by posttranslational modifications such as phosphorylation, ubiquitylation, and SUMOylation. There is evidence that oxidative damage upregulates both BER and NER (Cabelof et al. 2002; Kirkali et al. 2011), and gene expression profiling has shown that DNA damage upregulates many DNA repair genes (Friedberg et al. 2005). At a functional level, exposure of cells to low levels of DNA damage confers resistance to a subsequent higher dose, so-called adaptive responses (Huang et al. 2006;

Preston 2005). Adaptive responses are protective, which is beneficial for normal tissue, but can have the negative effect of conferring resistance of the tumor to treatment. Adaptive responses may reflect broad effects of damage on repair, checkpoint, and programmed cell death pathways, rather than upregulation of specific DNA repair pathways. For example, there is no clear evidence for upregulation of HR by DSB damage (Heyer et al. 2010), yet adaptive responses to ionizing radiation have been described (Preston 2005).

As noted above, DSB repair pathway choice is regulated by resection of DSB ends in a cell cycle dependent manner (Durant and Nickoloff 2005; Shrivastav et al. 2008; Symington and Gautier 2011). The upregulation of HR during S and G2 phase probably reflects the combined effects of end resection and availability of sister chromatid repair templates. When considering synthetic lethal or sensitivity approaches to cancer therapy, DSB repair illustrates an important principal: a single type of lesion can be shunted to multiple repair pathways that operate in hierarchical fashion (Fig. 2.1). In the case of DSBs, this hierarchy runs from cNHEJ to aNHEJ to HR. Moreover, DNA repair pathways display considerable functional overlap. As just one example of network connectivity, NER has known functional interactions with MMR, BER, HR, NHEJ, and TLS (Shaheen et al. 2011). There are several types of interactions among repair pathways, including shared repair factors, processing of specific lesions by multiple pathways, and common repair intermediates produced by different pathways.

DNA repair pathways are also tightly integrated with DNA damage checkpoint pathways. DNA damage checkpoints were originally defined as damage sensor, signaling and effector pathways that arrested cells in specific phases of the cell cycle, ostensibly to allow time for repair before resuming the cell cycle to reduce problems associated with replication or segregation of damaged DNA. However, studies in yeast demonstrated that artificially arresting certain checkpoint-defective mutants failed to rescue damage sensitivity (DeMase et al. 2005; Redon et al. 2003; Toh et al. 2006). There is now clear evidence that checkpoint factors, such as ATM and γ -H2AX regulate both checkpoints and DNA repair (Downey and Durocher 2006; Shrivastav et al. 2009; Smith et al. 2010; Xie et al. 2004).

The tumor microenvironment imposes considerable stress on tumor cells, including glucose and oxygen deprivation, low pH, replication stress associated with activated oncogenes, and of course, there is stress induced by genotoxic therapeutics (Bartkova et al. 2006; Gozuacik and Kimchi 2004; Karantza-Wadsworth et al. 2007; Mathew et al. 2007). There is substantial evidence that cells actively upregulate mutagenesis in response to stress by at least two mechanisms: DSB-induced gene amplification, which can increase expression of proteins that confer stress resistance, and switching from accurate to error-prone DSB repair mechanisms. These processes underlie “adaptive mutagenesis” which helps generate mutants that are better adapted to a stressful environment, and in essence, reflect “regulated evolvability” (Galhardo et al. 2007; Gonzalez et al. 2008; Hastings et al. 2009; Ponder et al. 2005). The inherent complexity of DNA repair networks and their regulation, coupled with the immense genetic heterogeneity of solid tumors, presents both challenges and opportunities for developing synthetic lethal and sensitivity approaches to cancer treatment.

