DNA Repair: From Genome Maintenance to Biomarker and Therapeutic Target

Shadia Jalal¹, Jennifer N. Earley¹, and John J. Turchi¹,²

Abstract

A critical link exists between an individual’s ability to repair cellular DNA damage and cancer development, progression, and response to therapy. Knowledge gained about the proteins involved and types of damage repaired by the individual DNA repair pathways has led to the development of a variety of assays aimed at determining an individual’s DNA repair capacity. These assays and their use in the analysis of clinical samples have yielded useful though somewhat conflicting data. In this review article, we discuss the major DNA repair pathways, the proteins and genes required for each, assays used to analyze activity, and the relevant clinical studies to date. With the recent results from clinical trials targeting specific DNA repair proteins for the treatment of cancer, accurate, reproducible, and relevant analysis of DNA repair takes on an even greater significance. We highlight the strengths and limitations of these DNA repair studies and assays, with respect to the clinical assessment of DNA repair capacity to determine cancer development and response to therapy. Clin Cancer Res; 17(22); 6973–84. ©2011 AACR.

Introduction

The human genome is subject to constant damage through a combination of endogenous and exogenous factors. Multiple pathways are required to restore the structure and the sequence of DNA once damage has occurred, and these systems are essential to maintain genomic integrity and stability. The response to DNA damage is exquisitely regulated, often specific to the type of damage incurred. Five main repair pathways have been described: the nucleotide excision repair (NER) pathway; base excision repair (BER) pathway; mismatch repair (MMR) pathway; and the 2 double-strand break (DSB) repair pathways, nonhomologous end-joining (NHEJ) and homology-directed repair (HDR). The HDR pathway is also involved in the repair of interstrand DNA cross-links (ICL) in conjunction with the Fanconi anemia pathway (1). Collectively, these pathways are orchestrated by more than 150 proteins, which enable the response to a wide array of DNA-damaging events.

Over the past decade, our knowledge about the roles of DNA repair pathways and how deficiencies or abnormalities in them affect the development of numerous disease processes has increased greatly. Mutations in DNA repair genes have been implicated in the development of neurologic diseases (2), aging (3), cancer risk (4), cancer therapy outcomes (5), inflammation, and other genetic syndromes with a variety of distinct phenotypes (6). Because of the importance of DNA repair in human disease, interest in the measurement or determination of an individual’s DNA repair capacity is great. Here, we review DNA repair pathways, the current assays available for analysis of DNA repair capacity, their strengths and limitations, and their clinical applicability.

DNA Damage Signaling

The wide variety of DNA damage that occurs necessitates a flexible and sensitive DNA damage response network to signal the presence of an insult and coordinate the cellular response to the damage. DNA damage response is initiated with the recognition of the damage and often results in the activation of cell-cycle checkpoints to arrest the eukaryotic cell-cycle progression (7). The cellular response to DNA damage is propagated through signal transduction and posttranslational modification of proteins involved in the various DNA repair pathways, as well as other signaling complexes that do not directly participate in repair reactions. As the DNA damage incurred typically disrupts nucleic acid metabolism, affecting either DNA replication or transcription, repair is often coupled to these pathways either directly or indirectly (8, 9). It should be noted that some DNA lesions escape repair and are bypassed during replication by error-prone polymerases in a process termed translesion synthesis. For a recent discussion of this pathway, see the review from McCulloch and Kunkel (10).
DNA mismatch repair

The MMR pathway is responsible for correcting replication errors that escape processing by the 3‘- to 5‘-proofreading exonuclease activity of replicative DNA polymerases. Mismatches and insertion–deletion loops arise from polymerase slippage. Defects or mutations that arise in certain MMR proteins have been ascribed to clinical manifestations (Fig. 1, refs. 11, 12). The most well described is hereditary nonpolyposis colorectal cancer, also known as Lynch syndrome, an autosomal dominant disorder characterized, in 90% of the cases, by germ-line mutations in one of the genes (13). The resulting failure to repair DNA mismatches is associated with the increased risk of development of colorectal, endometrial, ovarian, upper gastrointestinal, and genitourinary cancers (14, 15). The most common clinical assessment of MMR involves analysis of microsatellite instability, which serves as a robust and validated marker for MMR deficiency (16).

