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ROLE OF OXIDATIVE DNA DAMAGE IN GENOME INSTABILITY AND CANCER

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Mismatch repair defects are associated with tumors in the Hereditary Nonpolyposis Colorectal Cancer syndrome as well as in a substantial fraction of non-familial colorectal and other cancers. Inactivation of MMR is associated with a dramatic genomic instability that is observed experimentally as a mutator phenotype and microsatellite instability (MSI). In clinical samples, MSI is considered to be diagnostic for inactive MMR. The phenomenon of inactive MMR in tumors is therefore of interest to a wide cross-section of researchers as well as being of practical importance to clinicians. 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 particular, oxidized purine deoxynucleoside triphosphates - the precursors of DNA replication - are significant contributors to the mutator phenotype and genetic instability of MMR deficient cells. Since these findings have important mechanistic and clinical implications, we will extend our studies using biochemical and biophysical assays as well as *in vivo* animal models.

a) *In vitro* assays: Using template/primers and purified DNA polymerases, we will investigate how a template 8-hydroxyguanine (8-oxoG) in known target sequences influences the fidelity of DNA replication. Because we have identified the oxidized dNTP pool as a potential contributor to genetic instability in MMR-defective cells, we will also examine the influence of 8-hydroxy deoxyguanosine triphosphate on the efficiency and fidelity of replication in unmodified template/primer systems of similar sequences. to extend these studies to polymerases with different fidelities – particularly those suspected of involvement in lesion bypass. Although common 8-oxoG containing base pairs are not recognized by MMR, we plan to investigate, by standard band shift assays, whether a repetitive sequence context influences recognition by the MutS mismatch binding factor. Our previous work implicated oxidized adenine bases in oxidation-related mutations – either base substitutions or frameshifts in repeated A tracts such as *BAT26*. We plan to investigate the effects of template 2-hydroxyadenine (2-OH-A) within A repeats using the approach outlined above.

b) *In vivo* assays: We will address the contribution of reactive oxygen species (ROS) to mutator phenotype and microsatellite instability by expressing a transfected NADPH oxidase gene (*Mox)* in MMR deficient cells. Mox-expressing cells produce high levels of ROS and suffer high levels of oxidative DNA damage. The influence of Mox expression on mutation rates will be determined in established human tumor cell lines defective in the MutL MMR complex as well as in an *msh2*$^{-/-}$ mouse embryo fibroblast cell line established in our laboratory. The availability of Mox overexpressing cells also provides an opportunity to examine the abilities of known anti-oxidants (for example N-acetylcysteine or NSAIDs) to provide protection against genetic instability in MMR deficient human tumor cells.

c) Mouse models: In order to evaluate the contribution of the oxidized dNTP pool to the overall burden of DNA 8-oxoG and to tumorigenesis, we are currently constructing a new animal model in which *hMTH1* is overexpressed in the *msh2*$^{-/-}$ mouse. These mice will be used to examine the effects of improved pool sanitation on end-points such as tissue specific accumulation of DNA 8-oxoG and tumor incidence. Oxidative DNA damage is a by-product of tissue inflammation. It is implicated in colitis and colitis–associated colorectal cancer. Colitis can be induced in mice by short-term administration of dextran sulfate in the drinking water. The hMTH1 transgenic mice will also provide a useful tool with which to study the contribution of dNTP pool-derived oxidative DNA damage to inflammation and colitis–associated colorectal cancer.