Research Article

Transcriptomic Profiling of Human Placental Trophoblasts in Response to Infection with *Enterococcus faecalis*

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Increasing evidence suggests that *Enterococcus faecalis* strains can pass through placental barriers and cause adverse outcomes during pregnancy. However, the underlying molecular mechanism of the interaction between *E. faecalis* and the host placental barrier has yet to be fully elucidated. In this study, we have used DNA microarray analysis to investigate the response of human placental trophoblast-like BeWo cells to infection with *E. faecalis* OG1RF. These results indicate that a total of 2191 genes in BeWo cells are differentially expressed in the presence of *E. faecalis* OG1RF, with 1357 genes being upregulated and 834 genes being downregulated. These differentially expressed genes (DEGs) are involved in apoptosis, stress and stimulus response, placental and embryonic development, immune response, and cell adhesion. Therefore, these results provide information on the molecular mechanisms that *E. faecalis* invasion can trigger to cause adverse pregnancy outcomes.

1. Introduction

Enterococcus faecalis is a ubiquitous gram-positive bacterium that occurs widely in the foods, alimentary tract, and vagina [1], with certain *E. faecalis* strains regarded as commensal bacteria [2]. However, *E. faecalis* can readily cross host barriers, causing various nosocomial infections and diseases, including bacteremia and sepsis [3–6]. We have shown that *E. faecalis* OG1RF can cross the placental barriers of pregnant mice and translocate into the fetus [6]. Hence, food-borne *E. faecalis* may affect the quality and security of food, which has potentially serious implications for pregnant women. Many recent studies have focused on safety assessment of *E. faecalis* isolates from foods [7, 8]. However, knowledge of the molecular mechanisms that *E. faecalis* employs to traverse the host placental barrier remains unclear. The changes in gene expression of human placental trophoblasts are likely to investigate the effects of *E. faecalis* infection on maternal and fetal health.

Transcriptomic profiling has been widely applied for studying the molecular mechanisms underpinning bacteria-host interactions. Fan et al. have employed a purpose built

Bam4kOLI microarray to carry out transcriptome analysis of *Bacillus amyloliquefaciens* FZB42 in response to exposure to maize root exudates [9]. In addition, Chessler et al. have used DNA microarrays to show that *Trypanosoma cruzi* triggers an early type I IFN response *in vivo* at the site of intradermal infection [10].

The BeWo cell line has been shown to be a useful *in vitro* placental barrier model for studying adhesion, transport, and infection [11], with many researchers using BeWo cells as an *in vitro* barrier model of the placenta to explore the transport and toxicity of foreign agents or organisms [12, 13].

Food-borne *E. faecalis* was shown to cross host intestinal and placental barriers, thus affecting fetal and placental growth and development [6]. Consequently, we used BeWo cell to develop an *in vitro* placental barrier model to investigate the interaction, adhesion, and translocation of *E. faecalis* OG1RF in the placenta. In this study, we have used DNA microarrays to investigate the molecular mechanisms that *E. faecalis* OG1RF triggers in BeWo cells that can potentially cause adverse pregnancy outcomes. These include the differential expression of many genes involved in apoptosis, stress response, placental and embryonic development, immune

response, and cell adhesion in BeWo cells. Therefore, this study may contribute to understand and control the effects of food-borne *E. faecalis* on food quality and security.

2. Materials and Methods

2.1. BeWo Cell Line and Cell Culture. Human choriocarcinoma cell line BeWo was purchased from Action-award Biotech Co., Ltd (Guangzhou, China). BeWo cells were cultured in DMEM/F12 medium supplemented with 10% (v/v) FBS (Gibco, Grand Island, NY) and grown in 25 mm² cell culture flasks (NEST, Jiangsu, China) at 37°C under a 5% CO₂ atmosphere, until approximately 80–90% cell confluence (as verified by inverted light microscopy). BeWo cells were then liberated from cell culture flasks using 0.25% trypsin-EDTA (Solarbio, Beijing, China), before being resuspended in fresh medium containing 10% FBS, and then transferred to new flasks.

2.2. Bacterial Strain and Culture Condition. *E. faecalis* OG1RF (ATCC® 47077™) was cultured in trypticase soy broth (TSB) at 37°C for 24 h with shaking at 180 rpm. The bacteria were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with sterile PBS (0.01 M, pH 7.4), and reconstituted in cell culture medium DMEM/F-12 (Solarbio, Beijing, China) to give a concentration of 10⁹ CFU/mL before use.

