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Research paper

Cell cycle and histone modification genes were decreased in placenta tissue from unexplained early miscarriage

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ABSTRACT

Genetic defect is a major cause of early miscarriage, but still in many cases the etiology are not fully understood. Recent studies have shown that dysregulation of genes in placenta tissue are participated in the pathogenesis of unexplained early miscarriage. The aim of our study is to explore mRNA expression profile in placental chorionic villi and to reveal the underlying mechanism of unexplained early miscarriage. Chorionic villous were isolated and extracted from early miscarriage (n = 3) and control pregnancy (n = 3) placenta with normal chromosome karyotype using MLPA assay, and then mRNA expression profiles were determined by microarray. For verification the reproducibility of the microarray, three up-regulated genes and six down-regulated genes were chosen and examined by real-time PCR (n = 30). A total of 81 genes were up-regulated and 231 genes were down-regulated when compared to the control group, and the differences were reached statistically significances ($P < 0.05$). After Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, we found that almost down-regulation genes are associated with cell cycle and histone modification, and these genes are participated in several important physiological processes, such as cell proliferation, nuclear division, chromatic assembly, DNA packing and modification. These results indicated that cell cycle and histone modification genes, and related signaling pathway maybe contribute to the genesis and development of unexplained early miscarriage. Further studies and validations are necessary to elucidate the exact roles of these genes in miscarriage pathogenesis, which can develop tools for early detection and management.

1. Introduction

Miscarriage, also named as spontaneous abortion (SA), is defined as the involuntary termination of an intrauterine pregnancy before 20 weeks of gestation or the fetal weight < 500 g, nearly 80% occurred in the first-trimester (≤ 12 weeks' gestation), and the incidence is about 12–24%, actually, the true rate of miscarriage is higher because many pregnancy losses occur pre-clinically, before a menstrual period missed (Jurkovic et al., 2013, Saccone et al., 2017). Consequently, miscarriage is the most common complication of pregnancy. Some known causes of miscarriage were involved in structural and numerical chromosome abnormalities, uterine anatomical, endocrine disorders, thrombophilias, infectious and autoimmune diseases (Bettio et al., 2008, Jaslow and Kutteh, 2013, Kumar et al., 2015, Kaur and Gupta, 2016). However, in many cases the etiology and pathophysiological mechanism of miscarriage were largely remain unclear even after extensive epidemiological investigations (Ford and Schust, 2009, Branch et al., 2010).

Miscarriage is considered as one of the placenta-related diseases of pregnancy if the embryo karyotypes are normal, placental growth and development is essential for the maintenance of normal pregnancy. For normal pregnancy, the placenta development is under strictly time-spatial control, including differentiation and invasion of the trophoblasts, and specialized cells of the placenta. If this delicate balance was broken, some pregnancy complications, such as miscarriage, preeclampsia, and intrauterine growth restriction are occurred (Ji et al., 2013). The factors which affect intrauterine environment for placental development may be causes of unexplained miscarriage. However, exactly causes and underlying mechanisms of unexplained miscarriage remain undiscovered.

Recent studies showed that dysregulation of expression profile in

Abbreviations: UPL, unexplained pregnancy loss; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; SA, spontaneous abortion; MLPA, Multiplex Ligation Dependent Probe Amplification

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placenta tissue are participated in the pathogenesis of unexplained miscarriage (Krieg et al., 2012). Global gene expression profiling using microarray is a beneficial method to understand underlying pathophysiology of unexplained early miscarriage. Even though several causes of miscarriage have been identified, so far, whole genome understanding of altered pathways in the placental villi of unexplained early miscarriage has not been fully investigated. Therefore, in our study, we have performed microarray experiments to identify aberrantly regulated mRNA and related signaling pathways between the placental villi of normal developing pregnancy and unexplained early miscarriage.

2. Materials & methods

2.1. Placental tissue collection and ethics statement

From Jan. 2015 to Jan. 2016, sixty-four placenta chorionic villous samplings tissue were obtained from miscarriage patients who came to the hospital about gestational age 6–10 weeks after ART treatment due to termination of embryonic development. Samples were collected in sterile containers containing 10 ml of cooling PBS. The chorionic villi were dissected, selected and snap-frozen by trained technicians in the genetics laboratory in < 10 min after Dilatation & evacuation. Each sample was divided into two parts, one is for diagnosed of aneuploidy using MLPA methods, and the other is extracted RNA for microarray and real-time PCR examination. The placental villi which were deemed as having a normal karyotype and obtained from women having no definitive causes of miscarriage were recruited as unexplained miscarriage; finally, 30 patients were employed for the study. Three of 30 early unexplained chorionic villi were randomly selected for microarray analysis. This study was approved by the Research Ethics Committee of Shenzhen Zhongshan Urology Hospital, and all the women signed an informed consent prior to the study.

