Coordination of DNA Mismatch Repair and Base Excision Repair Processing of Chemotherapy and Radiation Damage for Targeting Resistant Cancers

Timothy J. Kinsella

Abstract

DNA damage processing by mismatch repair (MMR) and/or base excision repair (BER) can determine the therapeutic index following treatment of human cancers using radiation therapy and several classes of chemotherapy drugs. Over the last decade, basic and translational cancer research in DNA repair has led to an increased understanding of how these two DNA repair pathways can modify cytotoxicity to chemotherapy and/or ionizing radiation treatments in both normal and malignant tissues. This Molecular Pathways article provides an overview of the current understanding of mechanisms involved in MMR and BER damage processing, including insights into possible coordination of these two DNA repair pathways after chemotherapy and/or ionizing radiation damage. It also introduces principles of systems biology that have been applied to better understand the complexities and coordination of MMR and BER in processing these DNA damages. Finally, it highlights novel therapeutic approaches to target resistant (or DNA damage tolerant) human cancers using chemical and molecular modifiers of chemotherapy and/or ionizing radiation including poly (ADP-ribose) polymerase inhibitors, methoxyamine and iododeoxyuridine (and the prodrug, 5-iodo-2-pyrimidinone-2’-deoxyribose).

Background

Many of the commonly used chemotherapeutic drugs, as well as radiation therapy, target DNA for cytotoxicity. Indeed, the subsequent DNA damage response to these cancer treatments in both malignant and normal tissues determines the therapeutic index. The DNA damage response is a complex process involving multiple DNA repair, cell survival, and cell death pathways with both damage specificity and coordination of the DNA damage response to different types of DNA damage. These DNA damages include double-strand breaks, single strand breaks (SSB), base damages, bulky adducts, intra/interstrand cross links, and breakdown of replication fork lesions.

It is also now understood that human cancers typically arise after a long process of random mutations, accompanied by continual selection for more rapidly proliferating tumor cells. Some of these mutations that lead to cancers involve genetic changes in key DNA repair pathways (1). Additionally, epigenetic changes via DNA methylation and/or histone methylation and acetylation at DNA repair genes can lead to cancer (2). As such, cancer treatments that target a specific DNA repair defect can be selectively toxic (lethal) to cancer cells with different DNA repair capacities while sparing normal (DNA repair proficient) tissues.

In this article, I discuss the current understandings of two DNA repair pathways, namely DNA mismatch repair (MMR) and base excision repair (BER), with respect to damage processing after certain types of chemotherapeutic drugs, including monofunctional alkylators, bifunctional alkylators, and antimetabolites, and after ionizing radiation. Emphasis is placed on the potential cross-talk or coordination of these two DNA repair pathways and strategies to improve the therapeutic index for treating DNA repair deficient (damage tolerant) cancers. Although it is clear that the MGMT repair protein also plays a role, along with MMR and BER, in processing alkylation induced DNA damage, this overview will focus primarily on MMR and BER. Some pertinent principles of systems biology modeling will be addressed in the overview of these two DNA repair pathways, including their application to treatments targeting DNA damage tolerant cancers.

MMR

The MMR system repairs base-base mismatches that can give rise to point mutations and insertion/deletion loops that can give rise to frameshift mutations. Such mismatches result from DNA replication errors during S phase that escape proofreading by DNA exonucleases or homologous recombination (3, 4). The clinical implications of genetic stabilization by MMR are best illustrated by the fact that genetic defects in the human MMR pathway confer a strong predisposition to hereditary nonpolyposis colorectal (and
associated endometrial) cancers. Moreover, epigenetic silencing of key MMR genes may contribute to the development of 5% to 15% of sporadic cancers that have a microsatellite instability-high phenotype, including colorectal, gastric, endometrial, ovarian, breast, lung, and bladder cancers as well as high grade gliomas, leukemias, and lymphomas (5).