2.3. Invasion Assays. For invasion experiments, the liberated BeWo cells were transferred into 24-well plates (Corning, NY) and cultured for 48 h until a confluent monolayer was obtained. Culture supernatant was then removed and 5 µL of resuspended bacteria added to each well containing 495 µL DMEM/F12/10% FBS, with incubation then carried out under an atmosphere of 5% CO₂ at 37°C for 60 minutes.

The total number of invaded bacteria associated with BeWo cells (including adherent and internalized *E. faecalis*) was determined by twice dip-washing with sterile PBS to remove free bacteria, followed by lysis of BeWo cells using 500 µL 0.5% Triton X-100/PBS to release internalized bacteria [14]. Serial dilutions were then carried out to afford suspensions that were spread onto TSA plates and incubated at 37°C overnight to quantify the number of bacteria present.

The numbers of internalized bacteria present were determined using minor modifications of an antibiotic survival assay that has been described previously [15]. Therefore, bacteria and BeWo cells were incubated together for 60 min, unbound bacteria were washed away, and then 500 µL of DMEM/F12/10% FBS supplement containing 100 µg/mL gentamicin and 50 µg/mL penicillin was added. Bacteria and BeWo cells were then incubated for 60 min to kill any viable extracellular bacteria still present; then cells were washed twice and lysed, and the bacterial count was determined as described above.

2.4. RNA Preparation, DNA Microarray, and Data Analysis. BeWo cells were incubated in fresh medium for 4 h, with or without *E. faecalis* OG1RF before their total RNA was extracted using TRNzol Total RNA Reagent (Catalog number

DP405-02, TIANGEN, Beijing, China). RNA quality was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies, CA).

Beijing EMTD Technology Development Co. Ltd. carried out all microarray analysis using Illumina Human HT-12 v4 Expression BeadChip microarrays (Illumina, Inc., San Diego, CA). RNA preparation and hybridization were performed according to the manufacturer's instructions [16], with data assessed using a Bead-array Reader and permutation1.9.3EN. Illumina expression data was deposited in the NCBI Gene Expression Omnibus (GEO) database under the accession number GSE75626. Differential expression genes were analyzed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway databases.

2.5. Real-Time Fluorescent Quantitative PCR. First strand cDNA was synthesized using a Quantscript RT Kit Quant cDNA first strand Synthesis Kit (TIANGEN, Beijing, China). Real-time PCR was carried out using a SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) Kit (TaKaRa, Dalian, China) using the primer sets listed in Table 3. Amplification and detection were carried out using a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The real-time PCR conditions employed were as follows: 1 cycle at 95°C for 2 min, 45 cycles at 95°C for 20 s, heating at 60°C for 30 s, and heating at 68°C for 30 s. Melt curve analysis was carried out by heating samples for 1 min at 95°C and cooling to 55°C, followed by slow heating to 95°C whilst measuring any changes in fluorescence. Relative mRNA levels were determined using comparative critical threshold (Ct) real-time PCR (separate tube) analysis, as described in Applied Biosystems User Bulletin number 2 (P/N 4303859).

2.6. Statistical Analysis. Unless specified, all experiments were performed in triplicate. All data was analyzed using statistics programs contained in SigmaPlot 11.0 (Systat Software, San Jose, CA).

3. Results

3.1. Invasion Ability of *E. faecalis*. The number of *E. faecalis* OG1RF strain present in BeWo cells was determined using invasion assays. As shown in Figure 1, the total number of invaded bacteria was $6.32 \pm 0.10 \text{ Log}_{10} \text{ CFU/mL}$ (including adherent and internalized *E. faecalis* OG1RF), with a total cell count for internalized bacteria of $2.23 \pm 0.29 \text{ Log}_{10} \text{ CFU/mL}$. This assay confirmed that *E. faecalis* OG1RF can invade BeWo cells, suggesting that this species can readily cross the placental barrier, in accord with our previous study [6].

3.2. Differential Gene Expression Profile Analysis. DNA microarray techniques were used to compare the gene expression patterns of untreated BeWo cells with those infected by *E. faecalis*. After applying cut-off limits for induction (ratio > 2.0-fold) and suppression (ratio < 0.5-fold), 2191 genes (out of a total 47,231 genes) were found to be differentially expressed in the presence of *E. faecalis*. This included 1357 genes that had

FIGURE 1: Invasion assay of the human choriocarcinoma cell line BeWo with *Enterococcus faecalis* OG1RF. Values represent mean $\pm$ SD.

