Dual specificity protein phosphatase 1 (DUSP1) in normal pregnancy and preeclampsia

Zhi-Ling Yang, Hong-Mei Li, Wei Huang, Lei Han, Li-Li Yu, Li Li

Department of Obstetrics and Gynecology, Research Institute of Surgery and Daping Hospital, Third Military Medical University, Chongqing 400042, China

Received December 16, 2015; Accepted May 5, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Objectives: Preeclampsia is best described as a pregnancy-specific syndrome of reduced organ perfusion secondary to vasospasm and endothelial activation. It is severe and clinically important manifestations of placental insufficiency. Our purpose was to study DUSP1 expression in normal human pregnancy and preeclampsia. Study design: We used ELISA, RT-PCR and Immuno-histochemistry to characterize DUSP1 gene expression and protein levels in different ages of the first trimester pregnancy, late pregnancy and in severe preeclampsia. DUSP1 protein was detected in venous of every patient and villi of the first trimester pregnancy, placentas of normal pregnancy and preeclampsia pregnancy. Villi was gained from those women who terminated their pregnancy during the first trimester and the placenta was gained from normal and preeclampsia pregnancy during the cesarean. Results: DUSP1 protein was significantly lower in severe preeclamptic placentas compared to normal pregnancy (P<0.05). The express of DUSP1 was varied from different ages of pregnancy. There was a trend that DUSP1 level decreased in villi of first trimester with the gestational age increased. Conclusion: DUSP1 protein levels were significantly suppressed in severe preeclampsia. This suppression was the reason or result of preeclampsia, is still not clear. The express of DUSP1 was varied from different ages of pregnancy. So our study demonstrated that the level of DUSP1 protein and mRNA could be a factor of PE.

Keywords: Dual specificity phosphatase 1 (DUSP1), expression, preeclamptic, preeclampsia, ELISA, RT-PCR, immuno-histochemistry

Introduction

Dual specificity protein phosphatases are a superfamily, including DUSP1, DUSP2, DUSP5, DUSP9 etc, almost 30 types of DUSP [1]. All the DUSP participate in signal transduction pathways inactivating mitogen-activated protein kinases (MAP kinases). Researches have tested that downregulation of DUSP1 induced changes in the expression levels of genes involved in specific biological pathways, including angiogenesis, MAP kinase phosphatase activity, cell-cell signaling and tyrosine-kinase receptor activity [2]. Its role is very commonly recognized in tumor biology. But there is little about the relationship between DUSP1 and trophoblast and diseases. To understand more about the involvement of DUSP1 in normal pregnancy and preeclampsia, we performed these tests to analyze expression of DUSP1 in placenta of preeclampsia and normal late pregnancy and villi of the first trimester pregnancy woman and in venous of them. And the researchers have investigated that DUSP1 was an important negative regulator of the acute inflammatory response and it protected over-activation of hypoxia-inducible factor 1 (HIF-1) through inactivating ERK MAPK [3]. Another studies [4-8] show inflammatory response and HIF-1α play an important role in preeclamptic patients, so we care about how DUSP1 express in normal and abnormal placenta, and what is the role of DUSP1 in pregnancy and its' complications?

Therefore we tested DUSP1 expression in normal human gestation and in severe Preeclampsia (sPE). This study was one part of our foundation, the National Natural Science Foundation of China (No. 81070505).
Table 1. Clinical characteristics of normal term pregnant and preeclamptic patients

<table>
<thead>
<tr>
<th></th>
<th>Term pregnant patients (n=44)</th>
<th>Preeclamptic patients (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.65±3.30</td>
<td>30.11±5.98</td>
</tr>
<tr>
<td>No. of primiparas</td>
<td>32 (72.7%)</td>
<td>21 (80.8%)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>114.07±8.72</td>
<td>152.59±8.13*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.66±6.93</td>
<td>108.22±8.81*</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.2±2.1</td>
<td>35.2±2.4*</td>
</tr>
<tr>
<td>Fetal birth weight (grams)</td>
<td>3379.10±346.28</td>
<td>2303.22±810.03*</td>
</tr>
</tbody>
</table>

*P<0.05 stand for the difference between the two groups was significant. The systolic and diastolic blood pressure of preeclamptic patients was much higher than term pregnant patients, then the gestational age at delivery and fetal birth weight of preeclamptic patients were significantly lower than the other group.

Table 2. ELISA for DUSP1 protein levels in different group (conc: pg/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>OD 450</th>
<th>conc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first trimester women</td>
<td>94</td>
<td>3.82±0.83</td>
<td>991.42±386.23</td>
<td></td>
</tr>
<tr>
<td>The term pregnant women</td>
<td>44</td>
<td>3.59±0.78</td>
<td>929.62±336.17</td>
<td></td>
</tr>
<tr>
<td>The preeclamptic women</td>
<td>26</td>
<td>3.19±0.90*</td>
<td>853.19±444.76*</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

DUSP1 protein levels varied in different group. The results of the preeclamptic women were significant lower than the other two groups. *P<0.05.

