Current Research in Toxicology 3 (2022) 100082

Contents lists available at ScienceDirect

Current Research in Toxicology

journal homepage: www.journals.elsevier.com/current-research-in-toxicology

Aflatoxin B1 targeted gene expression profiles in human placental primary trophoblast cells

Rami El-Dairi, Jaana Rysä, Markus Storvik, Markku Pasanen, Pasi Huuskonen *

School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, FIN-70211 Kuopio, Finland

ARTICLE INFO

Keywords:
Aflatoxin B1
Estrogen signalling
Gene expression profiling
Pathway analysis
Placenta
Trophoblast

ABSTRACT

Aflatoxin B1 (AFB1) is a mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*. A high exposure (40 nM and 1 μM AFB1 for 72 h) was used to study mechanistic effects of AFB1 on gene expression patterns in human primary trophoblast cells, isolated from full term placentae after delivery. Gene expression profiling was conducted, and Ingenuity pathway analysis (IPA) software was used to identify AFB1-regulated gene networks and regulatory pathways.

In response to 40 nM AFB1, only 7 genes were differentially expressed whereas 1 μM AFB1 significantly dysregulated 170 genes (124 down- and 46 upregulated, ±1.5-fold, $p < 0.05$) in AFB1-exposed trophoblasts when compared to controls. The top downregulated genes were involved in endocrine signalling and biosynthesis of hormones, and lipid and carbohydrate metabolism. The top upregulated genes were involved in protein synthesis and regulation of cell cycle. The main canonical pathways identified by IPA were associated with endocrine signalling including growth hormone signalling, and corticotropin releasing hormone signalling. Furthermore, genes involved in aryl hydrocarbon receptor (AhR)-mediated estrogen receptor signalling were dysregulated in response to AFB1.

Our findings indicate that a high concentration 72 h AFB1 exposure caused relatively moderate number of changes on transcript level to human placental primary trophoblast cells. However, these preliminary results need to be confirmed with human-relevant concentrations of AFB1.

Introduction

Human placenta is an intermediary organ that is responsible for growth and development of the fetus and serves as a protective barrier that separates fetal blood from maternal blood (Burton and Jauniaux, 2015). Placenta is responsible for regulating fetal environment, providing immune protection, transfer of gases and nutrients from the maternal to fetal blood, and production of hormones, cytokines and growth factors necessary for maintenance of pregnancy (Guttmacher et al., 2014; Iliodromiti et al., 2012).

Aflatoxin B1 (AFB1) is a secondary metabolite produced by *Aspergillus flavus* and *A. parasiticus* mould and contaminates crops such as rice, maize, nuts, cereals, soybeans (Alshannaq and Yu, 2017) and even animal products such as meat, eggs and milk (Pier, 1992). Aflatoxins, especially AFB1 are known to cause acute and chronic toxicity such as liver failure and liver cancer (Ostry et al., 2017; Williams et al., 2004), teratogenicity (Gong et al., 2004; Smith et al., 2017), immunosuppression (Williams et al., 2004) and potential endocrine disruptive effects (Storvik et al., 2011).

AFB1 and its metabolites can be found in breast milk, neonatal cord blood and serum of pregnant women (Lamplugh et al., 1988). These toxins can be transferred and metabolized through the placenta in which aflatoxicol (AFL) is the only metabolite from AFB1 formed in the placenta (Partanen et al., 2010). However, the effects and molecular targets of AFB1 in human placental cells are not well-known. It has been previously demonstrated that AFB1 causes disturbances in placental hormone metabolism and normal oestrogen production in placental chorion carcinoma (JEG-3) cells (Huuskonen et al., 2013; Storvik et al., 2011; Wang et al., 2015; Zhu et al., 2016). In addition, previous gene expression profiling studies with AFB1 have been done on human hepatocytes which reported alternation in genes related to p53 signalling pathway, cell cycle, apoptosis and DNA repair (Josse et al., 2012). Yip et al., (2017) reported effects on cell growth, DNA synthesis and cell cycle progression in breast cancer MCF-7 cells. Other transcriptomic studies reported that AFB1 altered genes involved in gluconeogenesis, lipid metabolism, p53 signalling pathway (Lu et al., 2013) aryl

* Corresponding author.
E-mail address: pasi.huuskonen@ttl.fi (P. Huuskonen).

https://doi.org/10.1016/j.crtox.2022.100082
Received 31 December 2021; Received in revised form 29 June 2022; Accepted 30 June 2022
Available online 4 July 2022
2666-027X/© 2022 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

hydrocarbon receptor (AhR), nuclear factor erythroid 2-related factor 2 (Nrf2), glutathione-S-transferase (GSH), cell cycle, extracellular matrix, and cell differentiation networks (Merrick et al., 2013) in rat hepatocytes. Liu et al. (2020) identified AFB1 target genes in chicken liver and reported an upregulation in peroxisome proliferator-activated receptor gamma (PPARγ) signalling and apoptosis.

In this study, AFB1 targeted gene expression profiles were determined in human primary trophoblast cells isolated from full-term placentas immediately after delivery. Two concentrations, 40 nM and 1 μM of AFB1 were used to study mechanistic effects on placental gene expression at one time point, 72 h. To our knowledge, our microarray study is the first to report the transcriptomic changes in human primary trophoblasts in response to AFB1.

Materials and methods

Placental tissue

Four full term placentas were obtained from Kuopio University Hospital after a caesarean section. The study was approved by the ethics committee (Kuopio University Hospital, Finland) and the experiments were done according to the Declaration of Helsinki. All individual study participants signed the informed consent. The full-term placentas were from uncomplicated non-smoking pregnancies.

Isolation and culturing of human primary trophoblast cells

The isolation and culture of primary placental trophoblast cells was conducted according to Petroff et al. (2006) and described in detail in previous study by El Dairi et al., (2018). Briefly, the isolation of trophoblast cells started 20 min after caesarean sections by dissecting the placental soft villous tissue (50–60 g) and incubating the homogenate with digestion enzymes (Trypsin 2.5%, GIBCO, USA #15090–046). The cytotrophoblasts then were separated from the rest of the tissue using Percoll gradient (Sigma-Aldrich, USA, #P4973) and then transferred to 6-well plates (Thermo Scientific, USA, #140675) in a Cell-IQ incubator (Chip-Man Technologies Ltd., Finland). Primary trophoblasts (5×10^5 cells/well) were treated with 40 nM and 1 μM AFB1 (Sigma-Aldrich, #A6636) for 72 h and the control was 0.1% dimethyl sulfoxide (DMSO, Sigma-Aldrich, #D8418) (six replicates).

Cell viability test

The cell viability of AFB1 treatment on the primary trophoblast cells was assessed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) test (Sigma-Aldrich/Merck, #M2128) as previously described by Storvik et al. (2011). Human primary trophoblasts ($5x10^5$ cells/well) were incubated with 0.01–3 μM AFB1 for 72 h at 37 °C and then the MTT reagent was added for 4 h incubation, sodium dodecyl sulfate - N-dimethylformamide (SDS–DMF) (Sigma-Aldrich/Merck, #L3771 and #D4551, respectively) was added and the plate was maintained overnight at 37 °C. The optical density was measured using a BioTek ELx800 reader (BioTek, USA) at a wavelength of 570 nm. The results were expressed as a percentage of the control cells exposed to dimethyl sulfoxide to the same concentration used as the solvent for AFB1. The significance of the differences between exposures and respective controls was analyzed by one-way ANOVA followed by Tukey's multiple comparison post-hoc test.

DNA microarray

The extraction of total RNA from human primary trophoblast was performed with a Qiagen AllPrep mini kit (Qiagen Ltd, UK, #80284) followed by DNase treatment (Ambion Turbo DNA-Free Kit, Life Technologies, USA, #AM1907). The quality and integrity of RNA samples were monitored as previously described (El Dairi et al. 2018). DNA microarray assay with Illumina HumanHT-12 Expression BeadChip (Illumina, USA) was conducted according to the manufacturer's instructions at the Core facility of the Estonian Genome Center, University of Tartu. The raw intensity data for the transcripts was exported using the Illumina GenomeStudio software, and normalized to the same distribution in each chip by using Chipster (CSC, Finland). The differentially expressed genes were selected when the difference in the normalized expression was statistically significant ($p < 0.05$, Student's *t*-test with adjustment by the Benjamini-Hochberg multiple testing correction) and the fold-change between the AFB1 treated group was at least 1.5-fold as compared to the respective controls. A heatmap of the significantly up- and downregulated transcripts was created with Heatmapper (heatmapper.ca) with average linkage and Euclidian distance method. The complete data sets are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database and gene expression profiling data comply with the Minimum Information About a Microarray Experiment (MIAME) standard. The data can be obtained from the GEO database with the accession number GSE153590.

