Chapter 6
Targeting DNA Repair Pathways for Cancer Therapy

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Abstract DNA repair pathways maintain the integrity of the genome, reducing the onset of cancer, disease, and aging. The majority of anticancer therapeutics (radiation and chemotherapy) function as genotoxins, eliciting genomic DNA damage in an attempt to induce cell death in the tumor. However, cellular DNA repair proteins counteract the effectiveness of these therapeutic genotoxins by repairing and removing the cell death-inducing DNA lesions, implicating DNA repair proteins as prime targets for improving response to currently available anticancer regimens. To trigger a tumor-specific cell death response (with minimal normal cell toxicity), the level of genomic DNA damage must therefore surpass the DNA repair capacity of the tumor without overwhelming the DNA repair potential of normal tissue. Interestingly, cancer-specific DNA repair defects offer novel approaches for tumor-selective therapy. This has become highly relevant as it is suggested that most cancer cells are likely to be defective in some aspect of DNA repair. Herein, we describe the molecular pathways that participate in the repair of DNA damage induced by radiation- and chemotherapeutics and discuss strategies that are being developed to target DNA repair for cancer treatment and highlight key DNA repair inhibitors that can enhance response. Further, we present novel therapeutic strategies being considered to exploit inherent weaknesses in tumor cells such as defects in one or more DNA repair pathways or related processes that may provide the opportunity to selectively increase tumor-specific cell death.

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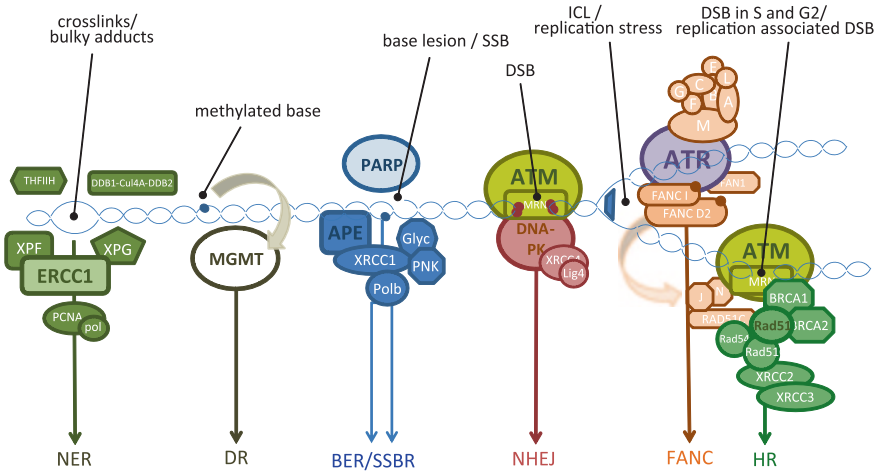

Fig. 6.1 Schematic representation of cellular DNA damage repair. This figure depicts cellular repair processes that deal with chemo- and radiotherapy-induced DNA lesions. Prevalent DNA repair targets for cancer treatment are highlighted in bold

6.1 Role of DNA Repair Pathways for Cancer Treatment

Human cells must repair tens of thousands of DNA lesions per day [1]. If they are not repaired, these lesions lead to mutations or genome aberrations that threaten cell survival and genomic integrity. To combat these threats, cells have evolved multiple DNA repair and DNA damage response (DDR) mechanisms that signal the presence of lesions and promote their repair or regulate cellular processes in response to the DNA damage (Fig. 6.1) [2]. Defects in these repair and response pathways can promote tumorigenesis and, indeed, are common in human cancers [3, 4]. On the other hand, current therapy options for cancer patients exploit the DNA-damaging properties of certain drugs and agents. The success of radiation exposure during radiotherapy and the success of most chemotherapy agents rely on the destructive nature that these agents have on cellular DNA, ultimately resulting in death and hopefully eradication of the tumor cells. Hence, DNA damage and repair mechanisms play a crucial role in determining treatment outcome. On a cellular level, resistance to treatment is profoundly determined by the capacity of the cancer cell to respond to and repair the individual DNA lesions that are induced by the chemotherapeutic agents or radiation.

Our increasing knowledge of these processes has led to the development of new concepts that target and exploit the cancer cell DDR [5]. Counteracting resistance to chemotherapy by targeting the appropriate DNA repair pathway is a promising strategy in cancer treatment. Another is to exploit the DNA repair and response defects that are present in cancer cells thereby specifically targeting tumor cells while sparing healthy cells from a high load of unrepaired DNA damage [6, 7]. Together, these strategies might provide promising avenues in the conquest against cancer.

Here, we will briefly describe essential cellular DNA repair and response mechanisms and illustrate novel concepts and promising strategies to exploit and target DNA repair for cancer treatment.

6.1.1 DNA Damage Response

The initial DDR of a cell involves the recognition of the DNA damage followed by the propagation of a series of signals ranging from alterations in RNA or protein expression and modification of protein function or stability through post-translation modification, among other signals. The cell's defense to genotoxic lesions is triggered and accomplished by a series of events that mediate and regulate proliferation, cell death, or DNA repair crucial to its survival [2, 4]. The initial steps for an appropriate response require detection of the lesion, signaling of its presence and promotion of repair. Cells act upon DNA damage not only by promoting and executing repair but also respond by halting the cell cycle or by promoting cell death mechanisms in order to prevent propagation of the damage. The DDR therefore has an impact on transcription, cellular metabolism, cell cycle regulators, as well as cell death, via apoptosis and senescence.

One of the most prominent members of the DNA damage signaling pathway that links DNA damage with cell cycle checkpoints is the protein ataxia telangiectasia-mutated (ATM) [8], a protein kinase that is recruited to DNA double-strand breaks (DSB) such as those induced by ionizing radiation. The formation of DSBs triggers the activation of ATM and activated ATM then phosphorylates a wide range of downstream substrate proteins thereby signaling the presence of the damage throughout the cell to facilitate repair [9]. Initial activation of ATM is promoted by its autophosphorylation that initiates a signaling cascade of further phosphorylation events that constitute the DDR [10]. With excessive unrepaired DNA damage present, this cellular response can culminate in an apoptotic response in which p53 is central but not necessarily always required.

Another key DDR signaling component is the ataxia telangiectasia-RAD3-related (ATR) kinase that gets activated after replication stress-induced DNA damage. Replication stress, caused, for example, by exposure to hydroxyurea (HU), results in the formation of large stretches of single-stranded DNA coated with replication protein A (RPA) that triggers activation of ATR. Similar to processes in ATM-mediated signaling, ATR signaling is promoted by regulatory proteins. ATRIP and TopBP1, together with RAD17-mediated 9-1-1 (Rad9-Rad1-Hus1) clamp loading, "sense" the damage and trigger the activation of ATR.

Further downstream of these initial events, the cell cycle checkpoint-regulating protein kinases CHK1 and CHK2 are among the most important targets (substrates) of ATM and ATR. Supported by the activation of p53 and mediated via multiple paths, this signaling cascade ultimately results in the reduction of cyclin-dependent kinase activity that drives cell cycle progression. The halt in cell cycle progression is thought to allow time for repair and, most importantly, if not successfully repaired,

to prevent propagation of the DNA damage. Cell cycle checkpoints are in place at the border from G1 to S, within S and at the G2/M border. The prevalent blocks and their extent depend on the damaging agent and the number and type of lesion. Another important downstream target of ATM and ATR is p53, an essential player in the induction of apoptosis upon DNA damage. Thus, DDR mechanisms have a crucial role in the protection against genome instability and chemo/radiotherapy response.

ATM/ATR signaling also enhances repair by recruiting repair factors to the site of the lesion and activating DNA repair proteins through phosphorylation or indirectly, by modulating acetylation, ubiquitylation, SUMOylation, or DNA repair gene transcription. These kinases also influence chromatin structure through phosphorylation of the histone H2A variant (γH2AX). Thereby, they facilitate recruitment of DDR factors and expedite DNA repair while amplifying DSB signaling that is crucial to cellular survival following exposure to DNA-damaging agents.

The role of the DDR is broad with respect to the type of cancer therapeutic. DDR activity is involved upon exposure of a whole range of chemotherapeutic agents and upon radiation. Indeed, DDR and cell cycle blocks are induced by radiation, topoisomerase I and II poisons, anthracyclines, alkylating drugs including platinum analogues and antitumor antibiotics. Interference in DDR by the use of inhibitors will likely affect the response and cellular survival in most cancer treatment options.

6.1.2 Direct-Reversal Repair and Mismatch Repair

One of the first DNA repair proteins to be considered as a viable target for improving chemotherapy was O^6-alkylguanine-DNA alkyltransferase (MGMT or AGT), a protein encoded by the O^6-methylguanine-DNA methyltransferase gene (*MGMT*) [11]. In depth, discussion on the function of MGMT and its role in cancer and chemotherapy can be found in many excellent reviews [12–14]. MGMT falls within the category of direct-reversal (DR) DNA repair proteins that also include the AlkB family of proteins [13, 15]. Unlike most other DNA repair pathways that correct lesions by removing the base containing the lesion [base excision repair (BER), see below], removing a short oligonucleotide containing the lesion [nucleotide excision repair (NER), see below] or removing long tracts of DNA (mismatch repair, MMR) followed by a DNA synthesis step (repair-directed DNA synthesis), DR proteins such as MGMT or the AlkB proteins reverse the damage to the DNA base directly, and the mechanism of repair does not involve a DNA synthesis step. This section will focus on the role of MGMT in DNA lesion repair and the subsequent role that the MMR proteins play in the cellular response when MGMT is unable to repair the O^6-alkylguanine lesion. Further discussion on the mechanism of action of AlkB proteins can be found elsewhere [13].