Base excision repair

The BER pathway repairs oxidative damage to the bases of DNA, which can be caused by reactive oxygen species (ROS). ROS can be produced by intracellular or extracellular processes, including therapeutic exposures and ionizing radiation. This pathway is orchestrated by DNA glycosylases, AP-endonuclease (APE) activity, DNA ligases, polymerases, XRCC1, PCNA, and other proteins (Fig. 1). PARP1 is also involved in BER, recognizing single-strand and double-strand DNA breaks. This protein has become a subject of considerable clinical interest in the past few years with the development of small-molecule inhibitors of this protein and the demonstration of anticancer activity (17, 18). Genetic diseases caused by mutations in BER genes seem less common than those described with other DNA repair pathways; however, increased levels of APE1 have been described in germ cell tumors (19). Mutations or overexpression of DNA polymerase β have also been linked to increased risk of multiple cancers, including colorectal, lung, breast, gastric, and prostate cancers (20). The analysis of BER activity is relevant in the context of cancer therapy; both temozolomide and dacarbazine induce base damage repaired by BER.

Nucleotide excision repair

The NER pathway recognizes larger, helix-distorting lesions that occur by chemical modification of DNA bases upon exposure to environmental mutagens such as UV light, tobacco smoke, ROS, radiation, and chemotherapeutic agents (21). The 2 subpathways of NER, transcription-coupled NER and global genomic NER, differ only in the initial recognition step and involve the assembly and coordination of more than 30 proteins (Fig. 1; ref. 22). Hereditary disorders related to defects in the NER pathway have been described and include xeroderma pigmentosum (XP), which predisposes affected individuals to certain cancers (6). Consistent with these findings, a subset of NER genes has been shown to have both prognostic and predictive value in the clinical assessment of certain cancers. NER is also relevant to therapeutic response as a function of the intrastrand DNA adducts formed by the platinum-based therapeutics cisplatin, carboplatin, and oxaliplatin being repaired via NER.

DNA double-strand break repair

Repair of DNA DSBs is mediated by the HDR and NHEJ pathways. HDR involves the Rad52 group of proteins, BRCA1/2 and XRCC2/3, in addition to EME1 and NBS1 (Fig. 1). Multiple hereditary disorders have been associated with defects in HDR, including mutations in BRCA1 or BRCA2, which have been associated with hereditary breast and ovarian cancer (23). In contrast, DSB repair via the NHEJ pathway is potentially more mutagenic and requires the coordinated assembly of a number of proteins at the DNA termini to facilitate end joining (Fig. 1). These proteins include the Ku heterodimer (Ku70/Ku80), DNA protein kinase catalytic subunit (DNA-PKcs), ligase IV, XRCC4, XLF, Artemis, and polymerases μ and λ (24). Mutations in NHEJ components have been described and confer radiation sensitivity and defective immune function via reduced V(D)J recombination (25), whereas complete abrogation of NHEJ components seems to be incompatible with life (26). The role of NHEJ and HDR in the repair of ionizing radiation–induced DNA damage has also made these pathways popular as targets for the development of radiosensitizing agents (27). DNA DSBs can also arise via the enzymatic processing of interstrand DNA cross-links. Repair of interstrand DNA cross-links involves the HDR pathway as well as the Fanconi anemia pathway, and mutations in these pathways confer increased susceptibility to cross-linking agents (1, 28, 29).
Figure 1. DNA damage repair pathways. The 5 DNA damage response pathways are presented with key proteins involved in mediating the repair listed. Also shown are representations highlighting the critical repair steps within each of the pathways.
Mutagen Sensitivity Assay

Treatment of cells with mutagen of choice

Verify with cell viability testing to determine results

Host Cell Reactivation Assay

Transfection of damaged (+) plasmid into a host cell line

Read-out based on reporter gene selection

Negative response: gene is not repaired

Positive response: gene is activated upon repair

Comet Assay

Cells are obtained then treated and fixed into a gel matrix

Electric field is applied across the gel; broken DNA escapes the cell

Positive response: DNA damage is repaired (no migration)

Negative response: DNA damage persists; migration out of the cell

SNP Analysis

A blood or tissue sample is retrieved from a patient and submitted for DNA sequencing

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CCR Reviews
Assessing DNA Repair in Cancer Development and Response to Therapy

Assays and Methods Analyzing DNA Repair Capacity

Hereditary disorders caused by defects in specific DNA repair genes, as described above, lead to reduced DNA repair capacity and distinct phenotypes. Although these disorders are exceedingly rare, they highlight the importance of DNA repair in influencing biologic and physiologic processes. The interindividual variations in DNA repair capacities in the general population are likely to be subtle; however, the impact of these subtle differences may be significant and a contributing factor for the predisposition to cancer and response to cancer therapy. As genomic instability is one of the hallmarks of cancer development (30), a large number of studies have aimed to compare DNA repair capacities between patients with cancer and healthy control subjects, with separate studies assessing DNA repair capacity as a predictor of response to chemotheraphy or radiation. Each of these studies uses assays of human tissue to analyze DNA repair capacity. The most popular assays are discussed below, along with the clinical trials highlighting the limitations of the assays and how these limitations affect the conclusions. The most important limitation for the biologic assays is the difficulty in adapting these methodologies to the clinical setting. Routine clinical use is often limited by the need for preparation of isolated, viable cells or extracts from patient samples, preparation of nonstandard reagents, and specialized, often expensive, instrumentation.

