Prenatal expression of thioredoxin reductase 1 (TRXR1) and microsomal glutathione transferase 1 (MGST1) in humans

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Article info
Article history:
Received 17 July 2014
Revised 7 October 2014
Accepted 8 October 2014

Keywords:
Thioredoxin reductase 1
Microsomal glutathione transferase 1
Fetus
Liver
Splice variants
DNA methylation

ABSTRACT

Thioredoxin reductase 1 (TRXR1) and microsomal glutathione transferase 1 (MGST1) are important redox and detoxifying enzymes in adult life. The aim of this study was to investigate the expression of these enzymes during fetal life. In addition, the role of gene methylation was studied since this might play an important role in the on-and-off switch of gene expression between fetal and adult life.

To this end, the expression of the TRXR1-encoding gene TXNRD1 and the MGST1-encoding gene MGST1 was studied in fetal tissues. The mean mRNA expression of TXNRD1 in fetal livers were seven times higher compared to the mean expression in adult livers (p < 0.001). Of the six studied splice variants of TXNRD1, four had a significantly higher expression in the fetal livers as compared to adult livers. The mean expression of MGST1 was twofold higher in adult compared to fetal liver tissue (p = 0.01). For MGST1 the alternative first exon 1B was the predominant splice variant in both fetal and adult liver samples. The highest mRNA expression of both TXNRD1 and MGST1 was found in fetal adrenals, whereas expression was lower in fetal liver, lungs and kidneys. There was a significant correlation between the hepatic expression of TXNRD1 and MGST1. Treatment with the demethylating agent 5-AZA resulted in decreased levels of TXNRD1 in human liver HepG2 cells but did not affect the expression of MGST1.

In conclusion, the expression of TXNRD1 is higher in fetuses than in adults and might be of importance during fetal life. Hepatic TXNRD1 and MGST1 are co-expressed in both fetuses and adults suggesting common regulatory mechanisms.

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1. Introduction

Thioredoxin reductase 1 (TRXR1) and microsomal glutathione transferase 1 (MGST1) are important enzymes for redox control and detoxification during adult life. Animal studies have shown that TRXR1 is important also in fetal life [1,2] but it has never been investigated in human fetal tissue. The aim of this study was to investigate the expression of TRXR1 and MGST1 in human fetal tissues and compare with the expression in adult liver.

The mammalian thioredoxin system consists of thioredoxin, thioredoxin reductase (TRXR) and NADPH [3]. This system is involved in many cellular processes including redox control of transcription factors, reduction of peroxides, regulation of apoptosis and synthesis of deoxyribonucleotides [4]. Mammalian TRXRs are selenocysteine-containing oxidoreductase flavoproteins with remarkable broad substrate specificity [4]. Three different human genes that encode TRXR enzymes have been identified; the classical cytosolic TRXR1, the mitochondrial TRXR2 and the mitochondrial TRXR3 (also known as TGR) mainly expressed in the testis [4]. The TRXR1 encoding gene TXNRD1 is widely expressed and is up-regulated by various stimuli such IL-1B, lipopolysaccharide, epidermal growth factors and hydrogen peroxide [4], whereas for example statins have been shown to inhibit the transcription of TXNRD1 [5]. However, the mechanisms of these transcriptional regulations remain unclear. In fact the transcriptional regulation of TXNRD1 is complex and involves several mechanisms such as alternative splicing and different transcriptional start sites [6]. In humans, 21 different mRNA forms have been identified, all differing in the 5'-end [7]. A putative CpG island in the TXNRD1 promoter
been identified, whereas one is predominantly expressed in the adrenal and pancreas [10]. Several alternative first noncoding exons have been identified, whereas one is predominantly expressed in the liver [10,11]. The ontology expression of MGST1 in humans has never been studied, but it is well known that cytosolic GSTs are functional in various human fetal tissues [12–14].

MGST1 is an enzyme that exerts both glutathione transferase activity as well as reduction of various hydroperoxides, being important in the protection against oxidative stress [9]. The expression of the MGST1 encoding gene MGST1 is abundant in most tissues, with the highest gene expression levels found in liver and pancreas [10]. Several alternative first noncoding exons have been identified, whereas one is predominantly expressed in the liver [10,11]. The ontology expression of MGST1 in humans has never been studied, but it is well known that cytosolic GSTs are functional in various human fetal tissues [12–14].

