

**S7 Transgenic Marine Medaka (*Oryzias Melastigma*): a Sensitive Sentinel for Estrogenic Pollutants.** Xueping Chen<sup>1</sup>, Masato Kinoshita<sup>2</sup>, Takashi Hirata<sup>2</sup>, Richard Man Kit Yu<sup>2</sup>, Shuk Han Cheng<sup>1</sup>. <sup>1</sup>Centre for Marine Environmental Research and Innovative Technology (MERIT), Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, <sup>2</sup>Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502.

Pollution caused by endocrine disruptor (ED) (especially estrogenic EDs) has become one of the most serious anthropogenic threats to biodiversity and ecosystem. Our lab has generated a transgenic medaka, the *O. melastigma*, which can be used for bio-monitoring of estrogenic activity in the marine and freshwater environment. Choriogenin genes, which encode precursors of the inner layer subunits of egg envelope, are regarded as sensitive biomarkers for estrogenic pollutants. We have shown that in the *O. melastigma*, expression of the choriogenin H (*omChgH*) appeared to be more sensitive to estrogen than the related gene member choriogenin L (*omChgL*). This observation highlighted the potential of using *omChgH* expression as a sensitive biomarker for estrogenic EDs in the developing *O. melastigma* embryos, juveniles and male adults. In our laboratory, a transgenic *O. melastigma* strain containing the reporter gene green fluorescence protein (*GFP*) which is regulated by the promoter region of the *omChgH* gene has been established. In this strain, *GFP* was expressed constitutively in the liver of mature female. Interestingly, the reporter gene expression could also be induced in the liver of embryos (initiated from stage 34), juveniles and male fish in response to 17 $\beta$ -estradiol (E2). By quantifying the intensity of the reporter gene expression, we found that low levels of E2 and 17 $\beta$ -ethanylestradiol (EE2) could induce *GFP* expression in a dose-responsive manner in yolk-sac larvae of this strain. Moreover, this transgenic strain also responded to different estrogenic compounds such as estrone (E1), estriol (E3), 4-nonylphenol (NP), bisphenol A (BPA), genistein and ethinylestradiol 3-methyl ether. This is a novel observation as most of the published transgenic fish only responded to E2 but not estrogen-like substances. Furthermore, preliminary study found that water samples collected in the Hong Kong Victoria Harbor could induce *GFP* expression in the transgenic larvae. Our data indicate that this transgenic *O. melastigma* is a sensitive sentinel for biomonitoring of estrogenic EDs in filed samples. The wide salinity adaptability of this strain of medaka enables its application as a sentinel for both freshwater and marine environment.

**S8 Pharmacological Application of Natural Toxin Peptides: Induction of Apoptosis of Cancer and Smooth Muscle Cells through Inactivation of NF- $\kappa$ B.** Jin Tae Hong<sup>1,2</sup>. <sup>1</sup>College of Pharmacy and CBITRC, Chungbuk National University, Cheongju, Cheongju, Korea, <sup>2</sup>College of Pharmacy, University of Kentucky, USA (present)

Natural toxins are the most powerful natural poisons, but are nevertheless used as therapeutic agents in an increasing number of indications and medical specialties. One of them, bee venom (BV) has been used in traditional medicine to treat diseases such as arthritis, rheumatism, pain, cancerous tumors, and skin diseases. In a series of our research on drug investigation with natural toxin peptides, it was found that BV and Melittin, a major peptide component of BV, has anti-inflammatory and anti-arthritis properties, and its inhibitory activity on NF- $\kappa$ B may be essential for the effects of BV. Based on the considering of the pharmacological roles of NF- $\kappa$ B, we have also investigated application of this toxin peptide to control of the growth of vascular smooth muscle cells (SMC) as well as colon cancer cells where NF- $\kappa$ B is implicated in the control of cell volume via apoptotic elimination. We found that BV and melittin inhibit several colon and prostate cancer cells as well as vascular VSMC proliferation via apoptotic elimination through suppression of NF- $\kappa$ B and Akt activation, and through enhancement of proapoptotic protein expression. We are also interesting to study the pharmacological application of Snake venom toxin (SVT). SVT also inhibited growth of prostate and neuroblastoma cancer cells in vitro and in vivo through inhibition of NF- $\kappa$ B which is also important to control resistance of cancer cells against chemotherapeutic agents. Detail and precise mechanisms and pharmacological applications of these natural toxin peptides will be discussed.

