

W1-3 Establishment of simple *in vitro* Comet assay and its validation

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The Comet assay has been widely used to detect DNA damage in cells exposed *in vitro* or *in vivo* to a variety of chemicals. The Comet assay is getting popular as a genotoxicity test in industries, because it is rapid, simple, visual, and sensitive. In the conventional protocol, a small number of cells are sandwiched between thin layers of agarose on a slide glass, lysed, electrophoresed in alkaline solution, stained with a fluorescent dye, and then the DNA remnants are visualized by fluorescent microscope. We now modified the Comet protocol that increase productivity and expectation for genotoxicity, and validated its *in vitro* system. To improve the ease of manipulation, we directly place the cell-agarose suspension on a special coated slide glass (Matsunami MAS coat) without agarose sandwich. We took alkaline version as well as neutral version in electrophoreses, which enable to distinguish types of DNA damage. According to the protocol, we examined gamma-irradiation (DSB and oxidative damage inducer), EMS (alkilating agent), hydrogen peroxide (oxidative damage inducer), and potassium bromate (oxidative damage inducer) using human lymphoblastoid TK6 cells. The improved *in vitro* Comet assay would be useful not only for measuring the DNA damage, but also for elucidating the genotoxic mechanism.

簡便な*in vitro*コメット試験法の確立とその評価

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W1-4 Analysis of 8-hydroxydeoxyguanosine and 7-methylguanine in human urine

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The urinary levels of 8-hydroxydeoxyguanosine (8-OH-dG), a marker of oxidative DNA damage and 7-methylguanine (7mG), a marker of DNA methylation, of 361 healthy men were simultaneously measured by HPLC-ECD method using anion-exchange- and reverse-phase columns. By univariate analysis of categorical variables, it was found that fruit consumption ($p=0.03$) and intensity of daily physical activity ($p=0.03$) reduced the 8-OH-dG level. Regarding continuous variables, cigarettes smoked day ($p=0.011$), Brinkman index ($p=0.012$), alcohol drinking ($p=0.037$) and rest index ($p=0.037$) showed positive association with 8-OH-dG, while energy consumption ($p=0.025$) and BMI (0.028) showed negative association. Based on multi-regression analysis of log-transformed values, alcohol consumed per day ($p=0.003$) correlated with 8-OH-dG, while daily physical activity ($p=0.001$) and meal combination (0.022) showed negative correlation. Urinary 7mG correlated most strongly with cigarettes smoked per day and Brinkman index. Nitroso compound NNK in cigarette smoke may be involved in the formation of 7mG in DNA.

ヒト尿中の8-ヒドロキシデオキシグアノシン及び7-メチルグアニンの測定

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