The MMR pathway is a multiprotein system that has three subprocesses (refs. 3, 4; Table 1). These subprocesses involve mismatch recognition by MutSα (a MSH2/MSH6 dimer) or MutSβ (a MSH2/MSH3 dimer); mismatch excision, which is initiated by the binding of MutLa (a MLH1/PMS2 dimer) or MutLβ (a MLH1/MLH3 dimer) to MutSα and the subsequent recruitment of an exonuclease (EXO1) that sequentially removes nucleotides between an adjacent SSB up to and beyond the mismatch on the daughter DNA strand; resynthesis by DNA polymerase along with at least two other proteins, proliferating cell nuclear antigen and replication protein A; and finally, the nick located in the daughter strand is sealed by a DNA ligase.

MMR is now recognized to be involved in the processing of DNA damage induced by several classes of clinically active (or experimental) chemotherapeutic drugs including the following: monofunctional alkylators such as temozolomide, dacarbazine, and N-methyl-N'-nitro-N-nitrosoguandine (an experimental drug); bifunctional alkylators such as the platinum analogues, cisplatin and carboplatin; and antimetabolites such as the thiopurines, 6-thioguanine (6-TG; Fig. 1) and 6-mercaptopurine, and the fluoropyrimidines, 5-fluorouracil and fluorodeoxyuridine. In experimental cell systems, MMR-proficient (MMR+) cells are 2- to 100-fold more sensitive to these drug types compared with isogenic MMR- cells (3, 6, 7), showing the counterintuitive fact that MMR processing of DNA lesions induced by these agents elicits a further set of cellular processing including activation of a prolonged G2 cell cycle delay (arrest) and subsequent activation of cell death pathways including apoptosis and autophagy (Fig. 2; refs. 3, 6–8).

In contrast, other antimetabolites such as the halogenated thymidine analogue, iododeoxyuridine (I UdR), have very modest differences in cytotoxicity in MMR+ versus MMR- isogenic cell systems (9, 10). MMR human colorectal and endometrial tumor cells/xenografts, however, show persistently higher I UdR-DNA levels compared with MMR+ (normal) cells after I UdR treatment as the resulting G:U mispairs are efficiently repaired in MMR+ cells but not repaired in MMR- cells. As I UdR is a known radiosensitizing drug, it can be combined with radiation therapy to selectively target MMR- (drug resistant) human cancers (Fig. 2; ref. 10). This potential therapeutic strategy is discussed in greater detail in the Clinical-Translational Advances section.

Using experimental systems, two different models of MMR processing of drug-induced damage have been proposed, the futile cycle model and the general damage sensor (or direct signaling) model (3, 4, 6). Each model attempts to link MMR processing with activation of a prolonged G2 checkpoint arrest and a later activation of programmed cell death pathways (both typically one to two cell cycles after drug treatment). In the futile cycle model, MMR has a single function with the MutSα/ MutLa/EXO-1 complex ultimately creating persistent DNA SSBs in the vicinity of chemically induced mismatches. For instance, when me6TG (after 6-TG treatment; Fig. 1) or O6-meG (after temozolomide treatment) are in the template during replication, the incorporation of a C or T into the newly synthesized strand creates another mismatch because the chemically modified base remains in the template strand after MMR-mediated excision (11, 12). Repair synthesis proceeds with regeneration of the meG-C or -T mismatches in a repetitive

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**Table 1: Protein complexes involved in MMR and BER DNA damage subprocessing of chemotherapy and ionizing radiation damage**

