

DNA Damage Repair and Response Proteins as Targets for Cancer Therapy

Howard B. Lieberman*

Center for Radiological Research, Department of Radiation Oncology, Columbia University College of Physicians and Surgeons, 630 W. 168th St., New York, NY 10032; Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, 60 Haven Avenue, Suite B1, New York, NY 10032, USA

Abstract: The cellular response to DNA damage is critical for determining whether carcinogenesis, cell death or other deleterious biological effects will ensue. Numerous cellular enzymatic mechanisms can directly repair damaged DNA, or allow tolerance of DNA lesions, and thus reduce potential harmful effects. These processes include base excision repair, nucleotide excision repair, nonhomologous end joining, homologous recombinational repair and mismatch repair, as well as translesion synthesis. Furthermore, DNA damage-inducible cell cycle checkpoint systems transiently delay cell cycle progression. Presumably, this allows extra time for repair before entry of cells into critical phases of the cell cycle, an event that could be lethal if pursued with damaged DNA. When damage is excessive apoptotic cellular suicide mechanisms can be induced. Many of the survival-promoting pathways maintain genomic integrity even in the absence of exogenous agents, thus likely processing spontaneous damage caused by the byproducts of normal cellular metabolism. DNA damage can initiate cancer, and radiological as well as chemical agents used to treat cancer patients often cause DNA damage. Many genes are involved in each of the DNA damage processing mechanisms, and the encoded proteins could ultimately serve as targets for therapy, with the goal of neutralizing their ability to repair damage in cancer cells. Therefore, modulation of DNA damage responses coupled with more conventional radiotherapy and chemotherapy approaches could sensitize cancer cells to treatment. Alteration of DNA damage response genes and proteins should thus be considered an important though as of yet not fully exploited avenue to enhance cancer therapy.

Keywords: DNA damage, DNA repair, cell cycle checkpoints, apoptosis, radiotherapy, chemotherapy, cancer.

INTRODUCTION

Cells have a multitude of molecular pathways capable of responding to DNA damage. These systems include DNA repair mechanisms, DNA damage tolerance systems, regulatory networks that coordinate cell cycle progression and damage repair, and apoptotic systems to mediate a suicide response when damage is excessive, not repairable, or intolerable with respect to permitting normal, critical transactions involving DNA. Radiotherapy and chemotherapy used to control or eradicate cancer most often inflict damage to DNA as the primary mode of action. As such, it seems logical that by manipulating elements mediating the cellular response to DNA damage, the effectiveness of therapy to treat individuals with cancer could be enhanced. This review summarizes facets of the cellular response to DNA damage, and strategies for manipulating those responses to serve as an adjuvant to current treatments based on the actions of radiation or chemicals that target DNA. This is not meant to be an exhaustive review of the relevant literature as the understanding of how cells respond to DNA damage is a very extensive, complex and fast-moving field of research. There are certainly excellent publications that cover these topics in depth [1, 2, 3]. In contrast, this article is meant to serve as an overview and introduction to DNA damage response pathways, their importance in the context of radiotherapy and chemotherapy, and to raise awareness of the potential for exploiting the ability to manipulate DNA damage response mechanisms as a logical strategy with translational potential to improve treatment of individuals with cancer.

DNA DAMAGE

The genomes of all living organisms are constantly subjected to conditions that induce damage to DNA. Some of the damage occurs spontaneously and is the result of normal metabolic processes. For example, deamination of cytosine in DNA can form uracil, which is an aberrant base that must be removed to permit DNA to resume normal transactions, such as during the synthesis of new DNA strands. The formation of uracil is estimated to occur 100-500 times per human cell per day, and is the most common aberrant deamina-

tion product in cells [4, 5, 6, 7]. Single and double strand DNA breaks are other examples of damage that can occur spontaneously [8]. These breaks form during intermediate steps in DNA replication as well as recombination, or due to the action of reactive oxygen species (ROS) generated by aerobic metabolic pathways. Aside from DNA strand breaks, a large variety of base damage can occur after exposure to ROS [9]. Errors in DNA replication can sometimes lead to insertion of the wrong base and thus result in nucleotide mismatches. DNA strand breaks, as well as inappropriate uracil moieties or base pair mismatches, are usually processed and the DNA mended quickly, thus avoiding adverse biological effects. These are just a few examples of the numerous types of DNA damage that can arise spontaneously.

Cells are also exposed to exogenous agents, chemicals or radiations, which can induce DNA damage. Individuals can be exposed to environmental contaminants or naturally occurring DNA damaging agents, such as radon or sunlight. On the other hand, radiotherapy or chemotherapeutic agents often target DNA and induce damage [10]. Uracil in DNA cannot only form spontaneously, but also after cytosine in particular is subjected to ionizing radiation exposure [11]. Moreover, ionizing radiation can cause single and double strand breaks in DNA as well, and less frequently base damage. Table 1 lists examples of commonly used chemotherapeutic agents that cause DNA damage, directly or indirectly, the types of cancers for which they are employed to eradicate, and the kinds of damage they induce. Interestingly as indicated, representatives of many different categories of chemotherapeutic agent cause DNA damage, and the exact damage induced can be different between groups as well as within the same category of agent. For example, alkylating agents can cause aberrant methylation of guanines in DNA, and DNA strand cross-links. Chemotherapeutic antibiotics, for example bleomycin, can bind DNA, inhibit DNA replication or transcription, and cause DNA strand breaks.

