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The unique characteristics of the placental transcriptome and the hormonal metabolism enzymes in placenta

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ABSTRACT

The placenta regulates the passage of both endogenous and xenobiotic compounds to the fetus during pregnancy. A small number of xenobiotic metabolizing CYP enzymes are constantly expressed in placenta, those include CYP19A1 which mainly converts androgens to estrogens, and CYP1A1 whose substrates include steroid hormones and xenobiotics. We performed an analysis of co-expression of xenobiotic metabolizing enzyme-coding genes and transcription factors in placenta and in 86 other tissues to discern the unique characteristics of the placental transcriptome. Transcription factors (TFs) driving the expression of proteins involved in phase I and II xenobiotic metabolism in the liver were not expressed in the placenta, nor were the bulk of xenobiotic metabolizing hepatic CYP enzymes. In contrast, TFs whose co-expression correlated with CYP1A1, i.e. AHR, PPARG, and CEBPB were highly expressed in placenta. The placenta is a hormonal tissue, and one needs to maintain the tissue-specific focus by removing the hepatic spectacles.

1. Introduction

The placenta connects two genetically distinct individuals, the mother and the fetus. Toxic or foreign compounds may interfere with placental function at many levels, e.g. there are many signaling cascades involved in the placental metabolism, production and release of steroid hormones [1] and enzymes, transport of nutrients and waste products, implantation, cellular growth and maturation and finally those systems triggered during the terminal phase of placental life, i.e. delivery (see Robins et al. [2] for a recent review). Any deviation from normal homeostasis may pose a threat to placental function, resulting in preterm delivery, congenital malformation or miscarriage in the worst-case scenarios.

The placenta is present only during the time of embryonic and fetal life; it has no later-life counter-part, and this placental structure exhibits a greater species diversity than any other mammalian organ [3]. These features define why the unique properties of placenta in hormonal regulation need to be considered carefully. For example, it is a challenge to examine the properties endocrine disrupters due the low concentrations at which effects occur, especially since most safety tests often only involve acute toxicity [4].

Certain placental CYP enzymes are very important for the synthesis and catabolism of steroids, fatty acids, as well as for the metabolism of many xenobiotics such as pharmaceuticals and pollutants. The CYP enzymes participate in oxidative and reductive metabolism; in fact they represent the core of phase I drug metabolism. However, recently it has emerged that CYP enzymes are responsible for a wide range of endogenous functions with different roles in tissues [5] being regulated in different ways from the CYP2 and CYP3 family enzymes. The majority of the 57 CYPs are involved in endogenous metabolism [6,7]. The human placenta at term expresses only a few functional cytochrome P450 (CYP) enzymes [8], e.g. CYP19A1 (aromatase) which converts androgens to estrogens. In addition, there are variations in the overall CYP expression profile in placenta depending on the developmental stage [9]. It is known that a number of factors can affect the net function of CYPs in placenta, exerting long-lasting health effects, even epigenetic changes. Maternal glucocorticoids [10] drugs of abuse [11] and health status [12] are also able to influence the expression of several CYP enzymes in placenta.

We propose that an examination of transcriptome-wide differential gene expression analyses can provide a broad and unbiased molecular perspective of placental and non-placental tissues. In order reveal the unique metabolic characteristics of the human placenta, we performed a bioinformatic study to evaluate in greater detail the transcription mechanisms present in placenta for the expression of the phase I and II enzymes. This analysis will focus

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on biologically relevant CYP, and phase II conjugative enzymes of GST, UGT, and SULT families. These were selected for the analyses in order to reveal the characteristics of CYP and phase II enzyme expression in placenta and other tissues. We performed a co-expression analysis based on human data obtained from public repositories, consisting of the expression profiles of the genes coding for the enzymes and ~900 TFs and coregulators in 87 tissues. The expression data were subjected to bioinformatic analyses to gain some insights into the hidden properties of the expression of enzymes involved in phase I and II xenobiotic metabolism across tissues, and more specifically in placenta.

The observed correlative co-expression patterns of enzymes and TFs expressed in placenta may be relevant if one is intending to undertake experiments evaluating placental function after exposure to medication, pollutants, and food components during pregnancy. Another intention is to alert the regulatory authorities to pay attention to the unique metabolic characteristics of extrahepatic tissues such as the placenta, since they differ so extensively from the classical view which has been largely obtained by studying liver.

2. Methods

2.1. Gene expression data

The data were extracted from the publicly available Human body index (Series GSE7307 by Roth and co-workers) from the Gene Expression Omnibus repository. The tissue phenotype data used were created by downloading the transcriptome of diverse human tissues, using the Affymetrix 133plus2 microarray (GPL570). All data of transformed cell-lines were excluded from this study.