Like many DNA repair proteins, MGMT repairs lesions from both carcinogenic compounds and from chemotherapeutic agents. As such, MGMT acts to suppress

cancer formation by removing lesions induced by carcinogens such as methylnitrosourea (MNU), the tobacco smoke lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK), and the colon carcinogen azoxymethane. Conversely, many chemotherapeutic agents, including Temozolomide (Temodar, TMZ), dacarbazine, streptozotocin, procarbazine, BCNU (camustine), CCNU (lomustine), and gliadel trigger cell death by inducing the formation of an alkyl lesion (methyl- or chloroethyl-) on the O^6 position of guanine bases in DNA [12–14, 16]. Upon MGMT binding to the DNA containing the alkyl lesion, the O^6-alkyl group is transferred from the guanine base onto a Cysteine (Cys) residue (amino acid residue Cys145 in humans) in the MGMT protein [12, 14]. Upon transfer of the alkyl group to MGMT, the protein undergoes a conformational change that both releases the protein from the repaired DNA and promotes the ubiquitylation and subsequent proteasome-mediated degradation. This suicide mechanism of MGMT has been taken advantage of clinically, as will be described below regarding the development and evaluation of MGMT inhibitors (see Sect. 6.3.2).

The chemotherapeutic agents mentioned above induce the formation of methyl or chloroethyl adducts on the O^6 position of guanine bases in DNA. If not repaired, the majority of the chloroethyl lesions are converted to G-C interstrand DNA cross-links. Such lesions are primarily repaired by a concerted effort of the NER, HR, and fanconi anemia (FA) pathways (see below). In general, interstrand DNA cross-links are highly genotoxic, inducing cell death. Conversely, if the methyl lesion (O^6-MeG) is not removed by MGMT, during cellular replication, the mispairing of O^6-MeG with thymine leads to the formation of a O^6-MeG:T mispair, a substrate for MMR. Repair mediated by the MMR pathway facilitates the removal of the DNA strand containing the newly synthesized "T" base. However, re-synthesis of the DNA in the process of MMR will regenerate the O^6-MeG:T mispair, perpetuating the O^6-MeG lesion and the presence of the mispair. As such, in the absence of MGMT-mediated repair, O^6-MeG is suggested to initiate a futile cycle of MMR or alternately to trigger ATR protein kinase activation through the action of several MMR proteins [17], leading to apoptosis and cell death [18–20]. Details on the MMR pathway can be found elsewhere [21]. However, for the purpose of this discussion, we should consider the MMR pathway as an essential sensor to trigger cell death from chemotherapeutic agents that induce the O^6-MeG lesion. Briefly, recognition of the O^6-MeG:T mispair by the MMR protein MSH2 induces recruitment and activation of ATR and subsequently CHK1 and CHK2 to activate an apoptotic response [22, 23]. In fact, much of the resistance to agents such as TMZ observed clinically is due to high expression of MGMT (and subsequent repair of the lesion) or loss of MMR (therefore preventing the initiation of apoptotic signaling) [24–26]. Currently, TMZ along with radiation and surgery are the standard of care for glioblastoma multiforme (GBM), the most common and aggressive primary brain tumor [27]. Median survival is less than two years [28–30], and unfortunately, almost all patients eventually recur with the disease and the large majority of recurrent tumors are resistant to chemotherapy [31, 32]. Inhibition of MGMT-mediated repair has been taken advantage of experimentally and in many clinical trials [33] since MGMT can be inhibited with the O^6-MeG

analogue O^6-benzylguanine [34] (Sect. 6.3.2). Improved prognosis has also been reported in tumors with loss of *MGMT* expression due to promoter methylation [35] whereas poor prognosis is observed when *MGMT* expression levels are high or MMR capacity is compromised. Hence, elevated expression of *MGMT* and/or a non-functional MMR pathway contribute much of the observed resistance to TMZ in many tumor cell lines and in clinical trials.

6.1.3 Base Excision Repair

As suggested in its namesake, the BER pathway is the primary mechanism to remove and repair base lesions. A special sub-pathway of BER, single-strand break repair (SSBR), is also essential for the repair of single-strand DNA breaks [13, 36, 37]. The types of base lesions repaired by the proteins of the BER pathway range from base deamination products (e.g., conversion of C to U or 5 meC to T) to oxidative modification of bases (8-oxo-7,8-dihydro-2′- deoxyguanosine; 8-oxodG), alkylation products such as N7-meG and N3-meA that are induced by chemotherapeutic agents such as TMZ [38] and many others [36, 37]. These and many other lesions are induced in genomic (and mitochondrial) DNA by a multitude of anticancer treatments including radiation, monofunctional alkylators such as TMZ, cisplatin, and 5FU, among others. There are over 20 proteins brought to bear to facilitate the complete process of BER [36]. Repair is initiated following recognition of the base lesion by one of the eleven DNA glycosylases in humans. This group of proteins is further subdivided into two classes: Bifunctional and monofunctional DNA glycosylases. A β-bifunctional glycosylase such as OGG1 excises the modified base and hydrolyzes the DNA backbone (via a β-elimination step) 3′ to the incised base, leaving a 3′ unsaturated aldehyde (after β-elimination) and a 5′ phosphate at the termini of the repair gap. Alternatively, a β,δ-bifunctional glycosylase such as NEIL1 hydrolyzes the glycosidic bond to release the lesion and then cleave the DNA backbone 3′ to the resulting apurinic/apyrimidinic (AP or abasic) site via β-elimination and 5′ to the abasic site via δ-elimination. More detail on the mechanism of these bifunctional DNA glycosylases and the specific BER proteins involved in processing the resulting repair gaps can be found elsewhere [36, 37].

For the purpose of describing the complete BER pathway, we will focus on the initiation of BER by monofunctional DNA glycosylases, with an emphasis on the methylpurine DNA glycosylase (MPG) (also called AAG or ANPG). MPG is the primary glycosylase for the repair of the chemotherapy-induced DNA lesions such as N7-meG and N3-meA. These lesions are removed by hydrolysis of the glycosidic bond, producing an abasic site, a substrate for AP-Endonuclease 1 (APE1). Given the highly toxic nature of the intermediates in BER [13], it has been suggested that the product of each BER reaction “hands off” the toxic BER intermediate to the next enzyme in the pathway likening the complete reaction to the hand-off of a baton in a relay

race [39]. Such a process or hand-off mechanism has the advantage of eliminating or avoiding the accumulation of free BER intermediates that are prone to induce cell death [13]. Once formed by the glycosylase, the resulting abasic site is then handed off to APE1 to be hydrolyzed on the 5′ end. The resulting single-nucleotide repair gap contains a 3′OH and a 5′deoxyribose-phosphate (5′dRP) moiety at the margins. It has been suggested that this BER intermediate (a single-strand break with a 5′dRP moiety) recruits poly(ADP)ribose polymerase (PARP)1 to the lesion site. Recruitment then triggers activation of PARP1. Activated PARP1 polymerizes NAD^+ to yield the polymer poly (ADP) ribose (PAR), an essential posttranslational modification. The first protein to be modified by PAR is PARP1 itself (auto-modification). Subsequently, it has been observed that XRCC1 and many other proteins are modified [40]. Once modified, activated PARP1 then facilitates chromatin relaxation (likely to provide access to the lesions for repair) [41, 42] and recruitment of the remaining BER proteins required to complete repair, including XRCC1, DNA Ligase III, and DNA polymerase β (Polβ). Whereas XRCC1 is a scaffold protein, Polβ carries out two essential enzymatic functions in BER. First, the repair gap is tailored by the 5′dRP lyase activity of Polβ. Next, Polβ fills the single-nucleotide gap, preparing the strand for ligation by either DNA ligase I (LigI) or a complex of DNA ligase III (LigIII), and XRCC1 [36].

Although some BER substrates (base lesions) induced by chemotherapeutic agents are cytotoxic [43], most are found to be mutagenic. However, essentially, every intermediate throughout the BER pathway (abasic sites, 5′dRP lesions, and single-strand DNA breaks) is toxic [13] and as such, there has been considerable interest in developing BER inhibitors to enhance the accumulation of the cytotoxic repair intermediates following chemotherapy or radiation treatment. This is discussed further in the sections below.

6.1.4 Nucleotide Excision Repair

Another multi-protein, highly complex DNA repair pathway is the NER pathway. NER plays an important role in the repair of DNA lesions induced by many genotoxins and chemotherapeutics including DNA cross-linking agents such as chloroethylating agents (see Sect. 6.1.2), cisplatin, carboplatin, and lesions induced by photodynamic therapy (PTD). Put simply, NER facilitates the removal of bulky DNA adducts that grossly distort the DNA double helix and those that cause a block to transcription. Molecular details on the proteins involved in NER can be found in several excellent reviews [44–47]. Overall, the pathway consists of two complementary sub-pathways that have some overlap. The two sub-pathways are distinct regarding the lesion recognition step but converge and utilize the same proteins to remove the oligonucleotide containing the lesion and for the steps involving new DNA synthesis.

The global genomic NER (GG-NER) pathway surveys the entire genome for DNA helix distorting lesions whereas the transcription-coupled repair NER

(TC-NER) pathway is recruited to facilitate removal of DNA lesions that block the elongating RNA polymerase and stall transcription. The GG-NER pathway utilizes the DDB1/DDB2 heterodimer, part of the DDB1-Cul4A-DDB2 E3 ubiquitin ligase, to facilitate lesion recognition and repair [48]. As such, targeting the proteasome (Sect. 6.4.2) or deubiquitinating enzymes (DUBs) (Sect. 6.5.4) would therefore indirectly impact NER function. The TC-NER pathway partners with the TFIIH transcription complex to recognize and repair lesions that halt transcription. Upon lesion recognition, XPG mediates cleavage of the DNA strand containing the lesion on the 3′ side of the lesion, and subsequently, the ERCC1/XPF heterodimer hydrolyzes the DNA strand containing the lesion on the 5’ side. Replication factors then facilitate DNA synthesis and ligation. Of all the proteins in this pathway, ERCC1 has emerged as a valuable biomarker of response to chemotherapeutic agents that induce DNA damage repaired by NER (e.g., cisplatin) and is under consideration as a drug target [49, 50]. Currently, biomarker measurements have included both mRNA and protein analysis. However, it is not yet clear whether protein levels of ERCC1 are a valid biomarker [51, 52].