CELLULAR RESPONSE TO DNA DAMAGE

Cells have multiple response pathways that can process DNA damage and restore genomic integrity, the normal DNA sequence and structure. DNA repair pathways can enzymatically reverse or mend the damage, and cell cycle checkpoint control mechanisms transiently delay cell cycle progression, thus providing extra time for proper repair to occur. In addition, apoptotic systems mediate

*Address correspondence to this author at the Center for Radiological Research, Columbia University College of Physicians and Surgeons, 630 W. 168th St., New York, NY 10032, USA; Tel: 212-305-9241; Fax: 212-854-5505; E-mail: lieberman@cancercenter.columbia.edu

Table 1. Chemotherapeutic Agents, the Kinds of Cancers for which they are Used, and their Mode of Action^a

Chemotherapeutic Agent (Class; Examples)	Examples of Cancers Treated	Mode of Action/DNA Damage
<u>Alkylating agents:</u> Nitrogen mustard derivatives (i.e., cyclophosphamide, chlorambucil, melphalan), ethylenimines (i.e., thiotepa), alkylsulfonates (i.e., busulfan), triazines (i.e., dacarbazine), piperazines (i.e., TFMPP, MCPP, MEOPP, and PFPP), nitrosoureas (i.e., BCNU, CCNU)	Lymphomas, chronic leukemia, multiple myeloma, solid tumors	<ul style="list-style-type: none"> Adds methyl or other alkyl groups to guanines. Causes DNA strand cross-links.
<u>Antibiotics:</u> Bleomycin, Dactinomycin, Doxorubicin	Choriocarcinoma, lymphomas, testicular carcinoma, Wilm's tumor, breast cancer	<ul style="list-style-type: none"> Binds to DNA, inhibits DNA replication, transcription. DNA strand breaks.
<u>Topoisomerase I and II inhibitors:</u> Irinotecan (Topo I), Etoposide (Topo II)	Colorectal cancers (Irinotecan); Lung cancer (Etoposide)	<ul style="list-style-type: none"> Effects recombinational repair.
<u>Spindle poisons:</u> Taxanes (paclitaxel and docetaxel)	Breast and lung cancers	<ul style="list-style-type: none"> Disrupts microtubule function.
<u>Miscellaneous:</u> Cisplatin, Hydroxyurea	Testicular, lung and ovarian cancer (Cisplatin); Chronic and acute leukemias (Hydroxyurea)	<ul style="list-style-type: none"> Cisplatin: intra-strand, inter-strand DNA cross-links. Hydroxyurea: inhibits ribonucleotide reductase, alters deoxyribonucleotide pools, delays cell cycle progression, causes DNA degradation.

^aA comprehensive list and description of commonly used chemotherapeutic drugs can be found in reference [10].

programmed cell death when damage is excessive, irreparable and/or severely interferes with standard critical transactions involving DNA. Other mechanisms are available to allow lesion tolerance, and thus still avoid significant interference with cellular reactions involving DNA. These processes are listed and their modes of action summarized in Table 2. Many of these processes are highly complex [2, 12, 13]. Table 3 lists the numerous genes known to participate in each of these mechanisms.

An underlying issue is that each gene in Table 3 is listed in association with a single repair pathway, usually one where the encoded protein function is most prominent or first established. However, many of the proteins participate in multiple DNA damage response pathways. For example, there is evidence that RAD9 is involved in cell cycle checkpoint control, apoptosis and BER [14]. BRCA1 is involved in homologous recombination, cell cycle checkpoint control and apoptosis [15]. There are many similar examples.

Another consideration is that some lesions can be mended by any of several pathways. For example, certain types of base damage can be repaired by base excision repair, nucleotide excision repair or homologous recombinational repair, and might also activate a cell cycle checkpoint or apoptosis. In addition, other types of DNA damage, such as inter-strand cross-links, can be repaired or processed by the coordinated effort of multiple pathways, such as homologous recombinational repair, nucleotide excision repair, and post-replication translesion synthesis mechanisms [16].

Reversal of DNA Damage

The simplest repair process involves an enzyme-catalyzed reversal of DNA damage. One example is the monomerization of two adjacent pyrimidine dimers in DNA, such as in the form of cyclobutane pyrimidine dimers or (6-4)pyrimidine-pyrimidone photo-products, which can be induced by exposure to UV light with wavelengths close to the absorption peak of DNA (260nm). DNA photolyase or photoreactivation enzyme, in the presence of light (>300nm wavelength), will reverse the dimerization by breaking the covalent bond joining the adjacent pyrimidines. Although this process has been studied extensively in *E. coli* and several other microorganisms, it remains controversial as to whether enzymatic photoreactivation is present in humans. Several investigators were un-

able to detect this process in human cell extracts [17, 18]. However, another group found such an activity in white blood cells [19], and a human gene encoding a protein showing extensive structural homology to the bacterial enzyme has been isolated [20]. In contrast, another relatively simple "damage reversal" repair process is evolutionarily conserved and well established in mammals. Alkylation damage in DNA, such as *O*⁶-methylguanine, can be repaired by the action of *O*⁶-methylguanine DNA methyltransferase. This enzyme can remove the inappropriate methyl group from guanine and transfer it to one of its own cysteine residues, thus restoring proper structure of the damaged guanine in DNA [21,22]. Similar reversals of damage can occur for *O*⁴-methylthymine, *O*⁶-ethylguanine and *O*⁶-chloroethylguanine. DNA ligase can rejoin the 3'-OH and 5'-P free ends in a single strand DNA break, although it is not clear how often this kind of simple repair occurs *in vivo*.

