**I-49**

Chemical screening for inhibition of Anthrax Lethal Toxin

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Anthrax Lethal Toxin (LeTx), a critical virulence factor for Bacillus anthracis, has been demonstrated to cleave and to inactivate mitogen-activated protein kinase kinases (MAPKKKS) that propagate proximal signals in macrophage. Anthrax toxins consist of three kinds of toxins, protective antigen (PA), lethal factor (LF), and edema factor (EF, 89kDa). As LeTx is used for bioterror, lots of investigation is concerned to develop chemicals for inhibition of LF. In this study, we purified recombinant LF, PA, and GST-MEK for testing activity of chemicals for LF inhibition. Herein, we screened about 50,000 chemicals from Korea Research Institute of Chemical Technology (KRICT) for inhibition of LF activity in vitro and in vivo. We selected some chemicals that showed activity to inhibit LF activity in vitro then treated into macrophage cell line, Raw 264.7 from mouse. The derivatives of chemicals showed inhibition of LF activity were also tested in vitro and in vivo. The activity of hit compound was compared with GMK001 which is already reported for inhibition of LF activity. We will discuss in terms of the chemical properties bound to the LF.

**I-50**

Characterization and molecular mechanism of hypopigmenting effect by Compound 18 in B16 melanoma cells

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To explore the hypopigmenting effect of Compound 18 (Comp18), tyrosinase, the key enzyme of melanogenesis, was examined by Western blot. Comp18 suppressed tyrosinase protein level. Since tyrosinase is involved in melanin synthesis, we measured melanin contents after Comp18 treatment in B16 cells. Comp18 showed melanin synthesis inhibitory effect in melanoma cells. In addition, at melanin inhibition concentrations of Comp18, it had no effect on cell viability assessed by MTT. To determine if Comp18 directly inhibits tyrosinase, we performed in vitro tyrosinase assay by using purified mushroom tyrosinase. However, Comp18 had no inhibitory effect on purified tyrosinase in itself while kojic acid almost completely inhibit mushroom tyrosinase activity. To determine if Comp18-induced hypopigmenting effect was caused by reduced gene expression of tyrosinase, we performed RT-PCR and DNA microarray. We also checked MITF level since MITF is key regulator of melanogenesis-related proteins such as tyrosinase and MC1R. To examine if MITF modification is required for its binding to E-box sequence in tyrosinase promoter region, a specific inhibitor responsible for MITF phosphorylation or ubiquilination was employed and determined its role in melanin synthesis. To determine if tyrosinase promoter region has another putative trans-acting factors other than MITF, we performed supershift assay (EMSA) by using the transcription factor antibody like CREB. In conclusion, MITF and its modulators which block binding interaction with DNA play a pivotal role in Comp18-induced hypopigmenting effect in B16 cells.

**I-51**

CG-140 inhibits VEGF-induced angiogenesis in HUVECs

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Angiogenesis is the process of new blood vessel formation by endothelial cells. It plays a critical role in various pathophysiologic conditions including the growth of solid tumors, diabetic retinopathy, and rheumatoid arthritis. Lately, numerous angiogenesis inhibitors have been developed with an aim to control angiogenesis-related diseases. We have discovered new angiogenesis inhibitors with anti-angiogenic activity using chemical genomics approach. From high throughput screening of angiogenesis inhibitors using natural and synthetic chemical libraries in our lab, CG-140 was identified to inhibit the proliferation of human umbilical vein endothelial cells (HUVECs) with no cytotoxicity. In angiogenesis assays, CG-140 inhibited the vascular endothelial growth factor (VEGF)-induced invasion and tube formation of HUVECs. These data demonstrate that CG-140 can be developed as an anti-angiogenic agent.

**I-52**

Cellular insulin sensitizing agent from Taxus cuspidata seed

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This study has been carried out to develop an anti-diabetes compound from natural resources. 3T3-L1 cell, a mouse-derived preadipocyte (fibroblastic) differentiating into adipocyte in the presence of insulin (10 mg/ml), isobutyl-1-methylxanthine and dexamethasone. We have screened cellular insulin sensitizing agent that enhanced differentiation of the cells at lower concentration of insulin (2.5 mg/ml). In the course of our screening program MeOH extract of Taxus cuspidata seed demonstrated a potent stimulatory activity in the differentiation of the cells. Isolation and purification—EDAC partition, silica flash, ODS flash and Prep HPLC—of the active agent afforded us to characterize a single compound. In the symposium, isolation and purification procedure, physico-chemical characterization, biological activity and mode of action of the active compound will be presented.

**I-53**

cDNA microarray analysis of liver tissues from diabetic Zucker diabetic fatty (ZDF) rats and Zucker lean control (ZLC) rats and ZDF rats that treated with anti-diabetic medicine

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Insulin resistance occurs early phase of type 2 diabetes. Therefore, the identification of molecules that contribute to insulin resistance and leading to type 2 diabetes are important to understand the molecular pathogenesis of the disease in response to anti-diabetic drugs. Metformine, a anti-diabetic drug, is decreases hepatic glucose production, and increases glucose uptake. Rosiglitazone is referred to as an insulin sensitizer and surnofurykna play a role in improvement of lipid metabolism. We characterized gene expression profiles from liver tissues of Zucker diabetic fatty (ZDF), a well characterized type 2 diabetes animal model, and Zucker lean control (ZLC) rats, and ZDF rats that treated with anti-diabetes medicine -metformine, Rosiglitazone and Surnofurykna, each treated rats named as ZFM, ZFR and ZFG. Gene expression profiles from diabetes ZLC rats (12 weeks) and ZDF rats (12 weeks) that treated with 3-different anti-diabetes drugs were compared with age- and sex-matched ZDF rats using 27k oligo chips (operon). Differentially regulated genes demonstrating over 1.5-fold change were identified and categorized through hierarchical clustering analysis. Our research will integrates the study of differentially regulated gene expression using the same 27k oligo DNA chips in other insulin-sensitive tissues including muscle, pancreas, and white adipose tissue. These profilings might provide better solutions to understand insulin resistance and development of type 2 diabetes.

**I-54**

Antiproliferation and induction of apoptosis by isomorellin on human cholangiocarcinoma cell line.

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Isomorellin is a caged xanthone derived from the fruit of Garcinia hanburyi Hook. f. (Guttiferae). Many caged xanthones have been shown to exhibit cytotoxic activities in several mammalian cancer cell lines, as well as antitumor and anti-tumor activities. In the present study, we examined the effects of isomorellin on the proliferation of a human cholangiocarcinoma cell line (KKU-100) by Sulforhodamine B assay and confirmed the isomorellin-induced apoptosis by cell morphology and DNA ladder formation. In addition, the expression of apoptosis-related genes such as Bax, Bcl-2 and Survivin were determined by real-time PCR. Treatment of KKU-100 cells with isomorellin (0.3-35 μmole) resulted in a dose-dependent inhibition of cell proliferation with IC50 of 2.5 μmole. KKU-100 cells treated with isomorellin exhibited the distinct morphological changes characteristics of cell apoptosis such as cell shrinkage, membrane blebbing, chromatin condensation, apoptotic bodies, and DNA fragmentations. An increase in expression of the pro-apoptotic gene Bax and a decrease in expression of the anti-apoptotic genes Bcl-2 and Survivin were also observed in a time-dependent manner. These data suggest a possible underlying molecular mechanism where isomorellin could induce the apoptosis signaling pathway in human cholangiocarcinoma cells by regulation of apoptosis-related proteins. This properties of isomorellin suggests that it could have a possible therapeutic potential in cholangiocarcinoma patients.