FIGURE 2: Logarithmic scatter plot of DEGs of treatment group. DEGs are represented by blue dots and non-DEGs by black dots. Upregulated and downregulated genes are segmented by a red slash. Treated group: BeWo cells were incubated in fresh medium for 4 h with *E. faecalis* OG1RF.

been upregulated and 834 genes that had been downregulated (Figure 2 and Table S1).

3.3. Bioinformatic Analysis of DEGs. The function of the DEGs that were affected by *E. faecalis* infection were characterized by comparison against the GO database which enabled their biological function to be identified. These results showed that 338 terms were responsible for “biological processes,” 59 terms were responsible for “cellular component,” and 68 terms were involved in “molecular function” (Tables S2–4). Most of the genes identified were classified under GO biological process categories using the terms “cellular process,” “regulation of cellular process,” “metabolic process,” “regulation of metabolic process,” “biosynthetic

process,” “regulation of biosynthetic process,” “gene expression,” “regulation of gene expression,” “transcription,” and “regulation of transcription” (Figure 3 and Table S5).

Of relevance to this study, 45 DEGs associated with placenta and embryonic development were identified, namely, “chordate embryonic development,” “embryonic development ending in birth or egg hatching,” “in utero embryonic development,” “placenta development,” and “embryonic placenta development” (Figure 3, Table 1 and Table S5). These results provide clues to the molecular mechanisms that *E. faecalis* OG1RF can employ to stunt placental and fetal growth and development [6]. Furthermore, other DEGs were categorized as being involved in cell adhesion, including “cytoskeleton organization,” “actin cytoskeleton organization,” “actin filament organization,” “negative regulation of cell adhesion,” “negative regulation of cell-substrate adhesion,” “negative regulation of cell-matrix adhesion,” and “negative regulation of intracellular transport.” These results agree with those of Peng et al., who have also reported on the range of molecular mechanisms employed by bacteria to cross the barrier [1]. Furthermore, we identified many other DEGs that are involved in “immune response,” “apoptosis,” and “stress and stimulus response” (Figure 3, Table 1 and Table S5). Being combined, these results indicate that *E. faecalis* infection can cause stress responses that stimulate host immune defense systems and induce cell apoptosis.

3.4. Signal Pathway Analysis of DEGs. DEGs were then mapped using the KEGG database for signal pathway analysis to gain further understanding of their biological function. These results reveal that these DEGs could be further categorized using 31 terms used by the KEGG database (Table S6). Close analysis revealed 13 main terms associated with differential gene expression of BeWo cells in response to *E. faecalis* invasion, namely, “Wnt signaling pathway,” “Jak-STAT signaling pathway,” “MAPK signaling pathway,” “mTOR signaling pathway,” “focal adhesion,” “regulation of actin cytoskeleton,” “adherens junction,” “pathogenic *Escherichia coli* infection,” “T cell receptor signaling pathway,” “B cell receptor signaling pathway,” “Toll-like receptor signaling pathway,” “apoptosis,” and “p53 signaling pathway” (Table 2).

According to descriptions used in the KEGG database (<http://www.genome.jp>), these descriptors are associated with the following biological processes. Activation of Wnt for basic developmental processes, such as embryonic and placental development [17]. The Jak-STAT pathway is the principal signaling mechanism for regulating cytokines and growth factors. Regulation of MAPK is important in cell proliferation, differentiation, and migration, which can be activated by diverse stimuli such as pathogen-associated molecular patterns and environmental stresses [18]. The mTOR pathway is a key intracellular signaling pathway that regulates the cell cycle that can respond to the presence of nutrients and mitogens. Focal adhesions are comprised of bundles of actin filaments anchored to transmembrane receptors that serve to connect membrane receptors to the actin cytoskeleton. Adherent junctions are the most common type of intercellular adhesions that are responsible for maintaining

FIGURE 3: Histogram presentation of representative terms of GO biological process category. The y-axis indicates percentage of genes in GO biological process category.

the integrity of tissue architecture. Activation of T and B cell receptors is necessary for an efficient immune response to occur, whilst Toll-like receptors are responsible for detecting pathogens and triggering an innate immune response. p53 is known to be induced by stress signals, which can result in genetically controlled cell death.

Importantly, these KEGG analysis results were largely in accord with results obtained from bioinformatic analyses (Figure 3, Table 1, and Table S5), thus providing an insight into the molecular events that occur when *E. faecalis* invades host placental cells.

