

cells transfected with pNF-kappa B-SEAP-NPT vector secreted the SEAPs into the culture medium as a time-dependent manner until 48h. The SEAPs were measured using fluorescent assay method. The treatment of transfected cells with antioxidants *N*-acetyl-L-cysteine (10 mM) and Vitamine C (10 mM) inhibit NF-kappa B activation up to 50% and 25% compared to a control, respectively. This system can be used for determining the effect of various chemicals and natural products to NF-kappa B activation in human HaCaT cells.

[PC1-4] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### Development of in vitro assay system for the screening of type specific 5 $\alpha$ -reductase inhibitors

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In many androgen-responsive organs, such as prostate and skin, testosterone is converted into 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) by 5 $\alpha$ -reductase. 5 $\alpha$ -DHT then binds to androgen receptors and functions in the nucleus to regulate specific gene expression. Human 5 $\alpha$ -reductase has at least two isoforms, designated types I and II. The type I 5 $\alpha$ -reductase expression predominates in skin, prostatic epithelia, and type II isoform predominates in human accessory sex tissues. Since 5 $\alpha$ -DHT promotes the development of acne, male pattern alopecia and benign prostatic hyperplasia, inhibitors of 5 $\alpha$ -reductase may be useful for treatment of these conditions. For the screening of 5 $\alpha$ -reductase inhibitors, human prostate cancer cell lines (LNCaP, DU145 etc) were used. But type-specific inhibitors were effective for the exclusion of side effects. We constructed cell lines that express the type specific 5 $\alpha$ -reductase. For type specific cell lines construction, DU145 and LNCaP were used respectively, type I and II. From each cell line, RNA were extracted and synthesized cDNA containing 5 $\alpha$ -reductase open reading frame (ORF) by using RT-PCR method, and then cloned into mammalian expression vector, pTarget T vector, 293 cell line, which don't express 5 $\alpha$ -reductase, were transfected by the electrophoration method. These cell lines were tested by using of a 5 $\alpha$ -reductase inhibitor, finasteride, is being evaluated as the chemoprevention agent of prostate cancer in a clinical trial and have been used successfully for treatment of benign prostatic hyperplasia. In these system, each cell line expressed type specific 5 $\alpha$ -reductase and was inhibited by finasteride effectively. These results suggest that these system are effective for the screening of type specific 5 $\alpha$ -reductase inhibitor for treatment of benign prostatic hyperplasia and alopecia.

[PC1-5] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### The anticoagulant activity of chondroitin sulfate proteoglycan derived from human placenta

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Chondroitin sulfates proteoglycans were isolated from human placenta. For the identification of enzymatic digestion products of isolated proteoglycan, strong anion exchange-high performance liquid chromatography (SAX-HPLC) was performed. By the action of chondroitin ABC and chondroitin B lyase, three unsaturated disaccharides 2-acetamide-2-deoxy-3-O-( $\beta$ -D-gluco-4-eneopyranosyluronic acid)-D-galactose ( $\Delta$ Di-OS), 2-acetamide-2-deoxy-3-O-( $\beta$ -D-gluco-4-eneopyranosyluronic acid)-6-O-sulfo-D-galactose ( $\Delta$ Di-6S) and 2-acetamide-2-deoxy-3-O-( $\beta$ -D-gluco-4-eneopyranosyluronic acid)-4-O-sulfo-D-galactose ( $\Delta$ Di-4S) were produced from the human placenta proteoglycan. The anticoagulant activity of chondroitin sulfate proteoglycan was evaluated by activated partial thromboplastin time (aPTT) assay and thrombin time (TT) assay. The clotting times of aPTT and TT were increased from

72 to 144 sec and 19 to 27 sec, respectively. With 0.5 M NaCl eluted fraction, we observed prolongation in clotting time (aPTT) in a dose-dependent manner and approximately three times prolongation was obtained at 200 µg/150 µl.

[PC1-6] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### Differentiation Inducer Activity of Magnolialide, a 1 $\beta$ -Hydroxyeudesmanolide Isolated from *Cichorium intybus* on Human Leukemia Cells

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*Cichorium intybus* contained two 1 $\beta$ -hydroxyeudesmanolides, magnolialide and artesin together with several constituents. Magnolialide exhibited a growth-inhibitory activity against several tumor cells and it appeared to induce differentiation of human leukemia HL-60 and U-937 cells to monocyte/macrophage-like cells. Another 1 $\beta$ -hydroxyeudesmanolide, artesin, and other constituents were not active. The content of magnolialide was shown to be highest in the leaves of Inje cultivar among the investigated cultivars in this study.

[PC1-7] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### Inhibitory effect of sophoricoside analogs on proinflammatory cytokines

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IL-5, IL-3 and GM-CSF are known to be involved in allergic inflammation, and their receptors are composed of ligand-specific alpha subunits associated with a common beta subunit. IL-1, TNF and IL-6 are well known as proinflammatory cytokines. Inhibitory effects of sophoricoside and its analogs (genistin, genistein and orobol), which were isolated from *S. japonica*, on the cytokine bioactivity have been investigated. Sophoricoside showed inhibitory effects on IL-5, IL-3 and IL-6 bioactivities but did not inhibit the GM-CSF, IL-1 and TNF bioactivities. Genistin inhibited the IL-5 and IL-3 bioactivities but not on the other cytokine bioactivities. Genistein and orobol showed inhibitory effects on IL-5, IL-3, GM-CSF and IL-6 bioactivities but did not inhibit the IL-1 and TNF bioactivities. Among the compounds, sophoricoside showed the highest inhibitory effects on IL-5, IL-3 and IL-6 bioactivities with IC<sub>50</sub> values of 1.9 µM, 6.9 µM and 6.0 µM, respectively and orobol did show on GM-CSF bioactivity with an IC<sub>50</sub> value of 18.0 µM. The result would provide an additional mechanism by which the compounds exert immunosuppressive and anti-inflammatory effects.

[PC1-8] [ 04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3] ]

### NO Produced by iNOS Mediates KH-1-Induced Differentiation In a Human Neuroblastoma Cell Line SH-SY5Y