**S9 Role of Molecular Markers in Environmental Health and Risk Assessment.** S.V.S. RANA. Toxicology Laboratory, Department of Zoology, Ch. Charan Singh University, Meerut-250004 (India).

Most diseases are thought to arise from the combined effects of genes and environment. While great strides have been made in our understanding of human genetics, the contribution of environmental exposures to disease such as cancer, respiratory problems and heart disease remains poorly understood. New concepts viz. Gene Environment Initiative (GEI) established in Feb. 2006 are working to seek biomarkers for diseases with known links to environment. Focus on inflammation and oxidative stress makes additional sense because both pathways are common to many ailments. New metabolic, proteomic and genomic methods offer new opportunities for biomarker development. DNA and protein adducts in particular could provide windows in the body's long term 'memory' of previous exposures, combined exposures and long term exposures. A new subject of "exposure biology" is ready to enter the bigger science of Environment and Health. Research initiatives in environmental sensors and biomarkers development are sure to address assessment needs for environmental pollutants, dust, physical activity and even psychosocial stressors. To sum up emerging advances in health risk assessment will allow us better use of our human resources and to create a healthier society. My laboratory during last three decades has witnessed the evolution of biomarkers. The days are not far when we shall add exposure biology in our study curriculum like occupational health or environmental medicine.

**S10 EST analysis of cDNA libraries from the entomopathogenic fungus *Beauveria (Cordyceps) bassiana*.** Eun-Min Cho, Nemat O. Keyhani. Department of Microbiology and Cell Science, University of Florida, Gainesville, USA.

*Beauveria bassiana* is an important entomopathogenic fungus that displays a broad host range able to target a diverse number of arthropod species. Strains of *B. bassiana* have been selected for control of insects and other arthropods that act as disease vectors, crop and household pests, and have been used as bio-control agents for limiting the spread of ecologically hazardous, invasive insects. *B. bassiana* has also been used as a model system to study fungal-mediated tick biological control, and recent studies have highlighted the potential for entomopathogenic fungi, including *B. bassiana*, as agents in combating the spread of malaria by controlling mosquito populations. Despite this interest, the molecular mechanisms of entomopathogenicity, endophyte establishment or rhizosphere competence, and *B. bassiana*-mediated biochemical transformations, are largely unknown. We have employed an expressed sequenced tag (EST) of *B. bassiana* cells in order to characterize the *B. bassiana* transcriptome. Analysis of EST transcripts expressed during discrete developmental and growth conditions represents an efficient means of characterizing the genome potential of an organism. *B. bassiana* produces at least three distinct single cell propagules including aerial conidia, vegetative cells termed blastospores, and microcycle conidia that can be isolated from agar plates, rich broth liquid cultures, and under conditions of nutrient limitation in submerged cultures, respectively. cDNA libraries were constructed from each *B. bassiana* cell type and a robust EST data set generated. Approximately 2,000 clones from each library were sequenced and a unique sequence set was constructed. Almost half (40-50%) of the sequences in each library displayed either no significant similarity (*e*-value >10<sup>-4</sup>) or similarity to hypothetical proteins found in the NCBI data base. Approximately 20-25% of the sequences in each library could be annotated by gene ontology terms. Comparative analysis of the expressed transcripts in each library indicated significant differences in gene expression pattern between the cell types in several broad categories including cell wall biosynthesis, secondary metabolism, and the production of proteases. These data will provide a basis for investigating the gene expression profile of distinct developmental stages as well as a data set for gene discovery and genome annotation. It also will facilitate gene identification for *B. bassiana* genome sequencing efforts. Further sequencing of the libraries and cloning of interesting clones are now under way.