<table>
<thead>
<tr>
<th>Subprocesses</th>
<th>MMR proteins</th>
<th>BER proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mismatch/Base damage recognition</td>
<td>MutSα</td>
<td>Damage-specific glycosylases and AP-lyase</td>
</tr>
<tr>
<td>2. Mismatch/Base damage excision</td>
<td>MutSα, MutLa, EXO-1</td>
<td>APE-endonuclease AP-lyase</td>
</tr>
<tr>
<td>3. Synthesis of DNA repair patch &amp; DNA ligation</td>
<td>Polδ, PCNA, RPA, DNA ligase</td>
<td>Short patch BER</td>
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<td></td>
<td></td>
<td>Long patch BER</td>
</tr>
<tr>
<td></td>
<td>PARP1, XRCC1, Polβ</td>
<td>PARP1, Fen1, PCNA, Polδ</td>
</tr>
<tr>
<td></td>
<td>XRCC1, Polβ, DNA ligase III</td>
<td>DNA ligase I</td>
</tr>
</tbody>
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*CCR Molecular Pathways*
model independent of the damage-induced G2 cell cycle delay. Of excision and resynthesis, however, could proceed in this mismatch processing through the downstream subprocesses for further processing of the mispair (3, 4). DNA directly activate the p53-ATR-Chk1 pathway without a requirement for extensive laboratory data using purified protein (in vitro) and cellular/tissue (in vivo) systems with appropriate time courses and levels of quantitation to test the model (22–24). As a first step in our hybrid modeling of the MMR processing of chemically induced mispairs, we recently published a study that integrated probabilistic mathematical models and experimental data on MMR versus MMR+ isogenic human colorectal (HCT116) cancer cells to describe the effects of IUdR-DNA incorporation on the cell cycle (25). Our goal is to use modeling data to increase IUdR-mediated radiosensitivity in MMR cancers (damage tolerant). These mathematical models are now being modified and used to attempt to maximize the therapeutic gain in MMR+ tumors (using xenograft models) versus MMR+ normal tissues by predicting the optimal dose of IUdR (or its produg, 5-iodo-2-pyrimidinone-2'-deoxyribose) and the optimal timing for ionizing radiation treatment to increase the synergistic action (26, 27).

A second example in which systems biology modeling can be applied to MMR processing of drug damage involves the development of acute myelogenous leukemia (AML) as a complication of thiopurine treatment (28). It is now recognized that secondary AML is a potential complication after treatment of acute lymphoblastic leukemia using either 6-TG or 6-mercaptopurine as well as after treatment of solid organ transplant patients with azathioprine for chronic immunosuppression. In both settings, the secondary AML cells often show microsatellite instability-high (MMR+) phenotypes, which are infrequent in de novo AML. Indeed, the probable cause for the observed MMR defects in thiopurine-related AML cells is the selective outgrowth of myeloid precursor "leukemic" stem cells in the bone marrow related to epigenetic silencing of hMLH1 or hMSH2 genes. Thus, our increased understanding of MMR processing of chemotherapeutic drugs such as antimetabolites (6-TG or IUdR) and alkylating agents as well as ionizing radiation using mathematical modeling can be applied to both cancer treatment and cancer prevention strategies (Figs. 1 and 2; refs. 25, 26, 28).

**BER**

BER is the major DNA repair pathway involved in the removal of nonbulky base damages induced by endogenous...
and exogenous adducts. A major source of endogenous base damage involves oxidative base modifications from reactive oxygen and nitrogen species during normal cellular respiration or during oxidative stress from ischemia or chronic inflammation (29). BER is also the major repair pathway for nonbulky damaged bases, abasic sites, and DNA SSBs after treatment with ionizing radiation, monofunctional alkylating drugs, and certain antimetabolites including the thiopurines, fluoropyrimidines, and halogenated thymidine analogues (30, 31). Thus, BER and MMR pathways are activated by similar types of DNA damage–targeted cancer treatments and are involved in damage (sub) processing (Table 1; Fig. 1).

BER is a complex multistep pathway initiated by damage-specific DNA glycosylases, which create abasic or apurinic/apyrimidinic (AP) sites by cleaving the N-glycosidic bond and holding the base onto the sugar-phosphate backbone (32). Next, AP endonuclease 1 recognizes the AP sites and cleaves the DNA phosphodiester backbone leaving a 3'-hydroxyl group and a 5'-deoxyribose phosphate group flanking the nucleotide gap. Subsequent repair proceeds by two subpathways, both initiated by DNA polymerase β, for 1 nucleotide repair (short-patch BER) or for 2 to 15 nucleotides repair (long-patch BER). Although these two subpathways use different subsets of enzymes, there is cooperation and compensation between the short-patch and long-patch pathways (32). It is generally held that short-patch BER accounts for most BER activity after chemotherapeutic and/or ionizing radiation treatments. Poly(ADP-ribose) polymerase (PARP) plays a key role in both BER pathways (Table 1), and chemical inhibitors of PARP are being used clinically as discussed later.