3.5. Real-Time PCR Analysis. Changes to the transcriptional level of selected genes were studied using real-time PCR. As shown in Figure 4, the expression levels of IL1, IL8, and TNF- α were significantly upregulated, whilst IL4, IL6, IL10, IL12, and IFN- γ were significantly downregulated, thus providing

further indication that infection with *E. faecalis* perturbs the host immune defense systems. Furthermore, expression of TLR1, 2, 3, 4, and 6 was significantly downregulated, suggesting that the host's ability to tackle this pathogen was being suppressed. Meanwhile, expression of claudin 1, E-cadherin, occludin, ZO-1, ICAM, and madCAM was significantly downregulated, indicating that infection with *E. faecalis* also results in disruption of the placental barrier.

4. Discussion

Previously, we have shown that *E. faecalis* can cross the placental barrier, resulting in fetal and placental growth and development being stunted [6]. However, little is known about the molecular mechanisms that occur during bacterial-host interactions that result in fetoplacental tropism. Hence, we have employed DNA microarray techniques to identify

TABLE 1: Classification of DEGs according to their GO biological process categories.

Term	Genes
Associated with apoptosis: GO:0042981~regulation of apoptosis GO:0006915~apoptosis GO:0006917~induction of apoptosis	RTN4, IER3, XRCC2, ZMAT3, TNFSF15, PRDX5, PRDX2, PRDX3, FOXO3, ITSN1, CITED2, MEN1, MAP3K7, CASP6, ZFP91, CDKN2A, FNTA, BAG1, BOK, AEN, ZNF443, NET1, IP6K2, ADAM9, EGFR, SOCS3, AIFM1, RELA, TP53, ARHGGEF17, JMY, ARHGFE1, CARD11, GRM4, SERPINB9, KRT18, NME3, PSEN1, TXNDC5, JUN, TNFAIP8, VEGFA, MAP3K10, KCNH8, DCUNID3, CDK5R1, YWHAZ, GSDMA, XIAP, ERBB3, ADAMTSL4, NFKBIA, COL2A1, BCL2L1, CHEK2, GCH1, DDX19B, PPP2CB, LHX4, CD2, BCL3, BCL6, TAF9, TRAF6, PCSK6, BMF, ERCC1, TERF1, PHLDAL, HIPI, IL6, TP53BP2, KLF10, CREB1, BAD, GASI, YWHAE, C14ORF153, NAE1, CDKN1A, TNFSF10, SON, NUPRI, BNIP3L, ID3, HSPD1, DNAJB6, BARD1, E2F1, SGPP1, ZNF346, CTNNB1, PAK2, GSN, RHOB, LOC100128086, ACIN1, EBAG9, SGK1, STAG3L3, DDIT4, PPM1F, EP300, MAPK7, GADD45B, GADD45A, GULP1, GPR65, DIDO1, TSC22D3, ECE1, PTPN6, PDGCD10, RYBP, DUSP22, TNFSF9, ASAH2C (117)
	XRCC2, PDGFB, PDGFA, RBM3, TIRAP, FOXO3, KIAA0652, CITED2, MUTYH, GP6, INSIG2, LTBR, CTGF, COL4A3BP, ZNF443, MAP2K7, ADAM9, EGFR, FIIR, ATG9B, SOCS3, USPI, RELA, NEIL1, GAGEI2G, SERPING1, GRHL3, DDIT4, PLAUR, RFC3, DCLRE1A, EP300, GAGE5, JUN, GAGEI2I, LILRB3, VEGFA, KIAA1715, RYR1, DSP, AMFR, COL1A1, PLA2G2D, CCL3, ACHE, BLM, ERBB3, ALDOB, MAP4K1, BCL2L1, CHEK2, PKX, ITGB1, ARNT, DUSP19, ECE1, TREC, C7ORF68, PTPN6, MAFK, RECQL5, CFB, MAP2K4, MYO1F, AGER, DDX58, HDAC5, APOL2, HYOU1, CDKN1A, ATF4, CXCL16, POLD2, PCNA, LOC732360, HSPD1, ENG, DNAJB6, ELF3, HPS6, FAMI75A, ZMAT3, PRDX5, PMS2L5, PRDX2, PRDX3, PMS2L1, OS9, PMS2L3, MEN1, HMOX2, FOS, ANKRD17, CASP6, MCM7, HSF1, GSN, SLC23A2, XYLT1, AEN, ILIRAP, ERRF1I, FANCC, NLRP7, NFKBIZ, SGK1, SP100, AIFM1, GENI, DDB1, CNPY3, LYZ, TP53, MMP14, WAS, JMY, C8G, CHST1, EYA3, RIF1, ADM, PSEN1, HIST2H2BE, MAP3K10, GADD45B, GADD45A, TTRAP, YWHAZ, XIAP, G17ORF70, WRNIP1, MNK2, EGLN2, EGLN1, COMT, VARS, TPM1, GCH1, IGSF10, CD9, CYP27B1, IL10RB, POU2F3, PPP2CB, BCL3, ENO3, NEATC4, BCL6, ERCC1, FEN1, HSPA8, IL6, ATRN, SIRT7, STAT3, NAE1, CCL11, VWF, NUPRI, TFRC, CSNK1E, TDPI, BNIP3L, ALKBH1, ID3, BARD1, SYTI, CTNNB1, FNTA, CHRNA5, RARA, PRKACB, BAIAP2, LIFR, SOCS7, GRB10, SLC25A36, PDGFRB, STC1, NFKBIA, IRAK3, NDRG1, TAF9, SRP54, KLF10, ABCG1, NRI2, TSCI, LDLR, LEPR, GPR81, RHOQ, ASAH1, VNIR2, AES, CYR61, ADAM10, UFDIL, SS18, G6PC, CTSC, GNB4, DGKD, LOC100128775, DDX19B, COL6A2, PIK3R3, PIK3R1, CREB1, CAPN3, LEP, ID2, ID1, GNG10, RSR1, ANXA11, MAPK15, TJP2, SBNO2, MAP3K7, ZFP91, AP2B1, PVRL2, SPG21, TRAF6, ATP6V1H, DUSP22, TRIM24, FOXPI, NPY5R, CARD11, NDE1, AP2A1, IRF4, NCOR1 (235)
Associated with stress and stimulus response: GO:0006950~response to stress GO:0042221~response to chemical stimulus GO:0048583~regulation of response to stimulus GO:0002237~response to molecule of bacterial origin	