6.1.5 Non-Homologous-End-Joining

One of the most cytotoxic lesions is a DNA double-strand break (DSB). If not repaired, DSBs lead to chromosome breaks, loss of genetic material, and gross genomic rearrangements. Whereas tolerance to the presence of DSBs might vary in different cell types and cellular states, only a few DSBs will cause cell death or prevent clonogenicity in most cells including cancer cells [53]. These lesions are induced by multiple agents such as ionizing radiation, bleomycin, and topoisomerase II inhibitors, but can also be induced indirectly at replication forks when converting DNA single-strand breaks (SSBs) induced by topoisomerase I inhibitors (camptothecin).

Two major cellular pathways deal with the repair of DNA DSBs. The use of homologous DNA for repair distinguishes those repair pathways. As indicated by the name, the non-homologous-end-joining (NHEJ) repair pathway does not require any homologous sequences. Proteins of the NHEJ pathway can repair the two ends in a DSB by simple end joining while the homologous recombination (HR) repair pathway (see Sect. 6.1.6) requires homologous DNA stretches as templates for DNA synthesis and repair.

After the initial recognition of the DSB that is held in place and stabilized by the binding of the MRN complex (MRE11, RAD50, NBS) and promoted by ATM (see above), DSB repair is executed by the DNA-dependent protein kinase (DNA-PK). DNA-PK is comprised of the catalytic subunit DNA-PKcs and the relatively small Ku proteins (Ku70/80). They promote the simple ligation of the two broken DNA ends. Damage-induced DSBs, in particular after ionizing radiation, are rarely re-ligateable, and some end processing might be required that is accomplished by other enzymes such as Artemis. The DNA ligase IV-XRCC4 complex

finally re-ligates the two ends. DNA PK–independent DSB end-joining activity has been observed in cells that have impaired NHEJ activity, the so-called alternative or B-NHEJ pathway. PARP and Ligase III activity appears to be implicated in this cellular DSB repair option [54, 55].

The role of ATM seems to be of particular importance in the repair of a certain proportion of DSBs, namely those in heterochromatic regions of the genome [56, 57]. These, judging from the repair kinetics, require more time to repair but influence survival substantially as indicated by the hypersensitivity to radiation of cells with impaired ATM function.

Based on the cytotoxic nature of DSBs, cells impaired in any step of the NHEJ process are highly sensitive to ionizing radiation. Genetic defects in or inhibition of NHEJ also profoundly affects survival of cells by other DNA-damaging agents that cause DSBs (directly or indirectly) such as DNA cross-linkers, bleomycin, and topoisomerase inhibitors.

6.1.6 Homologous Recombination DSB Repair and the Fanconi Pathway

The HR repair pathway, in contrast to NHEJ (see above), requires homologous DNA stretches as templates for DNA synthesis and repair [44, 58]. To assure accurate repair, HR tends to use the sister chromatid as a template, restricting this pathway to the S and G2 phase of the cell cycle. Together with the FA pathway, it has a crucial role in the surveillance of replication fork progression. As anticipated by its requirement for a homologous template, HR mainly determines survival of S- and G2-phase cells. HR's involvement upon radiation is not only required at directly induced DSBs but also at secondarily induced DSBs that result from replication attempts on nicked DNA [59]. In agreement with such a function, HR has been shown to determine radiosensitivity in a cell cycle phase-dependent manner. HR and the FA pathway are also crucial in resolving blocked replication forks [60]. Such blocks are, for example, caused by interstrand cross-links (ICLs) that tether the two DNA strands together and prevent separation during replication. Survival upon other cancer therapeutic agents that induce replication-blocking lesions such as alkylating agents and topoisomerase inhibitors is strongly determined by the functionality of HR. These blocks must be repaired or bypassed to allow cells to survive.

A complex multi-member process assures HR-driven repair. In brief, an ordered assembly of nucleoprotein filaments of RAD52, RAD51, and RAD54 upon RPA coating of the resected DNA promotes and catalyzes homologous DNA pairing. The extent of the resection at the break site is mediated by the MRN complex and appears to partly define the use of HR instead of NHEJ. Strand exchange is assisted by the RAD51 paralogs RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3. In concert, these proteins direct and provide the recombinase activity,

that is, crucial to resolve the complex-branched structures that arise in this process. Notably, the products of the breast cancer susceptibility genes BRCA1 and BRCA2 are involved in the FA and HR repair pathways, assisting DSB and cross-link repair.

To allow the resolution of blocked replication fork structures, in particular following exposure to DNA cross-linking agents, another replication-associated repair process is required, the FA pathway. Its members were discovered while analyzing FA patients, victims of a human genetic disease that is characterized, among other features [61], by extreme cellular sensitivity to drugs that produce ICLs. Subsequently, their role and actions in cellular cross-link repair was revealed.

The products of at least 15 genes have been currently implicated in this pathway [61–63]. This pathway constitutes a major signaling cascade upon replication fork stalling: the FA "core complex" consists of at least 8 FA elements (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and FANCM) and acts by realizing the mono-ubiquitylation of the FA ID complex (FANCD2 and FANCI). This activation of the ID complex allows chromatin binding and is thought to facilitate DNA repair, in particular HR. The FA-mediated recruitment of the RAD51 recombinase and the BRCA1-FANCJ helicase activity allows re-establishment of the replication fork. Resolution of stalled replication forks appears to be also supported by the translocase activity of FANCM that can remodel branched DNA structures. The FA core complex is regulated by ATR and cell cycle checkpoint elements (CHK1) allowing the activation of the pathway [64]. Importantly, any mutation upstream of the FA pathway that will disrupt the mono-ubiquitylation of FANCD2 will result in the cellular ICL hypersensitivity phenotype.

As illustrated above, upon exposure to DNA-damaging agents, it is the multitude of cellular repair capacities that ultimately determines survival. HR and FA have been shown to determine the cellular sensitivity to a wide range of cancer therapeutics. HR-defective cells are hypersensitive to cross-linkers, IR, topoisomerase inhibitors, and alkylators as they induce DSBs and replication stalling.

6.2 Strategies Targeting DNA Repair for Cancer Treatment

The requirement for DNA repair and genome maintenance in response to radiation and genotoxic chemotherapeutics implicates DNA repair proteins as prime targets for improving response to currently available anticancer regimens. In addition, frequent cancer-specific DNA repair and DDR defects offer tumor-selective therapy options. Thus, strategies targeting DNA repair pathways represent promising new avenues to improve outcome in cancer treatment.

Targeting DNA repair pathways in cancer treatment has been proposed in several settings (Fig. 6.2). Most evidently, inhibition of cellular DNA repair will cause increased sensitivity to chemotherapeutic agents or radiotherapy [65]. As illustrated above, cellular death upon exposure to most chemotherapeutic

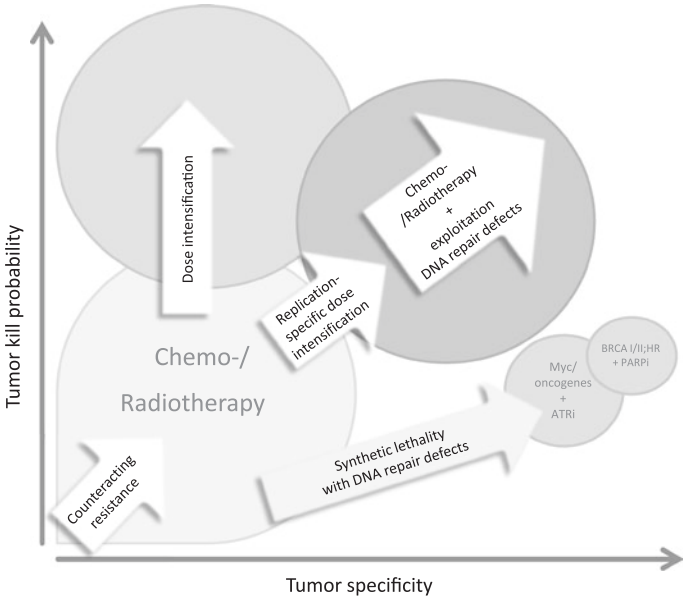

Fig. 6.2 Strategies to improve cancer treatment by targeting DNA damage response and repair. The overall goal is to increase tumor cell kill (*y-axis*) while sparing normal tissue by increasing tumor specificity (*x-axis*) of the cancer treatment. While some strategies will achieve increased cell death and thereby an increased probability to control the tumor (for example by applying DNA-PK inhibitors in combination with radiotherapy or by re-sensitizing chemotherapy-resistant tumors), others (such as those based on the exploitation of tumor-specific defects) will increase tumor specificity of cancer therapies. However, in the manner in which current chemo- and radiotherapy regimens are largely applied, each of the targeted approaches will be only beneficial in a small fraction of patients with tumors that harbor the respective DNA repair defects. Nevertheless, considering the wide range of combination possibilities and the high number of targets and exploitation opportunities, together they represent a promising avenue in cancer treatment

agents and ionizing radiation partly depends on the repair capacity for the respective lesions. HR, NHEJ, BER, and DR processes are responsible for the resistance to these agents. Hence, inhibitors to DNA repair elements might be useful as dose intensifiers, augmenting the cell-killing properties of many if not all therapeutic agents. This can be particularly useful in a setting in which cancer cells obtained resistance to certain agents due to an improved repair capacity. Thus, counteracting marked cancer cell resistance is one strategy in which DNA repair inhibitors are proposed to act as dose intensifiers. By lowering the tolerance and inhibiting alternative repair routes while augmenting cell kill, the application of "intelligent" dose intensification by DNA repair inhibition can also prevent the development of chemotherapy resistance.