2.4 DNA Repair and Genome Instability: Roles in Cancer Etiology, Tumor Progression and Resistance to Therapy

Genome instability was recognized early as a hallmark of cancer. Cancer arises when cells acquire altered cell growth properties including independence from growth signals, immortality, and defects in programmed cell death pathways. Tumor progression to a more aggressive state depends on alterations that affect tissue responses including angiogenesis, tissue invasion, and adaptability to new tissue environments which drive metastasis. Although it has long been known that cancer cells have unstable genomes, it was difficult to establish whether genome instability was an early driver of cancer, or a secondary manifestation of the cancer state. There are now several well-established cases where genome instability has been definitively shown to precede cancer (Hanks et al. 2004; Lengauer et al. 1998; Pikor et al. 2013; Shih et al. 2001; Weaver et al. 2007). Although the number and types of genetic changes required to initiate tumorigenesis and promote tumor progression vary among different types of cancers, a common theme is the early acquisition of defects in DNA repair systems that play critical roles in genome stabilization.

Genome instability manifests over a wide scale, from point mutations, trinucleotide repeat expansions and contractions, gene duplications, deletions, and inversions, to large-scale chromosome changes including translocations and whole chromosome gains and losses. Defects in DNA repair pathways contribute to specific instabilities (Fig. 2.4). Point mutagenesis is greatly increased by defects in BER, NER, and MMR. MMR also suppresses microsatellite repeat expansion or contraction reflecting replication slippage at short repeats such as trinucleotide repeats. cNHEJ and RAD51-dependent HR suppress translocations by aNHEJ and SSA, respectively.

Thus, DNA repair defects cause genome instability, which accelerates the acquisition of mutations in critical growth regulatory genes, including gain-of-function

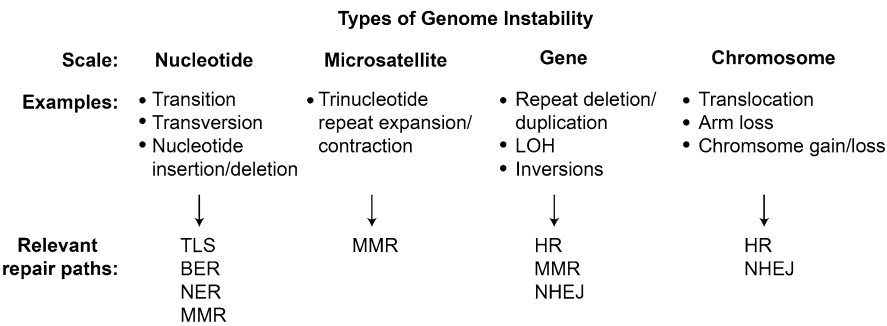


Fig. 2.4 Genome instability and DNA repair. Genome instabilities result in small, moderate, and large scale genome alterations. Indicated repair pathways suppress or induce different types of instabilities

mutations in proto-oncogenes and loss-of-function mutations in tumor suppressor genes (Wang 2005). Defects in specific DNA repair pathways are associated with heritable cancer syndromes such as colon cancer (MMR), skin cancer (NER), and breast cancer (HR) (Boulton 2006; Friedberg 2001; Gudmundsdottir and Ashworth 2006; Jass 2002; Jiricny 2006; Venkitaraman 2002).

Conversion of a normal cell to a metastatic cancer cell may require 3–10 mutations in key cell growth and tissue regulatory genes (Ilyas et al. 1999; Schedin and Elias 2004; Spurgers et al. 2006). Mutation rates in normal mammalian cells are low, $\sim 10^{-10}$ per base per cell generation, which translates to 10^{-6} to 10^{-8} mutations per gene per cell generation (Baer et al. 2007; Roach et al. 2010). Assuming mutations arise independently, even at the higher rate of 10^{-6} mutations per gene per generation, the odds of accumulating as few as five critical mutations in a single cell is vanishing small ($\sim 10^{-32}$). DNA repair defects greatly increase mutation rates and the odds of accumulating critical mutations.