Mutagen sensitivity assays
The most commonly used assay to indirectly measure DNA repair capacity is the mutagen sensitivity assay (Fig. 2). This cytogenetic assay quantifies chromatid breaks in cultured peripheral blood lymphocytes (PBL) after exposure to different mutagens (31, 32). Numerous studies have used mutagen sensitivity assays to evaluate DNA repair capacity in patients with a variety of malignancies (including head and neck cancer, bladder cancer, breast cancer, non–small cell lung cancer, and basal cell carcinoma), and in general, they showed higher mutagen sensitivity in patients with cancer compared with healthy control subjects (33–37). A strength of this assay is its reproducibility when conducted by different laboratories (38); however, a weakness is that the assay readout does not provide an analysis of the direct damage induced by the mutagen or its repair. The detection of chromatid breaks, which are indicative of cellular mutagenicity and hence risk for cancer development, can be influenced by many factors of which DNA damage and DNA repair are only a subset. The vast majority of studies use a single mutagen and, thus, limit the assessment of DNA repair to the pathway responsible for repair of the specific damage induced, which may or may not be clinically relevant. Thus, assessment of mutagen sensitivity is appropriate, although both positive and negative correlations must be viewed cautiously and in the context of the mutagen employed.

Host cell reactivation
The host cell reactivation (HCR) assay analyzes DNA repair capacity using a mammalian expression vector harboring DNA damage within a reporter gene that is transiently transfected into the host cell, typically PBLs from study participants, with DNA repair activity being measured via removal or repair of the damage to reactivator reporter gene expression (Fig. 2; ref. 39). Various DNA-damaging agents can be used in this assay and, thus, similar to the mutagen sensitivity assay, restrict the conclusions to a specific repair pathway. HCR assays are useful for assessment of minimal protein components necessary for repair activity, but they do not take into account important issues that can affect DNA repair, including chromatin effects and DNA damage signaling. Thus, results from these studies must be viewed as not the absolute activity but the repair activity possible under a limited set of conditions. As the damaged DNA is introduced to the cell, it does not necessarily initiate the same series of cellular responses and, therefore, although this assay is useful for assessing the mechanics of repair, the true repair capacity is a function of additional factors that cannot be measured in this type of assay. Despite these shortcomings, studies using the HCR assay in cancer patients have shown reduced DNA repair capacity (40–46).

Single-cell analysis
The comet assay (also known as single-cell micro gel electrophoresis assay) measures single-stand breaks and DSBs in a semiquantitative manner from whole cells (Fig. 2; ref. 47). Similar to HCR assays, comet assays require obtaining PBLs from patients, followed by treatment with a specific DNA-damaging agent that leads to strand breakage. The basic principle of interpreting this assay is that the more DNA damage induced, the farther the DNA migrates during electrophoresis. The DNA damage is introduced to the cell, and the less DNA remains in the “head” region, giving the appearance of a comet (48). This assay has been used to analyze damage and rate of repair in epidemiologic studies similar to those mentioned above, and it showed reduced DNA repair capacity in patients with lung cancer compared with control