Dose intensification will not be, in general, however, well tolerated, since chemotherapeutic drugs and radiotherapy doses are often administered at maximum-tolerated levels. Non-cancerous cells, with few exceptions, are as

much exposed to the chemotherapeutic agents as are the cancer cells. Indeed, it is the normal tissue response that defines the dose level and use of dose intensifiers. Some tumor properties could, however, provide a tumor-specific effect when targeting DNA repair. Further, in line with the rationale of most classical cancer therapeutics, the proliferative nature of tumors is exploitable. For example, targeting replication-associated repair pathways such as HR with novel targeted agents could be beneficial in radiotherapy regimens in which the healthy cells in the irradiated area are nonproliferative. A gain could also be expected if chemotherapy dose limits are not defined by the proliferative cells, allowing dose intensification by targeting replication-associated DNA repair. Other tumor-specific properties such as exposure to hypoxic conditions or the altered metabolic status in cancer cells can offer opportunities to achieve tumor-specific dose intensification and will be discussed in the following paragraphs.

Another implication of DNA repair-targeting strategies has, however, become highly relevant. It is suggested that most cancer cells are likely to be defective in some aspect of DNA repair. Considering the multitude of repair options of healthy cells, DNA repair defects in cancer cells can be exploited by targeting the remaining repair processes. The combined lethal effect of two genetic variations that are otherwise non-lethal is termed "synthetic lethality" [66]. In compliance with the synthetic lethality concept, cancer cells defective in the primary repair pathway are viable but rely heavily on secondary backup repair for survival. As this is not only restricted to repair of endogenously produced lesions, this concept also applies to cells exposed to exogenous damage by exacerbating the effects of chemo- and radiotherapy in the defective cancer cells only (also may be called synthetic sickness). Despite mutations and genetic defects, the differential expression of DNA repair proteins or the altered engagement of DNA repair sub-pathways can be a base of tumor-specific activities. Hence, these tumor-specific DDR and repair defects offer promising novel approaches to tumor-selective therapy.

Investigation of the functionality of the individual DNA repair pathways in cancer cells and the knowledge on which pathways are implicated upon the inhibition of DNA repair drug targets are necessary to combine these cancer therapy options in an intelligent manner while focusing on a differential effect in the cancer versus normal cells.

6.3 DNA Repair Targets

The recognition that DNA repair processes are prime targets for chemo- and radiosensitization has driven the development of specific inhibitors to elements of DDR, NHEJ, HR, DR, and BER. The more recent discovery of tumor-specific targeting opportunities by the inhibition of DNA repair processes fueled such attempts and has yielded a multitude of DNA repair inhibitors [5]. Some of these novel agents are currently being evaluated in the clinic while others are being tested preclinically. Novel DNA repair targets have been identified,

Table 6.1 Targets in DNA damage response and repair

DNA Repair protein target	DNA Repair pathway involved	Compounds (DNA repair inhibitors)	Context	Strategy	Development stage	Reference
PARP1/2	BER	Olaparib	Monotherapy	BRCA1 or BRCA2 deficiency	Clinical validation	[248]
PARP1/2	BER	Olaparib	Chemosensitizer	Combined with cisplatin and gemcitabine	Clinical validation	[249]
PARP1/2	BER	Olaparib	Radiation sensitizer	Combined with radiation	Preclinical	[250]
PARP1/2	BER	Veliparib	Monotherapy	BRCA1 or BRCA2 deficiency	Preclinical	[251]
PARP1/2	BER	Veliparib	Chemosensitizer	Combined with cyclophosphamide, carboplatin, temozolomide or topotecan	Clinical validation and preclinical	[134, 251–255]
PARP1/2	BER	Veliparib	Radiation sensitizer	Combined with radiation	Preclinical	[254, 256–258]
MGMT	DR	O^6-benzylguanine	Chemosensitizer	Combined with carmustine	Clinical validation	[259]
MGMT	DR	O^6-benzylguanine	Chemosensitizer	Combined with temozolimide	Clinical validation	[260]
MGMT	DR	Lomeguatrib	Chemosensitizer	Combined with temozolimide	Clinical validation	[261, 262]
CHK1	DDR	AZD7762	Radiation sensitizer	Combined with radiation	Preclinical	[263]
CHK1	DDR	AZD7762	Chemosensitizer	Combined with irinotecan	Preclinical	[115]
CHK1	DDR	SCH900776	Chemosensitizer	Combined with SN38	Preclinical	[264]

(continued)

Table 6.1 (continued)

DNA Repair protein target	DNA Repair pathway involved	Compounds (DNA repair inhibitors)	Context	Strategy	Development stage	Reference
CHK1	DDR	PF-0477736	Monotherapy	Myc-driven cancers	Preclinical	[265]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Chemosensitizer	Combined with pemetrexed	Preclinical	[142]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Chemosensitizer	Combined with temozolimide	Preclinical	[133–135]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Radio- and chemosensitizer	Combined with iododeoxyuridine + radiation	Preclinical	[136]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Chemosensitizer	Combined with BCNU	Preclinical	[137]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Chemosensitizer	Combined with manumycin A	Preclinical	[138]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Chemosensitizer	Combined with fludarabine	Preclinical	[139]
None (Abasic site in DNA)	BER	TRC102 (methoxyamine)	Radiation sensitizer	Combined with radiation	Preclinical	[140, 141]
ATR	DDR	NU6027	Chemosensitizer	Combined with PARP inhibitors	Preclinical	[151]
ATR	DDR	NVP-BEZ235	Monotherapy	Cyclin-E overexpressing cells	Compound screen	[152]
ATM	DDR	KU55933	Radiation sensitizer	Combined with radiation	Compound screen and preclinical	[266]
ATM	DDR	KU60019	Radiation sensitizer	Combined with radiation	Preclinical	[167]
ATM	DDR	CP466722	Radiation sensitizer	Combined with radiation	Preclinical	[153]

(continued)

Table 6.1 (continued)

DNA Repair protein target	DNA Repair pathway involved	Compounds (DNA repair inhibitors)	Context	Strategy	Development stage	Reference
DNA-PK	DDR	NU7026	Radiation sensitizer	Combined with radiation in EGFRvIII-expressing tumors	Preclinical	[164]
DNA-PK	DDR	NU7441	Radio- and Chemosensitizer	Combined with doxorubicin or radiation	Compound screen and preclinical	[267]
DNA-PK	DDR	DT01 (dbait, DRIIM)	Radiation sensitizer	Metastatic melanoma with relapsed cutaneous tumors	Compound screen and clinical validation	[158]
DNA-PK	DDR	DT01 (dbait, DRIIM)	Chemosensitizer	Combined with 5-fluorouracil or irinotecan	Preclinical	[268]
DNA-PK	DDR	HNI-38	Radiation sensitizer	Combined with radiation	Preclinical	[269]
DNA-PK	DDR	KU-0060648; A dual inhibitor of DNA-PK and PI-3 K	Chemosensitizer	Combined with etoposide	Preclinical	[159]
NAMPT	NAD^+ biosynthesis	FK866 (Apo866)	Chemosensitizer	Combined with various chemotherapeutics	Preclinical	[190–192]
NAMPT	NAD^+ biosynthesis	GMX1778	Chemosensitizer	Combined with various chemotherapeutics	Preclinical	[183]
NAMPT	NAD^+ biosynthesis	CB30865	Chemosensitizer	Combined with various chemotherapeutics	Preclinical	[184]
NAMPT	NAD^+ biosynthesis	CHS-828	Chemosensitizer	Combined with various chemotherapeutics	Preclinical	[185]

and compounds that specifically inhibit their activity are sought in order to apply tumor-specific anticancer strategies.

We will list currently explored DNA repair targets and some of the most advanced compounds according to their developmental stage (Table 6.1). Rationales and applied strategies will be discussed while pointing to opportunities on combinations and other DNA repair targets.

6.3.1 Poly(ADP)Ribose Polymerase

One of the most advanced and applied DNA repair target inhibitors to date are the PARP inhibitors [67–69]. Since the discovery that cells with defects in the BRCA genes are selectively killed by the inhibition of PARP, PARP inhibitors have rapidly made their way into the clinic [70, 71]. As tumors from carriers of mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 are almost exclusively composed of such BRCA-defected cells while normal cells of these carriers still carry a functional allele, hence are HR proficient, these PARP inhibitors achieve tumor-specific kill with little normal cell toxicity. The proposed mechanism that causes such selectivity points to the dependence of BER-inhibited cells on HR due to secondarily induced cytotoxic DSBs (Fig. 6.3a) [60]. Indeed, early synthetic lethality screens in yeast indicated such an opportunity revealing a crucial link between BER and HR for cellular survival [72]. Other hypotheses assume a direct role of PARP inhibitors in replication fork stalling [73]. Most compounds with PARP inhibitory activity target PAR generation by blocking the catalytic activity of the enzyme. In principle, these compounds compete with NAD^+ for the PARP catalytic site and are therefore not necessarily specific to PARP1 and could impact the activity of the other PARP isoforms [74]. Several PARP inhibitors are in clinical development. To date, the leading compounds Olaparib (AZD2281, AstraZeneca; originally developed by KuDos) and Veliparib (ABT-888, Abbott) are probably the two most extensively studied in the clinic whereas at least one compound, namely Iniparib (BSI-201; Sanofi-Aventis), has been reported to lack effective PARP inhibitory activity [75, 76].

