P365

STUDY ON COVALENTLY CLOSED CIRCULAR DNA OF HEPATITIS B VIRUS IN THE HEPG2 CELLS BY CRE-LOXP SYSTEM

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Viral persistence in chronic hepatitis B patients relies on maintaining a pool of the covalently closed circular DNA (cccDNA) of hepatitis B virus (HBV). However, how cccDNA is stably maintained is unknown. In this study, a stable clone (HR8) with the capacity to produce HBV cccDNA, which derived from HepG2 cells by transfecting with 1.3-fold HBV genome flanked by a directly repeated loxP sequence, was established. And we investigated the fate of cccDNA in the condition that integrated HBV genome was selectively removed. Following the introduction of the Cre recombinase-expressing plasmid that harbors a neo gene into HR8 cells, we found that the integrated HBV genome was selectively removed in that resulted in the lost of HBV gene expression. Re-transfected with 1.3-fold HBV genome into two such negative clones resulted in the detection of cccDNA production, suggesting that lost of HBV gene expression is not due to lost of the ability of cccDNA production in these cells. Taken together, our data suggest that cccDNA might not be able to maintain in culture cells.

P366

MUTATION ANALYSIS OF PCCB GENE IN CHINESE PATIENTS WITH PROPIONIC ACIDEMIA

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Propionic acidemia (PA, MIM 232000, 232050) is an inborn error of metabolism caused by propionyl CoA carboxylase (PCC, EC 6.4.1.3) deficiency. The functional PCC consists of two subunits, namely α and β subunit, which are encoded by PCCA and PCCB gene, respectively. Defect either in α or β subunit will cause PCC deficiency. In this study, 15 exons of the PCCB gene were PCR amplified and sequenced to analyze the mutation in Chinese PA families. Two novel mutations, designated c.491C>T and c.1301C>T, were identified in two PA patients. One of which was homozygote of c.491C>T mutation and the other one was homozygote of c.1301C>T mutation. Both patients were born in a non-consanguineous family. No other mutation was detected in the coding region and exon/intron boundary of PCCB gene for these 2 patients. Both c.491C>T and c.1301C>T transitions cause the substitution of Ala for Val, at codon 164 (A164V) and codon 434 (A434V), respectively. The c.491C>T and c.1301C>T transitions were not detected in 100 normal Chinese alleles. These data indicated the c.491C>T and c.1301C>T might be the disease causing mutations of PA patients. A STR marker, D3S3528 locating around 200kb upstream to PCCB gene, was analyzed to trace the transmission of c.491C>T and c.1301C>T in these two PA families. The heterozygosity of D3S3528 was found to be 37% in Chinese population. Both c.491C>T mutation in family 1 was linked to 272bp allele of D3S3528 while c.1301C>T mutation was linked to 270bp allele in family 2. The 270bp and 272bp allele of D3S3528 were found to be less frequent in normal Chinese population (11.0% and 6.1%, respectively). These data suggested that the c.491C>T and c.1301C>T mutation in Chinese PA patients might have founder effects.

P367

SPERMATOZOAN MORPHOLOGY OF FOUR SPECIES OF BIVALVE (HETERODONTA, VENERIDAE) FROM TAIWAN

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Using transmission and scanning electron microscopy, the mature spermatozoa of four bivalves of the family Veneridae *Gafrarium tumidum* and *Circe scripta* (Circinae), *Pitar sulfureum* (Pitarinae) and *Gomphina aequilatera* (Tapetinae) are described for the first time and compared with those of other bivalves, particularly other heterodonts. As our observations show the spermatozoa of these four species are of the primitive type or ect-aquasperm form. The head contains a slightly curved nucleus with a short cone-shaped acrosome. The structure of the acrosome is typical of heterodont bivalves and two major components of the acrosomal vesicle material can be distinguished. The midpiece exhibits four or five mitochondria which surround the proximal and the distal centrioles. Variation in the shape and dimensions of the acrosomal vesicle and nucleus is substantial in these four Veneroidea species. The sperm ultrastructure evidently may provides additional information to existing Veneridae phylogeny.

P368

A BACTERIOPHAGE AGAINST BURKHOLDERIA PSEUDOMALLEI IN SOILS

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Burkholderia pseudomallei, a causative agent of melioidosis, invade to human via people contact with infectious dust or soil. It has been demonstrated that incidence of melioidosis in endemic areas is correlated with isolation rate of *B. pseudomallei* from soil. In order to investigate the prevalence of *B. pseudomallei*, we accumulated and extracted total DNA from soils in southern Taiwan. With multiplex PCR assay, about 36% of total DNA were detected to be existence of *B. pseudomallei* DNA from soil samples in our studies. Nevertheless, only five strains of *B. pseudomallei* were isolated and identified from these PCR positive samples. We found a bacteriophage against *B. pseudomallei* specifically in soil filtrate, but not against *Burkholderia cepacia*, *Pseudomonas areuginosa*, *E. coil*, *Klbsiella oxycota* or *Citrobacter freundii*. This phage showed the temperature inducible bacteriolytic phenomena. These results may account for highly detection of *B. pseudomallei* DNA, but lacking isolation of this microorganism from soil in southern Taiwan

B21