Base Excision Repair

Multiple repair mechanisms function by excising damage from DNA, then mending the gap created by the process, essentially restoring DNA strands to their original, undamaged state, or at least to a condition that will not hinder normal activities. Base excision repair (BER) is one such pathway that removes damaged bases from DNA but can also repair DNA single strand breaks [23, 24, 25]. The process starts by excision of a damaged base *via* the activity of a DNA glycosylase cleaving the glycosyl bond between the base and the deoxyribose-phosphate backbone of DNA. The site missing the purine or pyrimidine (apurinic or apyrimidinic site; AP) is then recognized by an AP endonuclease, APE1, which causes a nick in the DNA 5' of the site. An associated lyase sometimes makes a nick 3' of the AP site. The terminal deoxyribose-phosphate residue generated from the 5' nick is removed by an exonuclease or DNA-deoxyribophosphodiesterase (dRpase). There are several variations of BER. In short patch BER, only the single nucleotide gap is filled in by repair synthesis mediated by POLB. This is the most frequently occurring type of BER activity in mammals. However, repair synthesis can extend beyond one nucleotide, in a process called "long patch" BER. During this activity, a DNA strand can become displaced and create a flap. When this occurs, a flap endonuclease (FEN-1) cleaves the displaced DNA strand. The free ends, generated by either version of BER, are then covalently joined by the action of LIG3.

Table 2. Mammalian DNA Damage Response Pathways

Pathway	Summary of Mode of Action	References
Reversal of DNA damage	<ul style="list-style-type: none"> Does not excise or allow tolerance of DNA lesion. Reverses damage to restore DNA integrity. Example: action of <i>O</i>⁶-MGMT (<i>O</i>⁶-methylguanine DNA methyltransferase) on methyl groups in <i>O</i>⁶-methylguanine, <i>O</i>⁴-methylthymine, <i>O</i>⁶-ethylguanine, and <i>O</i>⁶-chloroethylguanine. Example: rejoining a single strand break in DNA, bearing 3'-OH and 5'-P ends, by DNA ligase. 	See footnote ^a
Base Excision Repair (BER)	<ul style="list-style-type: none"> Repairs primarily non-bulky lesions, which do not appreciably distort DNA double helix structure. Examples: bases with alkylation, oxidation or ring saturation damage, deaminated bases, DNA single strand breaks. For base damage, a DNA glycosylase catalyzes hydrolysis of N-glycosyl bonds linking damaged or improper bases to the DNA deoxyribose-phosphate backbone, causing base excision. Sites missing a base (apurinic, apyrimidinic or abasic sites) in double stranded DNA recognized by apurinic/apyrimidinic (AP) endonucleases (APE). Incision (hydrolysis of the phosphodiester bond) immediately 5' to the AP site. Sometimes an AP lyase, associated with DNA glycosylase, nicks DNA 3' of the AP site. Incision at the 5' end produces a terminal deoxyribose-phosphate residue. Removed by exonuclease or enzyme with DNA-deoxyribophosphodiesterase (dRpase) activity. Single nucleotide gap filled in by repair synthesis mediated by a DNA polymerase (primarily Polβ). Sometimes repair synthesis goes beyond the single nucleotide gap and causes a flap in DNA, a displaced strand due to the action of DNA polymerase. The flap produced during "long patch" BER is removed by Flap endonuclease (FEN-1). Free DNA ends as part of "long patch" or "short patch" BER covalently joined by a DNA ligase. 	See footnote ^b
Nucleotide Excision Repair (NER)	<ul style="list-style-type: none"> Removes damaged or inappropriate bases in DNA as part of an oligonucleotide (usually 24-32 base pairs long), then mends the resulting gap. Processes bulky DNA lesions with potential to block DNA replication. Main pathway called "global genome" NER. DNA damage is recognized. Incision of the strand with damage at one site on either side of the damage. Excision of the oligonucleotide fragment caused by incision. Repair synthesis to fill in the gap left in DNA. Ligation to join the free ends. 	See footnote ^c
Alternative Excision Repair (AER)	<ul style="list-style-type: none"> Similar to nucleotide excision repair. Initiated by incision on only one side flanking damaged or inappropriate base. Nick can occur on either side of damage. Demonstrated in <i>E. coli</i> but not firmly established in mammals. 	See footnote ^d
Transcription Coupled Nucleotide Excision Repair (TC-NER)	<ul style="list-style-type: none"> Repairs damaged DNA specifically on the RNA PolIII transcribed strand of transcriptionally active DNA. Damage recognized when RNA polymerase II is stalled at a DNA lesion. Transcription repair-coupling factor moves RNA polymerase beyond damage, allowing transcription to resume. Sets the stage for nucleotide repair proteins to mend damage. Latter process similar to NER, and involves the same core proteins. Excision usually removes approximately 30 base pair oligonucleotide. Repair synthesis and ligation. 	See footnote ^e
Nonhomologous End Joining (NHEJ)	<ul style="list-style-type: none"> Repairs DNA double strand breaks. Probably the primary repair process in mammals that deals with this type of damage when it occurs outside a replication fork and not in S phase. Involves direct rejoining of broken, free ends of DNA. Does not depend upon long regions of homology. Might be influenced by short stretches of microhomology, from one to four base pairs, flanking the free ends. Procedure tends to result in loss of some DNA sequence from the free DNA ends. 	See footnote ^f
Homologous Recombinational Repair (HRR)	<ul style="list-style-type: none"> Repairs DNA double strand breaks and contributes to repair of DNA interstrand cross-links. In mammals, repairs double strand breaks at replication forks and when cells are in S phase. A 5'→3' exonuclease degrades DNA on one strand of the break creating a larger single strand free 3' end. Free end invades a homologous double strand DNA helix on a sister chromatid (interchromatid), a homologous or different chromosome (interchromosomal) or the same chromosome (intrachromosomal) where DNA structure and information remain intact. Displaces one of the original pairing DNA strands, forming a heteroduplex. Two resulting structures connected by a single strand of DNA form two "Holliday junctions". Structures can move along original double DNA helix by "branch migration" and extend the heteroduplex. Can lead to nonreciprocal transfer of DNA information, called "gene conversion". Holliday junctions resolved by single strand DNA cutting and rejoining by a "resolvase" protein complex. Positions of DNA scissions determine whether relative locations of DNA markers remain the same as in the original DNA strands or switch strand locations and form a "crossover". Can also occur by a synthesis-dependent strand annealing mechanism. Latter does not involve Holliday junctions or result in crossover recombinants. 	See footnote ^g

(Table 2). Contd.....