TABLE 1: Continued.

Term	Genes
<i>Associated with placenta and embryonic development:</i>	
GO:0043009~chordate embryonic development	XRCC2, COL2A1, EGLN1, BCL2L1, ITGB1, TPM1, CITED2, ARNT, MAP3K7, MEN1, OSR2, ECE1, HAND1, HSF1, COL4A3BP, PKD1, TRAF6, MAFK, ADAM10, MYOIE, GABPA, TP53, SPINT1, ICMT, MBNL1, GASI, DVL1, HES1, ACVR2A, NDEL1, EP300, TSCI, PSMC4, PSEN1, MNX1, TGIF1, PDGFRB, LOC652826, COL1A1, RBP1, VWF, CYP27B1, CCNE, PLCD3, PRDX3 (45)
GO:0007972~embryonic development ending in birth or egg hatching	
GO:0001701~in utero embryonic development	
GO:0001892~embryonic placenta development	
GO:0001890~placenta development	
<i>Associated with cell adhesion:</i>	
GO:0007010~cytoskeleton organization	
GO:0030036~actin cytoskeleton organization	ALDOA, CCL3, SHROOM4, PDGFB, VPS54, CALD1, SIPA1, CETN3, NEDD9, RHOQ, ITGB1, TPM1, TLL3, CTNNB1, SPC25, PTK2, PRR5, PACSINI, PAK2, ANK3, GSN, FBXO5, TEK2, BCL6, FGD5, ARHGAP8, SNHG3-RCCL, FGD6, TNXA, FSCN2, CKAP5, LOC100132288, ARHGEF17, CAPN3, WAS, ARHGEF11, NCK2, SSI8, NDEL1, LOC399942, SDCBP, HAUS8, NEURL2, CRK, GADD45A, NCOR1, DNAJB6, CDKN2A, ADAM10, ERBB3, MYOIE, COL1A1, MMP14, PIK3R1, TSCI, CYR61, RELL2, NFKBIE, NFKBIB, G3BP2, NFKBIA, BARD1 (62)
GO:0007162~negative regulation of cell adhesion	
GO:0010810~regulation of cell-substrate adhesion	
GO:0001953~negative regulation of cell-matrix adhesion	
GO:0032387~negative regulation of intracellular transport	

TABLE 1: Continued.

