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DNA Repair and Mutagenesis: From Molecular Mechanisms to Cancer Risk

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Genomic DNA is constantly damaged by both external and internal agents. Failure to repair DNA can cause severe biological consequences, including cancer, immunodeficiency, premature aging, and neurodegeneration. DNA lesions that have escaped repair are tolerated by translesion DNA synthesis (TLS), also termed translesion replication or error-prone DNA repair. This reaction is carried out by a novel class of specialized DNA polymerases, discovered in 1999 in our, and in other laboratories. The research in our laboratory focuses on the mechanism of TLS, on recombination repair, and on error-free DNA repair mechanisms and their involvement in cancer.

Translesion DNA Synthesis and Mutagenesis in Bacteria and in Humans

Mutagenesis by DNA damaging agents in *E. coli* is regulated by the SOS stress response. The *in vitro* reconstitution of SOS mutagenesis with purified components in our lab led us to the discovery of a novel DNA polymerase, termed DNA polymerase V, which has a remarkable ability to replicate lesions that block other DNA polymerases. Pol V was shown to be highly mutagenic when replicating undamaged DNA, with a particularly high level of transversions. Remarkably, pol V can replicate across non-DNA segments consisting of a hydrocarbon chain of 3-12 methylene residues. TLS by pol V requires the accessory proteins RecA and single-strand binding protein, and it is stimulated by the β subunit DNA sliding clamp. The mechanistic role of these proteins in the TLS reaction is being investigated.

Mammalian TLS, which involves multiple specialized TLS DNA polymerases, is studied using several approaches: (1) *In vitro* analysis with purified mammalian DNA polymerases. (2) *in vivo* analysis, using cells in culture. (3) Identification and analysis of proteins that interact with TLS DNA polymerases. A quantitative assay for TLS in cultured cells was developed. The assay is based on the transient transfection of cultured cells with a gapped plasmid, carrying a site-specific lesion in the gap region (Fig. 1). Using this method the basic features of *in vivo* TLS are being studies, including DNA damage specificity, and regulation of the activity of TLS DNA polymerases.

Bacterial and Mammalian Gap-Filling Recombination

Replication gaps caused by DNA lesions can be filled-in not only by TLS, but also by homologous recombination, depending on an intact sister

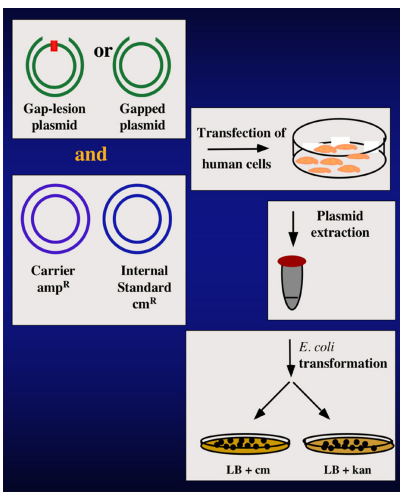

Fig. 1 *Quantitative translesion DNA synthesis assay for cultured cells. A gapped plasmid carrying a site-specific DNA damage (red square) is introduced into mammalian cells along with normalizing and carrier plasmids. After gap filling by TLS in the cells the plasmid content is isolated, and covalently closed circular plasmids are introduced into an E.coli tester strain, followed by plating in parallel on plates containing kanamycin (to select for the reporter plasmid), or chloramphenicol (to select for the normalizing plasmid).*

chromatid. This process of gap-filling homologous recombination repair (HRR) is investigated in *E. coli* and in mammalian cells. A bi-plasmidic assay system was developed, in which both TLS and HRR can be assayed simultaneously. Using this system evidence was provided, for the first time, that gaps are repaired in *E. coli* by HRR. Moreover, in this system HRR predominated over TLS, and was responsible for at least 85% of the gap-filling events. A similar system is currently used to examine whether HRR repairs gaps in mammalian cells.

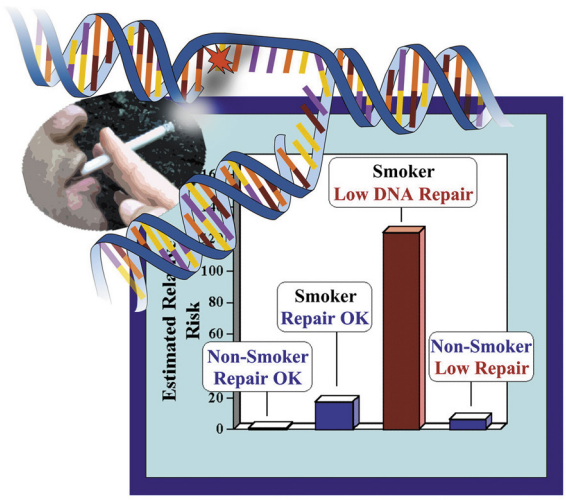

Fig.2 *Estimated relative risk for non-small cell lung cancer caused by low DNA repair OGG activity, smoking, or both. The combination of smoking and low OGG activity is associated with greatly increased estimated relative risk for lung cancer. DNA repair in the figure refers to OGG activity*

DNA Repair as a Risk Factor in Human Sporadic Cancer

DNA repair has emerged in recent years as a critical factor in cancer pathogenesis, as a growing number of cancer predisposition syndromes were shown to be caused by mutations in genes involved in DNA repair and the regulation of genome stability. These include the XP genes, DNA mismatch repair genes, the breast cancer BRCA1 and BRCA2 genes, and p53. However, there is a paucity of data on the role of inter-individual variations in DNA repair in susceptibility to sporadic cancer. We have developed a new blood test for the activity of the DNA repair enzyme 8-oxoguanine DNA N-glycosylase (OGG). Using this test we conducted a molecular epidemiology case-control study, and found that low OGG activity is a risk factor in non-small cell lung cancer. Moreover, a combination of smoking and low OGG caused extra-susceptibility to lung cancer. This test can be useful in prevention and early detection of lung cancer. Further studies are conducted in order to investigate the role of DNA repair in cancer risk.

Selected Publications

Avkin, S., Adar, S., Blander, G. and Livneh, Z. (2002) Quantitative Measurement of Translesion Replication in Human Cells: Evidence for Bypass of Abasic Sites by a Replicative DNA Polymerase. Proc. Natl. Acad. Sci. USA, 99, 3764-3769.

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