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FINE MAPPING OF LOH EVENTS IN SPONTANEOUS *TK*-MUTANTS OF THE HUMAN LYMPHOBLASTOIDE CELL LINE TK6.
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Loss of heterozygosity (LOH) is the major chromosomal mutational events of thymidine kinase (TK)-deficient human lymphoblastoide cell line TK6, which harbors the heterozygous thymidine kinase locus. Analysis of the LOH events with 10 microsatellite markers spanning over the whole chromosome 17 showed the LOH extended over half of 17q toward the terminal end (Honma, M., et al, Molecular Carcinogenesis, 28: 203 Ð 214, 2000). Here we report an improved system which allow to map more precisely the LOH events on the chromosome 17 of TK6 cells. To map the LOH events more detail, 38 microsatellite markers were selected from NCBI Gemome Database. The set of LOH markers allow us to map the LOH events on the chromosome 17 at average interval of 1.2 Mb. With the system, LOH events in 139 *TK*- mutants from the wild type cell line (20C) and 77 *TK*- mutants from the cell line deficient in *P53* function (5E) have been determined. The results can be summarized as followings; i) there was a hot region of LOH events showing ~3 times higher incidences than that of the over all average, ii) the *P53* function did not affect the spectrum of LOH, though the frequency of LOH was much higher in the *P53* deficient cell line (5E) than in the wild type cell (20C), iii) an unique hot-spot of LOH was found at the site 47.3 to 48.4 Mb, which is specific to the wild type cell line 20C. The results may suggest that there are preferred sites for LOH occurrence on the chromosome 17 which are independent of the cellular *P53* function.

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TESTS OF PARTICULATE NICKEL COMPOUNDS FOR GENOTOXICITY IN THE DROSOPHILA WING SPOT ASSAY.
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The metal carcinogen nickel was tested for genotoxicity in the somatic wing spot assay of *Drosophila melanogaster* in the form of three particulate compounds -- nickel oxide black, nickel oxide green and nickel subsulfide. Third instar larvae were exposed to the test compounds via a 6 hr acute exposure in which the larvae fed upon a cellulose powder and water slurry containing a particulate test compound. Nickel oxide green and nickel subsulfide were tested at a single concentration of 80 mM, while nickel oxide black was tested at 20, 40 and 80 mM concentrations. All experiments were conducted at 25°C. Both nickel oxide green and nickel subsulfide were evaluated as either negative or inconclusive in yielding mutant clones of any type. However, nickel oxide black was found to be a positive inducer of small single spots and total spots at 80 mM. A dose-response relationship also existed for nickel oxide black in its capacity to induce small single spots. Comparison of spot induction on wings of trans-heterozygous and inversion-heterozygous flies revealed that approximately 77% of the small single spots induced by nickel oxide black on trans-heterozygous wings involved a mitotic recombination event occurring between the marker genes *mwh* and *flr*. Nickel has previously been implicated in the spreading of heterochromatic regions in the genome via chromatin condensation. Twin spots in the wing assay arise from a mitotic recombination event occurring between the centromere and the nearby marker gene *flr*. The inability of nickel oxide black to induce twin spots in the wing assay may be due, at least in part, to such spreading of the heterochromatic centromere region.

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GENE EXPRESSION PATTERNS IN NORMAL HUMAN LIVER CELLS EXPOSED TO TETRACHLOROETHYLENE USING MICROARRAY ANALYSIS.
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Tetrachloroethylene (TCE), is an industrial solvent widely used in dry cleaning, textile-processing, degreasing fabricated metal parts and paint stripping. Occupational exposure to TCE can occur through inhalation, skin contact or ingestion during its use in dry cleaning and degreasing. Although there is limited evidence in humans for carcinogenicity of TCE, it is classified as a probable carcinogen to humans by International Agency for Research on Cancer. In this study, we have investigated TCE exposure and global gene expression pattern in normal human liver cells using microarray analysis. Exponentially growing cells were exposed to a final concentration of 200 and 400 μM of TCE for 12 h. Total RNA was used for the preparation of double stranded cDNA. Biotin labeled cRNA transcript was synthesized using cDNA, fragmented and hybridized to HuGeneFL GeneChip probe arrays representing more than 6800 human genes and expressed sequence tags. The arrays were stained with streptavidin-phycoerythrin and biotinylated anti-streptavidin antibodies. The differential gene expression data analysis was performed using GeneChip(r) 4.0 software. The 3'/5' cRNA transcript ratios for both GAPDH and α-actin were found to be consistent between control and treatment groups over a period of 12 h exposure. Altered gene expression patterns were observed in 194 RNA transcripts with at least a 2 fold change. Alterations in expression of some of the genes include *XRCC4, Rad51, Has2*, metalloproteinase, *MDM2* gene, TNF receptor, *RB1*, heat shock protein 70, TNF-receptor-1 associated protein (TRADD). The results indicate that TCE induces changes in expression of certain important cell cycle regulation genes. Whether these genes are directly involved in the process of TCE-induced carcinogenesis needs to be elucidated.

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GLOBAL PROFILING OF METHYLATION STATUS IN LUNG CANCER TISSUES.
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Aberrant DNA methylation pattern is an acquired epigenetic alteration causing inappropriate activation or silencing of a gene. Alterations in DNA methylation, mainly occurring in the CpG islands located in the 5' regulatory regions of genes, have been associated with cancers and represent one of the most consistent changes in neoplastic cells. To determine if alterations in global methylation may contribute to the development of lung cancer, we studied genome-wide aberrant methylation pattern in tissues obtained from 57 lung cancer cases. Methylation was carried out using methylation sensitive restriction DNA fingerprinting analysis. *BstU1* (sensitive enzyme for cytosine methylation at CpG site) and *Mse1* (non-sensitive enzyme for cytosine methylation) were used for restriction analysis. We found that 86% of all the lung cancer tissues were hypermethylated at various sites using short random primers. Many fragments appeared to be differentially methylated. Upon sub-cloning, sequencing and matching several common differentially methylated fragments using the available database (BLAST), we have identified the fragments to encode for human cyclin C (*CCNC*), Wilms tumor *(WT-1*) and nuclear factor-kappa B (*NF-kB*) genes. Analysis of fragments among tumor types revealed that 49% of the hypermethylated fragments were adenocarcinomas, 30% were squamous cell carcinomas and 21% belonged to other types. When age or sex was considered as a factor, no significant difference in any of these groups was observed. Methylation pattern was unrelated to smoking status of the patients. Our overall results indicate that hypermethylation seems to play an important role in the development of lung cancer. Further studies are in progress to elucidate the molecular mechanism(s) of hypermethylation in lung cancer.

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