Materials and methods

**Patient selection and sample (venous and placenta) collection**

Between January and December 2013, 94 first trimester villous tissues between 5-10 weeks of gestation, 44 normal term gestation and 26 women with severe preeclampsia were admitted to the Department of Obstetrics and Gynecology, Daping Hospital and Xi’an Hospital, Third Military Medical University were included in the study. Usually, there were about 800 pregnancies every month in these two hospital, we just get little part of them to be the inclusion because of the donation of their venous blood and placenta. Preeclampsia was defined according to the criteria stated in Williams Obstetrics (23rd edition), which included blood pressure of ≥140/90 mmHg for two or more tests after 20 weeks’ gestation but without previous hypertension history. In addition the diagnosis of preeclampsia required proteinuria, more than +1 on a dipstick. All these patients have no history of chronic hypertension, diabetes mellitus, nephropathy etc. The samples of the first trimesters of normal pregnancy were collected during the procedure of induced abortion with their agreement. All the placental tissues were obtained by cesarean section, which including placenta of the normal term pregnancy and preeclamptic women at the third trimester. At the same time, the cord blood of every patient was collected 10 ml in the anticoagulation tube. All the patients has signed informed consent to approve for donation of samples. Placental collection was performed as per protocol. Once collection was complete, the sample was sent to the seventh Department of Daping hospital and research institute of surgery for analysis. Ethics approval was granted and written informed consent was obtained from all the patients for donation of samples of their villi or placenta and blood to the hospital.

**Protein isolation**

The villi and placenta tissues were washed by 0.9% physiological saline three times till clear, then dried with filter paper and weighed by the microbalance. The weight of tissues was between 40 mg to 60 mg. Put the tissues in the Eppendorf Tubes/1.5 ml centrifuge Tubes. Protein were extracted in RIPA buffer (Beyotime Institute of Biotechnology, PR China, 50 mM TrisHCl, 150 mM NaCl, 1% Triton ×100, 1% Na Deoxycholate and 0.1% SDS) containing protease inhibitor Phenylmethanesulfonyl fluoride (PMSF, 100 mM, Beyotime Institute of Biotechnology, PR China). Protein concentration was determined by BCA Protein Assay Kit (Beyotime Institute of Biotechnology). All measurements were performed twice then get the mean values. Aliquots were stored at -80°C.

**ELISA for DUSP1**

ELISA assay procedure was performed according to the instruction of the DUSP1 (Human) Cell-Based ELISA Kit (96 assays, Version: 01, Abnova). Proteins were extracted as above.
DUSP1 in normal pregnancy and preeclampsia

Table 3. ELISA for DUSP1 protein levels in villi of the first trimester group of different ages (conc: pg/ml)

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>n</th>
<th>OD 450</th>
<th>conc</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>X≤6 weeks</td>
<td>19</td>
<td>3.87±0.72</td>
<td>999.36±316.16</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>6&lt;X≤7 weeks</td>
<td>51</td>
<td>3.83±0.75</td>
<td>993.95±322.27</td>
<td></td>
</tr>
<tr>
<td>X&gt;7 weeks</td>
<td>24</td>
<td>3.20±0.89</td>
<td>979.02±369.05</td>
<td></td>
</tr>
</tbody>
</table>

DUSP1 protein levels varied in different ages of the first trimester group. But the difference was not significant (P>0.05).

Table 4. DUSP1 mRNA of RT-PCR in placenta of the different groups (conc =2^{ΔΔCt})

<table>
<thead>
<tr>
<th>Group</th>
<th>ΔCt</th>
<th>DUSP1</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>The first trimester women</td>
<td>1.5</td>
<td>0.3535</td>
<td></td>
</tr>
<tr>
<td>The term pregnant women</td>
<td>5.2</td>
<td>0.0263</td>
<td></td>
</tr>
<tr>
<td>The preeclamptic women</td>
<td>7.33</td>
<td>0.0062</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

DUSP1 mRNA in placenta of the different groups varied. The DUSP1 mRNA of the the preeclamptic women was significant lower than the other two groups (P<0.05).

Every sample was tested twice then get the mean values.

ELISA for DUSP1 protein levels in different group (concentration (conc) pg/ml), Table 2.

ELISA for DUSP1 protein levels in villi of the first trimester group (concentration (conc) pg/ml) Table 3.