Figure 2. Commonly used assays to analyze DNA damage or repair. The mutagen sensitivity assay involves treatment of cells with a chemical or radiation and observation of the effect on the cells. In this depiction, cells are sorted using fluorescence-activated cell sorting to determine viable (green box) or apoptotic (red box) cells following treatment. The HCR assay takes advantage of the host cell’s DNA repair machinery to repair a pre-damaged plasmid DNA, which is transfected into the host cells. The damage is typically incorporated into a selection gene (i.e., luciferase), which remains inactive if not repaired, or active when repaired. The comet assay involves obtaining cells, which can be cultured from an animal or from a patient. The cells are then treated with DNA-damaging agents (i.e., radiation), then embedded into a thin layer of agarose. An electric field is applied across the gel, and broken DNA will migrate out of the cell. Single-nucleotide polymorphism (SNP) analysis involves obtaining a sample of cells from which DNA can be extracted and sent for sequence analysis. The sequencing information obtained will give the genotype for the marker selected.
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>SNPs</th>
<th>Reference SNP identifier</th>
<th>Clinical correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER</td>
<td>APE1</td>
<td>c.-656T &gt; G (promoter region)</td>
<td>rs1760944</td>
<td>Decreased risk of lung cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.-141T &gt; G (promoter region)</td>
<td>rs1760944</td>
<td>Decreased risk of lung cancer, Chinese populations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2197T &gt; G (p.Asp148Glu)</td>
<td>rs1130409</td>
<td>Reduced risk of bladder cancer</td>
</tr>
<tr>
<td>FEN1</td>
<td></td>
<td>c.-69G &gt; A (promoter region)</td>
<td>rs174538</td>
<td>Increased risk of lung cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.4150G &gt; T</td>
<td>rs4246215</td>
<td>Increased risk of lung cancer</td>
</tr>
<tr>
<td>MBD4</td>
<td></td>
<td>c.1036G &gt; A (p.Glu346Lys)</td>
<td>rs140693</td>
<td>Reduced risk of lung cancer, Chinese population</td>
</tr>
<tr>
<td>MUTYH</td>
<td></td>
<td>g.748-15C &gt; G</td>
<td>rs2072668</td>
<td>Increased risk of gallbladder carcinoma in females, northern India</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p.Ser326Cys</td>
<td>rs1052133</td>
<td>Increased risk of lung cancer, also noted in several places an increased risk of lung cancer</td>
</tr>
<tr>
<td>PARP1</td>
<td></td>
<td>c.2285T &gt; C (p.Val762Ala)</td>
<td>rs1136410</td>
<td>Increased risk of multiple malignancies, decreased risk of non-Hodgkin lymphoma in Korean patients</td>
</tr>
<tr>
<td></td>
<td>POLB</td>
<td>c.725C &gt; G (p.Pro242Arg)</td>
<td>rs3136797</td>
<td>Increased risk of breast cancer development</td>
</tr>
<tr>
<td></td>
<td>NEIL2</td>
<td>g.4102972C &gt; G</td>
<td>rs804270</td>
<td>Increased risk of squamous cell carcinoma of the oral cavity</td>
</tr>
<tr>
<td>XRCC1</td>
<td></td>
<td>c.580C &gt; T (p.Arg194Trp)</td>
<td>rs1799782</td>
<td>Platinum sensitivity</td>
</tr>
<tr>
<td></td>
<td>EXO1</td>
<td>c.1765C &gt; A (p.Glu589Lys)</td>
<td>rs1047840</td>
<td>Associated with increased risk of gastric cancer and breast cancer</td>
</tr>
<tr>
<td>MMR</td>
<td>MLH1</td>
<td>c.655A &gt; G</td>
<td>rs1799977</td>
<td>Increased risk of colorectal cancer but favorable outcome</td>
</tr>
<tr>
<td></td>
<td>MSH2</td>
<td>c.2006-6T &gt; C [gIVS12-6T &gt; C]</td>
<td>rs2303428</td>
<td>Poor prognostic factor in NSCLC</td>
</tr>
<tr>
<td></td>
<td>MSH3</td>
<td>c.693C &gt; A (p.Pro222Pro)</td>
<td>rs1805355</td>
<td>Increased risk of prostate cancer</td>
</tr>
<tr>
<td>NER</td>
<td>DDB2/XPE</td>
<td>g.457–314G &gt; C</td>
<td>rs830083</td>
<td>Possible association with lung cancer risk</td>
</tr>
<tr>
<td></td>
<td>ERCC1</td>
<td>C8092A &gt; CA</td>
<td>rs3212986</td>
<td>Poor prognosis in NSCLC patients</td>
</tr>
<tr>
<td></td>
<td>C8092A C/T,TT</td>
<td>rs11615</td>
<td>Better survival in ovarian cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ERCC2/XPD</td>
<td>c.934G &gt; A (p.Asp312Asn)</td>
<td>rs1799793</td>
<td>Increased risk of lung cancer; improved responses in patients with secondary AML</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2251A &gt; C (p.Lys751Gln)</td>
<td>rs13181</td>
<td>Increased risk of lung cancer; improved responses in patients with secondary AML</td>
</tr>
<tr>
<td></td>
<td>XPA</td>
<td>c.4A &gt; G (5' noncoding region)</td>
<td>rs1800975</td>
<td>Reduced risk of lung cancer</td>
</tr>
<tr>
<td></td>
<td>XPC</td>
<td>c.1496C &gt; T (p.Ala499Val)</td>
<td>rs2228000</td>
<td>Associated with bladder cancer risk</td>
</tr>
<tr>
<td></td>
<td>XPG</td>
<td>c.138T &gt; C (p.His46His)</td>
<td>rs1047768</td>
<td>Affects sensitivity to chemotherapy</td>
</tr>
<tr>
<td>HDR</td>
<td>ATM</td>
<td>c.111G &gt; A</td>
<td>rs189037</td>
<td>Increased risk of NSCLC in never smokers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.496+448G &gt; A</td>
<td>rs228597</td>
<td>Increased risk of NSCLC in never smokers</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.1803–355C &gt; A</td>
<td>rs228592</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.3078–77C &gt; T</td>
<td>rs664677</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NBS1</td>
<td>c.553G &gt; C (p.Glu185Gln)</td>
<td>rs1805794</td>
<td>Positive association with bladder cancer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.2016A &gt; G (p.Pro672Ala)</td>
<td>rs1061302</td>
<td>Decreased associated with bladder cancer, positively associated with liver cancer</td>
</tr>
<tr>
<td>Rad51</td>
<td></td>
<td>c.98G &gt; C (5' UTR)</td>
<td>rs1801320</td>
<td>Protective against breast and ovarian cancer in BRCA1 carriers</td>
</tr>
</tbody>
</table>