Although registration of these drugs is still awaiting approval, several studies have shown their beneficial application [77, 78]. One obstacle could be that these PARP inhibitors were expected to act in a fraction of tumors, those exhibiting HR defects due to BRCA1 & BRCA2 mutations only. It should be noted that those early clinical trials revealed that not all BRCA mutation carriers benefit, indicating that a certain degree and type of HR defect is required to be exploitable with PARP inhibition. The impact of individual BRCA mutations with respect to HR functionality and/or PARP inhibitor sensitivity could be variable [79]. The status and propensity to use the remaining DSB repair mechanism NHEJ, for example via 53BP1 channeling, also influences the extent of PARP inhibitor toxicity [60, 80]. In addition, other general drug resistance mechanisms such as increased compound rejection by

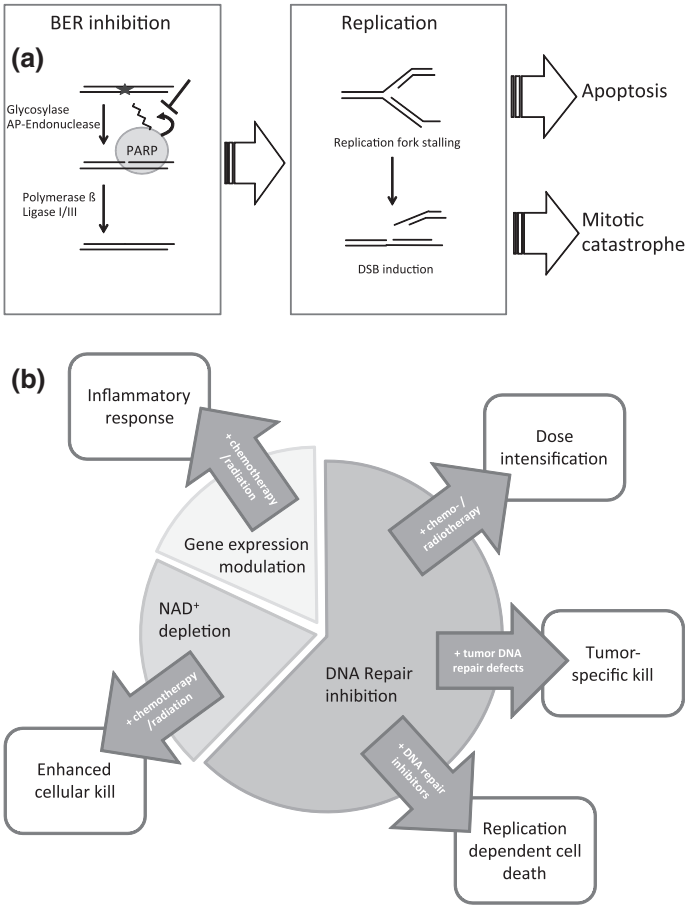

Fig. 6.3 Cellular functions of PARP and opportunities for cancer treatment. **a** The mode of action of *PARP* inhibitors is based primarily on inhibition of the poly(ADP)ribosylation activity of the enzyme *PARP*1. As a result, BER or SSBR cannot be executed. In addition, the lack of poly(ADP)ribosylation is likely to negatively impact chromatin and lesion accessibility. Auto-poly(ADP)ribosylation of *PARP*1 is thought to promote its repulsion from DNA. A failure to recruit downstream BER elements or an increase in lesion shielding by trapping *PARP*1 on the DNA further inhibits BER. BER intermediates accumulated upon chemo- and radiotherapy, however, will then cause replication problems that in turn will induce *DSB*s. Those will ultimately lead to cell death via apoptosis and/or mitotic catastrophe in particular if not repaired by HR. **b** PARP activity has several cellular roles that can be taken advantage of in cancer treatment. PARP promotes DNA repair and its inhibition, when combined with chemotherapy or with tumor-specific defects, enhances tumor cell kill. *NAD^+ depletion*, as a consequence of chemo- or radiotherapy-induced PARP activity, also induces cell kill. Agents depleting cellular NAD^+ levels could indirectly inhibit PARP. Conversely, PARP inhibitors can have the effect of lowering chemotherapy-induced *NAD^+ depletion*, thereby altering the mode of cell death. Lastly PARP also has regulatory functions regarding DNA damage–induced gene expression that is often connected to an inflammatory or fibrotic response. PARP inhibitors have been reported to exhibit anti-inflammatory properties

drug transporters, decreased tumor perfusion, cellular metabolism, or simply pharmacogenetics of a certain compound might also be responsible for a lack of benefit.

However, new studies indicate that other defects that supposedly result in impaired HR or DSB repair, such as when ATM is mutated, result in PARP inhibitor-mediated selective kill [81, 82]. Mantle cell lymphoma harbors ATM defects and preclinical studies demonstrate sensitivity of these tumors to PARP inhibition [83, 84]. Other genetic analyses indicated frequent ATM mutations in human cancer. These studies therefore warrant the application of PARP inhibitors for cancer treatment in a much larger patient population. In agreement with a synthetic lethal interaction with HR-driven processes, cells with an impaired FA pathway are hypersensitive to PARP inhibition, thus further enlarging the potential patient population that might benefit from such therapies [81]. Some studies indicated an impact of PTEN deletion on HR via a reduction of Rad51 or Rad51 paralogs, a condition that could be exploited by PARP inhibition [85, 86]. However, this could only be partly confirmed in a separate study [87]. HR impairment was observed when cells experience hypoxia [88, 89] which sensitized them to PARP inhibition. PARP inhibitor sensitivity in hypoxic cells or those with defects in FA, ATM, or PTEN is, however, generally less pronounced than when BRCA-defective. Yet, a benefit, in particular when combined with radio- or chemotherapy, can be anticipated since PARP inhibitors appear to enlarge the therapeutic window and could spare healthy cells from chemo- to radiosensitization.

Historically, prior to the discovery of specific killing of tumors with defective HR, PARP inhibitors have been actively studied in preclinical and clinical investigations to potentiate the cytotoxic effects of chemo- and radiotherapy (Fig. 6.3a). PARP inhibitors, due to their DNA repair-inhibiting properties, are radiation sensitizers and are highly effective in sensitizing cells to chemotherapeutic agents such as DNA alkylators (e.g., TMZ) and topoisomerase I inhibitors (irinotecan and topotecan). Combining PARP inhibitors with chemo-/radiotherapy could be beneficial in a large patient population with the added advantage of single-agent activity in a fraction of patients that happen to have tumors with exploitable DNA repair defects. As noted above, in such dose-intensification strategies, the benefit of tumor-specific killing needs to be evaluated against normal tissue effects that are connected to the use of DNA repair inhibitors. To exemplify, since the radiosensitizing effect of PARP inhibitors is most effective in proliferating cells, PARP inhibitors have been proposed in clinical radiotherapy settings in which normal tissue toxicity within the radiation field is not defined by a highly proliferating (stem) cell fraction such as in the treatment of lung cancer and glioblastoma [90, 91]. Radiation-induced lung toxicity is strongly determined by inflammatory and fibrotic processes. Therefore, PARP inhibition might impact lung toxicity in a beneficial way by altering the inflammatory DDR [92, 93].

Despite crude chemosensitization and synthetic lethal activity, other strategies exploit the BER inhibitory properties of PARP inhibitors. These strategies aim to prevent the quick development of resistance to alkylators such as temozolomide (TMZ). Resistance to TMZ is associated with MGMT expression and MMR defects (see above). PARP inhibitors, however, are able to (re-) sensitize

MMR-defective cells to the anti-tumor effect of TMZ [94–98] thereby counteracting the resistance to TMZ.

Other PARP inhibitor combinations have been pursued. Assuming that BRCA defects do limit HR functions but do not fully impair HR, BER inhibition and consequently increased engagement of HR might be the basis for the observed synergistic cytotoxicity of Olaparib and Cisplatin [99, 100]. Depending on the cytotoxic agents and genetic background, BRCA-defected cells die by apoptosis or mitotic catastrophe upon PARP inhibition.

DNA damage–induced and PARP-activation-mediated consumption of NAD^+ has been implicated in the increase in genotoxin-induced cell death [74, 101]. The PARP1 and PARP2 proteins [102] act as sensors of DNA damage such as DNA single-strand breaks and become hyperactivated, consuming NAD^+ as a substrate to synthesize PAR [103]. Consumption of NAD^+ after DNA damage leads to ATP depletion, likely due to continued re-synthesis of NAD^+ as well as ongoing cellular utilization of NAD^+ and ATP for metabolic functions [74, 101]. Following up on the observation that cell death due to BER inhibition and the accumulation of BER intermediates results in PARP hyperactivation [103], it was shown that the combination of BER and NAD^+ biosynthesis inhibition significantly sensitizes glioma cells to TMZ. Dual targeting of these two interacting pathways (DNA repair and NAD^+ biosynthesis) may prove to be an effective treatment combination for patients with resistant and recurrent GBM. Thus, in summary, several distinct roles of PARP including the promotion of DNA repair and its impact on damage-induced gene expression as well as the relationship to NAD^+ levels can be exploited for cancer therapy (Fig. 6.3b).

