ROLE OF OXIDATIVE DNA DAMAGE IN GENOME INSTABILITY AND CANCER

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Inactivation of mismatch repair (MMR) is associated with a dramatic genomic instability that is observed experimentally as a mutator phenotype and microsatellite instability (MSI). It has been implicit that the massive genetic instability in MMR defective cells simply reflects the accumulation of spontaneous DNA polymerase errors during DNA replication. We recently identified oxidation damage, a common threat to DNA integrity to which purines are very susceptible, as an important cofactor in this genetic instability. In addition MMR is capable to recognize and transform O6-methylguanine (O6-meG), a DNA adduct induced by methylating agents including several chemotherapeutic drugs, into a lethal lesion. This aberrant processing by MMR renders tumors with defects in this pathway extremely resistant to killing induced by methylating drugs. Since these findings have important mechanistic and clinical implications, we proposed to extend our studies on the role of MMR as well as of other DNA repair pathways in controlling genetic instability using *in vitro* as well as *in vivo* animal models.

Role of oxidized bases in mutation formation

2-Hydroxyadenine (2-OH-A), a product of DNA oxidation, is a potential source of mutations. We investigated how representative DNA polymerases from the A, B and Y families dealt with 2-OH-A in primer extension experiments. A template 2-OH-A reduced the rate of incorporation by DNA polymerase alpha (Pol alpha) and Klenow fragment (Kf(exo-)). Two Y family DNA polymerases, human polymerase eta (Pol eta) and the archeal Dpo4 polymerase were affected differently. Bypass by Pol eta was very inefficient whereas Dpo4 efficiently replicated 2-OH-A. Replication of a template 2-OH-A by both enzymes was mutagenic and caused base substitutions. Dpo4 additionally introduced single base deletions. Thermodynamic analysis showed that 2-OH-A forms stable base pairs with T, C and G, and to a lesser extent with A. Oligonucleotides containing 2-OH-A base pairs, including the preferred 2-OH-A:T, were recognized by the human MMR complex MutSalpha. MutSalpha also recognized 2-OH-A located in a repeat sequence that mimics a frameshift intermediate (Barone *et al*., 2007).

Overexpression of a non-phagocytic NADPH-oxidase complex: a model for studying chronic exposure to oxidative stress in mammalian cells

The production of reactive oxygen species (ROS) in mammalian cells is tightly regulated because of their potential to damage macromolecules, including DNA. To investigate possible

links between high ROS levels, oxidative DNA damage, and genomic instability in mammalian cells, we established a novel model of chronic oxidative stress by co-expressing the NADPH oxidase human NOX1 gene together with its cofactors NOXO1 and NOXA1. Transfectants of MMR-proficient HeLa cells or MMR-defective $Msh2^{-/-}$ mouse embryo fibroblasts overexpressing the hNOX1 complex displayed increased intracellular ROS levels. In one HeLa clone in which ROS were particularly elevated, reactive nitrogen species were also increased and nitrated proteins were identified with an anti-3-nitrotyrosine antibody. Overexpression of the hNOX1 complex increased the steady-state levels of DNA 8-oxo-7,8-dihydroguanine (8-oxodG) and caused a threefold increase in the HPRT mutation rate in HeLa cells. In contrast, additional oxidatively generated damage did not affect the constitutive mutator phenotype of the Msh2-/- fibroblasts. Because no significant changes in the expression of several DNA repair enzymes for oxidative DNA damage were identified, we suggest that chronic oxidative stress can saturate the cell's DNA repair capacity and cause significant genomic instability (Chiera *et al*., 2008).

Role of DNA repair pathways in the *in vivo* protection against oxidative DNA damage

Several human neurodegenerative disorders are characterized by the accumulation of 8-oxodG in the DNA of affected neurons. This can occur either through direct oxidation of DNA guanine or *via* incorporation of the oxidized nucleotide during replication. Hydrolases that degrade oxidized purine nucleoside triphosphates normally minimize this incorporation. hMTH1 is the major human hydrolase. It degrades both 8-oxodGTP and 8-oxoGTP to the corresponding monophosphates. To investigate whether the incorporation of oxidized nucleic acid precursors contributes to neurodegeneration, we constructed a transgenic mouse in which the human hMTH1 8-oxodGTPase is expressed. hMTH1 expression protected embryonic fibroblasts and mouse tissues against the effects of oxidants. Wild-type mice exposed to 3-nitropropionic acid develop neuropathological and behavioural symptoms that resemble those of Huntington's disease. hMTH1 transgene expression conferred a dramatic protection against these Huntington's disease – like symptoms, including weight loss, dystonia and gait abnormalities, striatal degeneration, and death. In a complementary approach, an *in vitro* genetic model for Huntington's disease was also used. hMTH1 expression protected progenitor striatal cells containing an expanded CAG repeat of the huntingtin gene from toxicity associated with expression of the mutant huntingtin. The findings implicate oxidized nucleic acid precursors in the neuropathological features of Huntington's disease and identify the utilization of oxidized nucleoside triphosphates by striatal cells as a significant contributor to the pathogenesis of this disorder (De Luca *et al*., 2008).

Role of DNA repair of methylated bases in tumor response to chemotherapy

Tumor resistance to cytotoxic chemotherapy drugs and their toxicity to normal cells are major clinical obstacles to anticancer therapy effectiveness. Alterations in various DNA repair pathways play a key role in the development of both mechanisms of drug resistance and toxicity. Since deregulation of the DNA damage response and alterations in DNA repair

pathways are relatively common in human cancer, the knowledge of these alterations in cancer cells would be an important predictive factor for the clinical response to chemotherapy and a useful guide in designing an appropriate therapeutic strategy. We examined how inactivation of MMR and the O(6)-methylguanine-DNA-methyltransferase (MGMT) repair protein might affect the response of tumor cells to chemotherapy, with a special emphasis on agents inducing methylation and oxidative DNA damage and interstrand DNA cross-links (ICLs). In addition, we provide novel experimental evidence indicating that MMR is required for efficient repair of ICLs via stabilization of RAD51 containing repair intermediates. Finally, we discuss possible emerging therapeutical strategies for treating MMR-defective tumors (Casorelli *et al*., 2008).

Publications of the project

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