Term	Genes
<i>Associated with immune response:</i>	
GO:0002682~regulation of immune system process	
GO:0002697~regulation of immune effector process	
GO:0045321~leukocyte activation	HIST1H4 K, FOXO3, CDKN2A, PVRL2, HIST1H4E, SPG21, RARA, ACINI1, CFB, KLF10, BAD, C8G, CDKN1A, ID2, JUN, HSPD1, RBM15, ATP6V1H, SERPING1, FOXPI, DDX58, AP2B1, AP2A1, YWHAZ, SBNO2, ITGB1, BCL3, NDRG1, ERCC1, PIK3R1, ADAM9, ADAM10, MYO1F, HDAC5, IRF1, KIR3DL1, BLM, TP53, WAS, PKNOX1, CD2, PATZ1, IRF4, KIF13B, STAT4, KRT18, SPI00, LIFR, STAT3, MAST2, NFKB1B, TIRAP, G3BP2, IRAK1BPI, EDARADD, VAPA, SLC20A1, PDGFB, EFNA1, CHMP5, LEPR, RGL3, FSTL3, TNFSF15, PRDX2, ITSN1, CITED2, CTNBN1, MAP3K7, MEN1, GPR89B, ZFP91, SPRY1, TBCID14, GIT2, GPR89A, NET1, EGFR, SOCS3, RELA, SOCS7, ARHGEF17, SOCS4, DDIT4, ARHGEF1, CARD11, SPAG9, GRM4, ACVR2A, RALGAPA2, NCK2, GRB10, NDEL1, PSEN1, VEGFA, MAP3K10, TGIF1, TBCID3G, SNX6, ERBB3, SIPA1, PPM1A, MAP4K1, TRIM13, TFG, NFKB1A, TIMP2, ARNT, TBCID3B, RASAL2, IRAK3, DUSP19, ECE1, CYP27B1, TSPYL2, SLC35B2, PPP2CB, BCL6, FGD5, TRAF6, PCSK6, FGD6, RASA2, IL6, TBCID10A, PAK1P1, DUSP22, ICMT, RGS17, GASI, TRIM24, DVLI, TBCID25, LER, TNFSF10, LRPI, TSCI, GRTPI, C6ORFI70, NCOR1, CRK, ENG, TOB1
GO:0032655~regulation of interleukin-12 production	
GO:0045075~regulation of interleukin-12 biosynthetic process	
GO:0045084~positive regulation of interleukin-12 biosynthetic process	
GO:0002711~positive regulation of T cell mediated immunity	
GO:0007253~cytoplasmic sequestering of NF-kappaB	
GO:0007249~I-kappaB kinase/NF-kappaB cascade	
GO:0042347~negative regulation of NF-kappaB import into nucleus	
GO:0009966~regulation of signal transduction	
GO:0002757~immune response-activating signal transduction

FIGURE 4: Fold-change of DEGs selected for validation by real-time PCR. The housekeeping gene β -2-microglobulin was used to normalize input amounts of RNA and the levels of gene expression between coinubation samples and individual bacteria compared. The relative expression ratio for each gene is presented as a log₂ value in the histogram. A ratio greater than zero (>0) indicates upregulation of gene expression and a ratio lower than zero (<0) indicates downregulation. Error bars represent the standard deviations from triplicate analysis of each sample. #Significant difference ($p < 0.05$). Values represent mean $\pm$ SD.

differential gene expression in placental trophoblasts in response to *E. faecalis* invasion. Whole-genome expression using BeadChip has been used to investigate differential gene expression of cells in response to infection, disease, and environmental stress [19]. In comparison with real-time PCR, the BeadChip technique can rapidly analyze microdoses in parallel using automated high throughput systems. Consequently, we employed the Illumina Human HT-12 v4 Expression BeadChip to investigate the biological effects that occur when placental cells are infected with *E. faecalis*.

According to the DNA microarray results, 45 DEGs were associated with placenta and embryonic development, which belonged to 5 terms within the GO “biological process” category. It is proposed that these changes to gene expression in placental trophoblasts in response to *E. faecalis* infection are likely to have a negative effect on placental and fetal health. This is in accord with our previous study, which found that *E. faecalis* infection resulted in a reduction in placental and fetal weights *in vivo* [6]. Furthermore, KEGG analysis revealed that exposure to *E. faecalis* resulted in DEGs that could affect numerous other biological pathways in placental trophoblasts. Among the KEGG annotated sequences, 18 genes were classified as being part of the Wnt signaling pathway, which is involved in multiple developmental events during embryogenesis [17, 20]. Indeed, Li et al. have previously shown that many of the genes involved in embryonic development that exhibit high ingenuity pathway analysis scores are responsible for cell death, which can lead to reduced fetal and placental weights in pregnant rats [21].