Mutations arise infrequently in undamaged DNA, e.g., by base mis-incorporation during DNA replication, but mutation rates are dramatically increased at or near sites of DNA damage as a result of inaccurate DNA repair or error-prone lesion bypass, including translesion DNA synthesis and recombinational mechanisms (Nickoloff 2002; Sale 2013; Shaheen et al. 2011). DNA lesions can arise spontaneously, reflecting the chemical lability of DNA (e.g., deamination of cytosine and 5-methyl cytosine to uracil and thymidine, respectively); damage caused by reactive oxygen species (ROS) formed during normal cell metabolism; and single- and double-strand breaks created by nucleases or when replication forks collapse (Allen et al. 2011; Barnes and Lindahl 2004; Caldecott 2008; Gates 2009). DNA oxidation by ROS is a major source of mutations as it produces many types of lesions including oxidized bases (e.g., 8-oxoguanine), abasic sites, bulky lesions (e.g., etheno and protein-DNA adducts), strand breaks, and DNA crosslinks (Waris and Ahsan 2006). Genotoxic agents are mutagenic because DNA repair does not always accurately restore the original DNA sequence.

When cells acquire a DNA repair defect, increased mutagenesis drives early stage tumorigenesis through alterations in key growth regulatory genes, and it also drives the rapid evolution of tumor cells that promotes tumor progression. As long as mutation rates remain below a critical “error catastrophe” limit (Fox and Loeb 2010), high mutation rates allow tumor cells to “test” mutations that allow them to adapt to various types of stress, including nutrient and oxygen deprivation, a common feature of tumor microenvironments, and to develop resistance to therapy. Most cancer patients are treated with DNA damaging agents, even when tumors are resectable, including chemotherapeutic drugs and/or ionizing radiation. These approaches exploit the fact that tumor cells divide more rapidly than normal cells, and cells actively replicating DNA are highly susceptible to the cytotoxic effects of DNA damage. This is because nearly all types of DNA damage block replicative DNA polymerases, causing forks to stall and eventually collapse to cell-lethal DSBs (Allen et al. 2011; Branzei and Foiani 2010). Nonetheless, tumor cells can be highly resistant to traditional therapies. It seems paradoxical that tumorigenesis can be driven by defects in DNA repair genes yet the resulting rapidly growing cells are

often resistant to DNA damaging agents. Although in certain cases this may reflect the use of genotoxins that create specific types of DNA damage that are repaired by pathways that remain functional in a particular tumor (i.e., failure to appropriately tailor the treatment to the specific DNA repair defect), there are many other ways to resolve this paradox. For example, resistance to therapy may reflect upregulation of other DNA repair pathways or drug efflux pathways, and/or defects in apoptosis and other programmed cell death pathways that are normally triggered by heavy loads of DNA damage. In this light it is noteworthy that ~50 % of tumor cells carry defects in p53, which plays critical roles in apoptosis (Carvajal and Manfredi 2013).

As noted above, the increase in genome instability associated with DNA repair defects promotes the acquisition of mutations, including those that drive changes in other DNA repair, drug efflux, and programmed cell death pathways. The realization that rapid evolution of tumor cells allows them to adapt to stressful environments and acquire resistance to genotoxic therapeutics, has forced cancer biologists to reevaluate therapeutic strategies. The traditional approach to induce DNA damage in tumor cells with chemo- and/or radiotherapy is fairly effective at killing bulk tumor cells, but this damage can also generate (and ultimately select for) a subpopulation of tumor cells that are resistant to therapy, and moreover, potentially generate more aggressive tumor cells leading to local tumor recurrence and progression to a more dangerous, metastatic state. These traditional approaches were initially promising because they provide clear short-term benefits, namely rapid and marked reduction in bulk tumor mass and increased median survival times, but they do not necessarily increase long-term patient survival (Fig. 2.5a). To increase long-term patient survival, i.e., to increase the “tail” of Kaplan-Meier survival curves (Fig. 2.5b), we must shift our focus toward strategies that kill or prevent proliferation of essentially 100 % of tumor cells. The significant difference between median survival time and the fraction of long-term survivors was elegantly explained by Stephen Jay Gould (1985). While the goal of improving long-term survival is clear, achieving this goal presents major challenges given the difficulty in eradicating tumor cells while minimizing the effects of chemo- or radiotherapy

