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Collaborative study on the toxicogenomics in JEMS/MMS: Quantitative RT-PCR analysis on the selected genes by the GeneChip

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As a collaboration of JEMS/MMS, we have studied the use of DNA microarrays for mutation research. After the last JEMS meeting, we studied the effect of three non-genetic carcinogens (DEHP, CCl_4, and trichloroethyrene) on mouse liver using Affymetrix GeneChip and selected genes responsible for genotoxicity. The primers for the selected genes were designed and quantitative RT-PCR analysis was performed to confirm the GeneChip data and applied for the samples obtained from dose-dependent study on the DEN and ENU. In general, the results of RT-PCR and GeneChip analysis were matching, but RT-PCR gave greater changes than GeneChip. Dose-dependent increase was observed in gene expression for the several selected genes when treated with DEN and ENU. From these results, it is clear that RT-PCR analysis is much easier and less expensive method for gene expression analysis once the responsible genes were selected. The usefulness of the quantitative RT-PCR analysis on selected genes for the screening of genotoxicity will be discussed.

トキシコジェノミクスに関する共同研究：GeneChipにより選択した遺伝子を用いた定量的RT-PCR解析
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Identification of major mutagens in surface soil in Osaka and Aichi prefectures

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Three and two surface soil samples were collected in Osaka and Aichi prefectures, respectively, to identify the major mutagens. Organic extracts were prepared from the soil samples with a Soxhlet apparatus. All of the soil extracts showed potent mutagenicity in *S.typhimurium* TA98 in the absence of S9 mix, 1240 – 233000 revertants/mg of organic extract. Each soil extract was separated by chromatography using a Sephadex LH-20 column and a silica gel column. The fractions with potent mutagenicity were subsequently separated into five fractions, Fr. 1 – Fr. 5, by an Ultra pack ODS column. The contribution ratios of these five fractions to the total mutagenicity of each soil extract were similar; Fr. 1: 45%; Fr. 2: 15%; Fr. 3: 25%; Fr. 4: 5; Fr. 5: 10%. These five fractions were further separated by HPLC on a COSMOSIL $5C_{18}$ AR-II column and a Luna 5 μ Phenyl-Hexyl column. In consequence, it was found that mutagenic potencies of Fr. 1 and Fr. 3 were attributed to dinitropyrene and 3,6-dinitrobenzo[*e*]pyrene, respectively. Major mutagens in Fr. 2, Fr. 4, and Fr. 5 were isolated from each soil extract. The chemical structures of these major mutagens are now under investigation.

大阪府及び愛知県の表層土壌中の主要な変異原性物質の同定
渡辺徹志[1)]、長谷井友尋[1)]、麻野間正晴[2)]、若林敬二[3)]、平山晃久[1)] (京都薬科大学[1)]、名古屋市衛生研究所[2)]、国立がんセンター研究所[3)])