2.2. Total DNA/RNA extraction and purification

RNA was isolated from the placental tissues using the Qiagen RNeasy Kit and QIAamp DNA/Blood Mini Kit according to the manufacturer's methods. The RNase-Free DNase Set was used to obtain high-quality RNA with DNAase digestion. The purity and concentration and quantified of all DNA/RNA samples were assessed by using nanoDrop spectrophotometer (Shimadzu, Japan), and then stored at − 20 °C for prepared.

2.3. MLPA for aneuploidy diagnose

DNA samples were detected by MLPA with SALSA P036, P070 and P181 probe mixes (MRC-Holland, The Netherlands) following the manufacturer's protocols for aneuploidy analysis. Copy number variation at both two subtelomeres and centromere of any individual chromosome indicated a whole chromosome aneuploidy. Increased or decreased dosage at one subtelomere and/or centromere of one chromosome indicated a segmental aneuploidy.

2.4. Microarray hybridization and data analysis

For microarray hybridization, a total of 200 ng of total RNA were labeled using the Low Input Quick Amp Labeling kit following the instruction manual and then were hybridized to Human whole genome 4 × 44 K array containing approximately 35,000 human transcripts with cRNA probes at the core facility of GenoCheck. After fragmentation, the labeled cRNAs were hybridized to the microarrays for 17 h at 65 °C and scanned as described in the manufacturer's protocol. Over-expressed mRNA was identified when the ratio of the mRNA expression level in unexplained miscarriage to the control group was 2 or more and under-expression was identified when the ratio was 0.5 or less.

2.5. Go and KEGG pathway analyses

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed. The P-value was calculated using a right-side hypergeometric test, and the Benjamini Hochberg adjustment was used to correct for multiple tests. An adjusted P-value of < 0.05 indicates a statistically significant deviation from the expected distribution, and the corresponding GO terms and pathways were enriched in the target genes. We analyzed all of the differentially expressed genes.

2.6. RT-PCR and real-time RT-PCR

To validate the results of expression profile, some genes were selected and analyzed by real-time RT-PCR. A total of 500 ng placental total RNA was subjected to reverse transcription using M-MLV reverse transcriptase kit (Toyobo, Japan) for cDNA synthesis. Synthesized cDNA was utilized for PCR with primers at optimized cycles (Table S1). Real-time RT-PCR was performed using ABI7500 cycler (USA) with QuantiTect SYBR Green PCR kit (Toyobo, Hilden, Japan). All PCRs were performed induplicate.

2.7. Statistical analysis

Statistical analysis was performed using Statistical Program for Social Science (SPSS Inc., Chicago, IL, USA) software. Student's *t*-test was performed to examine statistical significance (*P < 0.05; ** P < 0.01).

3. Results

3.1. The mRNA profile in chorionic villi of early miscarriage

To identify differentially mRNA expression in the chorionic villi of early miscarriage, we performed a Volcano Plot filtering between the two groups from the microarray database, the left Plot show the down-regulated genes and the right Plot show the up-regulated genes (Fig. 1A). After the mRNA profile have been normalized and analyzed, a total of 312 differentially genes were found to exhibit a 2-fold (P < 0.05) changes, when compared to the control group, including 81 genes were up-regulated and 231 genes were down-regulated, the results are displayed in Cluster and tree-view analysis (Fig. 1B).

3.2. Protein-protein interaction network analysis

The STRING 9.1 software (Search Tool for the Retrieval of Interacting Proteins), a database of protein interactions, was used to predict functional associations and generate networks of the differentially expressed mRNAs. We established a network containing 81 up-regulated genes and 231 down-regulated genes which functionally related to each other. The results demonstrated that interacting proteins which mainly focused on cell proliferation, DNA synthesis and histone modification (Fig. 2).

3.3. GO and KEGG analysis in target genes

The differentially expressed genes of all mRNAs were analyzed by the GO and KEGG pathway. GO analyses include biological processes, cellular components and molecular function. The differentially expressed genes were mainly related to following biological functions: cell cycle, DNA packing, cell and nuclei division, chromatic assembly, and histone modification (Fig. 3A); additionally, molecular pathways were annotated from the KEGG. Cell cycle, M phase and condensation of prophase chromosomes were the top three signaling pathway, these biological progress were blocked because of the relate genes are significantly down-regulated (Fig. 3B); the diseases enrich system