(Continued on the following page)
Candidate gene analyses

Finally, genetic-based assays have been used as surrogates to evaluate DNA repair capacity via quantitative real-time PCR of gene expression (56) and single-nucleotide polymorphism (SNP) analyses of DNA repair genes. The candidate gene approach for these studies has substantial advantages over genome-wide association studies and can support hypothesis-driven research. Multiple studies have correlated a variety of polymorphisms with the risk of development of different solid organ malignancies, and numerous DNA repair gene SNPs have been shown to be prognostic in patients with cancer (Table 1). SNPs are an important genetic tool, but the interpretation of studies evaluating individual SNPs as they relate to variables, such as therapeutic efficacy, cancer risk, or prognosis, is hindered by numerous limitations, including linkage disequilibrium, inadequate statistical analyses (e.g., small sample size, multiple testing, and reproducibility), and the publication bias of positive associations. Thus biologic, pathway-driven selection of candidate genes and independent corroboration of the effect of candidate SNPs on biologic activity should be the minimal requirement for studies measuring associations with therapeutic efficacy or prognosis.

Biomarkers of DNA damage and repair

A number of other assays are used occasionally, including measurement of the expression or activation of certain biomarkers or surrogates of DNA repair. The most common assays include γ-H2AX foci, which measure the expression of a histone variant (53–55). The analysis typically can generate a robust signal because megabases of DNA include this modification around a single DNA DSB. However, how gamma-H2AX foci relates to repair and, ultimately, sensitivity to DNA damaging agents remains to be determined. Similarly, the assessment of Rad51, 53BP1, and RPA foci is being investigated as they also indicate that recombination and repair machinery is accumulating at specific sites in the genome, likely in the vicinity of DNA damage. Although none of these analyses are routinely used in clinical practice, their potential as biomarkers is being investigated and should determine their effectiveness as prognostic or predictive factors.

Candidate gene analyses

Table 1. Clinical correlations of DNA repair gene single-nucleotide polymorphisms (Cont’d)

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene</th>
<th>SNPs</th>
<th>Reference SNP identifier</th>
<th>Clinical correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLM</td>
<td>c.2603C &gt; T (p.Pro868Leu)</td>
<td>rs11852361</td>
<td>Increased risk of rectal cancer</td>
<td></td>
</tr>
<tr>
<td>XRCC2</td>
<td>c.563G &gt; A (p.Arg188His)</td>
<td>rs3218536</td>
<td>Possible protective role against breast cancer in women that never breast fed</td>
<td></td>
</tr>
<tr>
<td>XRCC3</td>
<td>c.722C &gt; T (p.Thr241Met)</td>
<td>rs861539</td>
<td>Weak association with breast cancer risk</td>
<td></td>
</tr>
<tr>
<td>NHEJ</td>
<td>Ku70</td>
<td>T 991C</td>
<td>rs5751129</td>
<td>Increased susceptibility to oral cancer</td>
</tr>
<tr>
<td></td>
<td>Ku80</td>
<td>G1041T</td>
<td>rs828907</td>
<td>Increased risk of colon cancer</td>
</tr>
<tr>
<td></td>
<td>XRCC4</td>
<td>c.26C &gt; T (p.Thr9Ile)</td>
<td>rs1805388</td>
<td>Prognostic in NSCLC</td>
</tr>
<tr>
<td></td>
<td>c.894–1(7)G &gt; A</td>
<td>rs1805377</td>
<td>Prognostic in NSCLC</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AML, acute myelogenous leukemia; NSCLC, non–small cell lung cancer; SNP, single-nucleotide polymorphism; UTR, untranslated region.