Quantitative real-time (qRT)-PCR

Quantitative RT-PCR was performed as previously described (Huuskonen et al. 2008) using Taqman Real Time PCR assays (Thermo Fisher Scientific, USA) for cytochrome P450 family 1 subfamily A member 1 (CYP1A1) (Hs00153120_m1) and CYP19A1 (Hs00903411_m1), and the gene expression of was normalized with β-actin (Taqman assay #4316315E).

Pathway analysis

The list of differentially expressed genes was uploaded into Ingenuity pathway analysis (IPA) software (Qiagen, USA). IPA was used to analyse the main canonical pathways, diseases and functions and upstream regulators by clustering the differentially expressed genes (≥1.5-fold, p-value < 0.05, compared to controls). The calculation of P-values for the pathways using the Fisher Exact Test, and $p < 0.05$ was considered statistically significant. A Z-score with a value of ≥ 2 or ≤ -2 indicates significant activation or inhibition of the biological functions and upstream regulators. The full description of pathway analysis has been described by (El Dairi et al., 2018).

AFB1 dysregulated mRNA expressions in human primary trophoblast

Primary trophoblasts were exposed to increasing concentrations of AFB1 (0.01–3 μM) for 72 h, and cell viability was measured with the MTT-assay. Cell viability was not affected significantly up to 1 μM concentration of AFB1, and at 3 μM AFB1 cell viability was decreased significantly (87 % compared to control) ($p < 0.05$) (Fig. 1). Based on cell viability AFB1 concentrations of 40 nM and 1 μM were selected for the gene expression profiling study. In DNA microarray study, only 7 transcripts were differentially expressed in 40 nM AFB1-treated primary trophoblasts; all these transcripts were non-significantly downregulated. Only two of the transcripts, an amino acid transporter SLC43A2 and a histone protein coding gene HIST1H4C, correspond to protein coding genes, and other 5 transcripts were non-coding RNA sequences (LOC100008589, MIR1974, LOC441763, LOC100133565, LOC100008588). Due to minimal impact of 40 nM of AFB1 treatment on gene expression, a higher concentration of 1 μM AFB1 was used to perform further mechanistic analyses. The higher 1 μM AFB1 concentration significantly dysregulated 170 genes (46 up- and 124 down-regulated, ≥1.5-fold, p-value < 0.05) when compared to DMSO-treated controls (Fig. 2). The top ten up- and down-regulated genes in 1 μM AFB1-treated primary trophoblasts are listed in Table 1 and the complete list of differentially expressed gene sets is presented in Supplementary Table 1. Among the top down-regulated genes were several

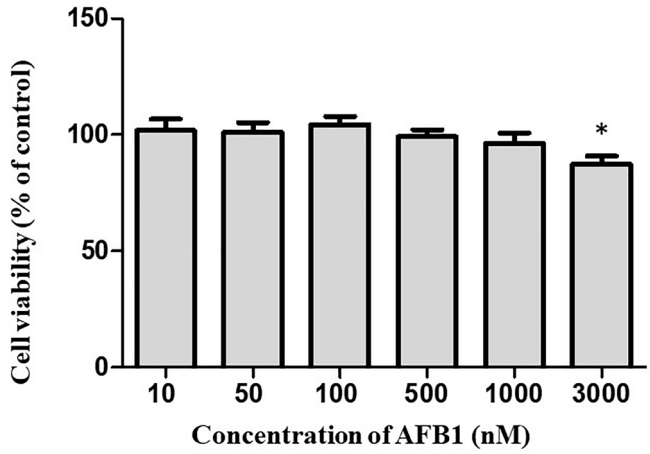

Fig. 1. Effect of aflatoxin B1 (AFB1) on the viability of primary human trophoblast cells. Cell viability was assessed by the MTT-assay in AFB1-treated (72 h) trophoblasts, expressed as a percentage of control cells treated with 0.25 % dimethyl sulfoxide. Results are the mean ± SD (n = 4). *p < 0.05 one-way ANOVA followed by Tukey's multiple comparison post-hoc test.

placental genes involved in endocrine signalling and biosynthesis of hormones, and lipid and carbohydrate metabolism whereas many cell cycle regulators and genes involved in protein synthesis were among the top up-regulated genes. We confirmed gene expression levels of CYP1A1 and CYP19A1 results by comparison with mRNA levels obtained by gene expression profiling with RT-qPCR. The normalized qPCR gene expression fold-change ratios for CYP1A1 and CYP19A1 after 72 h 1 μM AFB1 exposure were −2.9 and −1.5 respectively, when compared to controls. The corresponding microarray expression fold-change of CYP1A1 and CYP19A1 were −0.3 and −2.2, respectively.

Pathway analysis

To understand the mechanisms involved in AFB1 induced and repressed gene responses we explored the molecular pathways of differentially expressed genes in response to 1 μM AFB1 treatment in primary trophoblasts. First, we analyzed the canonical pathways affected by AFB1 treatment using IPA. Among the significant canonical pathways there were hormone signalling pathways inhibited including growth hormone, estrogen receptor, insulin secretion and corticotropin releasing hormone signalling pathways (Fig. 3).

IPA analysis exhibited that the main altered molecular and cellular functions in human placental trophoblast in response to 1 μM AFB1 were cell death and survival, cellular movement, development, growth and proliferation, and cell-to cell signalling and interaction (Fig. 4). Furthermore, the most significant diseases and disorders linked to the AFB1 regulated gene set in primary trophoblasts included those related to hyperlipidemia, transport of D-glucose and impaired glucose tolerance (Fig. 5). To further evaluate the effect of AFB1 on primary trophoblasts, IPA was used to determine the biological relationships among the differentially expressed genes. The top five most significant gene networks and the dysregulated genes involved in these networks are shown in Supplementary Table 2. The most significant network is related to development and function of endocrine and reproductive systems. In addition, there were also networks related to cancer, cell cycle and lipid metabolism among top five gene networks of AFB1 affected genes.

Furthermore, we used IPA to predict potential upstream regulators that could explain the observed changes in gene expression in AFB1-treated primary trophoblasts. The top predicted endogenous regulators of are shown in Table 2. Among the top predicted activated upstream regulators were tumour suppressor protein TP53 and several transcription factors including forkhead box O3 (FOXO3), FOXO4 and RUNX

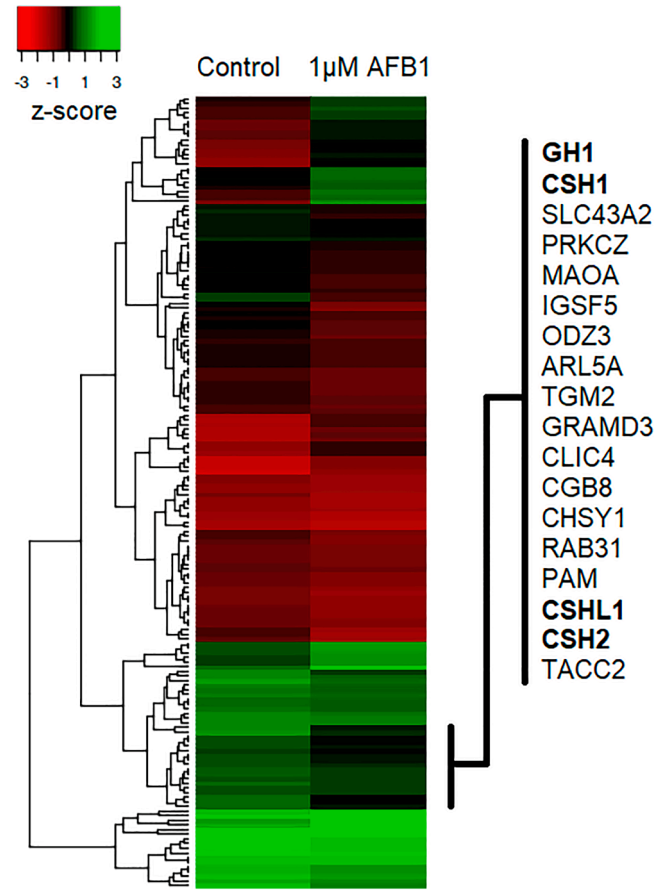

Fig. 2. A transcript heatmap of primary trophoblast cells, treated with 1 μM aflatoxin B1 (AFB1) or control treatments for 72 h. The green colour indicates transcripts with high expression levels and the red colour indicates low expression levels, when compared to controls. A prominent cluster of down-regulated transcripts related to four growth-hormone related transcripts (bolded) is marked on the right side of the image. The complete list of differentially expressed gene sets is presented in Supplementary Table 1. GH1, growth hormone 1; CSH1, chorionic somatomammotropin hormone 1; SLC43A2 solute carrier family 43A2; PRKCZ, protein kinase C zeta; MAOA, monoamine oxidase A; IGSF5, immunoglobulin superfamily member 5; ODZ3, teneurin transmembrane protein 3; ARL5A, ADP ribosylation factor like GTPase 5A; TGM2, transglutaminase 2; GRAMD3, GRAM domain containing 2B; CLIC4, chloride intracellular channel 4; CGB8, chorionic gonadotropin subunit beta 8; CHSY1, chondroitin sulfate synthase 1; RAB31, member RAS oncogene family; PAM, peptidylglycine alpha-amidating monooxygenase; CSHL1, chorionic somatomammotropin hormone like 1; CSH2, chorionic somatomammotropin hormone 2; TACC2, transforming acidic coiled-coil containing protein 2.

