These include LIMK1, STX1A, WBSCR1, RFC2, FZD3, GTF2I, and etc. No definitive role in the WS phenotype has been yet assigned to any of these genes. In this study, we present the results of deletion mapping in classic WS patients and SVAS patients with deletion but no other features of WS. To investigate the microdeletion at 7q11.23 in 8 WS and 9 SVAS patients, FISH was performed using 244H3 BAC clone that had been previously localized into the 500-kb region commonly deleted in WS. In FISH analysis, loss of heterozygosity (LOH) at ELN locus was found in eight WS patients and two SVAS patients. In addition, patial deletion in one SVAS patient, and no remaining deletion in six SVAS patients were also observed. RAG mouse cells were hybridized with lymphoblastoid cells of WS and SVAS patients to allow their deletion breakpoints to be more finely mapped. Genomic PCRs were carried out to test the sequence tagged site (STS) content of the hybrids using primer pairs designed from the microsatellite markers and genes on chromosome 7. Hybrids containing a chromosome 7 with deletion at the critical region from each patient were investigated for the presence of ELN, LIMK1 RFC2, D7S1870, and D7S489 by another round of PCR. These results make the genotype-phenotype matching possible and, therefore, provide valuable informations to fully understand the WS and SVAS pathogenesis.

F104

Construction of a Plasmid Vector Ensuring Site-Specific Integration and Stable Gene Expression

YoungJoon Moon*, YoonSung Kang, SungKook Jung and Kwang-Ho Lee
Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 156-756

Insertion of reporter constructs into the mammalian genome leads to variable gene expression due to position effects at the site of integration. This random integration has limited the gene therapy of human genetic disorders by its undesirable effects. We report here the newly constructed plasmid vector (pIRES-neo-YJ) based on the concepts of homologous recombination and position-independent promoter enhancing of beta-globin matrix attachment region (Glb-MAR). Chromosome 7 centromere-specific alpha satellite (alphoid) DNA sequence was cloned into pIRES-neo-YJ for homologous recombination of the cloned gene with the centromeric region of chromosome 7, which is genetically silent. Beta Glb-MAR sequence that allows high levels of transcription independent of the chromosomal site of integration was also inserted into pIRES-neo-YJ to ensure the stable and higher expression of the cloned genes. We expect that pIRES-neo-YJ would provide a valuable tool to eliminate random integration of cloned genes into the undesirable chromosomal region and their short-lived expression which often encounters during the construction of transgenic animals and human gene therapy.

F105

Xist Expression in Male-derived OTF9 Embryonal Carcinoma(EC) Cells

Sungkook Jung*, Yoonsung Kang, YoungJoon Moon and Kwang-Ho Lee
Department of Life Science, College of Natural Science, Chung-Ang University, Seoul, 156-756

The Xist (X inactive specific transcript) gene that resides at the putative X inactivation center (XIC) of the X chromosome is sorely expressed in female, but not in male somatic cells. Most researchers generally accept the fact that only the Xist allele on the inactive X chromosome is transcriptionally active. But it

has been reported that Xist is expressed in P10 female embryonal carcinoma (EC) cells with two active X chromosomes, and presence of the XIC is not responsible for the maintenance of X inactivation in female somatic cells. We investigated the expression of Xist in male mouse-derived OTF9 EC cells that presumably have only one active X chromosome. Surprisingly, Xist transcripts were observed in undifferentiated OTF9 cells by RT-PCR. Karyotype analysis showed that OTF9 has an inact X chromosome and a marker chromosome whose part looks like Xq. The marker chromosome was confirmed to be a translocated chromosome between an unknown chromosome and a part of Xq by the reverse painting FISH technique using total DNAs of microcell hybrids as a probe, which contain a mouse X chromosome on the chinese hamster background. In addition, it was found that the Xist sequence exists on the translocated fragment, demonstrating that the translocate breakpoint is more proximal from Xist. These results strongly suggest that the Xist transcript is not always derived from an inactive X chromosome and Xist per se functions as a sex counter to count the number of the X chromosomes within cells rather than as an indicator of X chromosome inactivity.

F106

Production and Characterization of The Monoclonal Antibodies to Human Pan-enteroviruses.

Sooyoun Shin[*1,2], Dooseong Cheon[2], Kisoon Kim[1,2], Yoonseok Chung[1,2], Youngmee Jee[2], Jaedeuk Yoon[2] and Kwang-Ho Lee[1]

Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 156-756[1]; Department of Virology, National Institute of Health, Seoul 122-701[2]

We have previously cloned the putative genes from enterovirus cDNA, which express the common antigen for pan-enteroviruses. In this study, the putative common antigen expressed in the prokaryotic system was purified and inoculated into the balb/c mice to produce monoclonal antibodies (mAbs) specifically reacting with the pan-enteroviruses. Splenocytes extracted from immunized mice were fused with murine myeloma cells (V653). After fusion, hybrid cells were selected by HAT supplement for two weeks. Twenty nine of hybridomas were cloned and confirmed for the producibility of specific antibodies against enterovirus-common antigen by using indirect immunofluorescent assay (IFA) and western blotting. Basic biochemical characteristics of the mAbs were analyzed and antigen recognition ability of the mAbs was also investigated for selection of putative common antibodies, cross-reacting with the broad spectrum of enteroviruses.

F107

Generation of Infectious cDNA of Cardiovirulent Coxsackievirus B3

Kisoon Kim[*1,2], Dooseong Cheon[2], Yoonseok Chung[1,2], Jeongbae Ahn[2], Youngmee Jee[2], Jaedeuk Yoon[2], Haewol Cho[2] and Kwang-Ho Lee[1]

Department of Life Science, College of Natural Science, Chung-Ang University, Seoul 156-756[1]; Department of Virology, National Institute of Health, Seoul 122-701[2]

An infectious cDNA of a cardiovirulent Coxsackievirus B3 (CVB3)-Car, was amplified by long-distance RT-PCR from whole viral genome of CVB3-ATCC (Nancy) strain and subsequently cloned into the plasmid containing the CMV IE (Cytomegalovirus immediate early) promoter. After transfection of the cDNA clone onto Buffalo green monkey kidney cell (BGMK), typical cytopathic effect (CPE) was