Tissue analysis and the use of surrogates

Another factor that will likely influence the results of studies evaluating DNA repair capacity would be the selected tissue used for analysis. A wide range of surrogates has been used, including PBLs (freshly isolated), cryopreserved Epstein–Barr virus (EBV)–transformed PBLs [also known as lymphoblastoid cell lines (LCL)], blood cultures (36), primary tumor samples, and, on rare occasions, metastatic sites (57). The effectiveness of LCLs as surrogates of cryopreserved lymphocytes for the analysis of DNA repair genotype–phenotype correlations is unclear, but they are continuously used in these studies. At least one study showed high variability and poor reproducibility of analysis of DNA DSBs using LCLs compared with PBLs, possibly because of chromosomal instability resulting from EBV transformation (58). Another study showed significantly increased 8-oxoG DNA glycosylase activity and expression in LCLs compared with PBLs (59). Even unmodified freshly isolated PBLs are unlikely to reflect DNA repair capacity of tumor tissue, as all cancers (unlike PBLs) will display some defect in DNA repair. In fact, one study compared the repair capacity of lymphocytes and colon tumor cells through measuring rates of removal of DNA cross-links induced by oxaliplatin using the comet assay and found significant differences (60). With recent data showing genomic differences between primary tumors and their metastases, in addition to the increased understanding of intratumor
heterogeneity, caution should be used when interpreting the available data evaluating DNA repair capacity. It is possible that newer circulating tumor cell technology will prove to be a very useful tool (61). Until then, tumor tissue should be used for analysis, as its DNA repair defects are more likely to be successfully exploited. Use of surrogates, though more easily obtainable, is of limited clinical value and should be avoided.

Impact of DNA Repair Capacity on Cancer Risk

Many retrospective studies have used the previously discussed assays to measure DNA repair capacity and, in general, have reported decreased DNA repair capacity in patients with numerous solid organ malignancies (Table 2). These studies had multiple limitations, including reverse causation biases due to their retrospective nature. A limited number of prospective studies have examined the possible association between cancer risk and reduced DNA repair capacity (31, 62, 63). The largest prospective trial was reported recently as part of the prostate, lung, colorectal, and ovarian (PLCO) cancer-screening trial. It compared prospective cases with lung cancer with their controls. HCR and comet assays were unrelated to lung cancer risk, but the mutagen sensitivity assay, which used bleomycin as the DNA-damaging agent, showed a positive association with an OR of 2.1 in the quartile with the highest chromatid breaks per cell (64). Cases and controls were well matched and stratified for pertinent variables, including age, gender, weight, and smoking history. Among the limitations of this trial was the use of EBV-transformed LCLs for the analysis, which, as stated above, might not be the best surrogate, in addition to the small sample size. The study again highlights the importance of the choice of DNA repair assay used.