DUSP1 mRNA detection by RT-PCR

Total RNA was extracted from the three groups: villous of the first trimester, placenta tissue of term pregnancy and Preeclampsia group. RNA was extracted by using the TRIzol Reagent extraction method (as per manufacturer’s instructions) (TRizol R0016, Beyotime Institute of Biotechnology, China). Reverse transcription and real-time PCR were performed on DNase treated RNA samples as previously described. Primers were designed based on the nucleocapsid gene of DUSP1 by Primer Express 5.0 software. The real-time RT-PCR assay had a detection limit of 5 copies, with a range of detection between 5×10^0-5 copies. GAPDH was chose to be the control. The standard curve was prepared based on the linear relationship between the amount of plasmid DNA and cycle threshold (Ct). Collected samples were detected with the real-time RT-PCR assay and every sample was tested three times, positive results were used for quantitative analysis. Comparative CT Method was used to analyze the results of real-time PCR. The expression of the DUSP1 gene was normalized to the geometric mean of the control genes GAPDH. All data was expressed relative to the control.

Immuno-histochemistry for DUSP1

4% Paraformaldehyde-fixed samples were performed overnight, then in 30% sucrose-based solution overnight. Cryosections was chosen for immuno-histochemical analysis of DUSP1 as previously described. SABC procedure was performed with SP-9001 Histostain™ Plus Kits (ZYMED, USA). Incubated overnight with primary anti-body (rabbit anti-DUSP1, 1:200 dilution, Sigma, Canada) at 4°C. The following day, PBS washed 3 times, then with biotinylated anti-rabbit immunoglobulin (1:200) was Incubated for 15 min at 37°C. PBS washed 3 times. S-A/HRP was Incubated for 15 min at 37°C. Then all slides were stained with DAB (Beyotime Institute of Biotechnology) in the same time (40 seconds). With the same time and solutions in order to minimize variation in intensity of stain. Negative controls included substitution of the primary anti-serum with non-immune serum to rule out non-specific binding.

Statistical analysis

The quantitative data are presented as mean ± SD. Means or medians were derived from duplicate values. A one-way ANOVA was used to determine differences in DUSP1 gene expression across gestation. The percentage data were presented in R×2 table and the comparison was analyzed by the χ² test. The inter-group comparison was analyzed by using one-way ANOVA. The analysis was performed with SPSS 13.0 Version. p-value of <0.05 were considered significant.

Results

Table 1 summarizes the clinical characteristics of the patients. The age of the NTP group was lower than the sPE group (28.65±3.30 and 30.11±5.98). The rate of primipara of the two groups were slightly different, 32 (72.7%) and 21 (80.8%). Systolic blood pressure was significantly lower in the NTP group (114.07±8.72 mmHg) compared to the sPE group (152.59±8.13, P<0.05). Diastolic blood pres-
DUSP1 in normal pregnancy and preeclampsia

Figure 1. Primers sequences used for RT-PCR. DUSP1: Forward: 5'-AGGACAACCACAGGAGCAC-3' 143 kb. Reverse: 3'-AAGGTAAGCAAGCCAGATGG-5'. GAPDH: Forward: 5'-GTCAAGGCTGAGAACGGGAA-3', 158 bp Reverse: 5'-AAATTGAGCCCCAGCTTCTC-3'.
Figure 2. Representative photomicrographs of DUSP1 immuno-histochemistry in the first trimester women (A), the term pregnant women (B) and sPE patients (C). Notable increased intensity of DUSP1 staining in first trimester villi vs placentas at term ones. DUSP1, primary antibody 1:200.

**Figure 2** shows the result of the Immuno-histochemistry for DUSP1 in villi and placenta of the different groups. Representative photomicrographs of DUSP1 immuno-histochemistry.
DUSP1 in normal pregnancy and preeclampsia

in the first trimester women (A), the term pregnant women (B) and sPE patients (C) in ×400 (left) and ×200 (right). Notable increased intensity of DUSP1 staining in first trimester villis vs placentas at term ones.

Discussion

Preeclampsia can result in maternal morbidity and mortality [9, 10]. It is a pregnancy-specific syndrome [11]. It usually occurs after 20 weeks of gestation and its clinical symptoms disappear after the termination of pregnancy [12]. The minimum criteria for the diagnosis of preeclampsia are hypertension plus proteinuria. Significant proteinuria is defined by 24-hour urinary protein exceeding 300 mg per 24 hours, or persistent 30 mg/dL (1+ dipstick) in random urine samples. The more severe the hypertension or proteinuria, the more certain is the diagnosis of preeclampsia. Similarly, abnormal laboratory findings in tests of renal, hepatic, and hematological function increase the certainty of preeclampsia. The decreased invasion capacity of trophoblasts in the first trimester may be crucial for the pathogenesis of preeclampsia [13, 14]. Many biochemical markers have been reported to be correlated with this disease, such as ET-1 (endothelin-1) [15], VEGF [16, 17] (vascular endothelial growth factor), PlGF [18, 19] (placental growth factor), HIF-1α [7, 8] et al. But there was no idea which biochemical marker was the most sensible one to predict the preeclampsia. It has been reported that hypoxia was a major contributor to the abnormalities seen in the preeclamptic plenta [20].