6.3.2 O^6-Methylguanine-DNA Methyltransferase

As described in Sect. 6.1 above, MGMT is the sole protein responsible for the repair of O^6-alkylguanine lesions (formed by chemotherapeutic agents such as TMZ). Tumors with elevated MGMT expression are resistant to TMZ and related chemotherapeutic agents, and so, an active area of investigation has been the development of MGMT inhibitors. If MGMT is inhibited (or MGMT is not expressed), the tumor becomes highly sensitive to the agent (provided the tumor cell is proficient in MMR—see Sect. 6.1.2). There have been multiple methodologies proposed to inhibit or overcome resistance mediated by MGMT expression in the tumor as well as to prevent sensitivity of normal tissue (primarily hematopoietic cells) [12]. As we alluded in Sect. 6.1.2, the mechanism of action of MGMT in the repair or de-alkylation of guanine suggested O^6-benzylguanine as an ideal inhibitor [104]. This inhibitor, also called BG, is an analogue of the O^6-alkylguanine base and contains a benzyl ring instead of an alkyl group. The BG compound readily reacts with MGMT, and the benzyl moiety is transferred to Cys145 as shown (Fig. 6.4), releasing free guanine and rendering MGMT inactive. In some cases, this has been shown to trigger ubiquitylation and proteasome-mediated destruction

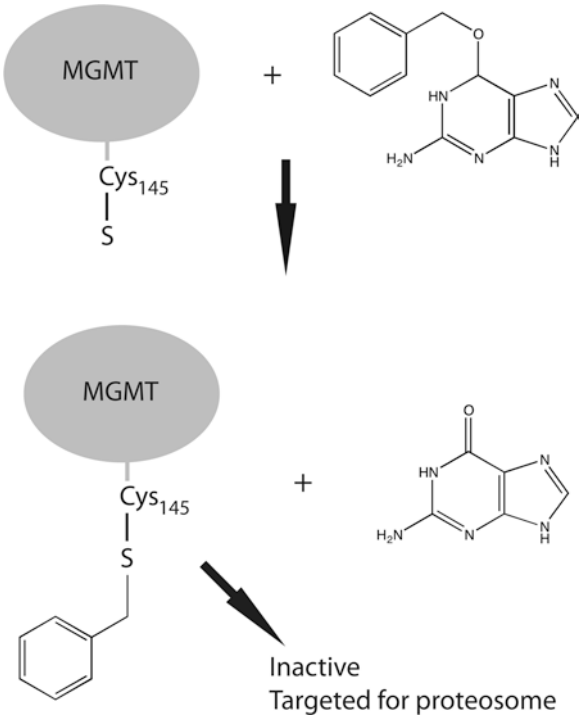

Fig. 6.4 Schematic representation depicting the mechanism of action of the MGMT inhibitor O^6-benzylguanine (*BG*). The benzyl moiety of BG is transferred to the *Cys*145 residue in *MGMT*, releasing free guanine, rendering *MGMT* inactive. In some cases, this has been shown to trigger ubiquitylation and proteasome-mediated destruction of *MGMT*

of MGMT [105]. Further details on BG and related analogues to inhibit or regulate MGMT can be found elsewhere [106]. A second inhibitor with greater inhibitory activity that has been widely tested is the alkylguanine analogue 6-[4-bromo-2-thienyl]methoxypurin-2-amine [107], also called lomeguatrib [108, 109].

A significant challenge with all or most chemosensitizers is the observed increase in normal cell/tissue sensitivity or cell death. This has been addressed with regard to MGMT by the use of an ex vivo gene therapy approach to express a mutant of MGMT in the bone marrow or hematopoietic stem cells (cells are modified ex vivo and re-delivered to the patient), as described [12]. This is feasible since the G156A or P140K mutants of MGMT are 60-fold and 500-fold more resistant to BG than the wild-type protein, respectively [110].

6.3.3 Cell Cycle Checkpoints

DNA damage activates checkpoints to arrest proliferation. Normal cells have intact G1, S, and G2 checkpoints that are mediated by the ATM/CHK2 and ATR/CHK1 pathways. Owing to mutations in the p53 or pRB tumor suppressor genes, cancer cells, however, lack a G1 checkpoint and as a result, rely on G2

checkpoints to prevent cell division and propagation of the damage. Thus, the S/G2 checkpoint is an attractive target for cancer-specific sensitization to DNA-damaging agents [111]. In order to exploit the cancer-specific defects, inhibitors have been developed that abrogate the G2 checkpoint. CHK1 has been a prime target for such attempts, as activated CHK1 mediates the arrest by phosphorylating Cdc25A and Cdc25C leading to their degradation and inactivation that otherwise promote S-phase progression and entry into mitosis. Loss of the G2 cell cycle checkpoint, despite the presence of unrepaired damage, is thought to provoke mitotic catastrophe and ultimately cell kill. Consistent with this idea, loss of intra-S or G2/M checkpoints increases the cytotoxicity of DNA-damaging agents such as ionizing radiation and cisplatin. CHK1 knockdown sensitizes cells to 5-fluorouracil, doxorubicin, and etoposide. Despite the proposed mechanism that CHK1 inhibition causes sensitization in p53-defective cancer cells due to the G2 block abrogation in a G1 block-deficient background, the data to support this are contradictory [112–114]. Xeno-transplant studies on human triple-negative breast cancer demonstrated the benefit of combining irinotecan with CHK1 inhibitors, inducing checkpoint bypass and apoptosis. A role of p53 was supported by the gain of CHK1 sensitization after p53 knockdown in the resistant tumors [115]. A more complicated rationale, however, argues that a series of DDR defects in tumors should be considered and could be exploited by the inhibition of CHK1. These are based on the secondary effects of CHK1 inactivation on replication and are discussed below.

A large battery of CHK1 inhibitors is available to support cancer treatment in combination with radio- and chemotherapy [116]. Older CHK1 inhibitors such as UCN01 were not very selective but did demonstrate potent chemosensitization to cisplatin and camptothecin. More potent and specific CHK1 inhibitors were developed; however, concomitant CHK2 inhibition to some degree is common to most CHK1 inhibitors. In general, cells appear to depend on a functional G2 checkpoint when exposed to agents that cause replication stress. Hence, potentiation is greatest to cross-linkers, topoisomerase I poisons and nucleoside analogues such as gemcitabine. Consistent with replication stress hypersensitivity when G2 arrest is abrogated, PARP inhibitors also cause problems. PARP inhibition can cause a G2 checkpoint dependence and the combination of PARP inhibitors with CHK1 inhibitors is synthetic lethal [117]. These cellular sensitivity features are the basis for the clinical trials testing the combination of older (UCN01) or later generation compounds (for example AZD7762, AstraZeneca; PF477736, Pfizer; LY2606368, Eli Lilly and SCH900776, Schering Plough) with cisplatin, topotecan, and gemcitabine. For a more detailed review, see [116]. Unfortunately, safety requirements as assessed in these initial studies were not met in at least one compound (AZD7762) [118]. Similar to chemotherapy regimens, CHK1 inhibitors prevent ionizing radiation (IR) induced S and G2 arrest and demonstrated some potential to radiosensitize in a p53-dependent manner. CHK1 is upregulated in Myc-overexpressing lymphomas, and single-agent activity is expected since CHK1 inhibition is cytotoxic to these cells.

Interestingly, recent data indicate that CHK1 activity might prevent replication-induced DNA damage or is implicated in DNA repair [119, 120]. A

conversion of halted replication forks (induced by the chemotherapeutics) into persistent and cytotoxic DSBs has been postulated. The CDK-driven unscheduled initiation of replication origins can be accounted for with regard to this DSB induction and explains chemosensitization beyond G2/M checkpoint abrogation [120]. The notion that CHK1 inhibition promotes replication stress merits their evaluation in synthetic lethal strategies exploiting tumor-specific DNA repair defects [121].

6.3.4 AP-Endonuclease 1

Targeted knockout (KO) of AP(apurinic/apyrimidinic)-endonuclease 1 (Ape1 or Ref1) in mice is lethal [122], and deletion of the *Ape1* gene in mouse cells induces apoptosis within 24 h [123]. Interestingly, granzyme A(GzmA)-mediated cell death is enhanced by GzmA-mediated cleavage of Ape1. It is suggested that the proteolysis of APE1 enhances GzmA-mediated cell death by promoting apoptosis [124]. In human cells, RNA interference–mediated depletion of APE1 suppresses cell and tumor growth [125]. These and many other studies therefore support the development of APE1 inhibitors to enhance radiation and chemotherapy response [126, 127].

The first such compound to be tested clinically that impacts APE1 function in BER is methoxyamine (TRC102; Tracon Pharmaceuticals). Methoxyamine hydrochloride (MX) was first suggested to be a mutator, inducing the formation of 5,6-dihydro-6-methoxyaminecytosine residues in DNA [128]. It was subsequently determined that MX traps aldehyde groups, forming a stable intermediate [129]. The AP site in DNA is not a chemically unique species but exists as an equilibrium mixture of the ring-closed cyclic hemiacetals and open-chain aldehyde, and hydrate forms [130]. The transient open-chain aldehyde form is reactive with aldehyde-specific reagents such as methoxyamine, allowing the trapping or quantitative measurement of AP sites in DNA [131]. It was subsequently determined that the reaction between MX and the open-chain aldehyde form of an AP site blocks repair of DNA base lesions by BER. The trapped AP site is a highly cytotoxic intermediate and sensitizes cells to the cytotoxic effects of alkylating agents [132–134] and other DNA-damaging agents that may give rise to spontaneous or enzyme-mediated AP sites, such as TMZ [135], iododeoxyuridine + radiation [136], BCNU [137], manumycin A [138], fludarabine [139], radiation [140, 141], and pemetrexed [142]. Interestingly, elevated expression of the downstream BER protein DNA polymerase β (Polβ) reverses MX sensitization of alkylating agents, suggesting that the cleaved open-chain aldehyde may be the preferred MX substrate [134]. MX is in the clinic under the brand name TRC102 and is undergoing clinical evaluation in Phase II trials for solid tumors as a sensitizer to Temodar® (TMZ) or Alimta® (Pemetrexed) and in hematologic malignancies with Fludara® (Fludarabine).