The specific aims of this study were to measure and compare the gene expression of TXNRD1 and MGST1 in adult and fetal livers, and to study the intra-individual gene expression profile of TXNRD1 and MGST1 in fetal livers, lungs, adrenals and kidneys. We also chose to co-investigate the gene expression and ontogenetic of TXNRD1 and MGST1 to investigate whether common regulatory mechanisms were involved. Moreover the effect of 5-AZA-2-deoxyxytidine (5-AZA), a demethylation agent, on TXNRD1 splice variants (sequences described in [16]), and 1A, 1B, 1C, 1D exon specific MGST1 primers (sequences described in [11]). All the reactions included 1µl cDNA template in a total volume of 15 µl. Thermal cycling conditions included activation at 95 °C (10 min) followed by 40 cycles each of denaturation at 95 °C (15 s) and annealing/elongation at 60 °C (1 min). Each reaction was performed in duplicates and no-template controls were included in each experiment. For the comparison between the mRNA expression in adult and fetal liver samples, an adult liver sample was employed as a calibrator and the delta CT-formula was used as described [17]. For intra-individual comparison between fetal tissues, a fetal liver sample was used as a calibrator. When different splice variants were compared, the TRXR1 x1/2 and MGST1 1B were chosen as calibrators, respectively.

2.3. Cell culture

Human liver cancer HepG2 cells were cultured in MEM supplemented with 5% FCS, 1% penicillin/streptomycin, 1% l-glutamine and maintained in humidified atmosphere at 37 °C and 5% CO2. No additional selenium source above that present in 5% FCS was added and the cells were thereby selenium starved but not selenium deficient [18]. Prior to 5-AZA exposure the HepG2 cells were split and plated in 12-well plates and pre-incubated for 2 days. 5-AZA were diluted in DMSO and added to the cells for 24 h at final concentration 5 mM. The non-treated controls were incubated with vehicle only. The experiments were performed in four independent experiments. The cells were harvested with Tri-zol (Invitrogen, UK) and RNA was extracted according the protocol and kept at −80 °C.

2.4. Statistical analysis

To investigate if there was any significant difference in relative hepatic expression between two groups (adult–fetal, smoking–non-smoking, 5-AZA-control) the nonparametric Mann–Whitney test was used since the measurements were few and could not be proven to be normally distributed. When looking for significant difference in splice variants and in tissue-specific (livers, kidneys, adrenals, lungs) expression of TXNRD1 and MGST1 the nonparametric Kruskal–Wallis test was performed followed by Dunn’s multiple comparison post-test for identification of which groups differed from the other. The results are presented as mean ± SEM and in box and whisker plots, i.e. the bottom and top of the box are the first and third quartiles, and the band inside the box is the median. The ends of the whiskers represent the 5–95th percentile. The correlation analyses were performed by the Spearman rank method. All statistical tests were performed using GraphPad Prism v. 5.00 and values of p < 0.05 were considered statistically significant.
3. Results

3.1. The mRNA expression of total TXNRD1 and MGST1 in adult and fetal liver

In order to compare the TXNRD1 mRNA expression between adult and fetal liver samples we used primers that bind to exon 5 and exon 6. TXNRD1 was detectable in 59 of 60 fetal and 17 of 20 adult liver samples. Significantly higher levels of TXNRD1 mRNA transcripts were observed in the fetal liver samples as compared to the adult liver samples. The mean TXNRD1 was 7 times higher in fetal liver compared to adult liver (mean 12.8 ± 0.82 vs mean 1.82 ± 0.48; \( p < 0.0001 \)) as shown in Fig. 1. In the adults specimens a larger inter-individual variation in TXNRD1 mRNA was found (90-fold) compared to the fetal specimens in which a 30-fold variation was found.

MGST1 was detectable in 59 (98%) and 18 (90%) of the fetus and adult samples investigated. There was a 2-fold higher expression of MGST1 in adults as compared to fetal expression (\( p = 0.01 \)) (Fig. 1). A large (30-fold) inter-individual variation of MGST1 gene expressions were noted both in adults and fetuses.

3.2. The mRNA expression profile of six TRXR1 splice variants in fetuses

Several isoforms of TRXR1 have been identified. Here we have studied the abundance of six splice variants previously described [16]. Of these, four splice variants (\( \alpha_{1/2}, \alpha_{6}, \alpha_{7/8}, \) and \( \alpha_{10/11} \)) were significantly higher in the fetal livers as compared to adult livers. The mean relative mRNA levels (±SEM) in fetal compared to adult livers were 21 ± 1 and 2.4 ± 0.66 for \( \alpha_{1/2}, 37 ± 2.4 \) and 1.4 ± 0.36 for \( \alpha_{6}, 15 ± 0.86 \) and 0.79 ± 0.10 for \( \alpha_{7/8}, 22 ± 1.6 \) and 1.0 ± 0.1 for \( \alpha_{10/11} \) respectively.