Other genes were identified, which control three signaling pathways involved in “focal adhesion,” “regulation of actin cytoskeleton,” and “adherens junction.” These pathways are associated with changes to the cell-extracellular matrix, cell-cell adhesion properties, and the maintenance of normal cellular structure and function (Table 2). Meanwhile, 62 genes were identified with cell adhesion-related terms relating to GO “biological process” categories (Table 1) that are likely to play important roles in maintaining the integrity of the placental barrier [22, 23]. Hence, the differential expression of these genes is likely to impair the normal structure and function of placental barrier, thus contributing to *E. faecalis* invasion and fetoplacental tropism. In this respect, a previous study by Peng et al. has revealed that *E. faecalis* enables actin to pass through the intestinal barrier [1]. Moreover, *E. faecalis* can also trigger the loss of E-cadherin, leading to impairment of intestinal and placental barriers [5, 6]. Interestingly, *Listeria monocytogenes* is a closely related pathogen that is also known to cross human intestinal and placental barriers via adhesion to E-cadherin [15, 24–26].

Large numbers of DEGs and signaling pathways that are closely related to the immune system response were also affected. Whilst changes in gene expression may activate or suppress host immune defense systems, disruption of the immune system in pregnancy can cause trophoblast death, resulting in placental insufficiency, spontaneous abortion, preterm labor, or stillbirth [27, 28]. In addition, many DEGs were identified, which are known to be involved in apoptosis, which could potentially cause preeclampsia, or intrauterine growth retardation [29]. Similarly, *Trypanosoma cruzi* has

TABLE 2: DEGs associated with signal pathways assigned by KEGG pathway analysis.

No.	Term	Count	Genes
1	hsa04310:Wnt signaling pathway	18	CTBP1, CTBP2, TP53, CTNBN1, DVLI, MAP3K7, EP300, PSEN1, PRICKLE1, CSNK1E, JUN, PPP2CB, NFAT5, FRAT1, CAMK2B, NFATC4, PRKACB, NFATC1
2	hsa04010:MAPK signaling pathway	31	FGFR4, PDGFB, PDGFA, PPM1A, MKNK2, MAP4K1, PPM1B, NFKB2, MAP3K7, MAX, FOS, PAK2, PLA2G12A, NFATC4, PRKACB, TRAF6, MAP2K7, HSPA8, RASA2, EGFR, RELA, MAP2K4, TP53, ATF4, JUN, PDGFRB, MAPK7, GADD45B, CRK, PLA2G2D, GADD45A
3	hsa04662:B cell receptor signaling pathway	15	PTPN6, IFITM1, NFKBIE, RELA, NFKBIB, NFKBIA, FOS, CARD11, LILRB3, JUN, NFAT5, NFATC4, PIK3R3, PIK3R1, NFATC1
4	hsa04660:T cell receptor signaling pathway	18	PTPN6, NFKBIE, RELA, NFKBIB, NFKBIA, MAP3K7, FOS, NCK2, CARD11, PAK2, JUN, PAK4, NFAT5, NFATC4, PIK3R3, MAP2K7, PIK3R1, NFATC1
5	hsa04620:Toll-like receptor signaling pathway	13	CCL3, IL6, RELA, MAP2K4, TIRAP, NFKBIA, MAP3K7, FOS, JUN, PIK3R3, TRAF6, MAP2K7, PIK3R1
6	hsa04630:Jak-STAT signaling pathway	20	PTPN6, IL6, SOCS3, LEPR, LIFR, SOCS7, SOCS4, BCL2L1, STAT3, LEP, ZFP91, TSLP, STAT4, SPRY1, EP300, IL10RB, IL5RA, MPL, PIK3R3, PIK3R1
7	hsa04510:focal adhesion	25	PDGFB, XIAP, PDGFA, COL2A1, ITGB1, CTNBN1, MYL9, VCL, PTK2, PAK2, PAK4, COL6A2, PDGFC, PIK3R3, PIK3R1, EGFR, TNXA, BAD, VWF, JUN, ITGA7, VEGFA, PDGFRB, COL1A1, CRK
8	hsa04520:adherens junction	12	MAP3K7, EGFR, PTPN6, PVRL4, EP300, PVRL1, BAIAP2, PVRL2, CTNND1, WAS, CTNBN1, VCL
9	hsa04810:regulation of actin cytoskeleton	23	EGFR, FGFR4, PDGFB, LIMK2, PDGFA, BAIAP2, ITGB1, WAS, MYL9, TTLL3, VCL, PTK2, ARPC3, PAK2, GSN, CFL2, PAK4, ITGA7, PDGFRB, PDGFC, PIK3R3, CRK, PIK3R1
10	hsa05130:pathogenic <i>Escherichia coli</i> infection	9	NCK2, YWHAZ, KRT18, ARPC3, LOC399942, WAS, ITGB1, CTNBN1, TTLL3
11	hsa04115:p53 signaling pathway	10	CDKN1A, CDKN2A, ZMAT3, BAI1, RPRM, TP53, CHEK2, GADD45B, CCNG2, GADD45A
12	hsa04210:apoptosis	14	XIAP, AIFM1, RELA, TP53, NFKBIA, BAD, BCL2L1, IRAK3, CASP6, TNFSF10, IL1RAP, PRKACB, PIK3R3, PIK3R1
13	hsa04150:mTOR signaling pathway	8	CAB39L, EIF4E, TSC1, STK11, VEGFA, PIK3R3, PIK3R1, DDIT4