Mismatch Repair (MMR)	<ul style="list-style-type: none"> Recognizes and repairs base-base mismatches and insertion/deletion loops that could arise during DNA replication. Repairs certain types of damage caused by reactive oxygen species and alkylating agents. Removes mismatched bases from newly synthesized strand of DNA to restore proper base pairing. Mechanism to distinguish mismatched from proper base in non-replicating DNA is not as well understood. Mechanisms of removal and strand integrity restoration are similar to what occurs in BER and NER. MMR proteins can interact with proteins in other repair pathways such as BER, NER and HRR, suggesting coordination of processes. 	See footnote ^b
Translesion Synthesis (TLS)	<ul style="list-style-type: none"> A damage “tolerance” mechanism. Permits DNA replication passed a damaged base, by either a single DNA polymerase or more than one. Correct or incorrect base inserted opposite the lesion during daughter strand synthesis. 	See footnote ⁱ
Cell Cycle Checkpoint Control	<ul style="list-style-type: none"> Delays cell cycle progression at specific phases in response to DNA damage. Thought to provide extra time for DNA repair before entry into critical cell cycle phases that may result in lethality if entered with DNA damage. Involves proteins that “sense” damage and “transduce” a signal to “effector” proteins, which in turn cause the cell cycle to cease progression. Some “effector” proteins regulate cellular damage response pathways unrelated to cell cycle progression. 	See footnote ^l
Apoptosis	<ul style="list-style-type: none"> Programmed cell death or suicide mechanism. Induced by abundant DNA damage and/or lesions that severely inhibit normal metabolic transactions of DNA. Characterized by chromatin condensation, cytoplasmic shrinkage, plasma membrane blebbing, outer mitochondrial membrane swelling and nuclear DNA cleavage. 	See footnote ^k

^areferences [21, 22].^breferences [23, 24, 25].^creference [26].^dreference [27].^ereference [28].^freference [30].^greference [31].^hreferences [32, 33].ⁱreferences [34, 35].^jreferences [36, 37, 38].^kreferences [39, 40].**Table 3. Mammalian Genes Involved in DNA Damage Response Pathways^{a,b}**

Pathway	Genes
Reversal of DNA damage	<i>O⁶-MGMT</i>
Base Excision Repair (BER)	<i>UNG, SMUG1, MBD4 (MED1), TDG, OGG1, MUTYH (MYH), NTHL1 (NTH1), MPG (AAG, ANPG, APNG, MDG), NEIL1, NEIL2, APEX1, APEX2, LIG3, XRCC1, PNKP, PARP1 (ADPRT), PARP2 (ADPRTL2), POLB, POLG, FEN1</i>
Nucleotide Excision Repair (NER)	<i>XPC, RAD23B (HR23B), CETN2, RAD23A (HR23A), XPA, RPA1, RPA2, RPA3, TFIIH, ERCC3 (XPB), ERCC2 (XPD), GTF2H1, GTF2H2, GTF2H3, GTF2H4, GTF2H5 (TTDA), CDK7, CCNH, MNAT1, ERCC5 (XPG), ERCC1, ERCC4 (XPF), LIG1, POLD1, POLE, PCNA</i>
Alternative Excision Repair (AER)	<i>ENDOV</i>
Transcription Coupled Nucleotide Excision Repair (TC-NER)	<i>CKN1 (CSA), ERCC6 (CSB), XAB2 (HCNP), DDB1, DDB2, MMS19L (MMS19)</i> , in conjunction with nucleotide excision repair proteins.
Nonhomologous End Joining (NHEJ)	<i>G22P1 (Ku70), XRCC5 (Ku80), PRDKC, LIG4, XRCC4, DCLRE1C (Artemis), XLF (NHEJ1), POLL, POLM</i>
Homologous Recombinational Repair (HRR)	<i>RAD51, RAD51L1 (RAD51B), RAD51C, RAD51L3 (RAD51D), DMC1, XRCC2, XRCC3, RAD52, RAD54L, RAD54B, BRCA1, BRCA2 (FANCD1, SHEM1 (DSS1), RAD50, MRE11A, NBS1, MUS81, EME1 (MMS4L), EME2</i>
Mismatch Repair (MMR)	<i>MSH2, MSH3, MSH6 (GTBP), MSH4, MSH5, PMS1, MLH1, PMS2, MLH3, PMS2L3, PMS2L4 (PMS6), POLD1, POLE, PCNA, EXO1 (HEX1)</i>
Translesion Synthesis (TLS)	<i>POLK (DINB1), POLI (RAD30B), REV3L (POLZ), MAD2L2 (REV7), REV1L (REV1), POLH (XPV), POLN</i>
Cell Cycle Checkpoints	<i>ATM, ATR, TP53, RAD1, RAD9, HUS1, RAD17 (RAD24), CHEK1, CHEK2, ATRIP (TREX1), TELO2 (hCLK2, KIAA0683)</i>
Apoptosis ^c	<p>1. Caspases: Caspase-1 (<i>ICE</i>), Caspase-2 (<i>ICH-1, NEDD-2</i>), Caspase-3 (<i>CPP32, APOPAIN, YAMA</i>), Caspase-4 (<i>ICH-2, TX, ICErel-II</i>), Caspase-5 (<i>ICH-3, TY, ICErel-III</i>), Caspase-6 (<i>MCH-2</i>), Caspase-7 (<i>MCH-3, ICE-LAP3, CMH-1</i>), Caspase-8 (<i>FLICE, MACH, MCH-5</i>), Caspase-9 (<i>MCH-6, ICE-LAP6</i>), Caspase-10 (<i>MCH-4</i>), Caspase-11, Caspase-12, Caspase-13 (<i>ERICE</i>), Caspase-14 (<i>MCE</i>), <i>CPP32</i></p> <p>2. Adaptor proteins: <i>APAF-1, APAF-2</i> (Cytochrome c), <i>CED-4, RAIDD, FADD (MORT1), RIP, FLIP_L</i></p> <p>3. Tumor necrosis factor receptor (TNF-R) super family: <i>TNF-R1, CD95, DR3, DR4, DR5, NGFR p75, TNF-R2, TNFR-RP, CD27, CD30, CD40, OX40, 4-1BB, GITR, RANK, ATAR, HVEM, DCR1, DCR2</i></p> <p>4. Bcl-2 family: <i>BCL-2, BCL-X_L, MCL-1, BCL-W, BCL-B (BOO, DIVA), A1A (BFL-1), BAX, BAK, BOK (MTD), MIL-1 (RAMBO), BCL-G, BIK (NBK), HRK (DP5), BIM (BOD), BAD, BID, PUMA, BMF, NOXA, NIP3 (BNIP3), NIX (NIP3L), BCL-L12, MAP-1, P193, SPIKE</i></p>

