Role of peroxiredoxin III in the pathogenesis of pre-eclampsia as evidenced in mice

Lianqin Li,1* Masuo Obinata2 and Katsuyoshi Hori3
1Obstetrics and Gynecology Center; Tsinghua University Second Hospital; Beijing, China
2Department of Cell Biology; and 3Department of Vascular Biology; Institute of Development, Aging and Cancer; Tohoku University; Sendai, Japan

As a member of peroxiredoxin (Prx) family, PrxIII has been demonstrated to play an important role in scavenging intracellular reactive oxygen species (ROS). Since PrxIII knockout mice exhibited oxidative stress in placentas resembling pathophysiologic changes in placentas of human pre-eclampsia, we measured blood pressure through the carotid artery and detected oxidative status by western blotting in pregnant mice. We did not notice hypertension in pregnant PrxIII knockout mice as compared with wild-type littersmates, although endothelin-1 was overexpressed in PrxIII-deficient placentas. Our results indicate that PrxIII is not involved in pre-eclamptic development. Instead, PrxIII is an indispensable antioxidant in placentas where oxidative stress exists.

Introduction

As a unique organ that connects mother and fetus, the placenta provides the place for materno-fetal exchange of gases, nutrients and metabolic products. Since placenta is abundant in mitochondria, large amount of reactive oxygen species (ROS) is continuously produced during metabolic processes. During pregnancy, ROS production is gradually elevated with the increase of energy demand due to both fetal and maternal requirements. Although small amount of ROS is considered to be a second messenger, excessive ROS would cause oxidative injuries on DNA, lipids, and proteins. Organisms have developed multiple antioxidant defenses against oxidative damage. For example, placental antioxidant activity was increased with gestation progression,1 and higher activity of superoxide dismutase was observed in maternal erythrocytes.2 More importantly, mitochondrion possesses a primary antioxidant chain that is composed of peroxiredoxin III (PrxIII), thioredoxin II and thioredoxin reductase.3

As a member of peroxiredoxin family, PrxIII was found to be responsible to oxidative stress in placenta.4,5 We have recently observed placental oxidative damage in PrxIII-deficient (PrxIII−/−) mice.6 Interestingly, our findings in PrxIII-deficient placentas were quite similar to the major pathophysiologic changes in placentas of pre-eclampsia (PE),7-9 a serious pregnancy complication in human beings. Since it is speculated that increased placental lipid peroxides and/or tumor necrosis factor alpha (TNFalpha) cause activation of leukocytes which serve as circulating mediators and contribute to endothelial dysfunction and subsequent hypertension,10 we have investigated whether placental oxidative damage causes hypertensive disorders in mice resembling PE.

Results

Blood pressure in pregnant mice. Since oxidative damage in PrxIII-deficient placentas of mice resembled pathological findings in pre-eclamptic placentas,6,9 we measured mean arterial blood pressure in pregnant mice trying to elucidate pathogenetic role of PrxIII in pre-eclamptic development. As indicated in Table 1, there was no significant difference of blood pressure...
Table 1. Blood pressure in PrxIII+, PrxIII+/- and PrxIII-/- mice before and after pregnancy

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>PrxIII+/+</th>
<th>PrxIII+/-</th>
<th>PrxIII-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Pregnancy</td>
<td>95 ± 2</td>
<td>95 ± 2</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>After Pregnancy</td>
<td>95 ± 6</td>
<td>93 ± 5</td>
<td>97 ± 5</td>
</tr>
</tbody>
</table>

Blood pressure in mice was measured as described in “Materials and methods” section (n = 10 for PrxIII+, PrxIII+/- and PrxIII-/- mice respectively). There was no significant difference of blood pressure among individual groups before or after pregnancy (p > 0.05).

Discussion

We recently reported that PrxIII-deficiency resulted in excessive lipid peroxidation and TNFalpha production in placentas of mice, which was quite similar to the pathological changes in pre-eclamptic placentas. We then measured blood pressure in pregnant mice and found serious oxidative stress in placentas of PrxIII−/− mice, which was in accordance with our previous report. Nevertheless, ET-1 expression was not significantly elevated in other PrxIII-deficient organs except kidney as compared with wild-type samples.

Systemic oxidative status in pregnant mice. To further understand oxidative status in pregnant mice, we detected expression of endothelin-1 (ET-1) systemically. As shown in Figure 1, ET-1 protein was evidently enhanced in placentas derived from PrxIII−/− mice, which was in accordance with our previous report. Nevertheless, ET-1 expression was not significantly elevated in other PrxIII-deficient organs except kidney as compared with wild-type samples.

Materials and Methods

Animals. Experimental mice were generated by mating C57BL/6 mice with PrxIII−/− mice to generate PrxIII−/− offspring, which were then intercrossed to produce PrxIII+, PrxIII+/- and PrxIII−/− littermates for experiments. PrxIII genotypes were determined by PCR analysis as previously described. Use of the experimental animals was approved by the institutional ethics committee of Tohoku University, Japan.

Measurement of blood pressure. Mean arterial blood pressure of mice was measured through the carotid artery. After anesthesia with diethyl ether the carotid artery of maternal mouse was isolated and a catheter was inserted into the artery through a tiny incision. Another end of the catheter was connected with a pressure transducer (Spectramed Medical Products, Singapore) whose output was fed into an amplifier (NEC-Sanei Co., Tokyo, Japan) and recorded. Age-matched non-pregnant female mice were used as controls.

Systemic detection of oxidative stress. Brains, hearts, lungs, livers, kidneys, skeletal muscles and placentas were removed from maternal mice that were pregnant for 18 days. Samples from age-matched non-pregnant mice were used as controls. The samples were homogenized through ultrasonic disruption and protein concentration was detected by Bio-Rad protein assay kit. After separation on 15% SDS-PAGE and transfer to Immobilon membranes (Millipore Corporation), proteins were recognized with primary antibody anti-ET-1 (Santa cruz). Visualization was achieved with ECL western blotting detection system (Amersham). Anti-β-actin antibody (Sigma) was used as internal control.

Statistical analysis. The value of blood pressure was presented as mean ± SEM and was compared among PrxIII+, PrxIII+-, and PrxIII−/− mice by analysis of variance. p < 0.05 was considered to be statistically significant.

References