In parallel, there are a number of groups developing direct APE1 active site inhibitors [143–146], and an overview of the development of APE1 inhibitors has just been reported [147]. As might be expected, many of these APE1 inhibitors are themselves cytotoxic and enhance the cytotoxicity of DNA-damaging agents such as TMZ [144, 145] or other alkylating agents [143]. Although Ape1 KO or GzmA-mediated Ape1 cleavage in mouse cells induces apoptosis, the cell death mechanism(s) induced by these recently developed APE1 inhibitors has yet to be resolved. Interestingly, the cell death mechanism triggered by some of these APE1 inhibitors may involve the accumulation of DNA DSBs since it was observed that the compounds are more cytotoxic in cells deficient in the HR proteins BRCA1 or BRCA2 [148].

6.3.5 Ataxia Telangiectasia-RAD3 Related

Similar strategies and rationales that apply to the CHK1 target also apply to ATR. A direct link connects ATR with CHK1 [121]. After initial Rad17 binding, Claspin-loaded single-strand DNA mediates the activation of CHK1 by ATR upon replication stress. ATR is essential in the surveillance of replication stress, especially when associated with exposed single-stranded DNA mostly connected to replication problems. Stalled replication forks can collapse which results in the formation of DSBs, a signal that mainly, but not exclusively, triggers ATM whereas the single-stranded DNA-induced damage response appears to be ATM independent. Endogenous and exogenous damage induces replication stress that requires a proficient ATR/CHK1 response for survival. One prevalent rationale to inhibit ATR for cancer therapy is to exacerbate the levels of replication stress that might be augmented in cancer cells due to inherited defects in the DDR and DNA repair pathways. Cancer cells are exposed to a higher load of replication stress compared to normal cells and will suffer most from targeting ATR. In addition, since targeting replication-associated processes, any selective killing property will be augmented in highly proliferative cells. Such a strategy is supported by two observations: (1) the activated DDR found in early stages of tumorigenesis [149, 150] indicates an increased load of “endogenous” DNA damage and (2) the discovery that a wide variety of oncogenes generate such damage.

Based on this proposed endogenous damage (replication stress)-induced killing mechanism, such ATR inhibitors have been proposed to act as single agents. However, combination strategies similar to those for the CHK1 inhibitor are envisioned. As noted before, the response of normal cells should be carefully taken into consideration. A few compounds have been pursued by industry. The ATR inhibitor NU6027 appears to impair HR and enhances the cytotoxicity of PARP inhibitors [151], a theme that is consistent to other DDR inhibitors. One compound (NVP-BEZ235) has been recently discovered and found to trigger preferential cell kill in cyclin-E overexpressing cells [152] and is awaiting entry into clinical trials for cancer therapy.

6.3.6 Ataxia Telangiectasia-Mutated

ATM inhibitors are less advanced in their clinical development than PARP or CHK1 inhibitors. Since involved in DSB repair, NHEJ, and HR, ATM inhibition results in reduced cellular DSB repair activity and cell cycle checkpoint defects. As a result, ATM inhibitors are highly potent radiosensitizers while exhibiting little toxicity on their own. Therefore, they have been proposed to be applied in this context [153]. Several ATM-inhibiting compounds have been identified and pursued in preclinical studies: KU55933 and KU60019 (KuDos/AstraZeneca) and CP466722 (Pfizer).

Combination strategies for ATM inhibitors have been proposed to enhance the cytotoxicity of PARP inhibitors (see above). Interestingly, p53 disruption in normal cells sensitized those cells to the combination of PARP and ATM inhibitors [83]. Due to its crucial role in DSB repair, the inhibition of ATM will radio- and chemosensitize most cells, including normal cells, with no evident DNA repair defects. However, synergistic cytotoxicity can be observed under certain conditions that could be exploited for tumor-targeted strategies. Cells with BER defects for example rely on secondary DSB repair pathways such as HR for survival upon damaging agents. Similar to the PARP inhibitor/HR-defect synthetic lethal interaction, the conversion of unrepaired SSBs to DSBs could be a mechanism that underlies such dependence. BER defects of different kinds have been reported in tumors, and it is suggested that inhibition of DSB repair processes will be beneficial in such a setting [154].

6.3.7 DNA-Dependent Protein Kinase

DNA-PK is crucial to DSB repair as well as cellular survival following radiation and topoisomerase II poisons, and so, DNA-PK has been a long sought target for drug development [155]. Kinase activities are relatively easy to target, and drugs inhibiting DNA-PK activity have been available for some time. One should note that a considerable fraction of first generation tyrosine kinase inhibitors do also inhibit DNA-PK to some extent. The radiosensitization phenotype by some of these agents should therefore be re-evaluated with respect to its origin (see below). Some examples of currently pursued inhibitors are NU7026 and NU7441 [156, 157]. Other targeting strategies apply short modified DNA molecules that are supposed to interfere in DNA-PK signaling (DT01, DNA therapeutics) or short peptides that resemble Ku80 (HNI-38) and disrupt the interaction with DNA-PK [158]. The dual DNA-PK and PI3-K inhibitor KU-0060648 takes advantage of the inhibition of two cellular processes central to promoting survival upon chemo- and radiotherapeutic insults. Surprisingly, this inhibitor enhanced etoposide-induced xenograft tumor growth delay substantially without exacerbating etoposide toxicity in mice indicating that the combination provided some tumor specificity [159].

Most DNA-PK-inhibiting agents are very potent radiosensitizers, enhancing clonogenic cell kill markedly [160]. Radiosensitization by one compound, the DNA-PK inhibitor BEZ235, has been shown to be associated with an accelerated p53-dependent senescence [161]. Although proposed as radiation dose intensifiers, DNA-PK inhibitors, similar to ATM inhibitors (though often to a greater extent), will sensitize normal tissue to radiation at least as much as it does sensitize the tumors. However, some DNA repair defects inherent to tumors might provide some therapeutic gain when applying low inhibitor doses that could spare normal tissue. Opportunities exist for example in the treatment of ATM-deficient tumors since DNA-PK inhibition induces tumor cell kill in ATM-deficient cells [162].

Considering that disruption of the catalytic activity of DNA-PK confers severe immunodeficiency, DNA-PK inhibitors will have to be evaluated carefully for toxicities in preclinical studies before entering clinical trial [163]. Tumor-targeted delivery strategies could, however, make use of these highly potent radiation dose intensifiers. Other strategies exploiting the differential expression or use of DNA-PK and Ku-dependent DNA repair in some tissues with respect to the tumors could apply these inhibitors successfully. An example for this is the sensitization of EGFRvIII-overexpressing cells with DNA-PK inhibitors [164].

6.4 Indirect DNA Repair Modulators

6.4.1 Signaling Pathways, EGFR and the PI3K/AKT Pathway

Targeting the cellular signaling pathways via EGFR, PI3K/AKT, or MAPK can modulate the DNA repair status of cells. The effects are multiple and involve different pathways. Cellular signaling influences DNA repair in multiple ways including changes in the expression levels of crucial DNA repair proteins such as RAD51 or the regulation of the activation or translocation of enzymes and kinases such as DNA-PK. For example, the inhibition of the MAPK pathway can lead to reduced DSB repair by both homologous and non-homologous pathways [165, 166]. Links between the AKT, MAPK, and EGFR pathways and DNA repair have been found, particularly with DSB repair by NHEJ or HR [166–171]. Hence, targeting these pathways can result in the concomitant inhibition of DNA repair.

Based on knowledge that the PI3K pathway promotes DNA repair and survival, inhibitors of AKT have been evaluated as chemosensitizers for alkylating agents [172, 173]. AKT inhibitors such as LY294002 and wortmannin radiosensitized and caused enhanced sensitivity to alkylating agents such as TMZ, or the cross-linkers cisplatin in various human tumor cell lines.

The suppression of ATR/CHK1 checkpoints has been observed upon treatment with wortmannin (a fairly non-specific PI3K inhibitor). For more details, see [174] and references within. Note that an influence on Rad51 expression or on NHEJ via DNA-PK has also been observed.

Co-targeting PARP and the PI3K pathway has been shown to synergistically decrease growth and further induce apoptosis [175]. The molecular mechanism of this combination effect is not completely clear but could originate from secondary effects on DNA repair pathways and the DDR from the inhibition of cellular signaling in human cancer cells. Further detail on AKT inhibitors is discussed in more detail in Chap. 13.

6.4.2 Proteasome Inhibitors

Proteasome-mediated destruction and removal of key DNA repair and DDR proteins are an essential aspect of the cellular response to genotoxins [4, 176]. Failure to remove critical DNA repair and DDR proteins in a timely fashion effectively halts the process, triggering a cascade of events leading to cell death [177]. As the endpoint in ubiquitin-mediated protein turnover, targeting the proteasome is a high-profile target to enhance DNA damage–induced cell death [178, 179]. Further detail on the proteasome pathway and proteasome inhibitors is discussed in more detail in Chap. 12.

6.4.3 NAD^+ Biosynthesis Inhibitors

The metabolite NAD^+ is an essential substrate for all PARPs [74]. Since PARP1, as well as PARP2 and PARP3, is a critical protein involved in DNA repair and the DDR [36, 103], we have included NAD^+ biosynthesis inhibitors as an indirect DNA repair modulator. All of the NAD^+ biosynthesis inhibitors developed to date target NAMPT, a pivotal and rate-limiting enzyme in the salvage pathway of NAD^+ biosynthesis [180]. NAMPT catalyzes the synthesis of nicotinamide mononucleotide (NMN) from nicotinamide to 5-phosphoribosyl-1-pyrophosphate [181]. The resulting NMN is then converted to NAD^+ by one of the three isoforms of NMNAT (1, 2 or 3), located in the nucleus, cytosol, or mitochondria, respectively [182]. The most studied inhibitor of NAMPT is FK866 (also referred to as Apo866) although several other NAMPT inhibitors have been reported including GMX1778 [183], CB30865 [184], and CHS-828 [185], and one group is actively screening for NAMPT inhibitors [186]. FK866 binds at the interface of the NAMPT dimer [187] and effectively inhibits NAMPT and depletes cellular NAD^+ levels within 24 h, inducing apoptosis [188] although the overall level of cell death may be offset by FK866-mediated induction of autophagy [189]. Further detail on the shift of cell death triggered by NAD^+ biosynthesis inhibitors is discussed in Chap. 2.

