Expression Profiling of Drug Metabolizing Enzymes in Rat Syncytiotrophoblast Cell Lines

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TR-TBT-18d-1 and -18d-2 are syncytiotrophoblast cell lines established from the placenta of a transgenic rat harboring a temperature-sensitive simian virus 40 large T-antigen (Tg-rat). To explore the functional roles of drug metabolizing enzymes in the syncytiotrophoblasts of the placenta, we analyzed the gene expression of both cytochrome P450 (CYP) and phase II conjugation enzymes in these TR-TBT cell lines. A relatively high level of expression of CYP1A1 and CYP2C11 was detectable in the 18d-1 cells whereas CYP2E1 was more strongly expressed in the 18d-2 cells. A moderate degree of expression of CYP2B2 and CYP3A, and low expression of CYP1A2, was observed in both cell lines, but no CYP3A2 was detectable in either. Relatively high levels of SULT2 family gene expression were also evident in these TR-TBT cells, although the SULT1 family genes were found to be either absent or present at only moderate levels. Strong expression of both the UGT1 and UGT2 subfamily, and also the glutathione S-transferase (GST) subfamily, was also observed in both cell lines. These results suggest that TR-TBT cells are a useful model system for the study of syncytiotrophoblasts, which act as a protective fetal barrier against harmful xenobiotics.

Key words —— cytochrome P450, phase II enzymes, placenta, syncytiotrophoblast

INTRODUCTION

The placenta separates the blood supply of the mother and fetus in mammals, whilst at the same time is simultaneously perfused by the circulation of both. The major function of the placenta is to transfer nutrients and oxygen from the mother to the fetus and to assist in the removal of waste products from the fetus to the mother.[1)] In addition, the placenta acts as a barrier that protects the fetus from xenobiotics that may be present in the maternal blood.[2)] Hence, in administering drugs to pregnant women, clinicians must always be cognizant of the potential for fetal exposure. However, the impact of placental metabolism and uptake on the effects of such agents has remained difficult to quantify.[1–3)]

Syncytiotrophoblasts, which form a continuous barrier between the maternal and fetal circulatory systems, play an essential role in the restriction of xenobiotics through the blood-placental barrier (BPB). To investigate the functional roles of syncytiotrophoblasts in the BPB, Kitano *et al*. have recently established syncytiotrophoblast cell lines (TR-TBTs) from a transgenic rat harboring a temperature-sensitive simian virus 40 large T-antigen (Tg-rat).[4,5)] These conditionally immortalized TR-TBT cells display a syncytium-like morphology, and express several syncytioblast specific markers and polarized glucose transporters.[4)] To further the characterization of syncytiotrophoblast cells as a BPB, we have analyzed the expression of drug metabolizing enzymes in these cells, including the phase I cytochrome P450s (CYPs) and phase II conjugation enzymes.

MATERIALS AND METHODS

Materials —— Reagents for RNA isolation were purchased from Daiichi Chemicals (Tokyo, Japan) and all other chemicals were obtained from Sigma, Japan.

Cell Culture —— TR-TBT cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 10 U/ml penicillin and 10 U/ml streptomycin at 33°C in a humidified atmosphere containing 5% CO_2.

RNA Isolation and Reverse Transcription-PCR (RT-PCR) —— Total RNA was isolated from cultured cells using the guanidium thiocyanate phenol-chloroform extraction method as previously described. First strand cDNAs were synthesized from 2.5 μg of total RNA using 1 unit M-MLV reverse transcriptase with oligo(dT) primers according to the manufacturer's protocol. PCR was carried out using the synthesized cDNAs as a template and AmpliTaq Gold polymerase (Perkin-Elmer). Cycling

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conditions (35 cycles) were 1 min at 94°C, 1.5 min at 58°C and 2 min at 72°C. The products were then separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and visualized by UV illumination. The band intensities were determined using image analysis software (Eastman Kodak Co., NY, U.S.A.). The quantities of cDNA used in the PCR amplifications were normalized to β-actin levels in each preparation and assessed by a quantitative PCR analyzer (ABI7700, Perkin Elmer, California, U.S.A.). The primers used for the amplification of CYPs were obtained from Takara Biochemicals, Japan. Primers for amplifying sulfotransferases (SULTs), uridine 5-diphosphate (UDP)-glucuronosyl transferases (UGTs) were designed from the Genbank cDNA sequences by Genetyx-Windows software. The Genbank accession numbers are as follows; ST1A1(X52883), ST1B1(D89375), ST1C1(L22339), ST2A18(M31363), ST2A2(M33329), ST2A58(D14989), UGT1A1(J05132), UGT1A6(J02612), UGT2B1(M13506). Primer sequences for GSTA1 and GSTA2 have been reported previously.[6]

