

E103 Screening of the novel cytolytic proteins from sea hare(*Aplysia*) in Cheju Offshore

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Sea hares of the species *Aplysia* belong to the subclass Opisthobranchia of the mollusca. They have only degenerated shells in their mantle cavity and expose their naked and soft bodies to the surrounding. However, there is no apparent predator which preferably preys on them. Various bioactive compounds such as toxins and antibiotic have been isolated from sea hares. Most of these compounds are lipophilic and low molecular weight compounds derived from their algal diets. Also, antibacterial and antitumor glycoproteins have been reported in *Aplysia*. In order to isolate the novel protein with cytotoxic activity, first of all, we are screening the proteins fractionated using FPLC from purple fluid and eggs in HL-60 cell lines, leukemia cell lines, using the MTT assay. We will present the biochemical characteristic of these candidate proteins.

E104 **Characterization of a Membrane-Associated Protein Containing Internal AARKAAEE Repeats In *Amoeba proteus***

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A monoclonal antibody (mAb, AHM8-2) reacting with proteins of different molecular masses (53, 66, 72, 85, 90 kDa) in *A. proteus* was obtained. In the indirect immunofluorescence microscopy, the antigens were located on the membranes of subcellular organelles whose sizes were 0.5 - 2.5 μ m in the cytoplasm. The mAb cross-reacted with a 30-kDa protein of *Tetrahymena pyriformis*, but didn't with proteins of *Xenopus laevis* embryo, *Drosophila melanogaster*, HeLa, or *E. Coli*. Using the mAb as a probe, we cloned a cDNA of 2.0 Kb. The cDNA had a poly-A tail, a putative polyadenylation site and an ORF(1.8 Kb) for a novel protein. Comparisons of the nucleotide and amino acid sequences in GenBank, EMBL, Protein database using FASTA III program showed that the protein coded in the cDNA was similar to KAP (kinetoplast associated protein) of *Trypanosoma cruzi*, TmpA and TmpB proteins of *Treponema pallidum*, and Tb-29 proteins of *T. Trypanosoma brucei*. The central portion of these proteins contained the amino acid sequence (A/LA/TRK/LAA/LEE) repeats in common. The protein of amoeba contained 7 repeats. In subcellular fractionation, the antigens were recovered in 100,000g pellet and the supernatant corresponding to the microsome and the cytosol, respectively. A topographical study of the antigens by chemical extraction and proteinase K digestion revealed the antigens as the peripheral proteins. In summary, one of the antigens recognized by the AHM8-2 was a noble peripheral protein of microsomal membrane containing internal A/LA/TRK/LAA/LEE repeats.