DNA repair: From genome maintenance to biomarker and therapeutic target.

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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 regarding 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 has 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 assess 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.
Statement of translational relevance:
An individual’s DNA repair capacity has major clinical implications. These include risk of development of various illnesses including cancer, the response to DNA damaging cancer therapies, and likelihood of therapy induced toxicities. Multiple assays assessing DNA repair capacity have yielded useful information but have limitations in applicability, reproducibility and interpretation. These assays assess different aspects of DNA repair capacity under specific conditions and are not necessarily a marker of an individual’s absolute DNA repair capacity. As we aim to personalize cancer therapy, a deeper understanding of DNA repair pathways and the specific assays used to assess their activity is critical when determining associations with complex molecular and cellular processes involved in disease susceptibility and response to therapeutics.
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 and include: the nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), and the two double strand break repair pathways: non-homologous end-joining (NHEJ) and homology directed repair (HDR) the latter of which is also involved in the repair of interstrand DNA crosslinks (ICL) in conjunction with the Fanconi Anemia (FA) 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 last decade, our knowledge of the roles of DNA repair pathways and how deficiencies or abnormalities in them impact the development of numerous disease processes has significantly increased. Mutations in DNA repair genes have been implicated in the development of neurological diseases (2), aging (3), cancer risk (4), cancer therapy outcomes (5), inflammation and other genetic syndromes with a variety of distinct phenotypes (6). Considering the importance of DNA repair in human disease, there is significant interest in the measurement or determination of an individual’s DNA repair capacity. Here, we review DNA repair pathways, the current assays available for assessment 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 (DDR) network to signal the presence of an insult and coordinate the cellular response to the damage. DDR 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 post-translational 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 impacting either DNA replication
and/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 Kunkel and colleagues (10).

**DNA mismatch repair:**

The MMR pathway is responsible for correcting replication errors that escape processing by the 3’-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 (11;12) (Figure 1). The most well described is hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, an autosomal dominant disorder characterized, in 90% of the cases, by germ line mutations in one of the alleles of the *MSH2* or *MLH1* genes (13). The resulting failure to repair DNA mismatches is associated with the increased risk of development of colorectal, endometrial, ovarian, upper GI (gastrointestinal) and GU (genitourinary) cancers (14;15). The most common clinical assessment of MMR involves analysis of microsatellite instability (MSI) 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 (Figure 1). Poly-(ADP ribose)-polymerase 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 last 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 appear 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 risks 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 repair 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, as well as radiation and chemotherapeutic agents (21). The two sub-pathways of NER, transcription-coupled (TC-NER) and global genomic (GG-NER), differ only in the initial recognition step and involve the assembly and coordination of over 30 proteins (22) (Figure 1). 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 have 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 HR and NHEJ pathways. HR involves the Rad52 group of proteins, BRCA1/2, XRCC2/3 in addition to EME1 and NBS1 (Figure 1). Multiple hereditary disorders have been associated with defects in DSB repair 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 (Figure 1). These include the Ku heterodimer (Ku70/Ku80), 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); while complete abrogation of NHEJ components appears to be incompatible with life (26). The role of NHEJ and HR in the repair of IR-induced DNA damage has also made these pathways popular as targets for the development of radiosensitizing agents (27). DNA double strand breaks can also arise via the enzymatic processing of interstrand DNA crosslinks. Repair of ICL involves the HRR pathways as well as the FA pathway, mutations is while confer increase susceptibility to crosslinking agents (1;28;29).