family transcription factor 1 (RUNX1). In top inhibited downstream regulators were several regulators related to hormone response such as androgen receptor, dihyrotestosterone, corticotropin releasing hormone (CRH) and beta-estradiol as well as to inflammatory and immune response (e.g., CD24 molecule, prostaglandin E2, interleukins 1, 1B and 6 as well as toll like receptor 2). In addition, AhR and aryl hydrocarbon receptor nuclear translocator (ARNT) were among inhibited upstream regulators. IPA was further utilized to evaluate the influence of AFB1 on AhR signaling in human primary trophoblasts. The analysis revealed that AFB1 dysregulated the following genes: adrenomedullin (ADM), Fos proto-oncogene, AP-1 transcription factor subunit (FOS), estrogen related receptor gamma (ESRRG), syndecan 1 (SDC1), solute carrier family 2A1 (SLC2A1, glucose transporter 1), cytochrome P450 subfamily 19A1 (CYP19A1, aromatase), actin alpha 2 (ACTA2), growth arrest and DNA damage inducible alpha (GADD45A) and cyclin-dependent kinase inhibitor (CDKN1A, P21), that are involved in AhR

Table 1
Top 10 up- and downregulated genes in aflatoxin B1-treated human primary trophoblasts compared to control cells.

Entrez Gene ID	Gene Symbol	Gene Name	Fold-change
Up-regulated			
92	ACVR2A	Activin A receptor type 2A	4.3
51065	RPS27L	Ribosomal protein S27 like	2.7
1026	CDKN1A	Cyclin dependent kinase inhibitor 1A	2.5
5430	POLR2A	RNA polymerase II subunit A	2.2
64752	ISG20L1	Interferon-stimulated 20 KDa exonuclease-like 1	2.0
6223	RPS19	Ribosomal protein S19	1.9
2876	GPX1	Glutathione peroxidase 1	1.9
51499	TRIAP1	TP53 regulated inhibitor of apoptosis 1	1.9
1647	GADD45A	Growth arrest and DNA damage inducible alpha	1.8
51693	TRAPPC2L	Trafficking protein particle complex 2 like	1.8
Down-regulated			
1442| 1443	CSH1/2	Chorionic somatomammotropin hormone 1/2	−3.3
2688	GH1	Growth hormone 1	−3.3
5069	PAPPA	Pappalysin 1	−3.0
25945	PVRL3	Nectin cell adhesion molecule 3	−2.9
8038	ADAM12	ADAM metallopeptidase domain 12	−2.9
1444	CSHL1	Chorionic somatomammotropin hormone like 1	−2.8
79987	SVEP1	Sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1	−2.7
5921	RASA1	RAS p21 protein activator 1	−2.6
84525	HOPX	HOP homeobox	−2.6
10761	PLAC1	Placenta enriched 1	−2.6

signalling, that in turn is predicted to be inhibited (Z-score −2.8, p-value 8.25E-04) in human term trophoblast (Fig. 6).

Gene expression analysis of IPA revealed that cell cycle, cellular compromise, DNA replication, recombination, and repair gene network were among the biological functions and gene networks that were dysregulated in AFB1-treated primary trophoblasts (Supplementary Fig. 1). Furthermore, IPA analysis revealed that cell cycle processes mitogenesis and mitosis were predicted to be inhibited due to downregulation of genes such as FOS, ADM, epidermal growth factor receptor (EGFR), protein kinase C zeta (PRKCZ), cholecystokinin (CCK) and upregulation of CDKN1A, GADD45A (Supplementary Fig. 2).

Discussion

In this study, AFB1 target genes were investigated in human primary trophoblasts with gene expression analysis and pathway analysis was performed to identify the main molecular and cellular functions with the dysregulated genes in the dataset. This study demonstrates that unlike the liver cells, primary trophoblast cells are resistant to AFB1-induced damage in *in vitro* platform. The downstream analysis data

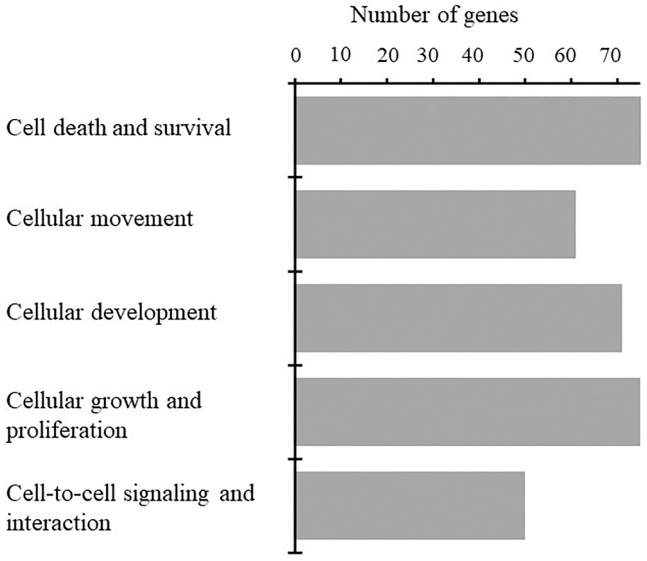

Fig. 4. Functional analysis of differentially expressed genes in aflatoxin B1-treated (1 μM) primary trophoblasts by the IPA software. The most significant biological functions with the highest number of significantly altered genes (≥1.5-fold upregulation or downregulation in AFB1-treated cells compared to controls; $p < 0.05$) are shown.

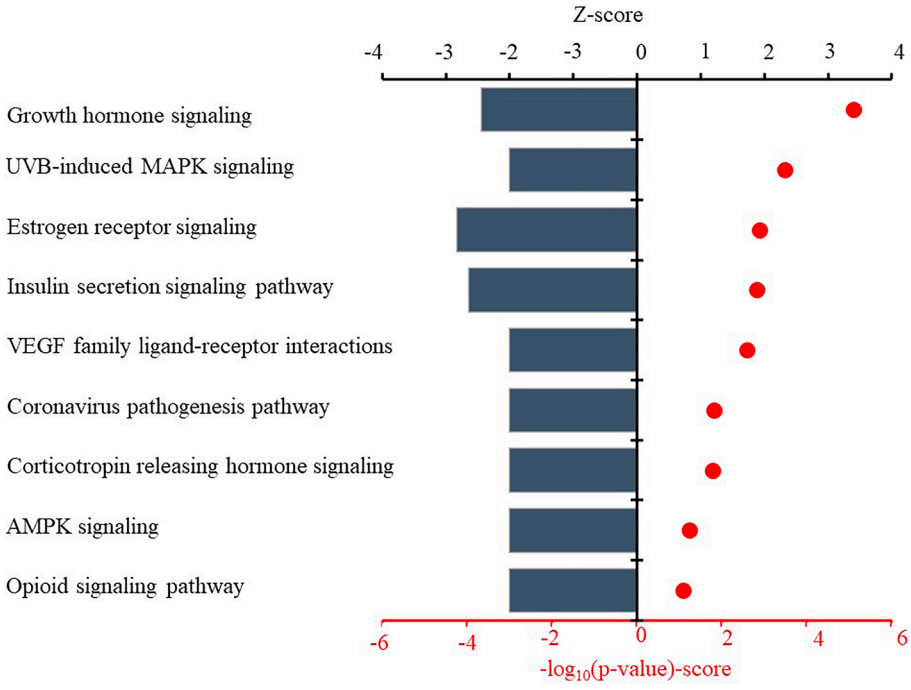

Fig. 3. The significant canonical pathways associated with differentially expressed genes in primary trophoblasts in response to 1 μM aflatoxin B1. The red dot represents -log10(p-value)-score of the canonical pathways. A positive or negative Z-score value (columns) indicates that a function was predicted to be either increased or decreased, respectively, with the value indicating standard deviations from the control mean. AMPK, adenosine monophosphate-activated protein kinase; MAPK, mitogen-activated protein kinase 1; UVB, ultraviolet B; VEGF, vascular endothelial growth factor.

