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.

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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

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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

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*

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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

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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