441 MUTAGENIC STUDY OF 4-AMINOPYRIDINEE IN CHO CELLS BY USING SISTER CHRONATID EXCHAMGE ASSAY

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In the present study,the mutagenicity of 4-Aminopyridine (4-AP) was compared with mitomycin C (MMC) in Chinese hamster overy (CHO) cells by using sister chromatid exchanges (SCEs) assay. In this study, the lower concentrations of 4-AP ($\leq$ 0.1mM) did not appear significant increment of frequency of SCEs as compared with the control. However,the higher concentrations of 4-AP ($\geq$ 0.3mM) caused significant increment frequency of SCEs as MMC. Since 4-AP is a potent K^+-channel blocker, the relationship between K^+-entry blockade and mutagenicity at higher concentrations of 4-AP is worthy to do more detail study.

Key words:4-Aminopyridine, SCEs, CHO cells.

443 THE EFFECTS OF PROGESTINS ON REGULATION OF HUMAN PAPILLOMAVIRUS GENE EXPRESSION.

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Cervical Cancer is the leading tumor of female in Taiwan. Infection of human papillomavirus (HPV) was reported to be highly associated with cervical cancer. Gene products of HPV, E6 and E7, are detected in the cancerial cervix. E6 and E7 gene expression is drived by the long control region (LCR) which contains a progesterone responsive element (or glucocorticoid responsive element). Thus, progesterone derivatives would affect the production of E6 and E7, resulting in deleterious effects on the cervix.

We have established a CAT assay to assess the effects of progestins on regulation of HPV expression using a chloramphenicol gene chimerized with the LCR of HPV. A series of progesterone derivatives will be tested.

442 USING HIGH MOLECULAR WEIGHT ALKALINE PHOSPHATASE FOR DETECTION OF HEPATIC METASTASIS IN PATIENTS WITH COLORECTAL CANCER.

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We had previously reported that the diagnostic sensitivity of high molecular weight alkaline phosphatase (HiMwALP) for primary colorectal cancer (n=72) was 63% and our present data further showed that HiMwALP is also a useful marker for hepatic metastasis in colorectal cancer because of its high sensitivity and specificity. In this study, the enzymatic activity of HiMwALP in serum samples from patients with hepatic metastasis in colorectal cancer (n=22) was quantitated after polyacrylamide gel electrophoresis (PAGE) separation from the other isoenzymes. Determination of carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) by enzyme immunoassay, a frequently used cancer assessment method, and immunosuppressive acidic protein (IAP) by radial immunodiffusion, were used for comparison. Sera from patients bearing with hemorrhoids (n=38) were used as a reference group. HiMwALP activities (17 U/L) twice those of pooled normal sera (8.5 U/L) from 52 individuals were adopted as cutoff values for positive and negative division. The diagnostic sensitivity of HiMwALP for 22 patients with hepatic metastasis was 77.3% vs 70.6% for the CEA method and 64.3%, 57% for the AFP and IAP methods, respectively. The diagnostic specificities of HiMwALP and CEA method were around 90-95%. The mean and standard deviation of HiMwALP activity in group of hepatic metastasis was 84.2±95.2 U/L. This HiMwALP value of the cancer group was significantly different from that of the reference group with P value of <0.001. The median value of HiMwALP in the cancer group was 24.7 U/L while that of the reference group was 7.2 U/L. In conclusion, the significance of increased HiMwALP levels in both primary and metastatic colorectal cancers revealed that it may be considered as a potential tumor marker.

444 ISOLATION OF HUMAN 6-PYRUVOYL TETRAHYDROPTERIN SYNTHASE cDNA

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Phenylketonuria (PKU) is an inherited metabolic disorder of phenylalanine hydroxylation. Defects in tetrahydrobiopterin (BH_4) metabolism, a cofactor required in phenylalanine hydroxylation, or phenylalanine hydroxylase itself may cause different forms of PKU. BH_4 deficient PKU were found to account for approximate 20% PKU in Taiwan. Defect in 6-pyruvoyl tetrahydropterin synthase (6PTPS), the second enzyme involved in BH_4 biosynthesis, was found to be the most frequent form of BH_4 deficient PKU. In attempt to study the molecular defects of 6PTPS deficiency in Chinese, the cDNA of human 6PTPS was isolated by reverse transcription combined with polymerase chain reaction (RT-PCR). According to the reported cDNA sequence of human 6PTPS, the sense and antisense PCR primers were designed to amplify the coding region of human 6PTPS cDNA. Total RNA, isolated from T2 hepatoma cell line, was subject to synthesis the cDNA primed by the antisense PCR primer. After PCR amplification, a RT-PCR product was subcloned into pT7Blue vector for further identification. This cDNA was 575bp in size and was confirmed to contain the entire coding sequence of human 6PTPS cDNA by DNA sequencing. This human 6PTPS cDNA could be used to clone the full length cDNA, to study the restriction fragment length polymorphisms of this gene, and to illustrate the molecular defects of 6PTPS deficiency.

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