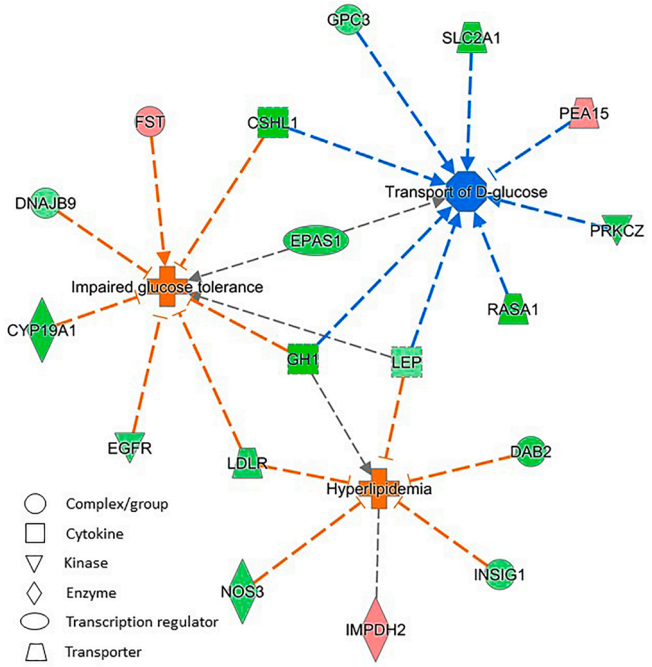

Fig. 5. Diseases and functions predicted by IPA to be associated to differentially expressed genes in aflatoxin B1-treated (1 μM) human primary trophoblasts. Green colour indicates downregulated genes and red colour upregulated genes, blue colour indicates inhibitory effects, and orange colour indicates predicted activation. Cross shape = predicted inhibition, hexagon shape = predicted activation. CSHL1, chorionic somatomammotropin hormone like 1; CYP19A1, cytochrome P450 family 19 subfamily A member 1; DAB2, DAB adaptor protein 2; DNAJB9, DnaJ heat shock protein family (Hsp40) member B9; EGFR, epidermal growth factor receptor; EPAS1, endothelial PAS domain protein 1; FST, follistatin; GPC3, glypican 3; GH1, growth hormone 1; IMPDH2, inosine monophosphate dehydrogenase 2; INSIG1, insulin induced gene 1; LEP, leptin; NOS3, nitric oxide synthase 3; LDLR, low density lipoprotein receptor; PEA15, proliferation and apoptosis adaptor protein 15; PRKCZ, protein kinase C zeta; RASA1, RAS P21 protein activator 1; SLC2A1, solute carrier family 2 member 1.

demonstrated that AFB1 disrupts endocrine signalling, lipid and carbohydrate metabolism, and cell cycle and DNA repair processes in human placental cells.

AFB1 sensitivity of human primary trophoblasts

Gene expression profiling analysis was performed for 40 nM and 1 μM AFB1-treated trophoblast cells. In response to 40 nM exposure, only 7 genes were changed, whereas at a dose of 1 μM AFB1 170 genes were differentially expressed. A moderate number of significant transcript changes with a high AFB1 dose could indicate low sensitivity of primary cells. AFB1 is mainly metabolised by the liver, especially by CYP1A2 and CYP3A4 enzymes into the carcinogenic DNA reactive epoxide, aflatoxin B1-8,9-epoxide (Rushing and Selim, 2019). However, these CYP enzymes are not expressed at protein level in human placenta at term. In addition, the CYP expression profile of term human placenta is restricted and only expresses a few CYP enzymes and differs from that present in the liver (Hakkola et al., 1998). Furthermore, only a less potent AFB1 metabolite, AFL, was detected in the human placenta (Partanen et al., 2010). This difference in CYP expression profile and activity between the liver and the placenta was also reported in rat in which no placental activities of CYP1A, CYP2B, 2C and 3A, UGT1, UGT2 and GST were found (Fabian et al., 2016). Therefore, because of placental cells lack the capacity to form DNA reactive AFB1 metabolites, the 1 μM dose of AFB1 did not affect cell viability on the MTT test. This is in line with results on JEG-3 cells where 1 μM of AFB1 did not decrease cell viability and a

Table 2
Upstream analysis of aflatoxin B1 (AFB1) regulated genes. The top endogenous upstream regulators that were predicted to be activated or inhibited in response to AFB1 exposure in primary trophoblasts are shown. Z-scores ≥ 2 or ≤ 2 indicate that the upstream regulator was predicted to be activated or inhibited, respectively. The p-value calculated by a Fisher's Exact Test was used to determine the significance of the overlap (p-value < 0.05 [i.e., −log10 ≥ 1.3] between the regulator and stretch-responsive genes. Only functional annotations that obtained a significant regulation Z-score (≥2) are presented.

Upstream Regulator	Description	Z-score	p-value
TFRC	Transferrin receptor	2.449	1.11E-07
TP53	Tumor protein P53	2.278	6.11E-10
FOXO4	Forkhead box O4	2.201	9.35E-05
RUNX3	RUNX family transcription factor 3	2.200	1.42E-03
IKZF1	IKAROS family zinc finger 1	2.200	2.52E-02
FOXO1	Forkhead box O1	2.077	2.57E-04
I	Inosine	2.000	4.24E-04
MLXIPL	MLX interacting protein like	2.000	1.42E-03
HIC1	HIC ZBTB transcriptional repressor 1	2.000	6.75E-04
AGT	Angiotensinogen	−3.221	1.27E-08
AR	Androgen receptor	−2.929	4.30E-05
DHT	Dihydrotestosterone	−2.896	4.11E-07
AHR	Aryl hydrocarbon receptor	−2.793	8.24E-04
Creb	CAMP responsive element binding protein 1	−2.589	1.81E-06
HGF	Hepatocyte growth factor	−2.513	6.48E-03
TCF4	Transcription factor 4	−2.449	4.82E-02
TEAD4	TEA domain transcription factor 4	−2.433	3.89E-06
CD24	CD24 molecule	−2.429	6.95E-06
PGE2	Prostaglandin E2	−2.419	6.34E-05
CRH	Corticotropin releasing hormone	−2.408	1.98E-05
FGF2	Fibroblast growth factor 2	−2.317	5.60E-06
LT3	L-triiodothyronine	−2.275	1.08E-07
ARNT	Aryl hydrocarbon receptor nuclear translocator	−2.229	2.45E-05
IL1B	Interleukin 1 beta	−2.223	8.49E-07
AURK	Aurora kinase B	−2.219	1.83E-05
EGFR	Epidermal growth factor receptor	−2.218	4.06E-05
IL13	Interleukin 13	−2.157	1.70E-01
PPARA	Peroxisome proliferator activated receptor alpha	−2.150	1.40E-02
LEP	Leptin	−2.148	7.99E-06
EGF	Epidermal growth factor	−2.093	9.48E-07
E2	Beta-estradiol	−2.087	4.43E-10
Vegf	Vascular endothelial growth factor A	−2.086	2.03E-03
IL6	Interleukin 6	−2.059	

(*continued on next page*)

Table 2 (*continued*)

Upstream Regulator	Description	Z-score	p-value
			5.91E-06
PI3K (family)	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	−2.043	9.37E-06
IL1	Interleukin 1	−2.021	1.48E-04
TLR2	Toll like receptor 2	−2.000	4.20E-02
MAP2K5	Mitogen-activated protein kinase 5	−2.000	7.63E-04
ELL2	Elongation factor for RNA polymerase II 2	−2.000	5.57E-04
FA	Fatty acid	−2.000	6.83E-03

significant reduction was seen only at the concentration of 3 μM (Storvik et al. 2011).

AFB1 dysregulated genes involved in AhR and estrogen receptor signalling

Our analysis revealed that estrogen receptor (ER) signalling is among the main pathways that were disrupted by 1 μM AFB1 in term human trophoblasts. Genes involved in ER signalling are highly expressed in the placenta (Su et al., 2004). ESRRG was downregulated in our analysis. ESRRG is highly expressed in the placenta, it plays a role in the placental development, and is induced during trophoblast differentiation and induction of CYP19A1 gene expression (Kumar and Mendelson, 2011).

Consistent with ESRRG repression, CYP19A1 was downregulated in 1 μM AFB1 exposed primary cells compared to controls. CYP19A1 plays a vital role in placental estrogen biosynthesis from C19 steroids (Nelson and Bulun, 2001). Conversely, an upregulation of CYP19A1 activity by AFB1 exposure with similar high dose in placental secondary cell line JEG-3 has been reported in our earlier studies (Huuskonen et al., 2013; Storvik et al., 2011). However, in this study primary trophoblast cells were utilized which can possess different characteristics compared to immortalized cell lines.

