DNA Repair & Mutagenesis: From Molecular Mechanisms to Cancer Risk

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Genomic DNA is constantly damaged by external, as well as 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, or by homologous recombination repair (HRR). TLS is carried out by a universal 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 Humans

The key feature of TLS DNA polymerases is their ability to replicate across DNA lesions that block replication. This capability is attributed in part to a loose and flexible active site, which makes the polymerases highly mutagenic. Therefore, the presence of multiple mutagenic polymerases in the cell requires tight regulation, to ensure their action only at the time, and place when they are required. Remarkably, this system evolved to provide tolerable mutation rate, and its malfunction can cause increased mutagenesis and cancer predisposition. This is highlighted by the human hereditary disease xeroderma pigmentosum variant (XPV), in which a deficiency in the TLS-specific DNA polymerase eta causes sunlight sensitivity, and a strong predisposition to skin cancer. The molecular mechanism of TLS and its regulation in mammals are studied using a combination of molecular biology, genetics, biochemistry and cell biology. A major and powerful tool is a quantitative assay for TLS in cultured cells, developed in our laboratory. The assay is based on the transient transfection of cultured cells with a gapped plasmid, carrying a site-specific lesion in the gap region. Using this method we found that the error-prone DNA repair system, despite its inherent mutagenic characteristics, is regulated in a manner that minimizes the burden of its mutational outcome. We suggested that this remarkable outcome is achieved by utilization of multiple mutagenic DNA polymerases, each having certain DNA damage-bypass specificity, which are all tightly regulated such that they act at the proper time and at the cognate damaged site in the DNA. Moreover we found that two of the main regulators of this activity are the tumor suppressor p53, and its target protein p21, which operate by controlling the access of the polymerases to the damaged site in DNA. This key regulation is mediated via interaction of p21 with the sliding DNA clamp PCNA, and the mono-ubiquitination of the latter, induced by DNA damaging agents (Fig. 1). In the absence of p53 and/or p21, error-prone repair gets out of control, and causes an increased mutation load. This has

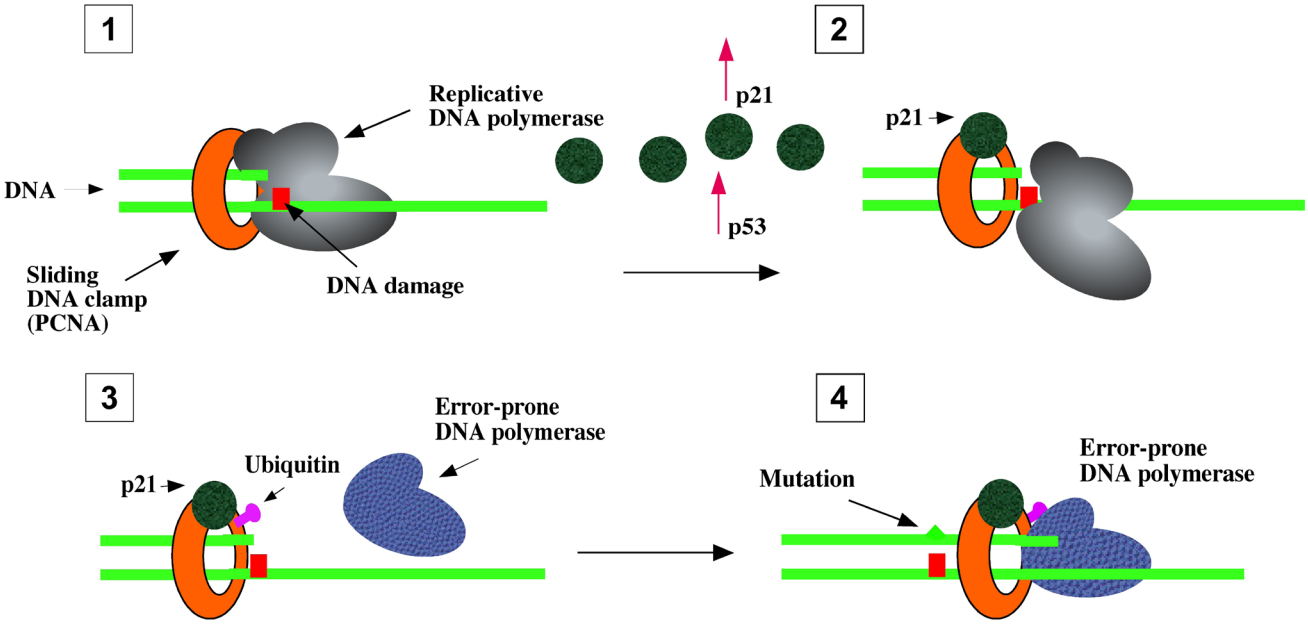

Fig. 1 *Regulation of translesion DNA synthesis (lesion bypass) by p53 and p21. The arrest of DNA replication at an unrepaired lesion leads to a stress signal that causes induction of p53. The p53 protein activates the synthesis of p21, which then binds PCNA, the sliding DNA clamp. This binding causes the dissociation of the replicative DNA polymerase from the lesion site, thereby exposing Lys164 in PCNA for monoubiquitination, and clears the way for binding of a TLS polymerase. The TLS polymerase then performs TLS across the damaged nucleotide.*

