ABSTRACT

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Title of Doctoral Thesis **Gene expression regulation of biotransformation enzymes and transporters in placental barrier**

Placenta is a unique organ facilitating the communication between mother and fetus. It serves as a respiratory, excretory, endocrine and metabolic organ during intrauterine development of the fetus and is necessary for maintenance of pregnancy and for the fetal protection. Syncytiotrophoblast is a single layer that represents the critical morphological and metabolic component of the placental metabolic and exchange barrier. The placental trophoblast contains multiple drug transporters and metabolizing enzymes that form placental metabolic barrier. The levels of the enzymes are not stable and fluctuate throughout pregnancy. Most of the enzymes are expressed at very low mRNA levels, however, in some cases there is absence of any relevant detectable protein or catalytic activity.

Cytochrome CYP1A1 is the only placental xenobiotic-metabolizing enzyme of the cytochrome P450 superfamily for which significant expression and catalytic activity have been conclusively demonstrated in placental trophoblasts throughout human pregnancy. Many researches reported significant induction of placental CYP1A1 activity in women exposed to cigarette smoke and in choriocarcinoma cell lines BeWo and JEG-3 exposed to Aryl hydrocarbon receptor (AHR) ligands. CYP1A1 is the prototype target gene of AHR receptor, that binds hydrophobic aromatic molecules including a wide variety of environmental contaminants (polycyclic aromatic hydrocarbons, halogenated aromatic hydrocarbons) generated through solid waste incineration, coal tar and wood burning and smoke exhaust.

Both synthetic and endogenous glucocorticoids have been shown to be involved in transcription regulation of CYP1A1. Transplacentally administered glucocorticoid therapy before 35 weeks of pregnancy has been distributed to pregnant women for several years to enhance fetal pulmonary surfactant production and prevent respiratory distress syndrome in premature newborns. Antenatal administration is associated not only with reduction in respiratory distress syndrome, but also with reduction of other pregnancy associated complications, such as intraventricular hemorrhage, necrotizing enterocolitis, and systematic infection in the first hours of life.

In this dissertation thesis we described AHR and ARNT (Aryl hydrocarbon receptor nuclear translocator) expression in human and rat placentas, and transcription activity of AHR in the human placental trophoblast in regulating its target genes CYP1A1, CYP1A2, CYP1B1, UGT1A1 and BCRP. These genes are involved in metabolism and transport of exogenous and endogenous substances. We showed that only CYP1A1 mRNA, but not CYP1A2, CYP1B1, UGT1A1, BCRP, AHR, ARNT and AHRR (Aryl hydrocarbon receptor repressor) mRNAs, was significantly induced in human placental trophoblast cultures after exposure to prototype AHR ligands. We also described for the first time placental localization of human AHR and ARNT proteins throughout pregnancy, which were present primarily in first-trimester and term placenta in syncytiotrophoblasts forming the so-called placental morphologic and metabolic barrier. Rat Ahr and Arnt proteins were mainly localized in placental trophoblasts and reached maximum expression during 15 and 21 gestation days, which might indicate a different response to Ahr ligands in placental Cyp1a1 induction during rat gestation.

The next study was focused on the effects of glucocorticoids on 3MC dependent induction of CYP1A1 and other AHR target genes in primary cultures of human trophoblast. We showed that dexamethasone alone had no significant effect on mRNA expression of AHR target genes (CYP1A1, CYP1A2, CYP1B1, UGT1A1 and BCRP) and AHR, ARNT AHRR mRNA expression. The basal level of CYP1A1 protein and basal CYP1A1 EROD (Ethoxyresorufin-O-deethylase) activity were not significantly

affected by dexamethasone. In contrast, we reported that 3MC-inducible CYP1A1 mRNA, but not CYP1A2, CYP1B1, UGT1A1, BCRP, AHR, ARNT and AHRR mRNAs, was significantly up-regulated by glucocorticoids in human placental trophoblast cultures of the term placentas. EROD activity of the trophoblast cultures treated with the combination of 3MC (3-methylcholanthrene) and dexamethasone (betamethasone) was higher when compared to the enzyme activity of 3MC-treated trophoblast cultures. Suprisingly, CYP1A1 protein level induced by 3MC was not significantly changed by usage of dexamethasone.

In conclusion, this dissertation thesis describes expression and transcription activity of AHR receptor in human and rat placentas and clearly demonstrates effects of glucocorticoids on gene regulation of AHR target genes in human primary trophoblast cultures.