

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