Editorial

Interrogating DNA Repair in Cancer Risk Assessment

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DNA is constantly injured by external stress such as exposure to tobacco-smoke constituents, sunlight, or dietary constituents, such as charbroiled meat products, and internal stress, such as free radicals associated with oxygen metabolism. DNA lesions interfere with replication and with transcription, and can trigger cell death, whereas mutations in critical regions can also trigger or promote carcinogenesis. These events are usually prevented by DNA repair mechanisms which remove or bypass the damaged site and restore the original sequence (1-3).

The DNA-repair system is complex, encompassing multienzyme pathways that work in a choreographed manner to repair specific base modifications or strand breaks. The system consists of low- and high-fidelity mechanisms, redundancy in some parts of the system, and surprisingly fragile components in other parts. The importance of interrogating this system in order to identify risk of neoplastic transformation in populations is amply shown in this month's issue of *Cancer Epidemiology Biomarkers and Prevention*. Of the 40 manuscripts published in this issue, 7 (18%) address associations between DNA-repair enzyme expression or genotype, and cancer risk. The cancers addressed are diverse—glioma (4), colon (5), breast (6), prostate (7), bladder (8), and head and neck cancers (9). Of this list, only two are studies of the most ubiquitous environmental carcinogen, tobacco smoke.

Recent comments and publications have highlighted the investigative challenges of detecting gene-environment interactions using DNA-repair genotypes or phenotypes as a model (10-13). To recognize the key known participants in the DNA-repair system, it is important to briefly review the key components of the process. This provides data on how best to interrogate the process with the goal of cancer-risk assessment or cancer detection profiles.

Biochemical Aspects of DNA Repair

Direct Damage Reversal. Direct damage reversal by a lesion-specific enzyme is the simplest DNA-repair strategy, and it is very efficient. However, because of its very high (DNA-lesion) specificity, it is limited to a very small number of DNA lesions. Humans correct the miscoding lesion O^6-methylguanine caused by alkylating agents through a specific methyltransferase (O^6-methylguanine-DNA-methyltransferase; MGMT) that removes the offending methyl group from the DNA guanine residue. The process is rapid and error-free, however, the methyl group is transferred to a cysteine residue on the methylguanine, resulting in inactivation of the enzyme.

Base Excision Repair. The base excision repair pathway manages damage involving small modifications of DNA bases (e.g., methylation and oxidation), which usually originate from endogenous events such as spontaneous loss of purine residues at 37°C, oxidation by reactive oxygen species, and reaction with active cellular metabolites. The pathway is initiated by excision of an altered DNA base in free form by 1 of at least 10 DNA glycosylases, each recognizing several structurally related altered bases. The glycosylases cleave the base-deoxyribose glycosylic bond of the damaged nucleotide residue, thereby generating an abasic site, which is further cleaved by either the lyase activity of bifunctional glycosylases or by APE1 endonuclease that cleaves the chain on the 5′ site of the abasic site. Next, XRCC1, which serves as a scaffold protein, recruits DNA polymerase β and DNA ligase, which complete the repair process. The DNA ligase III portion of the XRCC1-DNA ligase III heterodimer completes the repair for shorter DNA patches. Longer segments of DNA are repaired by DNA polymerase β, δ or ε, and DNA ligase I.

Nucleotide Excision Repair. Nucleotide excision repair is a multiprotein process, with a very broad DNA damage specificity. It manages primarily bulky, helix-distorting lesions, caused by external stressors such as UV light or chemicals [e.g., benzo(*a*)pyrene]. The damaged site is recognized by several proteins including the xeroderma pigmentosum-C (XP)C-HR23B, and the XPA-replication protein A complexes which, together with the TFIIH complex, transiently unwind the duplex creating an open bubble structure around the lesion. The TFIIH complex contains two DNA helicases, XPB and XPD, which catalyze this unwinding, creating a ssDNA which is cut by two incision enzymes: ERCC1-XPF which cuts the 5′ side, and XPG which cuts the 3′ side of the lesion. A 24- to 32-oligonucleotide is released, and the gap is filled by DNA polymerase δ or ε, with the help of replication factor C and proliferating cell nuclear antigen. The repair is sealed by DNA ligase I.

Repair of Strand Breaks. Single DNA strand breaks, commonly associated with reactive oxygen species, are detected by the poly(ADP-ribose) polymerases PARP1 and PARP2, and require nucleolytic processing of the DNA ends. PARP1 interacts with XRCC1 and recruits repair enzymes in the base excision repair pathway. Double-strand breaks (DSB) are commonly generated by ionizing radiation, and activate the DNA-damage response via the kinases ATM and ATR. They are repaired primarily by nonhomologous end joining, which requires DNA-dependent protein kinase, as well as DNA ligase IV and XRCC4. Double-strand breaks could also be repaired by homologous recombination in a process that requires the MER11-RAD50-NBS1 complex, the Rad51 recombinase, as well as the RAD52 and RAD54 proteins.