It is known that dual-specificity phosphates (DUSPs) played a very important role in the mitogen-activated protein kinase (MAPK) pathway [21]. A large amount of evidence demonstrated [22] that the phosphorylation and activation status of kinases in the MAPK system had crucial impact on the outcome of downstream events that regulate cytokine production. There were at least 13 members of the family display unique substrate specificities for MAPKs [23, 24]. DUSP1 was an important negative regulator of p38 MAPK activity [25]. Studies showed DUSP1 was to be related to the negative regulation of innate immunity, inflammatory responses, and signaling events downstream of p38 MAPK in mice [3, 26, 27]. DUSP1 was a potent inhibitor of MAPK activity through dephosphorylation of MAPK. Findings indicated that p53 was a transcriptional regulator of DUSP1 in stress responses [28]. DUSP1 was identified as a hypoxia responsive gene, but the roles of it in the response of hypoxia were not clear. Researches showed that hypoxia could induce DUSP1 expression and suppression of DUSP1 expression facilitated the interaction between HIF-1 alpha subunit [3]. DUSP1 may protect overactivation of HIF-1α through inhibiting ERK kinase activity. Just because of DUSP1 was identified as a hypoxia responsive gene, and there is some relationship between DUSP1 and HIF-1α. Hypoxia is a major contributor to the abnormalities seen in the preeclamptic placenta. So we suggest that DUSP1 would be a potential marker in preeclampsia. The ELISA of DUSP1 protein level of NTP group was higher than sPE group, and the difference between the two groups was significant. The result of RT-PCR of NTP group was much higher than that of the sPE group and the difference was significant (P<0.05). Our study showed, DUSP1 was significantly down expressed in the placenta of preeclampsia specimens in different methods. We also found that placental DUSP1 expression was higher in early gestation than in term gestation and a statistically significant correlation was found between preeclampsia and normal pregnancy (late pregnancy). But it was very striking result that there wasn't difference between preeclampsia and early pregnancy. Though there were more than 400 normal pregnancies and 5 sPE pregnancies admitted every month in our hospital, but not so many pregnancy persons want to donate their blood and placenta for the research, so the number of species was not so big.

In fact, during the clinical work, we can not keep the gestational weeks being the same between the normal and sPE pregnancy. Because we would chose cesarean section for the sPE group before 37 weeks, and if the patient was severe PE, we would do the cesarean section earlier, such as 34~36 weeks. But, if the patient was normal pregnancy, we would chose cesarean section for them after 39 weeks. Aslo we are not sure whether the low expression of DUSP1 in sPE was reason or result, and what is the function and key point of DUSP1 in placental development of preeclampsia patients, still be unknown. So there was too much need to learn. The mechanism mediating the significant suppression of DUSP1 in pre-
Eclampsia is unclear. Hypoxia caused significant down-regulation of DUSP1 gene expression in placenta tissue. There has been previously described that DUSP9 exerts its actions through the MAPK pathways ERK1/2 and p38. But what is the role of DUSP1, it need more study. So in our next study, we want to find out DUSP1 mRNA expression in villous trophoblast in early and term gestation and preeclampsia patients. We also want to find out whether there is any difference between the different times of gestation in the first trimester and third trimester. We also want use the DUSP1 knock-out mouse model where DUSP1 absent expression was associated with? What is the relation of DUSP1 and invasive capacity of trophoblasts?

We also need observing significant difference in the clinical symptoms between the preeclampsia patients with different level DUSP1 expression. Smets et al. have suggested that the DUSP2 may be one of the biomarkers for the prediction of preeclampsia. How about DUSP1? The different level of DUSP1 expression in normal early and term pregnancy and in preeclampsia may indicate that the examination of DUSP1 may be of great value in the prediction of preeclampsia at the clinic.

Acknowledgements

This study was supported by the grant from the National Natural Science Foundation of China (No. 81070505).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Li Li and Li-Li Yu, Department of Obstetrics and Gynecology, Research Institute of Surgery and Daping Hospital, Third Military Medical University, Chongqing 400042, China. Tel: +86-13808339118; Fax: +86-023-68767926; E-mail: cqili2011@163.com (LL); lililiyyu@sina.com (YLL)

References


DUSP1 in normal pregnancy and preeclampsia