**Assays and methods assessing 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. While these disorders are
exceedingly rare they highlight the importance of DNA repair in influencing biologic and physiologic processes. The inter-individual 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 controls with separate studies assessing DNA repair capacity as a predictor of response to chemotherapy or radiation. Each of these studies employs assays of human tissue to assess DNA repair capacity. The most popular assays are discussed below along with the clinical trials highlighting the limitations of the assays and how these impact the conclusions. The most significant limitation for the biological 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 non-standard 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 (Figure 2). This is a cytogenetic assay that quantifies chromatid breaks in cultured peripheral blood lymphocytes (PBLs) after exposure to different mutagens (31;32). Numerous studies 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, showed higher mutagen sensitivity in patients with cancer to healthy controls (33-37). A strength of this assay is the reproducibility when conducted by different laboratories (38) however, a weakness is that the assay read-out does not assess the direct damage induced by the mutagen or its repair. The detection of chromatid breaks, while 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 though 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 assesses 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 reactivate reporter gene expression (39) (Figure 2). Various DNA damaging agents can be used in this assay and thus similar to the mutagen sensitivity assay, restricts the conclusions to a specific repair pathway. HCR assays have good utility for assessment of minimal protein components necessary for repair activity but do not take into account important issues that can impact 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, while 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 short-comings, 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 SSBs and DSBs, in a semi-quantitative manner, from whole cells (47)(Figure 2). 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 further the DNA migrates during electrophoresis into a “tail” region, and less DNA remains in the “head” region giving the appearance of a comet (48). This assay has been used to assess damage and rate of repair in epidemiologic studies similar to the ones mentioned above, and showed reduced DNA repair capacity in patients with lung cancer compared to their controls (46;49;50). The comet assay has the advantage of being adaptable to be more specific to the type of lesion in question by using specific enzymes (51). However, performing the comet assay requires a significant amount of preparation and hands-on manipulation. These analyses have been plagued by issues with inter-lab reproducibility and variations in quantification and, while standardization is being addressed, no unifying standard has been established to date (52).
Biomarkers of DNA damage and repair.

A number of other assays are used occasionally including measuring the expression or activation of certain biomarkers or surrogates of DNA repair. The most common include γH2AX foci which measure the expression of a histone variant (53-55). The assay typically can generate a robust singly as megabases of DNA include this modification around a single DNA double stand break. However, how this relates to repair and ultimately sensitivity to DNA damage agents remains to be determined. Similarly the assessment of Rad51, 53BP1 and RPA foci are 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. While none of these assays are routinely used in clinical practice their utility as biomarkers is being investigated and should determine the utility of these markers as prognostic or predictive factors.

Candidate gene analyses:

Finally, genetic based assays have been used as a surrogate to evaluate DNA repair capacity via quantitative real time polymerase chain reaction (RT-PCR) of gene expression (56) and single nucleotide polymorphisms (SNP) analyses of DNA repair genes. The candidate gene approach for these studies has significant 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 SNP’s have been shown to also 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 including small sample size, multiple testing and reproducibility as well as the publication bias of positive associations. Thus biological, pathway driven selection of candidate genes and independent corroboration of the candidate SNPs effect on biological activity should be the minimal requirement for studies measuring associations with therapeutic efficacy or prognosis.

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 have been used, including PBLs (freshly isolated), cryopreserved EBV- transformed PBLs (also known
lymphoblastoid cell lines LCLs), blood cultures (36) primary tumor samples, and in rare occasions metastatic sites (57). The utility of LCLs as surrogates of cryopreserved isolated 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 to PBLs, possibly due to chromosomal instability resulting from EBV-transformation (58). Another study showed significantly increased 8-oxoG DNA glycosylase activity and expression in LCLs compared to 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 intra-tumor heterogeneity, caution should be used when interpreting the available data evaluating DNA repair capacity..

It is possible that newer technology using circulating tumor cells (CTCs) 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.

**The impact of DNA repair capacity on cancer risk**

A myriad of retrospective studies have used the previously discussed assays to measure DNA repair capacity and in general reported decreased DNA repair capacity in patients with numerous solid organ malignancies (Table 2). These studies had multiple limitations including suffering from ‘reverse causation biases due to their retrospective nature. A limited number of prospective studies have examined the possible association between cancer risk and a reduced DNA repair capacity prospectively (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, using bleomycin as the DNA damaging agent, showed a positive association with an OR of 2.1 in the quartile with the highest chromatid breaks/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.