Mismatch Repair. The mismatch repair system manages replication errors. Two heterodimers [MSH2/MSH6 (MutSα) and MSH2/MSH3 (MutSβ)] recognize single-base mismatches and small (1-40 bases) loops, respectively. Another

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Table 1. Summary of publications focused upon DNA repair in this issue of *Cancer Epidemiology Biomarkers and Prevention*

Author	Clinical target	Design	# Subjects	DNA repair target(s)	Assay technology
Wiencke et al. (4)	glioma	molecular association	260 cases	MGMT	genotype
Wark et al. (5)	colon	longitudinal cohort	929 cases/3,264 controls	hMLH1	immunohistochemistry
Kuschel et al. (6)	breast	pooled case-control	3,634 cases/3,340 controls	ERCC2	genotype
Ritchey et al. (7)	prostate	case-control	162 cases/251 controls	XRCC1, MGMT, XPD	genotype
Lin et al. (8)	bladder	case-control	89 cases/89 controls	nonspecific	functional
Huang et al. (9)	head and neck	pooled case-control	555 cases/792 controls	MGMT, XRCC1, XPD, XRCC2	genotype
Hu et al. (20)	multiple cancers	metaanalysis	11,957 cases/14,174 controls	XRCC1	genotype

heterodimer (MLH1/PMS2 primarily or MLH1/MLH3 secondarily) stabilizes the complex, and together with exonuclease 1, proliferating cell nuclear antigen and DNA polymerases δ and ε, completes excision and resynthesis.

Translesion DNA Synthesis. The translesion DNA synthesis system manages replication-blocking DNA lesions that have escaped error-free repair. It is carried out by low-fidelity DNA polymerases that are specialized for synthesis across DNA lesions, e.g., DNA polymerase η (product of the *XPV* gene) that effectively, and relatively accurately, replicates across UV light–induced pyrimidine dimers. Translesion DNA synthesis is fundamentally an error-prone DNA-repair pathway, but the presence of multiple translesion DNA synthesis polymerases and tight regulation seem to ensure that mutation frequencies due to translesion DNA synthesis are kept under control.

Translating DNA Repair Biology to Cancer Prevention in Humans

This brief review of DNA repair facilitates appreciation of the potential opportunities posed by this complex but biologically elegant system. The importance of mutations in key genes coding for DNA repair enzymes in the carcinogenesis process is emphasized by the accelerated development of cancer in the colon of individuals with hereditary nonpolyposis colon cancer who have inherited mutations in mismatch repair genes (14). Similarly, genomic instability phenotypes that result from defective helicases are associated with multiple malignancies in Bloom syndrome (15, 16), and the nucleotide excision repair–defective XPA to XPG forms of xeroderma pigmentosum are associated with skin and squamous cell cancers (17-19). Despite the scientific importance and clinical interest in rare cancer syndromes associated with highly penetrant mutations, the large majority of cancer incidence cannot be directly traced to a single gene product or a key mutation, but rather to a complex gene-environment interaction.

Table 1 summarizes the target enzyme, cancer site, and analytic method in the papers published in this issue of *Cancer Epidemiology Biomarkers and Prevention*. Four of the seven papers used genotyping methods to examine association between DNA-repair genes and cancer risk (6, 7, 9, 20). These join a large number of publications using a similar approach, including the application of refined quantitative tools to deal with the uncertainty of false-positives in genotype-based tests that necessarily miss other components in a complex system (11, 12). In essentially all these cases, associations between a particular single nucleotide polymorphism (SNP) in a DNA-repair gene and cancer were weak or nonexistent. In contrast, stronger associations are found between DNA repair and cancer risk when functional assays are used. This is illustrated by one paper published in this issue (8), which uses a modified host-cell reactivation assay (a measure of nucleotide excision repair), and by previous studies using the host-cell reactivation assay (21), or the OGG assay, which measures the removal of 8-oxoguanine from DNA by 8-oxoguanine DNA *N*-glycosylase (22). This raises the question of where should our efforts be directed, SNP analysis in DNA-repair genes, or functional tests of DNA repair?

The two main advantages of analyzing genetic polymorphism are simplicity and applicability to all tissues because the genetic change is in the germ line. These are powerful advantages that enable large-scale studies using high-throughput techniques. However, the SNP approach suffers from considerable disadvantages, which are often underestimated. Most importantly, a single mutation in a particular gene is often a poor predictor of the integrity of an entire repair pathway. Prior to embarking upon more large-scale genotyping projects aimed at establishing risk of a specific malignancy, it might be important to ask: what are the key or most critical components of the major known DNA-repair enzymes that, when altered or disrupted, have significant functional consequences in the DNA-repair system? Which enzymes are most critical to overall function of the system? Should multiple SNPs be analyzed simultaneously, and relative risk estimated for a combined genotype?

Functional DNA-repair tests are fundamentally more powerful, but they suffer from two main disadvantages: they are more complicated than SNP analysis, and are usually not done in the target tissue. Currently, there is a very limited number of DNA-repair assays available for epidemiologic studies. This situation could change, with the development of high-throughput, cost-effective functional assays that interrogate critical portions of the DNA-repair system. For example, the functional assay for OGG1 activity (22) could represent a paradigm for other functional assays of the activity of key DNA glycosylases (23, 24) and, ultimately, permit the development of a panel for the rational interrogation of the base excision repair pathway. Other approaches, based on analysis of the levels of specific DNA lesions in the genome (13), which evaluate the efficiency of DNA-repair processes, might also be effective.

Precise functional assays of DNA repair are urgently needed for two reasons: (*a*) they will enable genotype-phenotype correlation, thereby enabling the identification of those SNPs that are important, and (*b*) they may be used themselves as powerful screening tools in the quest to identify individuals at higher risk of common epithelial malignancies as the result of DNA-damaging environmental exposures.

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