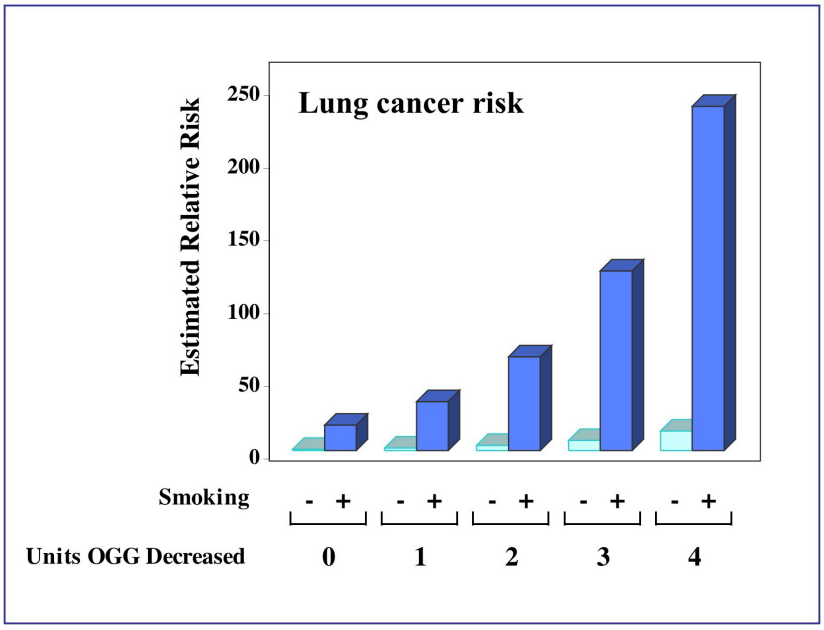

Fig. 2 *Estimated relative risk to develop lung cancer for smokers who have a low OGG DNA repair capacity. 'Units OGG Decreased' represents the decrease (in enzyme activity units) from the normal average OGG specific activity, which is 7.2 units/ug protein. Estimated relative risk for lung cancer is presented for smokers (dark blue bars) and non-smokers (pale blue bars) with low OGG activity.*

important implication for the regulation of carcinogenesis, suggesting that imbalances in the activity of error-prone polymerases facilitate mutagenesis and thereby carcinogenesis. It also defines a concrete mechanism for the 'guardian of the genome' function ascribed to p53.

The TLS assay is further used to study the operation principles of *in vivo* TLS, including DNA damage specificity, and regulation of the activity of TLS DNA polymerases. The study utilizes DNA substrates with single defined lesions, a variety of inhibitors, and genetically manipulated cells, via siRNA silencing, gene-knockout, and gene over-expression. Moreover, a novel method for studying TLS in the genome of mammalian cells is being developed.

DNA Damage Tolerance by Homologous Recombination Repair

DNA lesions that block replication or cause daughter strand gaps can be tolerated not only by TLS, but also by homologous recombination (HR), depending on the intact sister chromatid. If the impediment to replication leads to the formation of a double strand break (DSB), it is repaired primarily by non-homologous end joining (NHEJ), and to a lesser extent by homologous recombination repair (HRR). However, it is not known whether gaps or stalled forks can be repaired by HRR when the DNA is not broken. We investigate these topics using two main approaches. (a) We developed a bi-plasmidic assay system in which both TLS and HRR can be assayed simultaneously, and use it to examine whether HRR repairs gaps in mammalian cells. (b) We are developing a new method to study HRR at defined lesions in mammalian chromosomes.

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, MutYH, 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 blood test for the activity of the DNA repair enzyme 8-oxoguanine DNA glycosylase (OGG). Using this test we conducted two molecular epidemiology case-control study, and found that low OGG activity is a risk factor for non-small cell lung cancer, and for head and neck cancer. Moreover, a combination of smoking and low OGG caused extra-susceptibility to lung cancer (Fig. 2). Similarly, the combination of smoking and low OGG activity caused increased susceptibility to head and neck cancer. This test can be useful in prevention and early detection of cancer in general and lung cancer in particular. Current research is conducted to investigate the role of additional specific DNA repair pathways in cancer risk.

Selected publications

Paz-Elizur, T., Krupsky, M., Blumenstein, S., Elinger, D., Schechtman, E. and Livneh, Z. (2003) Reduced DNA repair activity for oxidative damage and the risk of lung cancer. J. Natl. Cancer Inst., 95, 1312-1319.