As an NAMPT inhibitor, FK866 does have a chemo-potentiating effect [190–192] that is more pronounced when combined with a second DNA repair inhibitor [133] or when combined with TRAIL [193]. Interestingly, it has been suggested that some tumors are deficient in the NAPRT1-mediated NAD^+ biosynthesis pathway

that is responsible for generating NAD^+ from nicotinic acid (NA) [183, 194]. In sum, these studies suggest that NAMPT is a potentially valuable target to impact both metabolism and DNA repair and may provide a type of synthetic lethality based on DNA repair and NAD^+ biosynthesis status [133, 195].

6.5 Promising Future Targets and Opportunities

6.5.1 ERCC1

An emerging DNA repair target protein is ERCC1, encoded by the excision repair cross-complementing rodent repair deficiency, complementation group 1 gene (ERCC1) [49, 50]. As an essential NER protein, elevated ERCC1 expression enhances the repair of cisplatin-induced genomic DNA damage [15] and decreased expression is suggested to improve response to platinum-based therapies [196]. As described above (Sect. 6.1.4), ERCC1 exists as an obligate heterodimer with the protein XPF to yield an essential NER structure-specific nuclease ERCC1/XPF [15]. The ERCC1/XPF heterodimer is recruited to function in NER by interaction with the NER protein XPA [197] although multiple interaction sites are found to facilitate recruitment of ERCC1/XPF to the NER complex [198]. In this regard, it has been suggested that the function of ERCC1 in NER can be disrupted by interfering with the interaction between ERCC1 and XPA [199]. Alternatively, since heterodimer formation (ERCC1/XPF) is needed to maintain the stability of ERCC1 [200], it might be feasible to interrupt the heterodimer complex and force ERCC1 proteolysis [201]. Finally, one might also consider deregulating expression of ERCC1 through modulation of the RAS kinase or ERK1/2 pathways [202]. Using the Raf kinase and VEGF receptor inhibitor sorafenib reduces ERCC1 levels and effectively enhances the response to radiation and chemotherapy [203]. Of course this treatment would have multiple effects on gene regulation.

6.5.2 DNA Polymerases

There are 15 DNA polymerases in the human cell [204] and several have been suggested to be viable drug targets either because they facilitate repair of DNA damage or may be overexpressed in cancer [205]. DNA polymerase β (Polβ) is a member of the X family of DNA polymerases [204, 206] and is an essential BER protein, as detailed above. Polβ has two active sites and several critical functional domains that may be considered as targets to inhibit Polβ and BER. Further, the central and pivotal role of Polβ in BER implicates this protein as a prime target to enhance the response to chemotherapeutic agents or radiation. Although many

inhibitors of Polβ have been developed and characterized very few, if any, show specificity and all are cytotoxic even in Polβ KO cells. Three recent reviews on Polβ inhibitors should be referred to for more detail [195, 207, 208].

Other polymerases have been also identified as potential targets. A siRNA screen and large-scale tumor sample analysis identified DNA polymerase theta (POLQ) as a target that determines radiosensitivity and is overexpressed in tumors. POLQ depletion radiosensitized these cells arguing for the development of POLQ inhibitors [209–211].

6.5.3 DNA Glycosylases

DNA glycosylases are the initiating enzymes in BER (see above). In general, DNA glycosylases probe the DNA helix for base lesions by a base-flipping mechanism, interrogating each base as it is flipped out of the major groove as the enzyme moves along the DNA helix. Identification of the lesion then promotes hydrolysis [212]. As discussed above, radiation and chemotherapeutic agents induce the formation of BER substrates and many of these base lesions block replication and are therefore cytotoxic. For example, the methylated base 3-methyladenine, induced by the chemotherapeutic agent TMZ and repaired by MPG, triggers lesion-induced apoptosis as well as sister chromatid exchange, chromatid and chromosome gaps and breaks, and S-phase arrest [213]. As such, it has been hypothesized that inhibition of MPG would enhance response to TMZ and other alkylators. Similarly, UNG, the primary glycosylase responsible for the removal of deoxyuracil, is reported to govern the efficacy of pemetrexed [142], and therefore, UNG might be considered a potential target to enhance the response to this chemotherapeutic agent. Finally, it was recently demonstrated that the DNA glycosylase OGG1 is in a complex with the BER protein PARP1. The OGG1–PARP1 complex promotes PARP1 activity whereas loss of OGG1 suppressed PARP1 activity [214]. From this study, it might be suggested that an inhibitor designed to disrupt the OGG1–PARP1 interaction may function as a pseudo- or indirect PARP1 inhibitor. However, there are multiple challenges to developing effective DNA glycosylase inhibitors such as the large overlap in enzyme substrate specificity [36, 37] and the requirement for some glycosylases in normal cell survival [215, 216] and immunoglobulin class-switch recombination [217]. To date, we know of no DNA glycosylase inhibitors with the exception of Ugi, a virally encoded UNG inhibitor [218].

6.5.4 DUBs

As with most posttranslational modifications, both the synthesis and removal of the ubiquitin modification is a highly regulated process [219, 220]. Recently, several studies have revealed the potential for targeting DUBs to enhance radiation

and chemotherapeutic response [221, 222]. Toward that end, several groups have reported the development of DUB inhibitors that are effective in preclinical studies [223–226].

6.5.5 HR/FA

HR and FA strongly determine cellular survival upon cross-linkers, topoisomerase poisons, alkylators, and radiation. These pathways are mostly engaged in a proliferation-dependent manner with less influence on damage repair and response in quiescent cells. Thus, targeting HR and FA can be an effective means to potentiate radio- and chemotherapy [227].

Novel agents targeting RAD51, albeit indirectly, are now emerging in clinical trials. MP470 (Supergen) is a tyrosine kinase inhibitor with RAD51 protein–suppressing properties. More specific inhibitors are sought in high-throughput screening efforts of which some provided some compounds with convincing specificity and potency [228].

The value of HR inhibition can be deduced from experiments in which elements of the HR or FA pathway such as RAD51 or BRCA2 have been downregulated, supporting the potential of HR/FA-targeting strategies. Downregulation of Rad51 or BRCA2 sensitized glioma cells to the cytotoxic effects of TMZ or nimustine in cells with low MGMT levels [229]. The marked resistance of gliomas to chemo- or radiotherapy regimens that might be partly defined by HR or increased Rad51 levels can be defined by targeting RAD51, indicating a similar strategy option to counteract resistance by HR inhibition [230].

FA- and HR-defected cells have been found to lack the increased radio-resistance that is observed in hypoxic areas of the tumors [231]. Inhibitors targeted to HR or FA will not only preferentially sensitize proliferating cancer cells but also hypoxic tumors to radiation. Owing to less-effective DSB induction under low oxygen concentration, radiotherapy often fails in patients with hypoxic tumors. Tumor specificity of HR-targeting drugs will be increased when applied to BER-defected cancer cells as illustrated above. BRCA2-knockdown radiosensitizes cells that express a dominant-negative Polβ mutant to a greater extent than isogenic controls [232]. Similarly, NHEJ-defected cancer cells are expected to rely on HR for survival upon genotoxic threats. Together, these interactions and data support strategies applying HR- or FA-inhibiting drugs for tumor-specific sensitization.

6.5.6 Poly(ADP)ribose Glycohydrolase

Poly(ADP)ribose glycohydrolase (PARG) [233] is an enzyme involved in BER and is the major factor responsible for removing the PAR molecules synthesized by PARP1 and PARP2, among other PARPs [234]. As seen with PARP1, PARG is also a substrate for caspase-3 [235], supporting the significance of PAR metabolism in cell death processes. Mouse KO studies or RNA interference studies to

knockdown PARG in human cells have suggested that blocking PAR degradation is an effective means to modulate chemotherapy and radiation response [134, 236, 237]. Pre-clinical evaluations using early phase small-molecule inhibitors such as gallotannin, tannic acid, and related small molecules have implicated PARG in genotoxin or chemotherapy sensitivity [238–240]. The inhibitor GPI-16552 (N-bis-(3-phenyl-propyl)9-oxo-fluorene-2,7-diamide) has shown early success in cell and mouse models as a chemotherapy sensitizer [97] but has mostly been effective to reduce inflammation [241–245]. Recent drug discovery efforts seem promising and have yielded PARG inhibitors with increased specificity and cell permeability [246, 247].

6.6 Summary and Concluding Remarks

Inducing tumor-specific or selective cell death is a significant challenge in the treatment of cancer. Although radiation and most chemotherapeutic treatments are designed to induce significant genotoxic damage to the tumor cells, a multitude of DNA repair and DDR gene products respond to the presence of genotoxic stress (DNA damage) by orchestrating a massively complex cellular response to survive and repair the genome insult. Most DNA repair pathways focus on specific damage types (e.g., DSBs), but there is considerable overlap and backup capacity when evaluating the overall involvement of DR, BER, MMR, NER, HR, and NHEJ proteins in response to these agents (radiation and chemotherapy). It has been observed that some cancer cells can survive the loss of key DNA repair proteins (e.g., BRCA1/2). In fact, we have come to realize that many cancer cell types are defective in one or more DNA repair or DDR genes/proteins, precipitating the need to identify the key stress nodes in these defective cells in response to radiation and classical chemotherapy treatments. With this in mind, we have described the molecular pathways that participate in the repair of DNA damage induced by radiation and chemotherapeutics. Further, we present novel therapeutic strategies being considered to exploit inherent weaknesses in tumor cells such as defects in one or more DNA repair pathways that may provide the opportunity to selectively increase tumor-specific cell death.

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