Based on IPA analysis, AhR was predicted as one of the most important regulators of AFB1 dysregulated genes. In this data set, AhR activity was observed to be among top suppressed upstream regulators inhibited by AFB1 although previous studies have reported that AFB1 caused a significant increase in CYP1A1/A2, AhR, constitutive androstane receptor (CAR) and pregnane X receptor (PXR) mRNA expression in human primary hepatocytes (Mary et al., 2015) and AhR-mediated increase of CYP1A1 activity and transcription in H4IIE rat hepatoma cells (Ayed-Boussema et al., 2012). CYP1A1 and AhR are highly expressed and inducible in the human placenta, whereas CAR and PXR are not remarkably expressed (Storvik et al., 2014). Furthermore, several studies have reported the involvement of AhR with steroid hormone receptors, mainly the AhR cross-talk with ERs (NR3A1/A2) (Ohtake et al., 2003; Pocar et al., 2005; Safe et al., 2018). The crosstalk between the liganded AhR and ER signalling was reported to be through the inhibition of estrogen signalling by inhibiting the binding of estrogen with ER (Klinge et al., 1999) or AhR binding to ER-chaperones and inhibiting the transcription of estrogen downstream target genes such as cathepsin D and c-fos (Duan et al., 1999). The AhR anti-estrogenic effects were also shown through the AhR-mediated activation of rapid proteasomal degradation of ER that leads to reduction in ER protein and transcriptional activity (Wormke et al., 2003). In line with this, our previous studies (Huuskonen et al., 2016, 2008) demonstrated that maternal smoking during pregnancy resulted in increased placental CYP1A1 activity but decreased CYP19A1 activity, thus confirming placental crosstalk between AhR and ER signalling.

ER signalling is predicted to be inactivated in this dataset, which supports the anti-estrogenic effects of AFB1-regulated AhR, however, the AhR-ER crosstalk also depends on the availability of estrogen that maybe linked to repressed CYP19A1 activity after AFB1 exposure. For instance, in line with the data, in the presence of estrogen, AhR possess anti-estrogenic effects and inhibit ER DNA-binding (Ohtake et al., 2003).

AFB1 disrupts endocrine signalling in human primary trophoblast

According to our analysis, growth hormone 1 (GH1) and corticotropin releasing hormone (CRH) signalling are predicted to be downregulated due to downregulation of chorionic somatomammotropin hormone 1 (CSH1), chorionic somatomammotropin hormone like 1 (CSHL1), FOS, GH1, PRKCZ, and nitric oxide synthase 3 (NOS3). Maternal GH1 gene is silenced in the pituitary gland during pregnancy, whereas placental syncytiotrophoblasts express GH1 (Handwerger and Freemark, 2000). GH1 is involved in fetal growth, placental development, maternal adaptation to pregnancy, and it stimulates maternal insulin-like growth factor (IGF1) production resulting in glucose and amino acids availability to the fetus (Lacroix et al., 2002; Pérez-Ibave et al., 2014). CRH is one of the vital hormones that is produced mainly by the placenta and involved in human pregnancy and parturition (Challis et al., 2000; Sasaki et al., 1987; You et al., 2014). The downregulated CSH1 and CSHL1 (placental lactogen) are members of

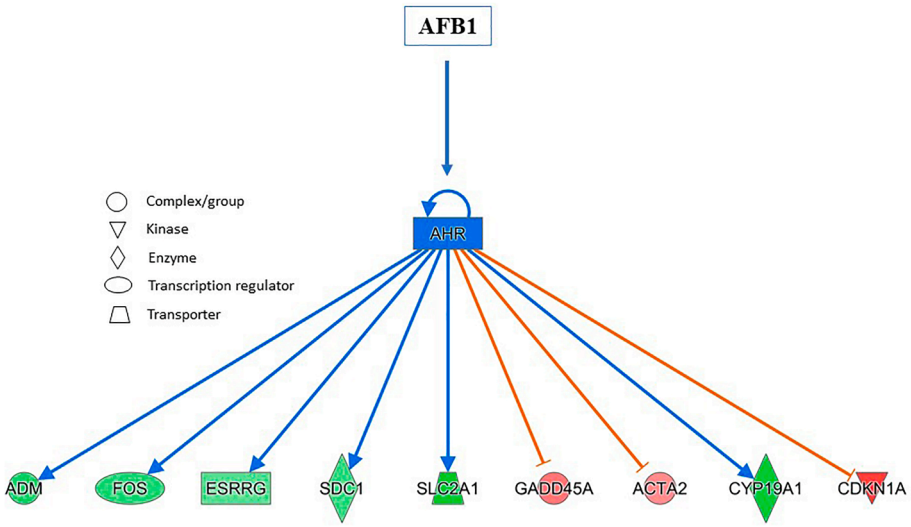

Fig. 6. The aryl hydrocarbon receptor (AhR) regulated genes in aflatoxin B1 (AFB1) treated (1 μM) term trophoblasts. Based on IPA analysis, AhR is predicted as one of the most important regulators of AFB1 dysregulated genes. Green colour indicates downregulated genes and red colour upregulated genes, blue colour indicates the inhibitory effects, and orange colour indicates predicted activation. ACTA2, actin alpha 2; ADM, adrenomedullin; CDKN1A, cyclin dependent kinase inhibitor 1A; CYP19A1, cytochrome P450 family 19 subfamily A member 1; ESRRG, estrogen related receptor gamma; FOS, Fos proto-oncogene; GADD45A, growth arrest and DNA damage inducible alpha; SLC2A1, solute carrier family 2 member 1; SDC1, syndecan 1.

somatotropin/prolactin hormone family which are solely expressed in the placental villi (Chen et al., 1989; Fagerberg et al., 2014) and play important role in fetal growth, regulation of maternal carbohydrate, lipid and protein metabolism (Handwerger and Freemark, 2000; Kim and Felig, 1971). The activity of placenta enriched 1 (PLAC1), which is important for placental development, has been reported to be reduced in human placentas in cases of fetal growth restriction (Sun et al., 2021). Interestingly, AFB1 downregulated PLAC1 in placental primary trophoblasts. Altogether, these findings suggest that a high AFB1 exposure among women of childbearing potential may result in poor prognosis for conception and further development of pregnancy and well-being of fetus.

To summarize, AFB1 affected the expression of genes involved in endocrine signalling in placental trophoblast cells. AFB1 have been demonstrated to cause fetal toxicity or teratogenicity (Smith et al. 2017) which can be expected by crossing the placental barrier (Partanen et al. 2010). These results suggest that decreased endocrine signalling in trophoblasts may contribute to AFB1 caused toxicity for feto-placental unit.

AFB1 disrupts carbohydrate and lipid metabolism in human placental cells

The gene expression analysis exhibited that AFB1 downregulated the expression of several genes involved in glucose and fatty acid homeostasis, including SLC2A1, pyruvate dehydrogenase (PDK4), leptin (LEP), low density lipoprotein receptor (LDLR) and insulin induced gene 1 (INSIG1), indicating AFB1 is interfering in regulation of placental carbohydrate and lipid metabolism as well as energy production. SLC2A1 is a major transporter in glucose uptake (Mueckler and Makepeace, 2008), whereas PDK4 is a mitochondrial protein that plays a key role in glucose and fatty acids metabolism and homeostasis (Abbot et al., 2005; Kulkarni et al., 2012). Leptin induces trophoblast cells proliferation and survival, and plays a key role in placental adaptation to stimuli such as hypoxia (Cervero et al., 2005; Dos Santos et al., 2015; Henson and Castracane, 2006; Schanton et al., 2018). INSIG1 is an important regulator in glucose homeostasis and lipid metabolism (Dong and Tang, 2010), similarly to LDLR (Dato and Chiabrando, 2018). Previous studies have also reported that AFB1 alters lipid, amino acid and carbohydrate metabolism in other experimental animal models (Baldwin and Parker, 1985; Cheng et al., 2017; Kiessl, 1986). So, in line with previous studies, our data indicates that AFB1 exposure during pregnancy may disturb also human placental lipid and glucose metabolism.

AFB1 disrupts cell cycle and DNA repair processes

Human trophoblastic cells at term cannot produce DNA reactive AFB1 metabolites via metabolic activation. However, our study demonstrates that AFB1 disrupts cell cycle and DNA repair process by dysregulating genes such FOS, ADM, CDKN1A, GADD45A and activating transcription factor 6 (ATF6) in human primary trophoblast. In the dataset, one of the downregulated genes was FOS, which is an oncogene that belongs to AP-1 family of transcription factors and it is implicated in many tumours (Curran and Morgan, 1987; Milde-Langosch, 2005). FOS plays significant role in cell proliferation, cell cycle entry and progression (Angel and Karin, 1991; Brown et al., 1998; Christmann et al., 2006; Kovary and Bravo, 1991), similarly to ADM (Miyashita et al., 2003; Ouafik et al., 2009; Shichiri and Hirata, 2003; Withers et al., 1996). CDKN1A, and GADD45A are known regulators of apoptosis, cell cycle progression and DNA repair (Barreto et al., 2007; Dutto et al., 2015; Kleinsimon et al., 2018). Also, ATF6 is known to play significant role in apoptosis, cell cycle arrest progression and steroid hormone production (Xiong et al., 2017). Consequently, our data supports the previous data that AFB1 plays a role on cell cycle arrest, progression, DNA damage checkpoint, and inhibition of DNA repair systems in different human and animal cell lines (Engin and Engin, 2019; Ricordy et al., 2002; Weng et al., 2017; Yin et al., 2016; Yip et al., 2017).