Because of the complexity of BER, mathematical modeling of the BER pathway has allowed basic and translational cancer researchers to gain insights into the association of genetic variances in BER protein expression and cancer risks (33). These data suggest that mild BER protein SNP variants have a minimal effect on BER capacity, whereas more severe defects in BER protein function or simultaneous variation in several BER proteins can lead to inefficient BER with enhanced lethality and increased mutagenesis/carcinogenesis. A direct cancer link has been established between familial colorectal cancer and biallelic mutations in the human MutY homolog gene (called MUTYH-associated polyposis; ref. 34). In this colorectal cancer–prone syndrome, the resulting MutYH glycosylase variants show decreased repair of 8-oxoG:A mismatches, leading to increased G:T transversions in the APC gene and inactivation of the APC protein. Although genetic mutations in MMR genes (typically hMLH1 and hMSH2) also lead to enhanced colorectal cancer (hereditary nonpolyposis colorectal cancer; ref. 3), the molecular mechanisms of carcinogenesis are quite different between DNA-repair defects in MMR versus BER. A second cancer-prone genetic syndrome related to mutations in another BER glycosylase, neil1, and an increased risk of familial gastric cancer is also recently reported (35).

Computational and mathematical modeling approaches have also been used to better understand BER processing of alkylating and antimetabolite chemotherapeutic damage as

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**Fig. 2.** DNA Damage Processing by MMR and/or BER. DNA damage processing by DNA MMR and/or BER of both drugs and ionizing radiation is illustrated.
well as ionizing radiation damage (Figs. 1 and 2; refs. 33, 35–37). These data support a model where BER protein levels are directly controlled by the type and extent of DNA damage. After repair is accomplished, the key BER proteins may be recycled or degraded by a proteosomal-dependent pathway if the BER protein levels exceed the levels of DNA damage. In contrast to MMR processing of these types of DNA damage, BER processing sometimes leads to chemotherapeutic and ionizing radiation resistance. It is well-recognized that BER removes a wide spectrum of DNA adducts such as those caused by alkylating drugs (particularly N7-methylguanine and N3-methyladenine) as well as some adducts caused by ionizing radiation (e.g., bleomycin; particularly 8-oxoguanine). Other BER glycosylases such as thymine-DNA glycosylase, uracil-DNA glycosylase, and methyl binding domain protein 4 (MBD4, also known as MED1) can efficiently process antimetabolite-DNA base damage, leading to antimetabolite drug resistance (38–40).

**Clinical-Translational Advances**

As discussed, both MMR and BER are central pathways in DNA damage processing of certain types of cancer therapy including the alkylating and antimetabolite classes of chemotherapeutic drugs and ionizing radiation (Figs. 1 and 2). An important therapeutically related distinction exists, however, between these two DNA repair pathways; i.e., MMR processing is required for the cytotoxicity of these drug types, whereas BER processing sometimes leads to reduced drug-related cytotoxicity. A similar distinction can be made for ionizing radiation damage processing by MMR versus BER, although nonhomologous end joining and homologous recombination are felt to be the major ionizing radiation repair pathways for double-strand breaks, which are the most lethal type of ionizing radiation-DNA damage (22, 23). It is also important to realize that human carcinogenesis can be associated with genetic and epigenetic perturbations of both MMR and BER pathways, with some human cancers arising after cancer treatment using alkylating/antimetabolite chemotherapeutic and/or ionizing radiation (3–6, 28, 29, 33, 35).