All the six splice variants were frequently abundant in the fetal samples. \( \alpha_{1/2}, \alpha_{6}, \alpha_{7/8}, \alpha_{10/11}, \alpha_{13} \) and \( \gamma_{2-4} \), were detected in 98%, 97%, 92%, 97%, 93% and 97% of the samples, respectively. In the adult samples however, occurrence of the splice variants was only observed in 65%, 40%, 90%, 20%, 60% and 25% of the samples analyzed.

In fetal liver samples \( \alpha_{7/8} \) was the most abundant transcript, three times higher than the wildtype \( \alpha_{1/2} \) (\( p < 0.001 \)), whereas the isoforms \( \alpha_{13} \) and \( \gamma_{2-4} \) were found at lowest levels (Fig. 2). In the adult liver samples \( \alpha_{13} \) was found at the highest levels, but with a large inter-subject variation (200-fold). Isoform \( \alpha_{6} \) was present at the lowest levels compared to the other isoforms (Fig. 2).

3.3. The mRNA expression profile of MGST1 splice variants

MGST1 1B and MGST1 1D were found in 93% and 88% of the fetal samples and in 70% and 60% of the adult liver samples investigated. Both in the fetal and adult liver samples the alternative first exon 1B was the predominant variant, the mean expression...
being 400 and 100-fold higher in the fetuses and adults, respectively. There was no difference in the expression of \textit{MGST1} 1B transcript between adults (mean 1.84 ± 0.58) and fetuses (mean 1.59 ± 0.24), whereas \textit{MGST1} transcript including the alternative exon 1D was 5-fold higher in adults (mean 4.92 ± 1.7) as compared to fetuses (mean 1.06 ± 0.13; \( p = 0.0006 \)). The other putative alternative exons 1 (1A and 1C) were not identified in any sample, further supporting that these exons are non-expressed.

3.4. Expression of \textit{TRXR1} and \textit{MGST1} in different fetal tissue

\textit{TXNRD1} and \textit{MGST1} transcripts were abundant in all adrenals, kidney and lung samples analyzed. There was no correlation between the extra-hepatic expressions of these genes. The highest mRNA expression of \textit{TXNRD1} was found in the adrenals whereas the liver, lungs and kidney had lower and about equal expression. For \textit{MGST1} also the adrenals had the highest expression, followed by the liver whereas the lowest expression was found in lungs and kidneys (Fig. 3).

3.5. Correlation between hepatic \textit{TXNRD1} and \textit{MGST1} mRNA expression

There was a significant correlation between hepatic \textit{TXNRD1} and \textit{MGST1} mRNA levels in both the fetuses (\( r = 0.49, \ p < 0.0001 \)) and adult samples (\( r = 0.60, \ p = 0.01 \)) (Fig. 4).

There was no correlation between the fetal gestational age and the mRNA expression of \textit{TXNRD1} or \textit{MGST1} in any specimens studied. Moreover, there was no apparent effect of maternal smoking on the gene expression of \textit{TXNRD1} and \textit{MGST1} in fetal liver (data not shown).

3.6. Methylation in HepG2 cells

HepG2 cells were treated with 5 mM 5-AZA and analyzed for the gene expression of \textit{TXNRD1} and \textit{MGST1} and were performed in four independent experiments. The mRNA expression of \textit{TXNRD1} was inhibited in every experiment and in average by 34% by the exposure of 5-AZA in the HepG2 cells (\( p = 0.03 \)), whereas 5-AZA did not affect the expression of \textit{MGST1} (data not shown).

4. Discussion

This is the first time the expression profile of both \textit{MGST1} and \textit{TXNRD1} has been studied in human fetal samples. Both genes were highly expressed in the first trimester. \textit{TXNRD1} mRNA was found at 7-fold higher levels in fetal than in adult livers. This is in agreement with previous studies performed in mice where embryos expressed higher levels of \textit{Txnrd1} compared to adults [2]. Our results, i.e. that \textit{TXNRD1} is highly abundant and consistently found in human fetuses indicates that TRXR1 is an important enzyme during human embryonic fetal development.

In contrast to \textit{TXNRD1}, but in agreement with several other phase II drug metabolizing enzymes, \textit{MGST1} transcripts seems to be more abundant in adults than in fetuses. This is in line with previous result showing that \textit{Mgst1} mRNA expression was detected prenatally (2 days before birth) and increased with age in newborn mice [19]. Moreover, the GST activity has been shown to be higher in cytosolic liver human samples obtained from adults as...
compared to samples from the first trimester [20] as was the case for microsomal GST activity in the rat [21].