Human outcome

This study focuses on the gene expression effects of AFB1 on cultured human placental trophoblastic cells only. The data shows that trophoblasts at term are much less sensitive to the toxic effects of AFB1 than hepatocytes. However, similarly to previous studies (Storvik et al., 2011; Huuskonen et al. 2013) the present data demonstrates that a high AFB1 concentration can transcriptionally alter genes related to placental endocrine functions and energy homeostasis, which are key processes for the fetal growth and development. These outcomes have been identified in several epidemiological studies in which maternal dietary exposures to much lower AFB1 concentrations resulted in impaired child growth and weight gain in sub-Saharan African countries (Gong et al., 2004; Gong, 2002; Turner et al., 2007), repressed immunoglobulin level in Gambian children (Turner et al., 2003) and aflatoxin-albumin adducts were inversely associated with serum IGF1 levels in Kenyan schoolchildren (Castelino et al., 2015). Alterations in the GH-IGF1 axis are a common pathophysiological feature associated with intrauterine growth retardation, type 2 diabetes, hypertension, and cardiovascular disease (Álvarez-Nava et al., 2017; Lupu et al., 2001) and it has been hypothesized that AFB1 retards child growth through effects on IGF1 (Woods et al., 1996). It is important to point out, that our data presents transcriptional pathways in cultured human placental trophoblastic cells in response to exposure of very high dose of AFB1, not present in food. However, this is the first report of gene expression data of AFB1 exposed human primary trophoblasts which may help in elucidating effects of AFB1 on placenta.

Other transcriptome studies

In line with present data, other microarray studies in different cell lines have reported changes in transcriptome that were associated with cellular growth and development, and energy homeostasis. Rieswijk et al. (2016) identified a set of AFB1 regulated genes associated with hepatocellular carcinoma that play a role in dysregulation of the DNA damage response and changes in cell growth and adhesion in human primary hepatocytes. In rat hepatic stem-like cells, AFB1 affected genes of cellular movement, adhesion and immune response (Yang et al., 2014). Furthermore, Tryndyak et al. (2018) treated human hepatic HepaRG cells with AFB1 and reported repressed lipid metabolism, energy production, carbohydrate metabolism and induction of genes related to cellular growth as well as DNA replication and repair.

Limitations

This study has several limitations such as the gene expression was studied only at one time-point and with one concentration which indicates that a dose–response relationship to different expression levels is missing. The lifespan of the trophoblasts after delivery does not allow any long-lasting studies using lower AFB1 concentrations. Furthermore, the used 1 μM AFB1 concentration is higher than the measured AFB1 concentrations in heavily contaminated areas (EFSA, 2020), however, the trophoblasts were not sensitive and even the high dose of AFB1 did not affect the cell viability. Furthermore, only the high dose of AFB1 induced the transcriptomic changes in primary trophoblasts in vitro. The preliminary results on cell viability and gene expression profiles need to be confirmed and serve as starting point for more detailed mechanistic studies.

Conclusions

In conclusion, gene expression analysis was performed in human term primary trophoblast cells after 40 nM and 1 μM AFB1 exposure for 72 h. Trophoblasts exhibited moderate number of significant transcript changes with a high AFB1 dose, which indicate low sensitivity of these primary cells. Based on pathway analysis of gene expression data AFB1

exhibited significant negative effects on placental endocrine function on transcript level including disruption of GH, CRH and ER-signalling. Although, the data is in line with previous studies reporting the endocrine disruptive effects of AFB1, the genes and the signalling pathways dysregulated by AFB1 in human term trophoblasts should be confirmed and studied further. In addition, these preliminary findings should be confirmed with concentrations reflecting real-life exposure to AFB1.

CRediT authorship contribution statement

Rami El-Dairi: Investigation, Funding acquisition, Writing – original draft. **Jaana Rysä:** Investigation, Supervision, Writing – review & editing. **Markus Storvik:** Data curation, Writing – review & editing. **Markku Pasanen:** Conceptualization, Supervision, Writing – review & editing. **Pasi Huuskonen:** Conceptualization, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This paper belongs to the studies carried out by Kuopio Birth Cohort consortium (www. KuBiCo.fi). We thank Ms. Pirjo Hänninen for expert laboratory assistance. We also thank the staff of the Department of Obstetrics and Gynecology in Kuopio University Hospital for skilful collection of the placental specimens.

Funding

This work was supported by the Finnish Cultural foundation.

Data availability

Complete microarray data sets are available at the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database, accession number GSE153590.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crtox.2022.100082.

References

Abbot, E.L., McCormack, J.G., Reynet, C., Hassall, D.G., Buchan, K.W., Yeaman, S.J., 2005. Diverging regulation of pyruvate dehydrogenase kinase isoform gene expression in cultured human muscle cells. FEBS J. 272, 3004–3014.
Alshannaq, A., Yu, J.-H., 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. Int. J. Environ. Res. Public Health 14, 632.
Álvarez-Nava, F., Lanes, R., Álvarez-Nava, F., Lanes, R., 2017. GH/IGF-1 Signaling and Current Knowledge of Epigenetics; a Review and Considerations on Possible Therapeutic Options. Int. J. Mol. Sci. 18, 1624.
Angel, P., Karin, M., 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. BBA - Rev. Cancer 1072, 129–157.
Ayed-Boussema, I., Pascussi, J.-M., Maurel, P., Bacha, H., Hassen, W., 2012. Effect of aflatoxin B1 on nuclear receptors PXR, CAR, and AhR and their target cytochromes P450 mRNA expression in primary cultures of human hepatocytes. Int. J. Toxicol. 31, 86–93.
Baldwin, S., Parker, R.S., 1985. Effects of dietary fat level and aflatoxin B1 treatment on rat hepatic lipid composition. Food Chem. Toxicol. 23, 1049–1055.
Barreto, G., Schäfer, A., Marhold, J., Stach, D., Swaminathan, S.K., Handa, V., Döderlein, G., Maltry, N., Wu, W., Lyko, F., Niehrs, C., 2007. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445, 671–675.
Brown, J.R., Nigh, E., Lee, R.J., Ye, H., Thompson, M.A., Saudou, F., Pestell, R.G., Greenberg, M.E., 1998. Fos Family Members Induce Cell Cycle Entry by Activating Cyclin D1. Mol. Cell. Biol. 18, 5609–5619.
Burton, G.J., Jauniaux, E., 2015. What is the placenta? Am. J. Obstet. Gynecol. 213. S6.e1-S6.e4.
Castelino, J.M., Routledge, M.N., Wilson, S., Dunne, D.W., Mwatha, J.K., Gachuhi, K., Wild, C.P., Gong, Y.Y., 2015. Aflatoxin exposure is inversely associated with IGF1 and IGFBP3 levels in vitro and in Kenyan schoolchildren. Mol. Nutr. Food Res. 59, 574–581.
Cervero, A., Horcajadas, J.A., Domínguez, F., Pellicer, A., Simón, C., 2005. Leptin system in embryo development and implantation: A protein in search of a function. Reprod. Biomed. Online 10, 217–223.
Challis, J.R.G., Matthews, S.G., Gibb, W., Lye, S.J., 2000. Endocrine and paracrine regulation of birth at term and preterm. Endocr. Rev. 21, 514–550.
Chen, E.Y., Liao, Y.C., Smith, D.H., Barrera-Saldaña, H.A., Gelinas, R.E., Seeburg, P.H., 1989. The human growth hormone locus: nucleotide sequence, biology, and evolution. Genomics 4, 479–497.
Cheng, J., Huang, S., Fan, C., Zheng, N., Zhang, Y., Li, S., Wang, J., 2017. Metabolomic analysis of alterations in lipid oxidation, carbohydrate and amino acid metabolism in dairy goats caused by exposure to Aflotoxin B1. J. Dairy Res. 84, 401–406.
Christmann, M., Tomicic, M.T., Origer, J., Aasland, D., Kaina, B., 2006. c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF. Nucleic Acids Res. 34, 6530–6539.
Curran, T., Morgan, J.I., 1987. Memories of fos. BioEssays 7, 255–258.
Dato, V.A., Chiabrando, G.A., 2018. The role of low-density lipoprotein receptor-related protein 1 in lipid metabolism, glucose homeostasis and inflammation. Int. J. Mol. Sci.
Dong, X.Y., Tang, S.Q., 2010. Insulin-induced gene: A new regulator in lipid metabolism. Peptides.
Dos Santos, E., Duval, F., Vialard, F., Dieudonné, M.N., 2015. The roles of leptin and adiponectin at the fetal-maternal interface in humans. Horm. Mol. Biol. Clin. Investig. 24, 47–63.
Duan, R., Porter, W., Samudio, I., Vyhlidal, C., Kladde, M., Safe, S., 1999. Transcriptional activation of c-fos protooncogene by 17beta-estradiol: mechanism of aryl hydrocarbon receptor-mediated inhibition. Mol. Endocrinol. 13, 1511–1521.
Dutto, I., Tillhon, M., Cazzalini, O., Stivala, L.A., Prosperi, E., 2015. Biology of the cell cycle inhibitor p21CDKN1A: molecular mechanisms and relevance in chemical toxicology. Arch, Toxicol.
EFSA Contam Panel (EFSA Panel on Contaminants in the Food Chain), Schrenk, D., Bignami, M., Bodin, L., et al., 2020. Scientific opinion – Riskassessment of aflatoxins in food. EFSA J. 18, 6040.
El Dairi, R., Huuskonen, P., Pasanen, M., Rysä, J., 2018. Peroxisome proliferator activated receptor gamma (PPAR-γ) ligand pioglitazone regulated gene networks in term human primary trophoblast cells. Reprod. Toxicol. 81, 99–107.
Engin, A.B., Engin, A., 2019. DNA damage checkpoint response to aflatoxin B1. Environ. Toxicol. Pharmacol.
Fabian, E., Wang, X., Engel, F., Li, H., Landsiedel, R., van Ravenzwaay, B., 2016. Activities of xenobiotic metabolizing enzymes in rat placenta and liver in vitro. Toxicol. Vitr. 33, 174–179.
Fagerberg, L., Hallström, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., Asplund, A., Sjöstedt, E., Lundberg, E., Szigyarto, C.-A.-K., Skogs, M., Takanen, J.O., Berling, H., Tegel, H., Mulder, J., Nilsson, P., Schwenk, J.M., Lindskog, C., Danielsson, F., Mardinoglu, A., Sivertsson, Å., von Feilitzen, K., Forsberg, M., Zwahlen, M., Olsson, I., Navani, S., Huss, M., Nielsen, J., Ponten, F., Uhlén, M., 2014. Analysis of the Human Tissue-specific Expression by Genome-wide Integration of Transcriptomics and Antibody-based Proteomics. Mol. Cell. Proteomics 13, 397–406.
Gong, Y., Hounsa, A., Egal, S., Turner, P.C., Sutcliffe, A.E., Hall, A.J., Cardwell, K., Wild, C.P., 2004. Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. Environ. Health Perspect. 112, 1334–1338.
Gong, Y.Y., 2002. Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. BMJ 325, 20–21.
Guttmacher, A.E., Maddox, Y.T., Spong, C.Y., 2014. The Human Placenta Project: Placental structure, development, and function in real time. Placenta 35, 303–304.
Hakkola, J., Pelkonen, O., Fasanen, M., Raunio, H., 1998. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto- placental unit: Role in intrauterine toxicity. Crit. Rev. Toxicol.
Handwerger, S., Freemark, M., 2000. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J. Pediatr. Endocrinol. Metab.
Henson, M.C., Castracane, V.D., 2006. Leptin in pregnancy: An update. Biol. Reprod.
Huuskonen, P., Amezaga, M.R., Bellingham, M., Jones, L.H., Storvik, M., Häkkinen, M., Keski-Nisula, L., Heinonen, S., O'Shaughnessy, P.J., Fowler, P.A., 2016. The human placental proteome is affected by maternal smoking. Reprod. Toxicol. 63, 22–31.
Huuskonen, P., Myllynen, P., Storvik, M., Pasanen, M., 2013. The effects of aflatoxin B1 on transporters and steroid metabolizing enzymes in JEG-3 cells. Toxicol. Lett. 218, 200–206.
Huuskonen, P., Storvik, M., Reinisalo, M., Honkakoski, P., Rysä, J., Hakkola, J., Pasanen, M., 2008. Microarray Analysis of the Global Alterations in the Gene Expression in the Placentas From Cigarette-smoking Mothers. Clin. Pharmacol. Ther. 83, 542–550.
Iliodromiti, Z., Antonakopoulos, N., Sifakis, S., Tsikouras, P., Daniilidis, A., Dafopoulos, K., Botsis, D., Vrachnis, N., 2012. Endocrine, paracrine, and autocrine placental mediators in labor. Hormones 11, 397–409.
Josse, R., Dumont, J., Fautrel, A., Robin, M.A., Guillouzo, A., 2012. Identification of early target genes of aflatoxin B1 in human hepatocytes, inter-individual variability and comparison with other genotoxic compounds. Toxicol. Appl. Pharmacol. 258, 176–187.