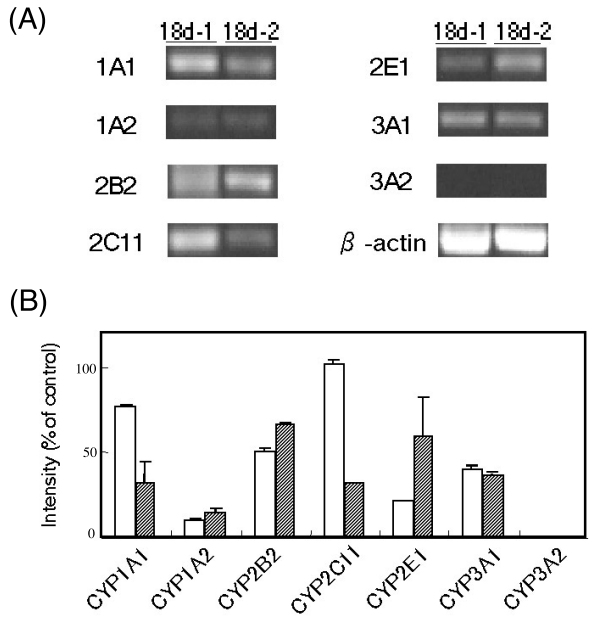

Fig. 1. Expression Profiling of CYP Genes in TR-TBT Cells

(A) Gene expression of the indicated CYP genes in the TR-TBT 18d-1 and 18d-2 cell lines, analyzed by RT-PCR. (B) The band intensities of each PCR product were measured using image analysis software and expressed relative to β-actin, which was set at 100%. Each bar represents the means of two independent measurements with error bars. Open bars, 18d-1 cells: hatched bars, 18d-2 cells.

RESULTS

The placenta plays a vital role in the maintenance of pregnancy as it can metabolize many foreign chemical compounds via both the cytochrome P450 (CYP) and phase II conjugation enzyme pathways. It is very important to clarify the expression of CYPs and conjugation enzymes in the rat placenta, as these animals are frequently and widely used in embryo- and feto-toxicity studies of foreign chemical compounds. To explore the functional roles of drug metabolizing enzymes in the placenta, we have measured their gene expression in two recently established rat TR-TBT syncytiotrophoblast cell lines, 18d-1 and 18d-2, by RT-PCR analysis. As shown in Fig. 1A, relatively high expression of CYP1A1 and CYP2C11 is detectable in 18d-1 cells, whereas a higher degree of CYP2E1 expression can be observed in 18d-2 cells. Moderate levels of expression for CYP2B2 and CYP3A1, and low CYP1A2 levels, were found in both of these cell lines. However, no CYP3A2 expression could be detected in either cell line. Moreover, densitometric analysis of the RT-PCR products revealed a 2–3 fold difference in the expression levels of the CYP1A1, CYP2C11 and CYP2E1 genes between the 18d-1 and 18d-2 cells (Fig. 1B).

Conjugation reactions are metabolic pathways that are in many cases followed by oxidative reactions catalyzed by CYP enzymes. Several conjugation enzymes such as SULT, UGT and glutathione S-transferase (GST) are now well characterized. These enzymes catalyze the bioactivation and inactivation of a wide variety of xenobiotics and endogenous compounds, including steroid hormones and eicosanoids.[7] Since most conjugated metabolites are transported outside the cells by specific transporters, conjugation enzymes have been suggested to play a crucial role in the detoxification of xenobiotics in the placenta.[2] To further explore the potential functional role of conjugation enzymes in the placenta, we measured their expression levels in the TR-TBT cells by RT-PCR. SULTs can be divided into two subfamilies, phenol-sulfating (SULT1) and hydroxysteroid-sulfating (SULT2).[8] In our present study, a relatively high level of expression of the SULT genes was observed in both cell lines (Fig. 2). In particular, the expression of SULT2A1 and SULT2A5 was found to be extremely high, comparable to the β-actin levels, whereas the expression of SULT1A1 and SULT1B1 was moderate, and no SULT1C1 expression could be detected.

UGTs are extensively involved in phase II metabolism where they conjugate glucuronic acid to

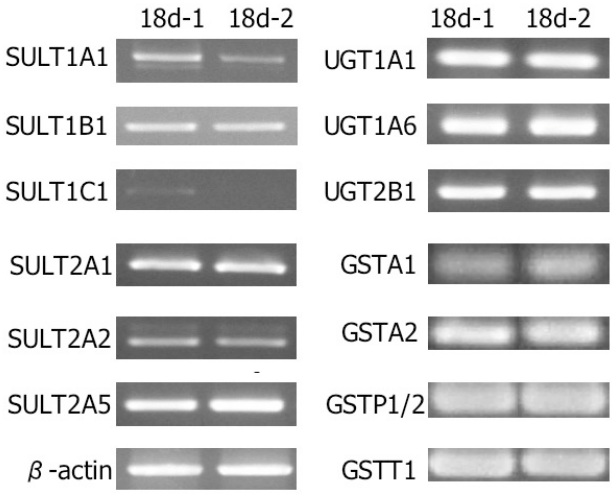

Fig. 2. Expression Profiles of Phase II Conjugation Enzymes in TR-TBT Cells

RT-PCR analyses of three phase II conjugation enzyme families (SULT, UGT and GST) were performed using isozyme-specific primers.