^aRefer to the following references for details [2, 12, 13].^bDoes not include genes that encode proteins capable of regulating nucleotide pools, altering chromatin structure, or selectively targeting proteins for degradation as part of the ubiquitination system, all processes that can impact on repair of damaged DNA. In addition, exonucleases whose activities have not been assigned to one or more specific pathways have also not been listed.^cThe apoptosis-related genes listed are separated into four categories. Splice variants of several corresponding mRNAs have been identified but are not listed [12].

Nucleotide Excision Repair

Nucleotide excision repair (NER) is another mechanism available for removing damaged DNA [26]. However, in contrast to BER, this process recognizes and mends DNA primarily with bulky, helix-distorting damage potentially capable of blocking DNA replication or transcription. Examples of agents that cause damage serving as a substrate for NER include 254nm UV light, cisplatin, benzo(a)pyrene and 2-acetylaminofluorene. When this mechanism repairs damaged sites in non-transcribing genomic DNA it is called "global" NER. Briefly, the repair process begins with the recognition of damage by RPA, XPA, and XPC. TFIIH (XPB and XPD) unwinds the duplex around the damage; then XPG and XPF-ERCC1 nicks the DNA at 3' and 5' sites, respectively, flanking the damage. The fragment is then excised. Repair synthesis fills in the gap through the action of RFC, PCNA, POLD1 and POLE. DNA ligase I (LIG1) then seals the free ends of the repair patch.

Alternative Excision Repair

A variation of NER (and BER), called alternative excision repair (AER), has been described [27]. These excision pathways are very similar, except in AER, endonuclease V (ENDOV) causes a nick on only one end of the damaged site, for example it hydrolyzes the second phosphodiester bond 3' to hypoxanthine or uracil in DNA. It is thought that a 5' endonuclease or a 3'-5' exonuclease possibly then removes the damaged base before repair continues, but the details have yet to be completely defined. AER has been demonstrated in the bacterium *E. coli*, but the pathway is not fully established for mammals.

Transcription Coupled Nucleotide Excision Repair

When bulky lesion type damage to DNA can be a substrate for NER and occurs in regions of the genome serving as a template strand for transcription, a specialized form of NER called transcription coupled nucleotide excision repair (TC-NER) functions to repair the damage [28]. When RNA polymerase II stalls during transcription because a lesion is encountered, a transcription repair-coupling factor moves the RNA polymerase beyond the damaged site and recruits repair factors. There are several proteins that play key roles in the coupling process, most notably Cockayne syndrome A and B proteins [29].

Nonhomologous End Joining

DNA double strand breaks are formed either directly or indirectly by the action of ionizing radiation, ROS and a large variety of chemotherapeutic agents such as bleomycin, cisplatin, ellipticine and etoposide as examples. This type of damage can be repaired by two mechanisms, nonhomologous end joining (NHEJ) or by a homologous recombination based process. In humans, the majority of DNA double strand breaks are repaired by NHEJ, in cells not in S phase [30]. Sometimes microhomology of the ends help alignment and ligation, and this is referred to as a micro-single strand annealing (micro-SSA) process. The details of NHEJ are not completely understood, but DNA-PK. Ligase IV and XRCC4 are major mediators of this pathway, although several other proteins certainly contribute to the process. DNA-PK is a protein complex of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and the targeting subunit composed of a Ku70-Ku80 heterodimer. The latter acts as a damage sensor, binds the free ends of DNA, and recruits DNA-PKcs and subsequently Artemis. The DNA ends may then be processed, either trimmed or extended, after recruitment of a polymerase or terminal deoxynucleotidyltransferase. XRCC4-ligase IV then joins the free ends.