Kiessling, K.H., 1986. Biochemical Mechanism of Action of Mycotoxins. Pure Appl. Chem. 58 (2), 327–338.
Kim, Y.J., Felig, P., 1971. Plasma chorionic somatomammotropin levels during starvation in midpregnancy. J. Clin. Endocrinol. Metab. 32, 864–867.
Kleinsimon, S., Longmuss, E., Rolff, J., Jäger, S., Eggert, A., Delebinski, C., Seifert, G., 2018. GADD45A and CDKN1A are involved in apoptosis and cell cycle modulatory effects of viscumTT with further inactivation of the STAT3 pathway. Sci. Rep. 8.
Klinge, C.M., Bowers, J.L., Kulakosky, P.C., Kamboj, K.K., Swanson, H.I., 1999. The aryl hydrocarbon receptor (AHR)/AHR nuclear translocator (ARNT) heterodimer interacts with naturally occurring estrogen response elements. Mol. Cell. Endocrinol. 157, 105–119.
Kovary, K., Bravo, R., 1991. The jun and fos protein families are both required for cell cycle progression in fibroblasts. Mol. Cell. Biol. 11, 4466–4472.
Kulkarni, S.S., Salehzadeh, F., Fritz, T., Zierath, J.R., Krook, A., Osler, M.E., 2012. Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus. Metabolism. 61, 175–185.
Kumar, P., Mendelson, C.R., 2011. Estrogen-related receptor γ (ERR γ) mediates oxygen-dependent induction of aromatase (cyp19) gene expression during human trophoblast differentiation. Mol. Endocrinol. 25, 1513–1526.
Lacroix, M.C., Guibourdenche, J., Frendo, J.L., Muller, F., Evain-Brion, D., 2002. Human Placental Growth Hormone—A Review. Placenta 23, S87–S94.
Lamplugh, S.M., Hendrickse, R.G., Apeagyei, F., Mwanmut, D.D., 1988. Aflatoxins in breast milk, neonatal cord blood, and serum of pregnant women. Br. Med. J. (Clin. Res. Ed). 296, 968.
Liu, X., Kumar Mishra, S., Wang, T., Xu, Z., Zhao, X., Wang, Y., Yin, H., Fan, X., Zeng, B., Yang, M., Yang, D., Ni, Q., Li, Y., Zhang, M., Zhu, Q., Chen, F., Li, D., 2020. AFB1 Induced Transcriptional Regulation Related to Apoptosis and Lipid Metabolism in Liver of Chicken. Toxins (Basel). 12, 290.
Lu, X., Hu, B., Shao, L., Tian, Y., Jin, T., Jin, Y., Ji, S., Fan, X., 2013. Integrated analysis of transcriptomics and metabonomics profiles in aflatoxin B1-induced hepatotoxicity in rat. Food Chem. Toxicol. 55, 444–455.
Lupu, F., Terwilliger, J.D., Lee, K., Segre, G.V., Efstratiadis, A., 2001. Roles of Growth Hormone and Insulin-like Growth Factor 1 in Mouse Postnatal Growth. Dev. Biol. 229, 141–162.
Mary, V.S., Valdehita, A., Navas, J.M., Rubinstein, H.R., Fernández-Cruz, M.L., 2015. Effects of aflatoxin B1, fumonisin B1 and their mixture on the aryl hydrocarbon receptor and cytochrome P450 1A induction. Food Chem. Toxicol. 75, 104–111.
Merrick, B.A., Phadke, D.P., Auerbach, S.S., Mav, D., Stiegelmeyer, S.M., Shah, R.R., Tice, R.R., 2013. RNA-Seq Profiling Reveals Novel Hepatic Gene Expression Pattern in Aflatoxin B1 Treated Rats. PLoS One 8, e61768.
Milde-Langosch, K., 2005. The Fos family of transcription factors and their role in tumourigenesis. Eur. J. Cancer 41, 2449–2461.
Miyashita, K., Itoh, H., Sawada, N., Fukunaga, Y., Sone, M., Yamahara, K., Yurugi, K., Nakao, K., 2003. Adrenomedullin Promotes Proliferation and Migration of Cultured Endothelial Cells. Hypertens. Res. 26, S93–S98.
Mueckler, M., Makepeace, C., 2008. Transmembrane segment 6 of the Glut1 glucose transporter is an outer helix and contains amino acid side chains essential for transport activity. J. Biol. Chem. 283, 11550–11555.
Nelson, L.R., Bulun, S.E., 2001. Estrogen production and action. J. Am. Acad. Dermatol. 45, S116–S124.
Ohtake, F., Takeyama, K., Matsumoto, T., Kitagawa, H., Yamamoto, Y., Nohara, K., Tohyama, C., Krust, A., Mimura, J., Chambon, P., Yanagisawa, J., Fujii-Kuriyama, Y., Kato, S., 2003. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 423, 545–550.
Ostry, V., Malir, F., Toman, J., Grosse, Y., 2017. Mycotoxins as human carcinogens—The IARC monographs classification. Mycotoxin Res. 33, 65–73.
Ouafik, L., Berenguer-Daize, C., Berthois, Y., 2009. Adrenomedullin promotes cell cycle transit and up-regulates cyclin D1 protein level in human glioblastoma cells through the activation of c-Jun/JNK/AP-1 signal transduction pathway. Cell. Signal. 21, 597–608.
Partanen, H.A., El-Nezami, H.S., Leppänen, J.M., Myllynen, P.K., Woodhouse, H.J., Vähäkangas, K.H., 2010. Aflatoxin B1 transfer and metabolism in human placenta. Toxicol. Sci. 113, 216–225.
Pérez-Ibave, D.C., Rodríguez-Sánchez, I.P., Garza-Rodríguez, M. de L., Barrera-Saldaña, H.A., 2014. Extrapituitary growth hormone synthesis in humans. Growth Horm. IGF Res. 24, 47–53.
Petroff, M.G., Phillips, T.A., Ka, H., Pace, J.L., Hunt, J.S., 2006. Isolation and culture of term human trophoblast cells. Methods Mol. Med. 121, 203–217.
Pier, A.C., 1992. Major biological consequences of aflatoxicosis in animal production. J. Anim. Sci. 70, 3964–3967.
Pocar, P., Fischer, B., Klonisch, T., Hombach-Klonisch, S., 2005. Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. Reproduction 129, 379–389.
Ricordy, R., Gensabella, G., Cacci, E., Augusti-Tocco, G., 2002. Impairment of cell cycle progression by aflatoxin B1 in human cell lines. Mutagenesis 17, 241–249.
Rieswijk, L., Claessen, S.M.H., Bekers, O., van Herwijnen, M., Theunissen, D.H.J., Jennen, D.G.J., de Kok, T.M.C.M., Kleinjans, J.C.S., van Breda, S.G.J., 2016. Aflatoxin B1 induces persistent epigenomic effects in primary human hepatocytes associated with hepatocellular carcinoma. Toxicology 350–352, 31–39.
