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Expression Profiles of the mRNA of CYP2B6, LGR-7 and Constitutive Androstane Receptor in Response to Individual and Different Combinations of Estrogen, TCPOBOP, and 9-cis Retinoic Acid in Cultured Human Cervical Adenocarcinoma Cells

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The purpose of this experiment was to investigate the expression profiles of CYP2B6, LGR7, and CAR mRNA when the human cervical carcinoma cell, HeLa, was incubated with individual or combinations of estrogen, TCPOBOP, and 9-cis retinoic acid. HeLa cells were maintained in DMEM supplemented with 5% calf serum, 1g/L glucose, 10ug/ml phenol, 4mM sodium bicarbonate, 100units/ml penicillin, and 100ug/ml streptomycin, and treated for 6 days with either individual or different combinations of 10nM 17-beta-estradiol, 5uM TCPOBOP, 1uM 9-cis retinoic acid. Total RNA was prepared from each sample by the method of cold lysis-phenol/chloroform/isoamyl alcohol, and mRNAs for CYP2B6, LGR-7, and CAR were reverse transcribed and amplified by polymerase chain reacion for 25 cycles and the level of expression was compared to the untreated control in 1% agarose gel electrophoresis. High levels of CYP2B6, LGR-7, and CAR were expressed in HeLa cells. Expression of CYP2B6 was inhibited by TCPOBOP, 9-cis retinoic acid, and estrogen plus 9-cis retinoic acid but not by estrogen or estrogen plus TCPOBOP. Whereas expression of LGR-7 was significantly inhibited by estrogen plus TCPOBOP, treatments with estrogen, TCPOBOP, 9-cis retinoic acid, or estrogen plus 9-cis retinoic acid did not influence its expression. In contrast, expression of CAR was inhibited by 9-cis retinoic acid, 9-cis retinoic acid plus estrogen, and estrogen plus TCPOBOP but not by estrogen or TCPOBOP. We conclude that diverse expression profiles of CYP2B6, LGR-7 and CAR genes by different hormone and ligand treatments suggest complex cross-talk of signal transduction pathways among steroid hormone receptors and transcriptional regulatory factors in human cervical adenocarcinoma cells.

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Expression of matrix metalloproteinase of metalloproteinase-1 from human gingival fibroblasts

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Matrix metalloproteinases (MMPs) plays an important role in the pathology of inflammatory periodontal disease. In this study, we investigated the influence of LPS from Prevotella intermedia, a major cause of inflammatory periodontal disease, on the enzyme activity and the mRNA expression pattern of MMP-1 and TIMP-1 in human gingival fibroblasts. LPS from P. intermedia ATCC 25611 was prepared by the standard hot phenol-water method. Cultures of gingival fibroblasts were established from gingival biopsies obtained from healthy individuals. The amounts of MMP-1 and TIMP-1 in the supernatant were measured by enzyme-linked immunosorbent assay (ELISA). The expression of mRNA for MMP-I and TIMP-I was assessed using the reverse transcription-polymerase chain reaction (RT-PCR). We found that human gingival fibroblasts constitutively produce MMP-1 and TIMP-1, and that P. intermedia LPS can augment both MMP-1 and TIMP-1 production in gingival fibroblasts. In addition, P. intermedia LPS significantly increased the levels of mRNAs encoding MMP-1 and TIMP-1 in cultured fibroblasts. Our findings demonstrate that the signal pathways COX-2, MAP-kinase, tyrosine kinase, and protein kinase C are involved in P. intermedia LPS-induced upregulation of MMP-1 and TIMP-1.