Table 2. Evaluation of DNA repair capacity in cancer patients

<table>
<thead>
<tr>
<th>Assay and/or agent</th>
<th>Patient population</th>
<th>Cases/Controls</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutagen sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleomycin</td>
<td>NSCLC</td>
<td>90/119</td>
<td>OR = 3.7 (95% CI, 1.4–9.4)</td>
<td>35</td>
</tr>
<tr>
<td>BPDE</td>
<td>Lung cancer</td>
<td>977/977</td>
<td>Higher mutagen sensitivity in lung cancer patients</td>
<td>36</td>
</tr>
<tr>
<td>BPDE</td>
<td>Squamous cell carcinoma of the head and neck</td>
<td>895/898</td>
<td>Higher frequency of BPDE-induced chromatid breaks in patients</td>
<td>33</td>
</tr>
<tr>
<td>BPDE</td>
<td>Breast cancer</td>
<td>100/105</td>
<td>Higher frequency of chromatid breaks in breast cancer patients; OR = 3.11 (95% CI, 1.72–5.64)</td>
<td>37</td>
</tr>
<tr>
<td>UV</td>
<td>Melanoma and nonmelanoma skin cancer</td>
<td>329/469</td>
<td>Higher frequency of UVB-induced chromatid breaks in nonmelanoma skin cancer patients</td>
<td>34</td>
</tr>
<tr>
<td>HCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPDE</td>
<td>Squamous cell carcinoma of the head and neck</td>
<td>744/753</td>
<td>Reduced DNA repair capacity; OR = 1.91 (95% CI, 1.52–2.40)</td>
<td>41</td>
</tr>
<tr>
<td>BPDE</td>
<td>NSCLC</td>
<td>467/488</td>
<td>Reduced DNA repair capacity; OR = 1.85 (95% CI, 1.42–2.42)</td>
<td>42</td>
</tr>
<tr>
<td>BPDE</td>
<td>Head and neck cancer</td>
<td>55/61</td>
<td>Reduced DNA repair capacity</td>
<td>43</td>
</tr>
<tr>
<td>BPDE</td>
<td>Lung cancer</td>
<td>51/56</td>
<td>Reduced DNA repair capacity</td>
<td>44</td>
</tr>
<tr>
<td>BPDE</td>
<td>Lung cancer</td>
<td>316/316</td>
<td>Reduced DNA repair capacity in patients; OR 1.8 (95% CI, 1.1–3.1)</td>
<td>45</td>
</tr>
<tr>
<td>UV</td>
<td>Basal cell carcinoma and/or squamous cell carcinoma</td>
<td>333/255</td>
<td>16% reduction in DNA repair capacity in patients</td>
<td>40</td>
</tr>
<tr>
<td>Comet assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O2</td>
<td>Lung cancer</td>
<td>30/90</td>
<td>Higher level of H2O2-induced DNA damage in lung cancer patients</td>
<td>49</td>
</tr>
<tr>
<td>BPDE</td>
<td>Patients with multiple versus single NSCLC</td>
<td>108/99</td>
<td>Higher BPDE-induced damage and repair in cases</td>
<td>50</td>
</tr>
<tr>
<td>Bleomycin and BPDE</td>
<td>Laryngeal carcinomas</td>
<td>52/56</td>
<td>Higher levels of mutagen-induced damage in patients</td>
<td>46</td>
</tr>
</tbody>
</table>

Abbreviations: BPDE, benzo(a)pyrene diol epoxide; CI, confidence interval; NSCLC, non-small cell lung cancer; UVB, ultraviolet light B.
have aimed to evaluate the impact of repair capacity in the context of cancer patients’ response to different cancer therapies. Although reduced innate DNA repair capacity is undesirable from a cancer risk perspective, reduced tumoral DNA repair capacity is desirable because it can be exploited therapeutically with DNA-damaging therapeutics, and via synthetic lethality (18, 65).

Platinum has been the most intensely studied anticancer agent due to the major role it plays in cancer treatment for a number of solid organ malignancies. Platinum efficacy is mediated by the formation of DNA adducts, the majority of which are repaired via the NER pathway (Fig. 1; ref. 66). ERCC1 is probably the most-studied protein as both a prognostic and predictive marker for the survival benefit from adjuvant platinum-based chemotherapy (67), although all NER-deficient cells display sensitivity to cisplatin. Despite the plethora of clinical data, skepticism persists about the usefulness of this marker due to multiple factors, including the retrospective nature of the trials and the known limitations of immunohistochemistry, including controversies around the optimal primary antibody for ERCC1 detection (68, 69). The definition of ERCC1 positivity is also arbitrary and varies between studies. DNA repair is a complex process that is unlikely to be measured by the expression of one protein. The prospective data using ERCC1 as a biomarker for platinum response, although statistically significant, were unimpressive. It is possible that some patients who were designated as DNA repair deficient (because of low ERCC1 expression) would, in fact, be considered DNA repair proficient if other DNA repair proteins had been measured. In addition, the mechanistic explanation for why ERCC1 would confer platinum sensitivity is unclear. The recognition step in NER is thought to be rate limiting, and ERCC1-XPF is the last factor recruited to the preincision complex (70). Does the likely role of ERCC1-XPF in the repair of interstrand cross-links explain its possible importance (71, 72)? Mutations in HDR genes confer extreme sensitivity to cisplatin, suggesting that HDR proteins are involved in some capacity in the cellular response to platinum lesions. These findings raise another interesting point that it may not be as simple as targeting one pathway, as the efficacy of platinum treatment may be the combination of the inter- and intrastrand cross-links that arise or cross-talk that may occur between different DNA damage pathways.