**DNA repair capacity’s impact on response to cancer therapies:**

In addition to the evaluation of DNA repair capacity in relation to risk of cancer development, multiple studies have aimed to evaluate repair capacity’s impact in the context of cancer patients’ response to different cancer therapies. While reduced innate DNA repair capacity is undesirable from a cancer risk perspective, reduced DNA repair capacity, especially in tumor cells, is desirable due to the fact it can be exploited therapeutically with the tumor’s increased sensitivity to DNA damaging therapeutics and to target the DNA repair defective tumors using a synthetic lethal approach (18;65).

Platinum has been the most heavily studied anti-cancer 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 (Figure 1) (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) though all NER deficient cells display sensitivity to cisplatin. Despite the plethora of clinical data, skepticism persists as to the utility of this marker due to multiple factors including the retrospective nature of the trials and the known limitations of immunohistochemistry (IHC), 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 measured by the expression of one protein. The prospective data using ERCC1 as a biomarker for platinum response though statistically significant was unimpressive. It is possible that some patients who were labeled as DNA repair deficient (due to low ERCC1 expression) were in fact DNA proficient had other DNA repair proteins been measured. In addition, the mechanistic explanation for why ERCC1 would confer platinum sensitivity DNA repair capacity is unclear. The recognition step in NER is thought to be rate limiting and ERCC1-XPF is the last factor recruited to the pre-incision complex (70). Does the likely role of ERCC1-XPF in the repair of inter-strand cisplatin crosslinks 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 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 intra-strand crosslinks that do arise or there may be cross-talk 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 APTX (aprataxin), a DNA repair gene, was recently found to regulate sensitivity to irinotecan in colorectal cancer where 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 pathological response to neoadjuvant chemotherapy was associated with lower Rad51 foci in tumor biopsies obtained post 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 due to 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 biological pathway.

**DNA repair proteins as druggable targets:**

Interest in targeting DNA repair proteins has significantly increased 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 due to their greater dependence on PARP and BER for maintenance of genomic integrity in the presence of HDR defects, establishing a synthetic lethal interaction.
Two genes are synthetically lethal if a mutation in either alone is compatible with cell survival while a mutation in both leads to cell death. The concept of synthetic lethality is very attractive since 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 (TNBC) due to the similarities between TNBC 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 regarding 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 Iniparib is a much less potent inhibitor of PARP1 (with approximately 0.1% 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 though 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, which govern genomic integrity necessitates 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 better understanding of DNA repair pathways since 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

None declared

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Figure Legends

Figure 1: DNA Damage Repair (DDR) pathways. Each of the five DDR pathways are presented with key proteins involved in mediating the repair listed. Also shown are schematic representations highlighting the critical repair steps within each of the pathways.

Figure 2. Commonly used assays to assess DNA damage or repair. The mutagen sensitivity assay involves treatment of cells with a chemical or radiation, and observing the effect on the cells. In this depiction, cells are sorted using fluorescence-activated cell sorting (FACS) to determine viable (green box) or apoptotic (red box) cells following treatment. The host cell reactivation 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 cultured or from an animal or 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. SNP analysis involves obtaining a sample of cells from which DNA can be extracted and sent out for sequence analysis. The sequencing information obtained will give the genotype for the marker selected.
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<td>XPC</td>
<td>c.1496C&gt;T (p.Ala499Val)</td>
<td>rs22228000</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>Impacts 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></td>
</tr>
<tr>
<td></td>
<td></td>
<td>c.1803-355G&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>NBS1</td>
<td></td>
<td>c.553G&gt;C (p.Glu185Gln)</td>
<td>rs1805794</td>
<td>Positive association with bladder cancer</td>
</tr>
<tr>
<td>Gene</td>
<td>Polymorphism</td>
<td>Reference</td>
<td>Association</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------------------</td>
<td>---------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Rad51</td>
<td>c.-98G&gt;C (5' UTR)</td>
<td>rs1801320</td>
<td>Protective against breast and ovarian cancer in BRCA1 carriers. *Results on this polymorphism seem to be highly variable based on introduction of given reference.</td>
<td></td>
</tr>
<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 bladder cancer risk</td>
<td></td>
</tr>
<tr>
<td>NHEJ</td>
<td>Ku70 T 991C</td>
<td>rs5751129</td>
<td>Increased susceptibility of oral cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ku80 G1041T</td>
<td>rs828907</td>
<td>Increased risk of colon cancer</td>
<td></td>
</tr>
<tr>
<td>XRCC4</td>
<td>c.26C&gt;T (p.Thr9Ile)</td>
<td>rs1805388</td>
<td>Prognostic in NSCLC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>c.894-1(*7)G&gt;A *two listed</td>
<td>rs1805377</td>
<td>Prognostic in NSCLC</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 2. Evaluation of DNA repair capacity in cancer patients

