Human Pathology (2014) 45, 1234–1242

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Targeted gene analysis: increased B-cell lymphoma 6 in preeclamptic placentas☆,☆☆

Frank Louwen MD, Cornelia Muschol-Steinmetz, Alexandra Friemel, Anne Kristina Kämpf MD, Eva Töttel, Joscha Reinhard MD[1], Juping Yuan MD*

Department of Gynecology and Obstetrics, School of Medicine, J. W. Goethe-University, Theodor-Stern-Kai 7, D-60590 Frankfurt, Germany

Received 23 October 2013; revised 17 January 2014; accepted 1 February 2014

Keywords:
Designed gene array;
Preeclampsia;
BCL6;
Angiogenesis and migration

Summary Preeclampsia is a leading cause for maternal and perinatal mortality and morbidity. Microarray-based transcriptional profiling has been widely used for identifying genes responsible for preeclampsia. These studies deliver multiple pictures of gene signatures, implying the complicated pathophysiology. In the present work, we designed our own gene array containing genes involved in various signaling transduction pathways and analyzed placental samples from patients with preeclampsia and controls. We verify that genes associated with angiogenesis and migration pathways are mostly altered in preeclamptic placentas. Interestingly, several genes including B-cell lymphoma 6 have been identified to be linked to preeclampsia. Increased expression of B-cell lymphoma 6 is correlated with enhanced FLT1 and LEPTIN, the hallmarks of preeclampsia. Moreover, the protein level of B-cell lymphoma 6 is elevated in preeclamptic placentas and is predominantly localized in the nucleus of villous cytotrophoblasts lying directly underneath the syncytial layer, suggestive of an involvement in the function of villous trophoblasts. Altered B-cell lymphoma 6, a key oncogene in B-cell lymphomagenesis, may be involved in the pathogenesis of preeclampsia, and further investigations are required to decipher the molecular mechanisms.

1. Introduction

Preeclampsia, characterized by new onset of hypertension and proteinuria in the second half of gestation, is a consequence of diverse pathophysiological processes associated with endothelial dysfunction and systemic inflammation [1–3]. It affects 2% to 8% of all pregnancies and is one of the leading causes of maternal and perinatal mortality and morbidity worldwide [3,4]. Preeclampsia is linked to abnormal placentation, uteroplacental vascular insufficiency, altered intervillous haemodynamics, placental oxidative stress, increased placental release of syncytiotrophoblast

☆ Competing interests: The authors declare no potential conflict of interest.

☆☆ Funding/Support: The work at our laboratory is supported by Deutsche Krebshilfe Nos. 108553 and 109672, Deutsche Forschungsgemeinschaft No. Yu 156/2-1, and der Verein zur Förderung der Reproduktiven Gesundheit im Alter E.V.

* Corresponding author. Department of Gynecology and Obstetrics, School of Medicine, J. W. Goethe-University, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany.

E-mail address: yuan@em.uni-frankfurt.de (J. Yuan).

[1] Present address: Department of Gynecology and Obstetrics, St Marien Hospital, D-60318 Frankfurt, Germany.

http://dx.doi.org/10.1016/j.humpath.2014.02.002
0046-8177/

debris, and antiangiogenic molecules, which cause dysfunction of maternal endothelial cells and a systemic inflammatory response [1,2].

Despite intensive research, a full understanding of the pathogenesis of preeclampsia remains to be defined. The placenta plays a central role in preeclampsia pathogenesis [2,5]. Preeclampsia originates in the placenta, as preeclampsia ends with delivery and removal of placental tissue. Placenta-based investigations are thus of importance for understanding the initiation and progression of the disease. Microarray-based transcriptional profiling has been widely used for identifying genes responsible for preeclampsia [6–8]. These studies have identified a number of differentially expressed genes associated with preeclampsia by delivering multiple pictures of gene signatures, highlighting the complicated pathophysiology and heterogeneous causes of this disease. Further investigations are required to corroborate these compelling results.