Rushing, B.R., Selim, M.I., 2019. Aflatoxin B1: A review on metabolism, toxicity, occurrence in food, occupational exposure, and detoxification methods. Food Chem. Toxicol. 124, 81–100.
Safe, S., Han, H., Goldsby, J., Mohankumar, K., Chapkin, R.S., 2018. Aryl hydrocarbon receptor (AhR) ligands as selective AhR modulators: Genomic studies. Curr. Opin. Toxicol. 11–12, 10–20.
Sasaki, A., Shinkawa, O., Margioris, A., Liotta, A., Sato, S., Murakami, O., Go, M., Shimizu, Y., Hanew, K., Yoshinaga, K., 1987. Immunoreactive Corticotropin-Releasing Hormone in Human Plasma During Pregnancy, Labor, and Delivery. J. Clin. Endocrinol. Metab. 64, 224–229.
Schanton, M., Maymó, J.L., Pérez-Pérez, A., Sánchez-Margalet, V., Varone, C.L., 2018. Involvement of leptin in the molecular physiology of the placenta. Reproduction 155, R1–R12.
Shichiri, M., Hirata, Y., 2003. Regulation of cell growth and apoptosis by adrenomedullin. Hypertens. Res.
Smith, L.E., Prendergast, A.J., Turner, P.C., Humphrey, J.H., Stoltzfus, R.J., 2017. Aflatoxin Exposure during Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. Am. J. Trop. Med. Hyg. 96, 770–776.
Storvik, M., Huuskonen, P., Kyllönen, T., Lehtonen, S., El-Nezami, H., Auriola, S., Pasanen, M., 2011. Aflatoxin B1–a potential endocrine disruptor–up-regulates CYP19A1 in JEG-3 cells. Toxicol. Lett. 202, 161–167.
Storvik, M., Huuskonen, P., Pehkonen, P., Pasanen, M., 2014. The unique characteristics of the placental transcriptome and the hormonal metabolism enzymes in placenta. Reprod. Toxicol. 47, 9–14.
Su, A.I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K.A., Block, D., Zhang, J., Soden, R., Hayakawa, M., Kreiman, G., Cooke, M.P., Walker, J.R., Hogenesch, J.B., 2004. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc. Natl. Acad. Sci. USA 101, 6062–6067.
Sun, D., Wu, H., Ping, Z., Zhu, H., Ai, L., 2021. PLAC1 Regulates the Occurrence of Fetal Growth Restriction by Inhibiting the Apoptosis of Trophoblast Cells. Ann. Clin. Lab. Sci. 51, 182–189.
Tryndyak, V., Kindrat, I., Dreval, K., Churchwell, M.I., Beland, F.A., Pogribny, I.P., 2018. Effect of aflatoxin B1, benzo[a]pyrene, and methapyrilene on transcriptomic and epigenetic alterations in human liver HepaRG cells. Food Chem. Toxicol. 121, 214–223.
Turner, P.C., Collinson, A.C., Cheung, Y.B., Gong, Y., Hall, A.J., Prentice, A.M., Wild, C. P., 2007. Aflatoxin exposure in utero causes growth faltering in Gambian infants. Int. J. Epidemiol. 36, 1119–1125.
Turner, P.C., Moore, S.E., Hall, A.J., Prentice, A.M., Wild, C.P., 2003. Modification of immune function through exposure to dietary aflatoxin in Gambian children. Environ. Health Perspect. 111, 217–220.
Wang, Y., Wenjuan, T., Wang, C.C., Leung, L.K., 2015. Aflatoxin B1 augments the synthesis of corticotropin releasing hormone in JEG-3 placental cells. Chem. Biol. Interact. 237, 73–79.
Weng, M.W., Lee, H.W., Choi, B., Wang, H.T., Hu, Y., Mehta, M., Desai, D., Amin, S., Zheng, Y., Tang, M.S., 2017. AFB1 hepatocarcinogenesis is via lipid peroxidation that inhibits DNA repair, sensitizes mutation susceptibility and induces aldehyde-DNA adducts at p53 mutational hotspot codon 249. Oncotarget 8, 18213–18226.
Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., Aggarwal, D., 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80, 1106–1122.
Withers, D.J., Coppock, H.A., Seufferlein, T., Smith, D.M., Bloom, S.R., Rozengurt, E., 1996. Adrenomedullin stimulates DNA synthesis and cell proliferation via elevation of cAMP in Swiss 3T3 cells. FEBS Lett. 378, 83–87.
Woods, K.A., Camacho-Hübner, C., Savage, M.O., Clark, A.J.L., 1996. Intrauterine Growth Retardation and Postnatal Growth Failure Associated with Deletion of the Insulin-Like Growth Factor I Gene. N. Engl. J. Med. 335, 1363–1367.
Wormke, M., Stoner, M., Saville, B., Walker, K., Abdelrahim, M., Burghardt, R., Safe, S., 2003. The aryl hydrocarbon receptor mediates degradation of estrogen receptor alpha through activation of proteasomes. Mol. Cell. Biol. 23, 1843–1855.
Xiong, Y., Chen, H., Lin, P., Wang, A., Wang, L., Jin, Y., 2017. ATF6 knockdown decreases apoptosis, arrests the S phase of the cell cycle, and increases steroid hormone production in mouse granulosa cells. Am. J. Physiol. Physiol. 312, C341–C353.
Yang, L., Ji, J., Chen, Z., Wang, H., Li, J., 2014. Transcriptome profiling of malignant transformed rat hepatic stem-like cells by aflatoxin B1. Neoplasma 61, 193–204.
Yin, H., Jiang, M., Peng, X., Cui, H., Zhou, Y., He, M., Zuo, Z., Ouyang, P., Fan, J., Fang, J., 2016. The molecular mechanism of G2/M cell cycle arrest induced by AFB1 in the jejunum. Oncotarget 7, 35592–35606.
Yip, K.Y., Wan, M.L.Y., Wong, A.S.T., Korach, K.S., El-Nezami, H., 2017. Combined low-dose zearalenone and aflatoxin B1 on cell growth and cell-cycle progression in breast cancer MCF-7 cells. Toxicol. Lett. 281, 139–151.
You, X., Liu, J., Xu, C., Liu, W., Zhu, X., Li, Y., Sun, Q., Gu, H., Ni, X., 2014. Corticotropin-Releasing Hormone (CRH) Promotes Inflammation in Human Pregnant Myometrium: The Evidence of CRH Initiating Parturition? J. Clin. Endocrinol. Metab. 99, E199–E208.
Zhu, Y., Tan, Y.Q., Leung, L.K., 2016. Aflatoxin B1 disrupts transient receptor potential channel activity and increases COX-2 expression in JEG-3 placental cells. Chem. Biol. Interact. 260, 84–90.