<table>
<thead>
<tr>
<th>Assay/agent</th>
<th>Patient population</th>
<th>cases/controls</th>
<th>outcome</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutagen Sensitivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bleomycin</td>
<td>Non-small cell lung cancer</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 non-melanoma skin cancer</td>
<td>329/469</td>
<td>Higher frequency of UVB induced chromatic breaks in nonmelanoma skin cancer patients</td>
<td>34</td>
</tr>
<tr>
<td><strong>Host cell reactivation</strong></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>Non-small cell lung cancer</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 CI 1.1-3.1)</td>
<td>45</td>
</tr>
<tr>
<td>UV</td>
<td>Basal cell carcinoma/squamous cell carcinoma</td>
<td>333/255</td>
<td>16% reduction in DNA repair capacity in patients</td>
<td>40</td>
</tr>
<tr>
<td><strong>comet assay</strong></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>
### Base Excision Repair (BER)

**Key Players:**
- APE1
- MBD4
- NUDT1
- SMUG1
- APE2
- MPG
- OGG1
- TDG
- APTX
- MUTYH
- PARP1
- TDP1
- DNA2
- NEIL1
- PARP2
- UNG
- FEN1
- NEIL2
- PNKP
- XRCC1
- LIG1
- NEIL3
- POLB
- LIG3
- NTHL1
- POLG

### Mismatch Mediated Repair (MMR)

**Key Players:**
- EXO1
- MSH6
- HMBG1
- PCNA
- LIG1
- PMS1
- MLH1
- PMS2
- MLH3
- POLD
- MSH2
- RFC
- MSH3
- RPA

### Nucleotide Excision Repair (NER)

**Key Players:**
- CEN2
- ERCC4/XPF
- XPA
- CSA
- HR23B
- XPC
- CSB
- LIG1
- XPG
- CUL4A
- LIG3
- XRCC1
- DDB1
- POL D/E
- DDB2/XPE
- RPA
- ERCC1
- TFIIH

### Homology Directed Repair (HDR)

**Key Players:**
- ATM
- FANCF
- Rad50
- XRCC2
- ATR
- FANCM
- Rad51
- XRCC3
- BLM
- FANCN
- Rad52
- BRCA1
- GEN1
- Rad54
- EME1
- MRE11
- RecQ4
- EXO1
- NBS1
- RPA
- FANCD/BRCA2
- WRN

### Non-Homologous End-Joining (NHEJ)

**Key Players:**
- ARTEMIS
- POL4
- DNA-PKCs
- Ku70
- Ku80
- LIG4
- ATM
- XRC4
- ATX
- XLF/cernunnos
- Ku70-Ku80
- DNA-PKCs
- LigaseIV/XRCC4-XLF
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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Clinical Cancer Research

DNA repair: From genome maintenance to biomarker and therapeutic target

Shadia Jalal, Jennifer N Earley and John J Turchi

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