Here we show for the first time that mRNAs specific for TXNRD1 and MGST1 are correlated in the liver, both in fetuses and adults. The reason for this correlation is not known, but the genes share some common transcriptional regulatory mechanisms. Both genes comprises functional Sp1 sites in their proximal promoter regions [8,19]. Both MGST1 and TXNRD1 genes also display elements for Oct-4, a transcription factor known to drive the gene expression in early embryogenesis [22]. So this may be a putative factor for high correlation observed in the first trimester. Future studies are warranted to further understand how these genes are co-regulated in different settings.

We did not note any correlation between gestational age and expression level. However, all our samples were collected during the first trimester. It is possible that the expression profile of TXNRD1 and/or MGST1 is altered during fetal development. Unfortunately, sex was not determined at the collection of fetus specimens.

As in the case of TXNRD1 and MGST1, the mRNA expression profile during the development is often different than in adults. This developmental switch may be driven by different transcriptional mechanisms such as alternative splicing. Here we noted different distribution of the TXNRD1 isoforms between adults and fetuses, indicating that there may be some switches in the splicing events during ontogeny. Most splice variants investigated here were found at higher levels in the fetal liver samples. α7/8 was the predominant isoform during first trimester. The same splicing profile has been reported in adult malignant mesothelioma cells, where α7/2 and α7/8 were the major transcripts, whereas α6 was found at the lowest levels [16]. For MGST1, our results indicate that the alternative first exon 1B is preferred in both fetuses and adults. This is in agreement with previous findings [10,11]. This first exon is the exon that gives rise to the predominant mature mRNA transcripts in human tissues [10].

In addition to TXNRD1 and MGST1 gene expression in livers, we performed studies in fetal samples obtained from adrenals, kidneys and lungs. The expression of TXNRD1 was most pronounced in the adrenal tissue suggesting an important role for the enzyme in this tissue during fetal life. To our knowledge a comparison of the expression of TRXR1 in different tissues in adults has not been done. MGST1 was most abundant in adrenals and livers during the first trimester. In adults MGST1 transcripts have been found at highest levels in livers and pancreas [10].

Epigenetic events such as DNA methylation of CpG sites has been suggested to play a role in the developmental switch in gene expression of several genes [22,23]. Methylation of CpG islands may result in inhibition of transcriptional rate or in some cases activation. Even though a CpG island was identified in silico in the proximal promoter of TXNRD1, no one has investigated the methylation grade of the TXNRD1 promoter in vivo. Our 5-AZA results indicate that methylation of TXNRD1 may be a transcriptional determinant in liver cells. We showed that de-methylation leads to decrease in TXNRD1 expression in HepG2 cells. Even though up-regulation is more common, several genes have been shown to be repressed by 5-AZA exposure in HepG2 cells [24]. It is possible that de-methylation increases access of repressor binding sites. Alternatively, the availability of trans-acting factors may decrease. It would be of interest to further assess the CpG island in TXNRD1 and study whether or not the methylation grade differs throughout development.

MGST1 expression was not affected by 5-AZA indicating that DNA methylation does not play an important role in MGST1 gene expression, at least not in HepG2 cells. This is consistent with an in silico analysis using UCSC Genome Bioinformatics (http://genome-euro.ucsc.edu/index.html), which did not identify any CpG islands in the MGST1 gene.

Many proteins that are highly expressed during fetal life are often overexpressed also in malignant cells. Such proteins can be used as biomarkers for different types of cancer, for example alfa-fetoprotein that is a biomarker for liver cancer. TXNR1 is over-expressed in many cancer cells and plays an important role during carcinogenesis [25–27]. This is in accordance with the results presented here showing high expression of TXNR1 during fetal life. Indeed, it has been discussed whether this enzyme could be used as a biomarker for different cancers [25,28].

In conclusion we have found that the expression of TXNRD1 is higher in the first trimester than in adult life and might be of importance during fetal life, whereas MGST1 was more abundant in adults. Interestingly, hepatic TXNRD1 and MGST1 are expressed in the fetuses as well as in adults suggesting common mechanism for regulation. In addition, DNA methylation may be important in the regulation of the transcriptional activity of TXNRD1 but is probably of minor importance for MGST1.

Author contributions

LBB, MJ, RM, AR and LE conceived and designed the study. LBB, MJ and LE acquired and analysed the data. LBB, MJ, RM, AR and LE interpreted the data and wrote the paper.

Acknowledgments

This study was financially supported by Grants from The Swedish Research Council, Magnus Bergwall Stiftelse, Stockholm County Council (SLL/ALF) and Karolinska Institutet. The authors have no conflict of interest to declare.

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