Homologous Recombinational Repair

DNA double strand breaks in cells undergoing DNA replication (i.e., in S phase) can be mended by homologous recombinational repair (HRR). Before repair actually begins, ATM or ATR, the latter in the case of stalled replication forks, act as part of a sensing mechanism to recruit repair proteins to the damaged site. For example, the MRE11-RAD50-NBS1 (MRN) complex recruits ATM to the break site, which in turn activates a variety of downstream target effectors that mediate not only homologous recombinational repair but also other responses to DNA damage such as cell cycle checkpoint control and apoptosis. The γ -H2AX protein aids in the recruitment process. HRR makes use of undamaged DNA strands in a sister chromatid (interchromatid), a homologous or different chromosome (interchromosomal), or the same chromosome (intra-chromosomal). A basic outline of the classical process involving Holliday junctions and production of crossover recombinants is presented in Table 2. The actual repair process is complex and involves many proteins working coordinately. Key players include RAD51, RAD51B, RAD51C, RAD51D, XRCC2, XRCC3, RAD52, RAD54 and BRCA2. An alternative process is referred to as "synthesis-dependent strand annealing". This mechanism does not involve Holliday junctions and is characterized by formation of noncrossover recombinants. The process involves transient invasion of a free 3'-end (part of the double strand break), which primes DNA synthesis and is then displaced. The newly synthesized strand then anneals to the other DNA double strand break end, DNA synthesis occurs, and subsequently the free ends are ligated. Sung and Klein [31] recently reviewed these processes in detail.

Mismatch Repair

Base pairing mismatches and insertion/deletion loops that could arise during DNA replication, as well as damage caused by ROS and alkylating agents can be fixed by the mismatch repair system. Recent reviews [32, 33] describe mechanistic aspects of the process. Briefly, a mismatch is detected in DNA; MUTLalpha makes a single-strand break close to the problematic site; then an exonuclease (EXO1) degrades the strand containing the mismatch. Table 3 lists the many different proteins that mediate MMR. This process not only repairs induced DNA damage but also is critical for maintaining genomic stability. Defects in components of the pathway are the cause of typical and atypical hereditary nonpolyposis colon cancer.

Translesion Synthesis

DNA synthesis can stall when DNA damage is encountered at a replication fork. During the process of translesion synthesis (TLS), any one of five low stringency polymerases can still carry out DNA synthesis past lesions [34, 35]. Each polymerase has DNA damage substrate specificity. PCNA plays an important role in the engagement of the DNA polymerases to the damaged site and the switch from normal replicative to translesion activity.

Cell Cycle Checkpoint Control

Cell cycle progression can be temporarily delayed in response to DNA damage by a process called cell cycle checkpoint control [36, 37, 38]. This process occurs at specific sites within the cell cycle and is thought to provide extra time for repair before cells progress through critical phases that might be lethal if attempted with DNA damage. This is a signal transduction pathway involving the "sensor" MRE11-RAD50-NBS1 (MRN) protein complex recruited to sites of damage, and subsequent recruitment of other proteins such as γ -H2AX, BRCA1, 53BP1 and MDC1. For double strand DNA breaks, ATM protein functions as a "transducer" and activates "effector" proteins such as p53, MDM2 and CHK2. When DNA replication forks are stalled or single strand DNA breaks are

formed, ATR, ATRIP, RPA, RAD9-RAD1-HUS1 (9-1-1) heterotrimer, RAD17-RSR and claspin are involved. The effectors activate downstream targets to mediate cell cycle progression, as well as DNA repair and apoptotic pathways as part of the cellular response to DNA damage.

Apoptosis

A programmed suicide pathway, called apoptosis, can be activated when cells incur irreparable, excessive DNA damage that interferes with normal functions of DNA, such as during replication fork progression. The apoptotic process is complex and has been reviewed [12, 39]. Interestingly, other genetically distinct, non-apoptotic, stress-induced programmed cell death pathways have been described [40], but those will not be considered. Many types of DNA damage can induce apoptosis, including alkylation of bases, DNA cross-links, bulky lesions and DNA double strand breaks. If damage is not properly repaired a cascade of events is triggered and ultimately leads to cell death. Cells undergoing apoptosis display hallmark characteristics, such as abnormal chromatin condensation, cytoplasmic shrinkage, plasma membrane blebbing, outer mitochondrial membrane swelling and nuclear DNA cleavage (Table 2). Many of the key genes/proteins that mediate apoptosis are listed in Table 3 and grouped for ease of understanding into four categories: 1. Caspases; 2. adaptor proteins; 3. tumor necrosis factor receptor super family members; and 4. Bcl-2 family members [12].

DNA DAMAGE REPAIR AND RESPONSE PROTEINS AS TARGETS FOR CANCER THERAPY

Rationale

The goal of all therapies aimed at eradicating cancer is to selectively destroy cancer cells while sparing normal tissue. Most chemotherapeutic or radiotherapeutic agents function by damaging DNA or interfering with DNA replication. The cellular responses to DNA damage, especially related to repair or tolerance of the damage as well as the activation of apoptosis, are thus critical elements in determining the effectiveness of most cancer therapies. Furthermore, rapid advances in recent years have contributed significantly to identifying and understanding the roles of key proteins in DNA damage response pathways. Logically then, it is reasonable to assume that by modulating the cellular response to DNA damage, perhaps through manipulating genes or proteins related to DNA repair, cell cycle checkpoint control, damage tolerance or apoptosis, the effectiveness of conventional cancer therapy can potentially be enhanced [41-47]. Of course, also selectively sensitizing cancer cells relative to normal tissues should improve the therapeutic ratio for anti-cancer treatment.

Proteins to Target as an Adjuvant to Standard Therapies

There is a large array of genes/proteins involved in modulating the cellular response to DNA damage, as summarized in Table 3. Theoretically, any single one can serve as a target for inhibition to enhance current therapeutic modalities that rely on damaging DNA. Therefore, some strategy must be applied to prioritize the wide, extensive selection of potential targets for testing and use in the clinic.