Given our current understanding of these two DNA repair pathways, it is now possible to design novel cancer treatments to directly target human cancers in which one or both of these DNA repair pathways are perturbed. In this targeted treatment approach, it is assumed that all dose-limiting normal tissues are fully DNA repair competent, which would eliminate cancer patient populations with germ line mutations in either DNA repair pathway. This proposed direct or targeted treatment strategy requires cancer therapeutics that are modulated (resulting in enhanced tumor cytotoxicity) by the cause of the cancer (e.g., BER- and/or MMR- deficiency). The overall complexity and coordination between these two DNA repair pathways, however, as discussed in the previous sections, present major obstacles to the design and analyses of clinical trials using such targeted treatment.

One of the key proteins in the BER pathway is PARP1 (Table 1), which functions in both short patch and long patch BER. PARP I forms complexes with XRCC1, DNA polβ, and DNA ligase III involved in short patch BER and forms complexes with XRCC1, flap endonuclease 1, and DNA ligase I involved in long-patch BER (41). PARP I expression/activity increase significantly in human normal and cancer cell lines after chemotherapeutic DNA damage from monofunctional and bifunctional alkylators, topoisomerase I inhibitors such as irinotecan, antimetabolites including gemcitabine, as well as after ionizing radiation (41–44). High PARP I levels are also found in a variety of human cancers (43, 44). As such, chemical inhibition of PARP 1 as a strategy to enhance chemotherapeutic- and ionizing radiation-mediated cytotoxicity in human cancers by inhibiting BER has been undergoing preclinical and clinical development for several decades (41–44).

The first generation PARP 1 inhibitors, such as 3-aminobenzamide, were simple analogues of nicotinamide (43, 44). Although showing enhanced cytotoxicity to ionizing radiation and alkylating drugs in preclinical testing in a variety of human cancer cell lines, the clinical trials showed conflicting data (42–44). The second generation PARP 1 or PARP 1/2 inhibitors are currently undergoing clinical testing in phase 0 to I/II trials (30, 43–45). The second generation compounds have further structural modifications of benzamide to include an electron-rich aromatic or polyaromatic heterocyclic moiety at the 3-position and a carbamoyl group with an amide protein for hydrogen binding (42). Most of these compounds are currently undergoing standard phase I testing, whereas the Abbott Pharmaceuticals drug (ABT-888) is in phase 0 testing at the National Cancer Institute (45). The only reported phase II study of these second generation PARP inhibitors using the Pfizer PARP inhibitor (AG-014699), however, showed dose-limiting myelosuppression when combined with temozolomide (46). Some more recently developed PARP 1/2 inhibitors are reported to have reduced bone marrow toxicity while maintaining enhanced cytotoxicity to temozolomide and irinotecan in several different *in vitro* and *in vivo* human cancer cell models (47).

The concept of synthetic lethality using monotherapy with second generation PARP 1 inhibitors is also being tested clinically in *BRCA1* and *BRCA2* carriers with cancer using both the AstraZeneca compound (AZD2281) and the Pfizer compound (AGO14699; ref. 48). Synthetic lethality arises when a mutation in either of two related genes results in cell viability but mutations in both genes leads to cell death (49). It follows that by inhibiting the gene products (proteins) that are synthetic lethal to cancer-causing mutations, there will be an enhanced therapeutic index as the mutated cancer cells will be targeted for cell death, whereas sparing normal cells/tissues. The scientific basis for these clinical trials of second generation PARP 1 inhibitors in *BRCA1* or *BRCA2* carriers with cancers involved the demonstration of cytotoxicity in *BRCA1-* and *BRCA2*-deficient cells after siRNA interference of PARP 1 expression levels with no significant cytotoxicity to cells heterozygous for *BRCA1* or *BRCA2* mutations, which is analogous to normal tissues in these *BRCA* carrier patients with cancers (50). The proposed mechanism of this type of synthetic lethality involves the initial creation of DNA SSBS by the PARP inhibitors with subsequent creation of double-strand breaks when encountered by a replication fork. In the absence of *BRCA1* or *BRCA2* proteins, both homologous recombination and nonhomologous end joining repair of double-strand breaks is faulty, leading to cell death (48). A similar approach to synthetic lethality with PARP 1 inhibitors as monotherapy might be effective clinically in the 10% to 15% of sporadic breast and ovarian cancer patients with promoter methylation.
of BRCA1, where all normal tissues should have wild-type BRCA1 (48).

