Role of Axl in Preeclamptic EPCs Functions

Ying HU (胡颖), Xiao-ping LIU (刘小平), Xiao-xia LIU (刘晓夏), Yan-fang ZHENG (郑艳芳), Wei-fang LIU (刘维芳), Ming-lian LUO (骆名恋), Hui GAO (高慧), Ying ZHAO (赵颖), Li ZOU (邹丽)

Department of Obstetrics and Gynecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

© Huazhong University of Science and Technology and Springer-Verlag Berlin Heidelberg 2016

Summary: Axl encodes the tyrosine-protein kinase receptor, participating in the proliferation and migration of many cells. This study examined the role of Axl in functions of endothelial progenitor cells (EPCs). Axl was detected by RT-PCR and Western blotting in both placentas and EPCs from normal pregnancy and preeclampsia patients. The Axl inhibitor, BMS777-607, was used to inhibit the Axl signalling pathway in EPCs. Cell proliferation, differentiation, migration and adhesion were measured by CCK-8 assay, cell differentiation assay, Transwell assay, and cell adhesion assay, respectively. Results showed the expression levels of Axl mRNA and protein were significantly higher in both placentas and EPCs from preeclampsia patients than from normal pregnancy (P<0.05). After treatment with BMS777-607, proliferation, differentiation, migration and adhesion capability of EPCs were all significantly decreased. Our study suggests Axl may play a role in the function of EPCs, thereby involving in the pathogenesis of preeclampsia.

Key words: Axl; endothelial progenitor cells; preeclampsia

Preeclampsia is one of the main causes of maternal and perinatal mortality, which seriously threatens the health of both mothers and fetus[1]. It is clinically described as the occurrence of high blood pressure and proteinuria after the 20th gestational week[2]. Despite years of advancements, causes of preeclampsia remain ill-defined. However, there is a unified notion that early onset cases show deficient placentation, which causes the placenta to be poorly perfused, followed by formation of a relative hypoxic uteroplacental environment[3] where preeclampsia-associated changes in placental production of vasoclagenic/angiogenic substances take place[4]. Substances entering maternal blood are the foundation of the vasoclagenic activities, including endothelial cell migration, activate coagulation, and reduce vascular integrity, resulting in pathophysiological changes of preeclampsia[5]. Therefore, vascular endothelial dysfunction is a key point in the pathogenesis of preeclampsia, and endothelial progenitor cells (EPCs) are believed to have the capacity for endothelial repair[3] and can be specific markers reflecting endothelial injury[6].

It has been nearly two decades since Asahara et al characterized the isolation of EPCs from peripheral blood. EPCs are a heterogeneous group of endothelial cell precursors[6], and are divided into early EPCs and late EPCs. Early EPCs appear at the 7th day of culturing with spindle shapes, proliferate and migrate to the vessel formatting location, and secrete paracrine factors which promote the subsequent angiogenic process, thereby contributing to vascularization. Late EPCs appear after 2–3 weeks in cobblestone shapes exhibiting high proliferative capacity and differentiate into mature endothelial cells[7]. They are thought to be essential in vascular remodelling and endothelial homeostasis. Studies have found that both bone marrow-derived and circulatory EPCs are beneficial to the recovery of ischemic tissues[8]. In response to tissue ischemia, EPCs mobilize from bone marrow into circulation[9]. Circulatory EPCs can then be recruited to vascular lesion sites and may play a critical role in blood vessel damage repair, and the restoration of endothelial function or neoangiogenesis after the fetal period[6, 10]. Preeclampsia can be seen as a specific placental ischemic disease due to endothelial dysfunction[11]. Various researches find the number of EPCs increases during normal pregnancy[12, 13], but decreases in patients with preeclampsia[12–15]. Furthermore, EPCs in preeclampsia patients show cell senescence and function diminution[14, 15]. The decrease of EPCs in quantity and their function impairment weakens endothelial regeneration potential, which may contribute to the pathogenesis of preeclampsia[14].

Axl (UFO/ARK/Tyro7) is a receptor tyrosine kinase (RTK) stimulated by growth arrest specific 6 (Gas6)[16]. Axl is widely present in normal human tissues, e.g. hippocampus and cerebellum, mononuclear macrophages, platelets, endothelial cells, myocardium, colon mucosa, liver, thyroid, kidney, testis, skeletal muscle, etc[17, 18]. In various tumours, Axl abnormal expression is detected and is related to tumour proliferation and invasion caused by angiogenesis, affecting the malignant degree, metastatic ability and prognosis[17, 18]. In endothelial cells, Axl is the central factor in the regulation of various vasogenic activities, including endothelial cell migration, proliferation and tubal formation[19]. Gas6-Axl interac-

Ying HU, E-mail: huying99999@126.com

Corresponding author, E-mail: xiehezouli@126.com
tions can protect endothelial cells from apoptosis\(^2^0\). However, Gallicchio et al. found the activation of Axl signalling pathway can inhibit the vascular endothelial growth factor receptor 2 (VEGFR-2)-mediated endothelial cell angiogenic program\(^2^1\). The aforementioned studies proved the importance of Axl in the angiogenic program of endothelial cells. However, little is known about EPCs. In addition, studies existing show the concentration of Axl in serum (sAxl) in preeclampsia patients is significantly higher than that in normal pregnancy\(^2^2\). This phenomenon suggests Axl may play a role in the pathogenesis of preeclampsia.

