observed. Supernatants were then inoculated onto fresh monolayered BGMK cells and resulting CPEs were reconfirmed. No difference of antigenicity was observed between CVB3-Car and the parental CVB3-ATCC by the neutralizing test. CVB3-Car showed the cardiovirulent phenotype when inoculated into the balb/c mice as did CVB3-ATCC, reflecting that CVB3-Car still retains the cardiovirulence. The infectious cDNA described here provides valuable information on cardiovirulent-determining factor(s) of CVB3 through molecular manipulation of the clone.

F108

Cloning and Expression of the 56kDa 3D^{pol} of Coxsackievirus B3 in *Escherichia coli*

Doonseok Chung[*1,2], Kisoon Kim[1,2], Dooseong Cheon[2], Jeongbae Ahn[1,2], Youngmee Jee[2], Jaedeuk Yoon[2], Kwang-Ho Lee[1] and Chulyong Song[4]

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]

Coxsackievirus B3 (CVB3) is a member of enteroviruses in the *Picornaviridae* family and the major etiological agent for myocarditis and meningitis. The genome of CVB3 is a single-stranded positive (+)-sense RNA molecule approximately 7,400 nucleotides in length. The genome has single open reading frame (ORF) which encodes 11 kinds of structural and non-structural proteins. Non-structural protein coding region (P3) is subdivided into four functional subunits. Among them, RNA dependent RNA polymerase (3D^{pol}), which involves in the replication of negative and positive strand RNA by initially interacting with 3′ and 5′ UTR of viral genome, is localized in 3′proximal end of the viral genome flanked by 3′ UTR. The 3D^{pol} of CVB3, which is known to play a critical role(s) in the CVB3 replication, was cloned and expressed in *E. coli*. The molecular mass of the polyhistidine-tagged fusion protein was 56kDa. Sequence analysis indicated that the 56kDa protein contains YGDD (amino acid 327 to 330) motif, which is a catalytic site of 3D^{pol}. The 56kDa protein was then purified by metal affinity chromatography and nucleotide polymerization assay was performed to ascertain its RNA polymerization activity. Negative (-)-sense RNA was detectable by strand-specific RT-PCR when the in vitro transcribed (+)-sense CVB3 viral RNA was used as a template for the RNA polymerization. Taken together, the 56kDa protein expressed in *E. coli* has RNA polymerizing activity and would be used for investigation of replication kinetics of CVB3.

F109

Development of Cancer Cell-Specific Synthetic Regulatory Elements

June Ho Shin, Mi Ae Park, Young Jin Lee, Dae Hwan Kim, Ae Rie Kim and Chul Geun Kim*

Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791

The fundamental characteristics of cancer cells are categorized as an unrestricted proliferation, the formation of new blood vessels, and metastasis. Even though there have been lots of effort to develop early diagnoses and therapies for specific cancers at gene level, no solid protocol is not yet obtained partly since the prognosis of each cancer is not known at molecular level. One promising protocol for the cancer therapy depends on the expression of toxic materials only in specific cancer cells using natural regulatory regions of genes expressed in the specific cancer type, but the limitation of this

strategy is that the level of expression of target gene is not sufficient to kill specific cancer cells and regulatory elements expressing the target gene only in cancer cells are hard to obtain. As a means to obviate this critical limitation, we demonstrated the isolation of synthetic regulatory elements showing cancer-cell specific expression from the shuffles of various combinations of the regulatory elements in several well-known cancer cell-specific genes. Out of 7 selected regulatory elements in T cell factor 1 α (TCF1a) gene enhancer, c-Ha-ras gene promoter, transcription factor E2F binding site in cyclin E and cdc25 phosphatase genes, hexokinase gene promoter, and telomerase catalytic subunit (hTERT) gene promoter, more than 200 combinatory regulatory elements were constructed by random ligation or random PCR. Cancer specific high expressivities of these synthetic regulatory elements were monitored in the transiently transfected cancer cell lines (HeLa, CV1, and HepG2) or normal fibroblasts after subcloning these elements individually into pGL3-Luc vector along with a control pRL-TK vector. Several clones showed promising luciferase activities in HeLa cell line and two of them also showed high luciferase activities in HepG2 cell line.

F110

Expression of Poly (ADP-ribose) Polymerase During Apoptosis Induced by Ultraviolet Radiation in HeLa S3 Cells

Kyu Seon Oh*, Dong Wook Lee, Jeong Hyun Chang and Kyung Il Um

Dept. of Biology, Dong-A University, Pusan 604-714

The present investigation has been performed to elucidate the expression of poly (ADP-ribose) polymerase (PARP) during apoptosis induced by ultraviolet radiation (UV) in HeLa S_3 cells. Four assays were employed in this investigation; Gel electrophoresis of isolated DNA, quantitative assay of fragmented DNA, morphological assay of apoptotic cells and western blot analysis. Alteration of DNA level on apoptosis was determined by DNA ladder pattern. DNA ladder pattern in HeLa S_3 cells irradiated with UV was observed from 6 hrs to 18 hrs after incubation. The cleavage of PARP and DNA fragmentation during apoptosis was shown from 6 hrs after incubation. And the cleavage of PARP pretreated with 2 J/m^2 UV and subsequently treated with 50 J/m^2 UV was lower than treated with 50 J/m^2 UV alone. And the cell viability pretreated with 2 J/m^2 following treated with 50 J/m^2 is higher than treated with 50 J/m^2 alone.

F201

Construction and Characterization of Garlic Bacterial Artificial Chromosome Library.

Eun-mi Eum[1], Hye-ran Lee[1], Ji-young Park[2], Yong-Pyo Lim[2] and Dong-Hee Lee[1]

Dept. of biology, Ewha Womans Univ., Seoul, 120-750[1]; Dept. of Plant Science and Resource, Chungnam National Univ., Taejon, 305-764[2]

We had constructed two BAC libraries of Danyang garlic cultivar. First one has been constructed using the pIndigoBAC536 vector. HMW garlic DNA was extracted from leaf protoplasts. Total 129 clones were obtained from transformation of 1/50 fraction of the ligation mixture. Among 129 clones, 47 clones contained relatively large inserts and average size was about 97 kb. Seventeen BAC clones out of 47 showed strong positive hybridization signals on Southern blot analysis using garlic genomic DNA as a probe. Second BAC library was constructed using the pBAC1SACB1 vector, which is designed to give zero background. HMW