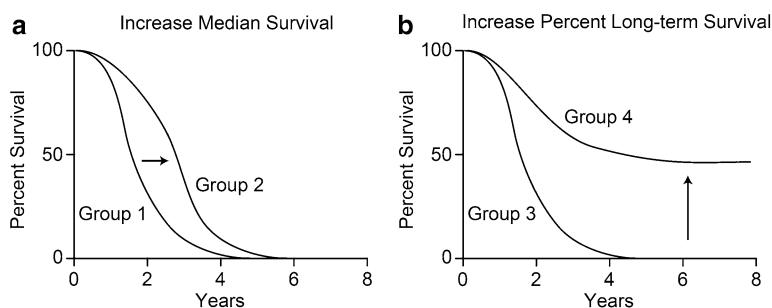


Fig. 2.5 Idealized Kaplan-Meier survival curves showing (a) increased median survival time of treatment group 2 vs group 1, but no increase in long-term survival, and (b) increased percentage of long-term survivors in treatment group 4 vs group 3

on normal tissue. In addition to causing serious immediate side effects such as neurological and gastrointestinal problems, fatigue, fever, liver and kidney failure, traditional cancer therapies can cause a wide range of serious late effects including cardiac disease, nephrotoxicity, infertility, hearing loss, neurological problems, and secondary tumors (Gururangan 2009). These late effects are a more serious problem for pediatric patients and others with potential for long-term survival. Therapeutic interventions that target tumor weaknesses through synthetic lethal and synthetic sensitivity approaches that exploit known DNA repair, checkpoint, or programmed cell death defects (or target these pathways with inhibitors) could be more effective at eradicating tumor cells while minimizing harm to normal tissues. Because DNA repair plays such a prominent role in tumorigenesis and tumor response to therapy, a deep understanding of DNA repair networks holds significant promise for improving cancer therapy.

2.5 Synthetic Lethality and Sensitivity in Targeted Cancer Therapy

The key to effective cancer treatment is to target tumor cells while sparing normal tissue. Although there have been notable successes identifying tumor-specific targets, such as the bcr-abl fusion protein in chronic myelogenous leukemia that can be inhibited with Imatinib (Gleevec) this approach may not be generally applicable to far more genetically heterogeneous solid tumors (Fox et al. 2009). This has led to the idea that the search for cures should focus on “*disrupting the broader biological pathways that support cancer growth*” (Hayden 2008). DNA damaging agents do indeed disrupt a critical biological pathway required for growth (DNA replication), but systemic chemotherapy, and even well-targeted radiotherapy, can cause significant normal tissue damage. Normal tissue tolerance limits the doses that can be delivered to tumors, and increases the chance that some tumor cells will survive. After therapy, surviving tumor cells are likely to have suffered considerable DNA damage which could drive mutagenesis and promote tumor progression upon recurrence. It is therefore imperative to develop targeting strategies that selectively kill tumor cells. Given that DNA repair defects are early drivers of many solid tumors, there is great interest in developing therapeutics that exploit these potential weaknesses based on synthetic lethality and sensitivity. Because unrepaired DSBs are generally lethal to cells, there has been significant focus on DSB repair pathways and genotoxins that directly or indirectly induce DSBs. However, we should not restrict our thinking to just these pathways and agents, as there are many pathways to death or even senescence, which achieve the same goal of preventing tumor growth and spread.

Early *Drosophila* geneticists first defined the concept of synthetic lethality and redundant genetic pathways in terms of genetic compensation for the loss of a required function by dependence on a redundant pathway (Dobzhansky 1946). The concept was formalized for cancer drug discovery by Hartwell and colleagues (1997) which led to an early genetic screen to assess drug sensitivities of yeast with