DNA repair is also the focus of predicting responses to other DNA-damaging agents. For example, both MMR- and BER-related protein levels are thought to correlate with clinical response to alkylating agents (dacarbazine and temozolomide) in patients with metastatic melanoma (73, 74). Expression of aprataxin (APTX), a DNA repair protein, was recently found to regulate sensitivity to irinotecan in colorectal cancer, in which low tumor levels of APTX correlated with good response (75). Survival benefit in glioblastoma multiforme patients treated with temozolomide was only noted in the subset with methylated MGMT promoter leading to its silencing (76). In a recent breast cancer study, complete pathologic response to neoadjuvant chemotherapy was associated with lower Rad51 foci in tumor biopsies obtained after chemotherapy (77).

Finally, various polymorphisms of DNA repair genes have been studied in association with sensitivity to platinum or other DNA-damaging agents (Table 1). However, the significance of the SNPs identified to date must be further investigated because of conflicting observations and a general lack of follow-up linking specific SNPs to specific pathway deficiencies. The specific defect conferred by each SNP should be tied to a molecular phenotype and functional significance within a biologic pathway.

**DNA Repair Proteins as Drug Targets**

Interest in targeting DNA repair proteins has increased greatly over the past decade. The impact of DNA repair on resistance to cisplatin is well documented in numerous cancers. The holy grail of reversing either innate or acquired cisplatin resistance is being pursued by targeting specific proteins that are involved directly in the repair of the cisplatin lesions (78–81) or in the pathways responsible for signaling DNA damage (82–84). Currently, the most advanced inhibitors of DNA repair in the clinical setting are inhibitors of PARP. PARP is activated by DNA breaks and is involved in multiple DNA damage responses including BER, HDR, NHEJ, and replication restart, in addition to its role in transcription (85, 86). PARP inhibition was noted to be most effective, in preclinical studies, in BRCA-deficient or mutant tumor cell lines, possibly because of their greater dependence on PARP and BER for maintenance of genomic integrity in the presence of HDR defects, establishing a synthetic lethal interaction (87, 88). Two genes are synthetically lethal if a mutation in either alone is compatible with cell survival, whereas a mutation in both leads to cell death. The concept of synthetic lethality is very attractive because normal cells should be less likely to be affected, reducing toxicity. Iniparib (BSI-201), a PARP inhibitor, was recently evaluated in combination with chemotherapy in triple-negative breast cancer because of the similarities between triple-negative breast cancer and BRCA-deficient breast cancers (89). The combination showed improvement in progression-free and overall survival in a phase II trial (90); however, preliminary reports of a phase III trial evaluating the same combination were negative with a lack of improvement in overall survival in women with triple-negative metastatic breast cancer who received iniparib combined with chemotherapy (91). Importantly, PARP is not directly involved in the repair of platinum DNA lesions, which raises many mechanistic questions about the reasons for benefit initially reported with the addition of the PARP inhibitor in conjunction with cisplatin. Another possible reason for the lack of survival improvement could be related to the fact that iniparib is a much less potent inhibitor of PARP1 (with approximately 0.1% of the potency) than most other agents of this class (92). Both olaparib and iniparib are currently being studied in a number of solid organ malignancies and seem to be especially promising in...
relapsed ovarian cancer, either in combination with chemotherapy or as maintenance therapy. The negative phase III study in triple-negative breast cancer, nonetheless, highlights the importance of a deeper understanding of DNA repair and the intimate connection between the damage induced and the pathway responsible for its repair. Only armed with this information can rational combinations of therapies be developed, tested, and, ultimately, be effective in the treatment of complex diseases such as cancer. The activity of the topoisomerase II inhibitor, etoposide, in cancers with inactivation of the retinoblastoma tumor suppressor protein is another example of synthetic lethality (93).

Conclusions

Genome instability is a hallmark of cancer, and accurate determination of one’s capacity for maintaining genome stability holds the potential to assess risk of cancer development. The complexity and specificity of the pathways that govern genomic integrity necessitate accurate analyses of each specific pathway to ultimately determine risk. Perhaps more important than assessing an individual’s risk of malignancy is the potential to personalize therapy with a better understanding of DNA repair pathways, because DNA damage continues to be the mainstay of cancer therapy. The development of reproducible, patient- and laboratory-friendly assays for the analysis of DNA repair is critical. Limitations with current methodologies provide the impetus for improvement, miniaturization, and automation to bring to fruition the goal of assessing individual DNA repair capacity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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Shadia Jalal, Jennifer N. Earley and John J. Turchi

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