Maor-Shoshani, A. Ben-Ari, V. and Livneh, Z. (2003) Lesion bypass DNA polymerases replicate across non-DNA segments. Proc. Natl. Acad. Sci. USA, 100, 14760-14765.

Covo, S., Blanco, L. and Livneh, Z. (2004) Lesion bypass by human DNA polymerase mu reveals a template-dependent sequence-independent nucleotidyl transferase activity. J. Biol. Chem., 279, 859-865.

Avkin, S., Goldsmith, M., Velasco-Miguel, S., Geacintov, N., Friedberg, E. C. and Livneh. Z. (2004) Quantitative Analysis of translesion DNA synthesis across a benzo[a]pyrene-Guanine Adduct in Mammalian Cells: The Role of DNA polymerase kappa. J. Biol. Chem. 279, 53298-53305.

Paz--Elizur, T., Krupsky, M., Elinger, D., Schechtman, E. and Livneh, Z. (2005) Repair of the oxidative DNA damage 8-oxoguanine as a biomarker for lung

cancer risk. Cancer Biomarkers 1, 201-205.

Livneh, Z. (2005) UmuC, D Lesion Bypass DNA Polymerase V. In: Encyclopedia of Biological Chemistry , (eds. W. Lennarz and M. D. Lane), Elsevier Inc. San Diego.

Paz-Elizur, T., Brenner, D. and Livneh, Z. (2005) Interrogating DNA Repair in Cancer Risk Assessment. Cancer Epidemiology Biomarkers & Prevention. 14, 1585-1587.

Livneh, Z. (2006) Mechanism of translesion DNA synthesis in E. coli. In: DNA Damage Recognition, (eds. W. Siede, Y. W. Kow and P. W. Doetsch), Marcel Dekker Inc. New York, pp. 495-506.

Adar, S. and Livneh, Z. (2006) Translesion DNA Synthesis Across Non-DNA Segments in Cultured Human Cells. DNA Repair, 5, 479-490.

Avkin, S., Sevilya, Z., Toube, L., Geacintov, N., Chaney, S., Oren, M. and Livneh, Z. (2006) p53 and p21 regulate error-prone DNA repair to yield a lower mutation load. Mol. Cell, 22, 407-413.

Livneh, Z. (2006) Keeping mammalian mutation load in check: Regulation of the activity of error-prone DNA polymerases by p53 and p21. Cell Cycle, 5, 1918-1922.

Paz-Elizur, T., Ben-Yosef, R., Elinger, D., Vexler A., Krupsky, M., Berrebi, A., Shani, A., Schechtman, E., Freedman, L. and Livneh, Z. (2006) Reduced repair of the oxidative 8-oxoguanine DNA damage and risk of head and neck cancer. Cancer Res., 66, 11683-11689.

Paz-Elizur, T., Elinger, Blumenstein, S., Krupsky, M., Berrebi, A. Schechtman, E. and Livneh, Z. (2007) Development of an enzymatic DNA repair test for molecular epidemiology studies: distribution of OGG activity in healthy individuals. DNA Repair, 6, 45-60.

Izhar, L., Goldsmith, M., Dahan, R., Geacintov, N., Lloyd, R., and Livneh, Z. (2008) Analysis of strand transfer and template switching mechanisms of DNA gap repair by homologous recombination in E. coli: predominance of strand transfer. Submitted.

Arad, G., Hendel, A., Curth, U., Urbanke, C. and Livneh, Z. (2008) Single-Stranded DNA-Binding Protein Recruits DNA polymerase V to Primer Termini on RecA-Coated DNA. J. Biol. Chem. , 283, 8274-8282.

Paz-Elizur, T., Sevilya, Z., Leitner-Dagan, Y., Elinger, D., Roisman, L., and Livneh, Z. (2008) DNA repair of oxidative DNA damage in human carcinogenesis: Potential application for cancer risk assessment and prevention. Cancer Letters. In press.

Shachar, S., Avkin, S., Adar, S., Wittschieben, J., Chaney, S. G., Friedberg, E. C., Wang, Z., Carell, T., Geacintov, N., Wood, R. D. and Livneh, Z. (2008) Evidence for a general and REV3L-dependent two-polymerase error-prone DNA repair pathway in mammalian cells. In preparation.

Hendel, A*., Ziv, O*., Gruenger, Q., Geacontov, N. and Livneh Z. (2008) Reduced efficiency and increased mutagenicity of TLS across a T-T cyclobutyl pyrimidine dimer, but not a T-T 6-4 photoproduct in human cells lacking DNA polymerase eta. Submitted.

Covo, S. and Livneh, Z. (2008) Hierarchy in processing complex double strand breaks in mammalian cells. In preparation.

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