As a reference representing smoking and non-smoking mother placentas, we used our own previously published data (GSE7434 record) [13], created with the same microarray platform as GSE7307. All data were pre-processed by using the robust multi-array average algorithm which is a standard method for working with the Affymetrix microarray, and normalized by median normalization in GeneSpring 7.2 (Silicon Genetics, USA). The replicates for each tissue were averaged to represent the expression of each gene in each tissue. The expression values of 963 genes coding for TFs and co-regulators, and the expression values of the CYP, GST, UGT, and SULT genes, were evaluated across the 87 tissues. The classification of genes was obtained by the David functional annotation tool [14]. The official human gene nomenclature committee symbols were used to indicate genes and their products.

We then compared the expression of enzymes and transcription factors between placenta and other tissues. Naïve inspection for the high-expressed enzymes and TFs in placenta was performed by comparing the expression values of the genes in liver, placenta and one other extrahepatic tissue, lung [15], i.e. sites for xenobiotic metabolism. A co-expression intercorrelation matrix was analyzed by using the hierarchical clustering algorithm to reveal possible groups of enzymes being expressed in the same tissues as certain TFs throughout the 87 tissues. An intercorrelation matrix was created by calculating the Pearson correlation coefficient between each TF-gene, and the expression of each gene for phase I and phase II enzymes throughout the normalized gene expression data in all 87 tissues. The highly positive and negative co-expression values between the enzymes expressed in placenta, and the co-expressed TFs, were then observed. We then aligned the expression values of the enzymes in the 87 tissues to the same order as in the previous matrix, to display the difference between the hepatic and placental expression of these enzymes. By comparing the expression of both TF and enzymes in high numbers of tissues, it was hoped to evaluate the availability of TFs in those tissues in which certain key enzymes are expressed in order to identify likely candidates which may be key TFs for the transcription of the discussed enzymes. Finally, the analyses were completed by creating a heat map deciphering the correlation coefficients across tissues of 9 selected genes coding enzymes expressed highly in liver or placenta, and TFs relevant for their expression as postulated by earlier analysis steps.

3. Results

The initial evaluation of the reliability of the microarray datasets was based on the similarities between the placenta data from the publicly available Human Body Index dataset (GSE7307) and our previously published (GSE7434) placenta gene expression data [13]. Despite the semiquantitative nature of the microarray data, the observed placental expression values were in a synchronisation with each other showing a high intercorrelation between the samples. The placental data on the expression atlas GSE7307 resembled somewhat more data obtained from smoking mothers ($R = 0.942$) than the data from verified non-smoking [13] mothers ($R = 0.937$). This is probably due to the fact that certain genes with high expression in placenta, such as CYP19A1 appear to be downregulated by smoking [13], and the smoking status on samples in GSE7307 was not verified as being negative. We observed that the weakest correlation between the expression data between placenta and other tissues was found to be that between placenta and liver ($R = 0.18$) (Fig. 1).

3.1. The expression levels of genes coding phase I and II enzymes and transcription factors in placenta

One of the best known inducible enzymes in placenta is the estrogen metabolizing CYP1A1 that was expressed marginally in the present data but in a parallel dataset with a sample from smoking mothers it was the only enzyme to be induced to a moderately high level [13]. The few enzymes which are expressed in placenta at high level were those associated with placental physiological functions, such as CYP19A1, CYP11A1, and CYP2J2. All of these enzymes have at least one degree of magnitude higher expression in placenta than the main hepatic CYPs involved in xenobiotic metabolism such as CYP2 or CYP3 families. In addition, only a few phase II enzymes, mostly glutathione S-transferases were expressed in placenta.

Due to the absence of most hepatic CYPs and Phase II enzymes, and their lack of induction [13] the expression profiles of genes coding for TFs were compared between the placenta and other tissues, since the expression of enzymes requires the presence of different TFs. We observed that the RNA for many liver or xenobiotic associated TFs [16], especially nuclear factors, such as NR1I3 (also known as constitutive androstane receptor) and other common hormonal targets, e.g. AR were virtually absent from placenta.

Only a few genes associated with nuclear receptor activity were among the 150 most highly expressed TFs and coregulators in placenta (NR3C1, NR2F6, ESRRG, PPARD, NR2F1, PPARG, NR2F2, RXRA). However, also the basic helix-loop-helix factors AHR and HIF1a were highly expressed (Supplementary Table 1). ESRRG, PPARG and PPARD were expressed in the placenta ten-fold higher than that in liver. With respect to the leucine-zipper transcription factors, MAFF, CEBPB and FOS were expressed more extensively in the placenta than in liver. Due to the distinct pattern of expressed nuclear factors observed in the initial evaluation, the expression of all nuclear factor family TFs in liver and placenta were clustered in order to show that the distinction is obvious also in a data driven analysis (Fig. 2), separating the hepatic TFs on the top of the top of the heat map and placental TFs on the bottom of the heat map.