checkpoint and DNA repair defects (Simon et al. 2000). A recent example of a much larger scale synthetic lethal/sensitivity screen used ~110,000 yeast double knockout mutants in a synthetic genetic array (SGA) approach to identify growth defects and sensitivity to three genotoxins, the alkylating agent methylmethane sulfonate, the radiomimetic zeocin, and the TopI inhibitor camptothecin (Guenole et al. 2013). This study generated ~1,800,000 data points that reveal important DNA repair, checkpoint, and replication interactions clustered into “interaction maps” that suggested novel roles for known proteins including the RTT109 histone acetyltransferase, and roles for previously uncharacterized proteins in DNA repair and checkpoint functions. This and similar datasets will no doubt reveal many new targets to explore for novel synthetic lethal/sensitivity approaches. One of the advantages of the yeast SGA system is the ability to generate large numbers of double-mutant combinations that can be tested in largely unbiased screens. A second advantage (yet to be pursued) is that interesting double-mutants can be backcrossed to large sets of mutants to create triple mutants that can rapidly screened to determine if the double-mutant lethal or drug-sensitive phenotype can be suppressed by a third mutation, and this approach can be iterated to create strains with four or more mutations. Identifying suppressors of the original synthetic lethal or sensitivity phenotype may provide important insight into how cancer cells might evolve resistance to treatments that exploit a particular synthetic interaction, and this might lead to the development of robust protocols that “anticipate” and thereby prevent the development of resistant tumors. Many synthetic lethal or sensitive phenotypes are based on genetic interactions observed with gene inactivating mutations, but the approach is not limited in this way. For example, lethal interactions can arise with gain of function mutations as well, such as cells with activated RAS depending on STK33 and TKB1 kinases for viability (Barbie et al. 2009; Scholl et al. 2009). Yeast again provides a means to efficiently screen for such interactions with available overexpression libraries, offering a means to identify “synthetic dosage lethality” (Jones et al. 2008; Kroll et al. 1996). These approaches may be useful in regard to sensitization of mammalian cells to DSB damage because overexpression of human RAD51 or RAD52 can have dominant negative effects on DSB repair (Kim et al. 2001), and RAD51 is overexpressed in a wide variety of tumor cell lines (Raderschall et al. 2002).

The most dramatic discovery of a synthetic lethal interaction in human cancer was made when the Helleday and Ashworth labs independently tested the hypothesis that HR defects in BRCA1- or BRCA2-defective breast cancers would be synthetically lethal with PARP1 inhibitors (PARPi) because PARP1 functions in BER, and inhibiting the repair of single-strand damage would increase the frequency of replication fork collapse, and there would be strong requirement for a functional HR system to restart the many collapsed forks (Bryant et al. 2005; Farmer et al. 2005). There was great excitement about these findings for several reasons. BRCA-defective tumor cells were exquisitely sensitive to PARPi relative to matched BRCA-proficient cells, with differences ranging from ~50- to nearly 1,000-fold, and BRCA-defective tumors were effectively eradicated by PARPi treatment in mouse models. Thus PARPi displayed impressive therapeutic gain. Most importantly, the

PARP1i chemotherapy approach with BRCA-defective tumors was unique among cancer therapy strategies that exploit DNA damage sensitivity of tumor cells, in that *no exogenous DNA damaging agents were applied*. Instead, the strategy depended only on the spontaneous damage that is normally present in all cells. Thus, the PARP1i-BRCA interaction is truly synthetically lethal, rather than synthetically sensitive. Nonetheless, for certain tumors (e.g., those resistant to PARP1i alone) it is worthwhile exploring synthetic sensitivity by combining PARP1i with traditional chemotherapeutics. One example of this approach combined the PARP1i AZD2281 with cisplatin and carboplatin, which gave improved treatment outcomes in mouse models (Rottenberg et al. 2008).

The excitement surrounding PARP1i-BRCA synthetic lethal discovery quickly led to clinical trials with several PARP1i candidates; unfortunately, some early trials employed candidates with weak PARP1i activity, which not unexpectedly, gave poor results, but nonetheless slowed the field significantly until the weak compounds were revealed as such (Garber 2013). Additional challenges emerged when it became apparent that BRCA-defective tumors can gain resistance to PARP1i by several mechanisms including loss of PARP1, reactivation of HR, and increased expression of the P-glycoprotein efflux pump (Lord and Ashworth 2013).