One consideration is the coupling of a DNA damage response process with the type of damage induced by the primary therapeutic agent. For example, if radiotherapy, which primarily generates DNA strand breaks, is to be used, then homologous recombination or nonhomologous endjoining proteins should be targeted, not those involved in mismatch repair as the primary ideal choice to sensitize cancer cells. For example, G22P1 (Ku70) and XRCC5 (Ku80), part of the DNA-PK protein complex is important for promoting radioresistance and plays a key role in nonhomologous endjoining.

Thus, inhibition of any member of the complex could be effective for increasing sensitivity of tumors to radiation [48]. Likewise, it is reasonable to first test elements of DNA damage response pathways having the most dramatic effects on repair, to maximize therapeutic gain through reduction of protein levels or activity. If multiple damage response pathways participate in promoting survival after the application of a particular anticancer agent, then inhibition of all responsible repair-related mechanisms could be pursued. Likewise, proteins that participate in multiple DNA damage response pathways could be good targets. Helicases or proteins such as BRCA1 and RAD9 function in multiple repair and related pathways, and could thus serve as good targets for inhibition with the goal of enhancing the effectiveness of more conventional therapies involving exposure to radiation or chemicals [14, 49, 50].

Proteins that participate in maintaining genomic integrity often have multiple functions. All of these functions must be considered when planning to inhibit the activity of a protein since neutralizing repair activity may result in the unwanted elimination of or reduction in desired functions. Therefore, damage resistance proteins without other functions unrelated to DNA damage processing should be examined as a priority. However, when a protein target is desirable but has multiple functions, strategies that include inhibition of a specific functional domain of interest may be planned.

A variety of strategies can be used to inhibit specific key components of damage response pathways, and thus form the basis for the radiosensitization or chemosensitization of cancer cells. These agents can be small molecule inhibitors and include peptides or oligonucleotides [42, 51]. There are inhibitors of components of virtually every major DNA damage response pathway, and some are in clinical trial. Reports describe inhibitors of elements of BER [52-56], NHEJ [47, 57, 58], HRR [59], damage reversal [21, 60], cell cycle checkpoint control [61-64] and mismatch repair [65], or agents that restore endogenous cell death mechanisms [66, 67].

Inhibition of some damage response components can affect multiple pathways. Helicase inhibitors can alter MMR, NER, BER, and the repair of DNA double strand breaks as well as DNA cross-links [50]. Inhibition of γ H2AX affects multiple DNA repair pathways and the cell cycle checkpoint response [68]. Targeting MSH2 for inhibition impacts on DNA repair, cell cycle control and apoptosis [69]. Strategies to modulate BRCA1-associated proteins might also affect multiple damage response pathways since BRCA1 is involved in DNA repair, cell cycle checkpoints and chromatin remodeling [49].

Exploitation of Pre-Existing Alterations of DNA Repair in Tumors

The hallmark of cancer cells is that they are genomically unstable, and in some cases that may be due to deficiency in a particular DNA repair process. This is also consistent with the finding that often cancer cells are unexpectedly sensitive to radiation or DNA damaging chemicals. This characteristic might make these cancer cells rely on backup repair processes [42, 47]. If these alternative damage response pathways could be identified, they can be selectively targeted for inactivation. Often tumor cells have deficiencies in BRCA1 and BRCA2, which would diminish repair through homologous recombination-based systems, and thus make the cancer cells rely on other pathways, such as NHEJ or BER to mend certain types of DNA lesions. Thus, inhibitors that target the alternative pathways should selectively sensitize tumor cells, and have much less of a deleterious effect on noncancerous surrounding tissues where both the primary and alternative repair/response pathways would presumably be intact. Therefore, specific inhibitors of DNA-PK (i.e., Wortmannin, NU 7026, OK-1035, etc.) or PARP (i.e., NU 1025, AG 14361, CEP 6800, etc.) could be applied as required to selectively reduce NHEJ and BER activity, respectively. This same

strategy can be applied to tumor cells with certain defects in Fanconi anaemia (FA) genes. Heterozygosity of group III FA genes predispose female carriers to breast cancer. In fact, the FANCD1 gene is allelic with *BRCA2* while the FA genes *FANCN* and *FANCI* are identical to the *BRCA* genes *PALB2* and *PRIP1*, respectively. All three of these FA genes likely effect recombinational repair of damaged DNA, and as such individuals with breast tumors having heterozygous alterations in these genes also might be good candidates for NHEJ and BER inhibition to enhance the efficacy of radiotherapy. A review of FA genes and their relationships to breast cancer as well as DNA repair has recently been published [70]. Mutations in *p53* are also found frequently in tumors. The encoded protein functions in several major DNA damage response mechanisms [71], and a careful analysis of those functions in the context of cancerous versus noncancerous tissues might also be exploited to increase the response of tumors to treatment. As an ideal treatment strategy, the genetic profile of tumors versus noncancerous tissues should be obtained to make rationale decisions about which DNA damage response inhibitors and which DNA damaging agents should be used to selectively sensitize cancer cells to therapy. The use of proteomics and genomics, especially in microarray formats, can be pursued in this regard [72-76]. The development of high throughput screens for deficiencies in specific DNA repair pathway activities would certainly be valuable, and could lead to rational custom-designed patient-specific therapy.

