Peripheral Blood 6-Thioguanine Resistant (TG^R) Mutant T Cells Are Biomarkers for Monitoring Human Chemoprevention Trials

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Abstract The TG^R mutagenesis assay has proven useful to monitor genetic damage arising *in vivo* in human and rodent T lymphocytes. The assay detects mutation events at the X-linked *hprt* gene, the product of which is important for purine salvage. Mutants at the *hprt* locus are unable to convert TG to toxic derivatives and survive in concentrations of TG that kill wild-type cells. Molecular changes in $HPRT^-$ mutants include deletions, insertions, rearrangements, and point mutations involving base substitutions, frameshifts, and deletions or insertions. Therefore, molecular mutational spectra can be defined, as can the chemopreventive reversion toward background of induced mutations. Mutant frequencies (Mfs) at the *hprt* locus increase after human exposure to environmental mutagens such as cigarette smoking, cytotoxic chemotherapeutic agents, ionizing radiation, or occupational chemicals. Thus, measurement of mutation in somatic recorder genes may be a convenient surrogate for the measurement of other cancer-causing genetic mutations. Recently, our laboratory compiled its experience with the *hprt* clonal assay in 232 normal individuals ranging in age from 19–80 years. This large data base was used to identify elements which should be considered in studies of populations exposed to mutagens and to quantify the interdependent relationship between three important factors: namely Mf, age, and cloning efficiency (CE) ($\ln Mf = 1.99 - 1.13\ CE + 0.016\ age$, $p < 0.001$). Further, we used the TG^R mutagenesis assay in a prospective, longitudinal study to identify factors that modulate genetic damage in breast cancer patients. A total of 107 women (49 with breast cancer, 52 with benign breast masses, and 6 normal women) were enrolled. Mf in the *hprt* locus increased with age and a history of cigarette smoking. As expected, TG^R mutants were induced by adjuvant chemotherapy, especially regimes containing alkylating agents. However, these induced Mfs were inversely related to serum folate levels, and women with folate levels in the deficient range were more likely to have striking elevations of Mf. Subsequent *in vitro* studies with CHO cells indicated that folate deficiency acts synergistically with alkylating agents to increase Mf at the *hprt* locus because folate deficient cells are unable to fully repair DNA damage. Therefore, correction of vitamin deficiency by folic acid supplementation may be a cancer control strategy relevant to large populations. To test this possibility, we are proposing a clinical trial to the Eastern Cooperative Oncology Group in which patients with early stage breast cancer are randomized to folic acid or placebo. Blood folate levels and Mf at the *hprt* locus in lymphocytes will be measured before and after adjuvant chemotherapy. Folate status and the effect of folic acid supplementation will be correlated with measurements of *hprt* Mf to identify a relationship between folate levels and frequency of somatic mutation. A positive outcome of this chemoprevention trial would indicate that a non-toxic nutrient (folic acid) can ameliorate the genetic damage caused by mutagens. Similar measurements of mutations arising *in vivo* in the *hprt* "recorder" gene may provide the biomarker for other chemoprevention trials and thereby overcome the difficulty of studying prevention of diseases that are infrequent or have long incubation periods. This has already been done in rodents, where the radiation-induced increase in TG^R mutant lymphocytes was reduced substantially by a radioprotective agent which also protects the animals from cancer. © 1993 Wiley-Liss, Inc.