Another example of a clinical application using a chemical inhibitor of BER is methoxyamine (also known as TRC-102; Tracon Pharma; refs. 30, 51). Methoxyamine is an alkyloxamine derivative that blocks short patch BER by reacting with an aldehyde-sugar group of the AP site, causing a methoxyamine-AP adduct. Methoxyamine-AP adduct formations in tumor and normal granulocytes are measured at multiple time points to assess the potential therapeutic index of methoxyamine in addition to standard clinical end points.

As mentioned previously, my laboratory has recently focused on the preclinical and now clinical use of IUdR or the oral IUdR prodrug as a radiosensitizing drug for MMR- (damage tolerant) human cancers (9, 10, 25, 26). Such a targeted approach to MMR cancers can be extended to also targeting BER in these damage-tolerant cancers with the use of methoxyamine. The highlights of this targeted approach follow. First, we have shown that specific IUdR-DNA mismatches (i.e., G:IU but not A:IU) are recognized and efficiently repaired by MMR (9, 10, 55). Consequently, MMR tumors retain significantly higher IUdR-DNA levels compared with proliferating MMR+ normal tissues. Because the level of IUdR-DNA incorporation directly correlates with the extent of tumor radiosensitization, MMR tumor cells and xenografts can be selectively targeted to increase ionizing radiation cytotoxicity. Second, using our probabilistic model of the cell cycle (25), we noted faster cell cycling in MMR- versus MMR+ cells and we can now predict from the model when tumor cells with higher IUdR-DNA levels should be irradiated as tumor cells accumulate in more ionizing radiation–sensitive cell cycle phases. This concept (or prediction) is currently being tested in a human colorectal (HCT116) tumor xenograft model using isogenic MMR− and MMR+ human tumor cells. Third, intermediate end points of the potential therapeutic index with this treatment strategy can be assessed by measuring IUdR-DNA incorporation levels in biopsy specimens of MMR− tumor versus dose-limiting MMR+ normal tissues (bone marrow; gastrointestinal mucosa) by routine immunohistochemistry and flow cytometry with anti-IUdR antibodies or by high-performance liquid chromatography approaches. Indeed, a proof-of-principle human tumor xenograft study of this approach is already published, showing persistently higher IUdR-DNA incorporation in tumor compared with normal tissues (26). A phase I/Ii clinical trial of IUdR-mediated radiosensitization in MMR− (damage tolerant) gastrointestinal and gynecologic cancers is being planned in which single-photon emission computed tomography/positron emission tomography scanning using 123I- or 124I-labeled IUdR will noninvasively monitor IUdR-DNA levels in tumor versus normal tissues will also be tested. In this clinical trial, the oral prodrug of IUdR, which has been shown to have improved pharmacokinetics compared with i.v. infusions of IUdR, will be given similar to its administration schedule in the published xenograft studies (26, 27).

Two additional therapeutic modifications to enhance IUdR-mediated radiosensitization in MMR− tumors are also proposed for future clinical trials. First, a logical extension of this initial clinical trial of targeted IUdR (IPdR)-mediated radiosensitization of MMR− (drug tolerant) cancers is to next include cotreatment with methoxyamine. Again, preclinical data have already confirmed enhanced IUdR-DNA incorporation and enhanced IUdR-mediated radiosensitization in MMR− tumor versus MMR+ normal cells (31, 53). Second, because we and others have also shown that methoxyamine can also enhance temozolomide-related tumor cytotoxicity (51, 52), then a three-drug combination of IUdR, methoxyamine, and temozolomide, along with concomitant radiation therapy is another future targeted treatment strategy. This more complex three-drug–ionizing radiation treatment will be used for drug- and ionizing radiation–resistant cancers, such as high-grade brain tumors and high-grade sarcomas, where the combination of continuous i.v. of IUdR and radiation therapy has already shown some efficacy (56–58).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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