xenobiotics.[9] UGTs are divided into two subfamilies, UGT1 and UGT2, and we determined the expression of a representative number of these genes, UGT1A1, UGT1A6 and UGT2B1. High expression of these three UGT genes was observed in both TR-TBT cell lines (Fig. 2) and these were again almost comparable to the β-actin levels. We next measured the expression of the GST family of genes for which four classes have been identified in mammals, α, μ, π, and θ.[10] We selected several glutathione *S*-transferase (GST) isoenzymes for expression analysis and detected high transcript levels for GSTA1, GSTA2, GSTP1/2 and GSTT1 gene in both TR-TBT cell lines (Fig. 2). We found no differences between the expression levels of the UGT and GST genes that we tested in the two TR-TBT cell lines.

DISCUSSION

In our current study, we report the gene expression analysis of a number of crucial drug metabolizing enzymes in the syncytiotrophoblastic cell lines, TR-TBT 18d-1 and 18d-2, established from a Tg-rat placenta. Relatively high levels of expression could be observed for CYP1A1, the CYP2 genes and CYP3A1 in both cell lines but these levels differed between these two cell types. These differences in CYP expression might be related to the difference in cellular origin; these two cell lines are thought to be derived from different syncytiotrophoblast layers (I and II, respectively), based upon the glucose transporter expression profile.[4] Although many studies have failed to detect significant levels of CYP in the mammalian placenta,[2,3] both a wide variety and significant amount of CYP expression might be characteristic of syncytiotrophoblasts. This feature would be beneficial for cells which act as a barrier against the entry of xenobiotics from the placenta into the fetal bloodstream.

Relatively high levels CYP1A1 expression were found in the TR-TBT cells and the transcription inducibility of CYP1 enzymes is known to be related to the levels of both aryl hydrocarbon (Ah) receptors and the Ah receptor nuclear translocator (ARNT). Since our preliminary data indicate that there are high levels of expression of both of these transcription factor genes in TR-TBT cells (data not shown), CYP1A genes might be induced by specific xenobiotics in the syncytiotrophoblasts. Indeed, the induction of CYP1A1 by β-naphthoflavone in the rat placenta has been reported.[11] Although most previous studies have failed to detect any significant expression of the CYP2 subfamily of genes in either the rat or human placenta,[3,12] we have detected significant levels of CYP2 gene expression (CYP2B2, CYP2C11 and CYP2E1) in the TR-TBT cells. Expression of CYP2 genes might therefore be a specific marker of syncytiotrophoblasts and this possibility should be clarified in future experiments.

We also analyzed the expression of phase II conjugating enzyme genes in the two TR-TBT cell lines. SULTs are of great importance in the sulfate conjugation of steroids, particularly estrogens, and also of catecholamines in the placenta, but little is known about their involvement in placental drug metabolism.[2,3] In our present analysis, we show relatively high expression of the SULT1 and SULT2 subfamily of genes in rat syncytiotrophoblasts. These results suggest that sulfation in syncytiotrophoblasts may be a significant biotransformation pathway for endogenous substances and specific drugs. The lack of SULT1C1 expression in the TR-TBT cells might also be beneficial for the protective role of the placenta since the activity of SULT1C1 has been thought to be involved in the activation of some procarcinogens such as *N*-hydroxy-2-acetylaminoflurene.[13,14]

Relatively high expression of the UGT genes, UGT1A1, UGT1A6 and UGT2B1, in the TR-TBT cells was also observed. Glucuronidation is generally considered to be a detoxification mechanism that produces metabolites from both exogenous and endogenous substrates that have a higher polarity and

are thus more readily eliminated. Since UGT activity is not observed in the fetal liver as a major pathway of xenobiotic and endobiotic detoxification,[15] the presence of UGTs in the syncytiotrophoblasts may play a protective role during gestation through the metabolism and clearance of such compounds.

High expression of the GST subfamily of genes was also detectable in both TR-TBT cell lines. GSTs can catalyze glutathione conjugation with various electrophiles, many of which are toxic. The π-class GST (GSTP) is known as an extrahepatic GST, and a number of studies have reported that the _-class GST is substantially elevated during the early stages of rat liver carcinogenesis.[16] High expression of GSTP1/2 genes in the TR-TBT cells indicates that the syncytiotrophoblasts exhibit one of the known characteristics of the placenta.

Recent studies have shown that the induction or inhibition of drug metabolizing enzymes can occur following exposure to different drugs, environmental chemicals and food constituents.[17–19] If these events occur in the placenta, this might interfere with the normal fetal growth and development and TR-TBT cells may thus be useful in future studies to analyze the effects of such exposures on drug metabolizing enzymes in the placenta.

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