Epigenetic Gene Expression Modulators as Part of a Treatment Strategy to Alter Damage Response Pathways

Epigenetic mechanisms can cause heritable changes in gene expression without altering DNA sequence. Thus, another approach to modulate DNA damage response gene expression for enhancing cancer therapy could be through manipulation of epigenetic regulatory elements [77], perhaps by the use of inhibitors [78]. Hypermethylation of promoter regions, especially in cytosines within CpG dinucleotide islands, can alter gene expression and is a major mechanism of epigenetic control. This type of DNA modification is mediated by DNA methyltransferases (DNMTs), and four different ones have been identified in humans. DNMT inhibitors, which function as nucleoside (i.e., 5-azacytidine, 5-aza-2'-deoxycytidine, 5-fluoro-2'-deoxycytidine, 5,6-dihydro-5-azacytidine, zebularine) or non-nucleoside (i.e., procaine, (-)-epigallocatechin-3-gallate, hydralazine, RG108, antisense oligonucleotides) analogues of cytidine, are available. They function by either incorporating into DNA or not, respectively, and differ in the extent to which they inhibit methylation [79]. They could be used to alter DNA damage response gene transcription to enhance cancer therapy.

Chromatin structure can also impact on gene expression, and is considered another important epigenetic regulatory mechanism. Acetylation status of histones is intimately involved in regulating chromatin structure and consequently gene expression [80]. Histone deacetylase (HDAC) is one key enzyme responsible for removing acetyl moieties from histones as well as several other proteins [81]. Histone acetyltransferases (HAT) are enzymes that acetylate conserved lysine amino acids on histones and can also modulate chromatin structure [82]. Inhibitors of HDAC, which fall into several categories including short chain fatty acids, hydroxamic acids, cyclic tetrapeptides and benzamides, vary in activity and can modulate chromatin structure [78], thus leading potentially to altered gene expression that would favor hypersensitization to radiation or chemicals used to treat cancer patients. Of note though, since HDAC enzyme targets are not limited to histones, inhibitors might alter gene expression at least in part *via* mechanisms that do not involve chromatin structure. A similar strategy can be used to inhibit HAT enzymes. There are already numerous reports in the literature demonstrating that inhibition of HDAC or HAT activity can sensitize tumor cells to ionizing radiation [83-90]. However,

whether the activity is due to inhibition of DNA damage response genes remains to be fully assessed.

The advantage of these epigenetic approaches is that there are drugs available to alter gene expression by this mechanism, and the activity of some have shown promise with respect to promoting radiosensitivity of human tumor cells. Moreover, modulating gene expression in this way will change expression of a battery of genes, which in the end could further enhance chemotherapy or radiotherapy. However, caution must be exercised since such an approach is untargeted in the sense that all the affected genes will not be known and thus the process could activate genes capable of diminishing the efficacy of therapy or cause unwanted deleterious side effects *in vivo*. Further testing is obviously needed.

Considerations of Delivery and Specificity of Treatments

One important consideration for any cancer treatment is the optimization of selective killing of tumor cells while sparing normal tissues and thus reducing unwanted toxicity. As mentioned previously, the use of advanced molecular screening techniques to identify unique repair deficiencies in tumor cells could allow targeting of functions that would maximize radiosensitization or chemosensitization of cancerous tissue. An inherent benefit of radiotherapy for localized tumors is that treatment can be targeted to diseased areas, especially by exploiting advances in techniques such as Intensity-Modulated Radiation Therapy and 3D Conformal Therapy [91], so involvement of normal tissue is minimized. In addition, this targeted approach can be combined with a gene therapy strategy whereby replication-defective shuttle vectors bearing sequences that encode therapeutic or DNA damage repair protein inhibitors can be introduced into cancerous regions of interest. The sequences can be under the control of promoters that are inducible by radiation or specific chemicals. As such, the effectiveness of radiotherapy or even chemotherapy in a localized region can be enhanced because those treatments concomitantly activate production of a second therapeutic agent or a sensitizer. These kinds of approaches are in clinical trials [92].

SUMMARY

Radiotherapy and chemotherapy to treat cancer patients are based mainly on the effectiveness of the agents involved to damage DNA, hopefully more selectively in the diseased tissues than in surrounding normal cells, and kill cancerous cells predominantly. There are a multitude of mechanisms that constitute the cellular response to DNA damage and include DNA repair, damage tolerance, cell cycle checkpoint and apoptotic pathways, which together determine whether the damage incurred will be deleterious. Therefore, since these cellular pathways are critical for determining therapeutic outcome, it seems reasonable to develop approaches to modulate those responses, preferably in tumors, to enhance therapy. A promising approach is to take advantage of the deficiencies in specific damage response pathways often found in tumors. Genomics and proteomics can be coupled to aid in the identification of those deficient pathways, and back up alternative pathways could be targeted for inhibition. Since normal, noncancerous tissues should have all mechanisms intact, this strategy will selectively sensitize cancer cells to radiotherapy or chemotherapy. Thus, tumor-specific custom designed therapy could be achieved. Given the current state of advances in understanding the molecular mechanisms by which cells respond to damaged DNA, it is somewhat surprising that this abundance of information has not been translated more aggressively into the clinic. Nevertheless, numerous clinical trials are progressing in this direction. Clearly, the coupling of conventional, established therapies to gene expression profiling, especially with respect to damage response elements, might prove extremely valuable for treating cancer patients, and perhaps individuals with other diseases as well.

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ABBREVIATIONS

AP	=	Apurinic/aprimidinic
AER	=	Alternative excision repair
APE	=	AP endonuclease
BER	=	Base excision repair
HAT	=	Histone acetyltransferase
HDAC	=	Histone deacetylase
HRR	=	Homologous recombinational repair
MMR	=	Mismatch repair
NER	=	Nucleotide excision repair
NHEJ	=	Nonhomologous end joining
O ⁶ -MGMT	=	O ⁶ -methylguanine DNA methyltransferase
TC-NER	=	Transcription coupled nucleotide excision repair
TS	=	Translesion synthesis
UV	=	Ultraviolet

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