As previously mentioned, preeclampsia patients possess impaired endothelial function and increased expression of sAxl. Moreover, both Axl and EPCs are involved in endothelial function. Therefore, our study assumed that Axl signaling pathway may play a role in the pathogenesis of preeclampsia by affecting EPCs functionality. To test this assumption, researches were conducted concentrating on the change of Axl expression in preeclampsia.

1 MATERIALS AND METHODS

1.1 Study Population and Blood Collection

The study was conducted at the Department of Obstetrics and Gynecology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST) from September 2014 to February 2015. The study groups consisted of 15 women with uncomplicated pregnancy (controls) and 15 women with preeclampsia. All subjects were in the third trimester of pregnancy. Preeclampsia was diagnosed according to the following criteria: hypertension (blood pressure higher or equal to 140/90 mmHg) and proteinuria (300 mg/24 h) that occurred after 20 weeks of gestation in women with previously normal blood pressure\(^3\). Controls consisted of healthy pregnant women without chronic problems or pregnancy complications. Exclusion criteria included history of cardiovascular disease, diabetes mellitus, other significant preexisting metabolic disorders, rupture of membranes and multifetal gestation. The relevant clinical characteristics of the two groups are presented in Table 1. All the subjects underwent cesarean section (controls undergoing cesarean section due to their own demands, cephalopelvic disproportion or breech presentation). After the delivery of placenta, cord blood (40–60 mL) was collected into sterile tubes containing heparin immediately and processed within 2 h of collection for cell culture. Ethical approval for the study protocol was obtained from the Ethics Committee of Union Hospital, Tongji Medical College, HUST.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (n=15)</th>
<th>Preeclampsia (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>30.00±2.23</td>
<td>31.07±2.37</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI in pregnancy</td>
<td>25.24±0.79</td>
<td>25.80±0.69</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gestation weeks at delivery</td>
<td>38.21±1.31</td>
<td>37.03±1.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SBP</td>
<td>112.60±5.96</td>
<td>166.50±7.94</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DBP</td>
<td>73.53±4.00</td>
<td>101.9±4.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>100% (15/15)</td>
<td>0 (0/15)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are expressed as X±s or percentage (number/total).

BMI: body mass index (kg/m\(^2\)); DBP: diastolic blood pressure; SBP: systolic blood pressure

1.2 EPCs Isolation and Culture

Heparinized cord blood was used to isolate peripheral blood mononuclear cells (PBMCs) by employing the method described previously\(^2^3\). First, cord blood was diluted with the equal volume of phosphate buffer solution (PBS), then isolated using Ficoll-Paque density gradient centrifugation (400 g, 30 min, 20°C) in a lymphocyte separation solution (TBD Sciences, China), washed twice with PBS, and resuspended in endothelial basal medium-2 (EBM-2, Lonza, USA) supplemented with EGM-2-MV-SingleQuots (Lonza) containing 5% fetal bovine serum, 50 ng/mL human vascular endothelial growth factor (VEGF), 50 ng/mL human insulin-like growth factor 1, 50 ng/mL human epidermal growth factor, 100 mg/mL penicillin, and 100 mg/mL streptomycin. After the above treatment, PBMCs at a density of 10^7 cells/mL were seeded on fibronectin-coated (Sigma–Aldrich, USA) six-well culture dishes (NEST). After 3 days of culture, non-adherent cells were removed and the medium was replaced every 2 days. After 3 days, attached cells appeared and formed small round EPC clusters, then the round cells started elongating and had a spindle shape similar to that of the EPCs firstly reported by Asahara. After 7 days of culture, they gradually turned to clusters consisting of round cells centrally with multiple spindle-shaped cells sprouting from the central core, which we called colony-forming units (CFU), was also regarded as early EPCs\(^2^4\). The identification of EPCs had been performed in our previous experiments\(^2^5\) and all experiments were performed with early EPCs.

1.3 Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated from placental tissue or cultured cells using RNAiso Plus (Takara, Japan) according to the manufacturer’s protocol, and cDNA was synthesized from total RNA by employing Prime Script\textsuperscript{TM} 1st RT Master Mix (TaKaRa, Japan). The expression of Axl gene was detected by reverse transcrip-