INTER-INDIVIDUAL VARIABILITY OF THE MUTATOR PHENOTYPE IN CELLS DERIVED FROM MUTYH-ASSOCIATED POLYPOSIS PATIENTS

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Oxidative stress causes different kinds of DNA damage, including double and single strand breaks and base modifications; 8-oxo-7,8-dihydroguanine (8-oxodG) is the most extensively studied type of oxidative damage because it is potentially mutagenic and it is implicated in carcinogenesis. DNA 8-oxodG codes ambiguously during replication and directs incorporation of C or A with almost equal efficiency, leading to G:C → T:A transversions. The MUTYH DNA glycosylase counteracts the mutagenic effects of 8-oxodG by removing A opposite the oxidized purine. Biallelic germ-line mutations in *MUTYH* cause the autosomal recessive MUTYH-associated adenomatous polyposis (MAP).

We previously identified a large variability in the spontaneous mutator phenotype associated with inactivation of the *MUTYH* gene in lymphoblastoid cell lines (LCLs) derived from MAP patients harbouring different mutations. Our aim is to investigate whether this variability depended on specific *MUTYH* mutations or the genetic background, so we characterized LCLs derived from MAP patients expressing the same variant. We focused our attention on two mutations, the Y179C and R245H variants. Six LCLs derived from homozygous and four heterozygous carriers were analysed for spontaneous and oxidant-induced mutation frequencies at the *PIG-A* gene. In addition levels of oxidative damage to DNA were provided by measurements of DNA 8-oxodG. Homozygous inactivation of MUTYH by either Y179C or R245H mutations resulted in increased spontaneously or $KBrO_3$-induced mutations frequencies. A certain degree of inter-individual variability was identified in LCLs expressing the same Y179C variant, with increases in spontaneous mutation frequency ranging from 2- up to 7.6-fold over wild-type cells. In contrast the two cell lines expressing the R245H mutation showed a similar 10-fold increase in spontaneous mutagenesis. Once exposed to $KBrO_3$-induced oxidative stress, the increase in mutations was similar in Y179C or R245H expressing LCLs. Finally inter-individual variability in the spontaneous mutator phenotype was also observed in LCLs expressing a single mutant Y179C allele, while both R245H carriers displayed mutation frequencies intermediate between wild-type and homozygous LCLs. We are presently setting up an enzymatic MUTYH assay in cell-free extracts from heterozygous carriers to clarify whether the level of MUTYH DNA glycosylase activity might be influenced by the presence of a mutant protein.