Because HR proficient cells are fairly resistant to PARP1 inhibitors, these drugs are well-tolerated by patients, leading to the suggestion that they may be used to prevent cancer in cancer-prone populations including carriers of *BRCA1* or *BRCA2* defective alleles (Vinayak and Ford 2010). There are a wide range of human tumors with known or suspected defects in HR. The best studied of these have defects in *BRCA1* or *BRCA2*, but defects in many other HR factors are known or suspected to occur in cancers including ATM, ATR, each member of the MRN complex, RAD51, RAD51 paralogs XRCC2 and XRCC3, and members of the Fanconi anemia family (FANCF, FANCF, FANCC, FANCA, and FANCG). Tumors harboring these HR defects include women's cancers (breast, ovarian, endometrial, and cervical cancer), men's cancers (prostate, male breast cancer) and others including pancreatic, head and neck, brain, thyroid, lung, gastrointestinal, and melanoma (Cerbinskaite et al. 2012). Interestingly, HR defects have also been found in blood tumors including leukemia, multiple myeloma, and lymphoma (Cerbinskaite et al. 2012). Thus, PARP1i could have broad applicability for treating tumors that exhibit "BRCAness" (Bast and Mills 2010; Turner et al. 2004). If a tumor doesn't exhibit BRCAness, this state can be induced by inhibiting HR, for example with proteasome or HSP90 inhibitors, or siRNA downregulation of *BRCA2* (Gudmundsdottir et al. 2007; Noguchi et al. 2006; Yu et al. 2008).

Genetic screens and other approaches continue to identify novel synthetic lethal and synthetic sensitive interactions. siRNA screens in mammalian cells have identified additional gene targets that when repressed result in synthetic lethality or sensitivity to PARP1i. Such screens identify HR factors including *BRCA1* and *BRCA2*, as expected, but interestingly, genes that operate in DNA repair and other pathways are also recovered, i.e., NER proteins DDB1 and XAB2, and the PI3K regulator PTEN (Lord et al. 2008; Mendes-Pereira et al. 2009). PARP1i are also synthetically lethal with ERCC1 defects associated with lung cancer (Postel-Vinay

et al. 2013). Many other DNA repair-based synthetic lethal interactions have been found, such as BRCA1 and Tankyrase 1, MMR proteins MSH2 or MLH1 with DNA polymerases POLB and POLG, ATM and p53, and ATR and p53 (Jiang et al. 2009; Martin et al. 2010; McCabe et al. 2009; Nghiem et al. 2001). These and other examples point to the rich opportunities that lay ahead in the search for more effective, targeted cancer therapies.

2.6 Concluding Remarks

The exploration of DNA repair pathways to develop new synthetic lethal/sensitivity approaches has great potential for identifying novel targeted cancer therapies. Naturally, these investigations have largely taken a genetic approach, using gene knockouts, gene knockdowns, and chemical inhibitors. It is important to remember that there are many ways to target cancer and thereby increase therapeutic gain. For example, radiotherapy provides a physical approach to targeting tumors and improvements in beam focusing and “dose painting” continue to increase the ratio of dose delivered to tumor volumes relative to surrounding normal tissue. Localized drug treatment to sensitize tumors to radiation could provide significant advantages, especially if such drugs were themselves targeting tumor-specific synthetic lethal interactions. An interesting example of this type of approach is based on the observation that the complex DNA damage caused by carbon ion radiation is poorly repaired by NHEJ, thus tumor cells rely on HR to repair this damage (Okayasu et al. 2006). In effect, carbon ion radiation damage mimics an “NHEJ-defective” state, and when HR is inhibited, either by targeting BRCA2 with siRNA, or by downregulating RAD51 with HSP90 inhibitors, carbon ion radiotherapy efficacy is substantially increased (Noguchi et al. 2006; Yu et al. 2008). This approach is analogous to familiar synthetic lethal approaches as it restricts one pathway (NHEJ) for repair, and then targets the remaining pathway (HR). Because cancer cells are highly proficient at adapting to stress, combining, or “layering” multiple targeted approaches may offer the best opportunities to enhance therapeutic gain and prevent rare surviving tumor cells from developing resistance to subsequent therapy (Kon et al. 2012; Nickoloff 2013).

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