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Title of research project	Dose-response analysis in low doses of oxidative stress-inducing genotoxic substance
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【Abstract】

Reactive oxygen species (ROS) are produced through normal cellular metabolism, and the formation of such ROS is further enhanced by exposure to either ionizing radiation or various chemicals. ROS attack DNA and its precursor nucleotides, and consequently induce various oxidized forms of bases in DNA within normally growing cells. Among such modified bases, 8-oxo-7, 8-dihydroguanine (8-oxoG) is highly mutagenic lesion. Mechanisms for preventing *in vivo* mutagenesis/carcinogenesis are known to include suppression of metabolic activation, translesional DNA synthesis and DNA repair.

Nrf2 is an essential transcription factor for the expression of phase II drug-metabolizing enzymes and antioxidant proteins, and enhancement of ROS generation is observed in the organs of *Nrf2*-knockout mice. To examine the relationship between ROS generation and practical threshold, we assessed mutagenicity induced by potassium bromate ($KBrO_3$), a typical ROS generator, in the intestine of *gpt* delta (*Nrf2*(+/+)) mice and *Nrf2*-knockout (*Nrf2*(-/-)) *gpt* delta mice, which we bred by mating *gpt* delta mice with *Nrf2*-knockout mice. After administration of 0, 0.06, 0.2 and 0.6 g/L $KBrO_3$ in drinking water for 90 days, the significant increase of total mutant frequency (MF) was observed at a dose of 0.6 g/L in both *Nrf2*(+/+) and *Nrf2*(-/-) *gpt* delta mice. 0.2 g/L $KBrO_3$ was considered to be a practical threshold. Transversion of G:C to T:A increased in parallel to dose-dependent enhancement in production of 8-oxodG in *Nrf2*(+/+) mice. Generation of ROS was suggested to be a driving force to enhance in vivo mutagenesis, being a possible determinant of threshold.

MUTYH is a DNA glycosylase that excises adenine incorporated into opposite to 8-oxoG, thus preventing G:C to T:A transversions in mammalian cells. The Mutyh-deficient mice showed a marked predisposition to spontaneous tumorigenesis in various tissues when examined at 18 months of age. The incidence of adenoma/carcinoma in the intestine significantly increased in Mutyh-deficient mice, as compared with wild-type mice. This high susceptibility of the intestinal tumor-development was well correlated with the condition observed in MAP (MUTYH-associated polyposis) patients. The intestinal tumor susceptibility of Mutyh-deficient mice was further enhanced by treatment with $KBrO_3$, a known oxidative renal carcinogen associated with 8-oxo-G accumulations. Oral administration of $KBrO_3$ at a dose of 0.2% in drinking water dramatically increased the formation of intestinal tumors in Mutyh-deficient mice. Using this experimental system, we have been investigating the tumorigenic effect of $KBrO_3$. With relevance to the assessment of health risks, the exposure to lower dose in the range of 0.05% to 0.1% of $KBrO_3$ reduced the frequency of intestinal tumor formation in Mutyh-deficient mice. These results suggest that cells are able to correctly repair oxidative DNA lesions resulting from exposures to a certain level of low doses of endogenous and exogenous

chemicals with oxidizing property, and thus are less likely to be transformed to the neoplastic phenotype.

DNA polymeraseζplays an important role in DNA synthesis across DNA damage, i.e., translesion DNA synthesis (TLS), and thus its genetic modifications may have significant effects on genotoxic threshold. In this study, we engineered human cells, i.e., D2781N cells expressing a variant form of DNA polymeraseζthat possesses much lower DNA polymerase activity than the wild-type enzyme, and L2618M expressing another variant form of DNA polymeraseζwhose fidelity of DNA replication is much lower than the wild-type enzyme, and compared the sensitivity to the genotoxicity of oxidative mutagens, i.e., $KBrO_3$ and sodium dichromate($Na_2Cr_2O_7$). D2781N exhibited the highest sensitivity among three cell types for the gene mutation and micronucleus formation induced by $KBrO_3$ and $Na_2Cr_2O_7$ while L2618M was the highest for sister chromatid exchange formation. These results suggest that DNA polymeraseζplays an important role in threshold of genotoxicity induced by oxidative mutagens and also that factors affecting the threshold may be different depending on the indexes of genotoxicity. Roles of the variant forms of DNA polymeraseζin the oxidative genotoxicity were discussed.