also been reported to induce apoptosis in human chorionic villi [30].

5. Conclusion

E. faecalis infection induces stress and stimulus response activation, apoptosis, immune response disorder, and cell adhesion in human placental trophoblasts. It is likely that these effects may combine to damage placental trophoblasts, potentially leading to placental insufficiency and retardation of embryonic development. Therefore, this study provides important results on biological pathways that could potentially be targeted to develop interventions for the prevention/treatment of maternofetal infections caused by pathogenic enterococci.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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TABLE 3: Primers used for real-time PCR gene expression assays.

Target gene	Sequence (5'-3')
IL1	ACCTTCAAGGAGAGCATGGTG GGTGCTGACCTAGGCTTGATG
IL4	GCTATGACTAAAGCAGGGACAA CACTCTCCTCAGCAAACACAGA
IL6	TGCTCCTGGTGTTCCTG GCCGTCGAGGATGTACCG
IL8	GGCTCTCTTGGCAGCCTTC GCAGTTGGTCCACTCTCAATC
IL10	CCAGGCAACCTGCCTAACA TCTCAGACAAGGCTTGCAAC
IL12	GTCTCCTGGACCACCTCAG TCAATCTCTTCAGAAGTGCAAGG
TNF- α	CACCACGCTCTTCTGCCTG GTTCAGCCACTGGAGCTGC
IFN- γ	GCAGGTCATTTCAGATGTAGCG TTCCTTGATGGTCTCCACACTC
TLR1	CAGTGTCTGGTACACGCATGGT TTTCAAAAACCGTGTCTGTAGAGA
TLR2	CACTCAGGAGCAGCAAGCAC GCAGGAACAGAGCACAGCAC
TLR3	CCTGGTTTGTAAATTGGATTAACGA TGAGGTGGAGTGTGCAAAGG
TLR4	CAGAGTTTCCTGCAATGGATCA GCTTATCTGAAGGTGTTGCACAT
TLR6	GAAGAAGAACAACCCTTTAGGATAGC AGGCCAAACAAAATGGAAGCTT
Claudin 1	TGCTGGAATTGAACTACTCCAC TACTCTTTCCGGTTGGTGTGA
E-cadherin	GAATGATGATGGTGGACAAT TACCACATTTCGTCACTGCTA
Occludin	GGCACCTGCATACTCACCC CTGGGAGAGCAACTCATCCTC
ZO-1	CAACATACAGTGACGCTTCACA CACTATTGACGTTTCCCCACTC
ICAM	TTGTCCTGCCAGCGACTC AAGGAGTCGTGCCATAGGTG
madCAM	GCAGGAGGAGGAGGAGGAGGAG TCCAAGCCAGGCAGCC
β -2-microglobulin (housekeeping gene)	GGCTATCCAGCGTACTCCAAA CGGCAGGCATACTCATCTTTTT

Supplementary Materials

Table S1: differential gene expression profile of BeWo cell in response to *Enterococcus faecalis* OG1RF invasion. Table S2: differentially expressed genes classified with biological process in GO database. Table S3: differentially expressed genes classified with cellular component in GO database.

Table S4: differentially expressed genes classified with molecular function in GO database. Table S5: differentially expressed genes, containing representative terms of GO biological process category. Table S6: differentially expressed genes classified into different signal pathway of KEGG database. (*Supplementary Materials*)

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