In the present study, we designed our own gene arrays targeting several critical pathways and analyzed placental samples from patients with preeclampsia and controls.

2. Materials and methods

2.1. Sample collection

This study was approved by the Ethic Committee of Frankfurt University Hospital. Informed written consent was obtained from patients with preeclampsia and controls. Preeclampsia was diagnosed as described [9]. Placenta samples (0.5 cm^3) were taken from the fetal side of the placenta within 30 minutes after delivery, frozen immediately and stored at −80°C until use.

2.2. Gene array design, RNA extraction, real-time polymerase chain reaction and data analysis

We designed our own gene array in a TaqMan Array Fast 96-well format, manufactured by Applied Biosystems (Darmstadt, Germany). The array plate contained 92 genes, 1 reference gene *18S*, and 3 endogenous controls: *SDHA* (succinate dehydrogenase complex, subunit A), *YWHAZ* (tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide), and *TBP* (TATA box-binding protein). Based on the published data, we focused on key molecules involved in several signal transduction pathways related to preeclampsia, such as migration/invasion and angiogenesis. In particular, we integrated cell-cycle regulation, cell survival, and checkpoint control into the array, as trophoblasts in preeclamptic placenta encounter possibly various stresses and damages.

Total RNAs were extracted using RNeasy kits with column DNase digestion according to the manual instructions (Qiagen, Hilden, Germany). Reverse transcription was performed using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems). Real-time polymerase chain reaction (PCR) was performed with a StepOnePlus Real-time PCR System (Applied Biosystems). All primers and probes were obtained from Applied Biosystems. The data were analyzed with StepOne Software v2.2.2 (Applied Biosystems). Using the comparative C_T method [10], the gene expression was represented as ΔCt, which is normalized to endogenous controls and is inversely related to the amount of target molecules in the reaction. The mean value of expression levels of 3 genes *SDHA*, *YWHAZ*, and *TBP* served as endogenous controls. The final results were represented as relative quantification (RQ), the difference in gene expression level between preeclampsia samples and normal placenta samples, by setting the expression value of normal placentas as 1, in mean with minimum and maximum range. Because the RQ of a group is defined as $2^{-(\Delta Ct_{group}-\Delta CT_{control})}$, the RQ value for the control group itself leads to the value RQ = 1 without variation. In the bar chart, the RQ value of the control group is omitted and served just as a benchmark.

2.3. Immunohistochemistry and Western blot analysis of placenta tissues

Formalin-fixed, paraffin-embedded placenta tissue sections were deparaffinized and further treated with 4% (vol/vol) hydrogen peroxide in Tris-buffered saline (TBS) for 15 minutes. Antigen retrieval was performed by exposure to pressure cooker conditions for 20 minutes with target retrieval solution (Dako, Hamburg, Germany). Nonspecific binding was blocked with blocking buffer (Santa Cruz Biotechnology, Heidelberg, Germany) for 1 hour at room temperature. Sections were then incubated with rabbit polyclonal antibody against BCL6 (1:150; Atlas, Stockholm, Sweden) overnight at 4°C and an additional 45 minutes at room temperature. Nonimmune rabbit IgG and secondary IgG (Santa Cruz Biotechnology) were taken as negative controls. After washing with TBS, sections were incubated with biotinylated goat antirabbit antibody (Santa Cruz Biotechnology) for 40 minutes at room temperature, followed by washing with TBS. After incubation with a streptavidin-peroxidase conjugate, the antibody complexes were visualized through exposure to 3-amino-9-ethylcarbazole substrate (Dako) for 6 minutes. Sections were counterstained with hematoxylin, mounted, and examined using an Axio Imager 7.1 microscope (Zeiss, Göttingen, Germany). Quantitative analysis was performed based on the nuclear positive staining of BCL6 in villous cytotrophoblasts. At least 700 cytotrophoblasts from each sample were counted for quantification.

Cellular lysates from placental tissues were prepared using total protein extraction kits from Millipore (Schwalbach,