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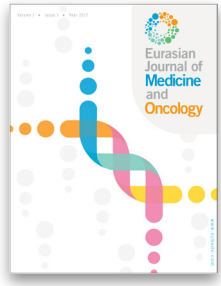

Research Article

Elevated Expression of Glucocorticoid-Induced Leucine Zipper in Placental Endothelial Cells and Trophoblasts in Preeclampsia

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Abstract

Objectives: The objective of this study was to investigate the association between normal and preeclamptic glucocorticoid-induced leucine zipper (GILZ) levels in the placenta.

Methods: Placental paraffin sections (5 µm) were obtained from gestational age-matched normal (n=9) and (PE) (n=9) pregnancies. Tissue sections were immunostained with rabbit monoclonal anti-human GILZ antibodies. Staining intensity of GILZ was evaluated with a histologic scoring (HSCORE) system. Parametric (t-test) and non-parametric tests (Mann–Whitney U) were used for statistical analysis, and a p value of <0.05 was considered significant.

Results: Trophoblasts and endothelial cells were shown to be the source of GILZ release. Compared with the control, PE placental samples displayed significantly increased GILZ immunostaining HSCOREs in trophoblast cell nucleus (mean±SEM, 106.8±12.1 vs 167.9±14.9; p=0.006) and endothelial cells (95.0±8.1 vs 147.2±12.3; p=0.003).

Conclusion: This study suggests that GILZ release by placental trophoblasts and endothelial cells may contribute to PE pathogenesis.

Keywords: Endothelium, glucocorticoid-induced leucine zipper, placenta, preeclampsia, trophoblast

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Preeclampsia (PE) is a pregnancy complication, characterized by systemic widespread inflammation related to the placenta and other fetus-originated tissues, which causes infant and maternal morbidity and mortality.[1] A significant clinical and biochemical improvement is seen with separation of the fetus and placenta subsequent to birth in most cases.[2] Although many extensive studies have been conducted, the exact cause and treatment remain unknown. Vascular inflammation and endothelial cell damage are some of the main mechanisms involved in PE formation mechanism.[3, 4] Increased cortisone levels are closely related with hypertension and endothelial damage, which are symptoms frequently observed in PE patients.[4]

Glucocorticoid-induced leucine zipper (GILZ) is a protein that was first identified in 1987.[5] GILZ gene expression is increased by IL-10 and dexamethasone.[6] GILZ was first detectedin T cells.[5] It is also secreted by mesenchymal stem cells as well as immune cells such as monocytes, macrophages, mast cells, and dendritic cells. This steroid and chemokine has been observed to play a key role in anti-inflammatory and immunosuppressive processes. GILZ may be an alternative to treatments in which glucocorticoids are used because it increasesthe efficacy of glucocorticoids andinhibits the harmful effects of glucocorticoids during treatment.[6] GILZ interacts with intracellular signaling pathways such as C-RAF, nuclear factor kappa B subunit 1 (NFKB1), and NFKB 2 in the cell. Moreover, GILZ is required for the release of IL-8 in dexamethasone-dependent respiratory epithelial cells and of cyclooxygenase 2 (COX-2) in bone marrow-originated mesenchymal cells.[6] Therefore,

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GILZ has an important role in regulating inflammation in various cells and tissues, suggesting that GILZ may be involved in PE and can be utilized in PE treatment. In this study, we anticipate that increased GILZ level is involved in PE pathogenesis in endothelial cells and trophoblasts.

Methods

In this prospective, experimental laboratory work, placental samples obtained from normal pregnant women who have never received steroid treatment before, who were matched for their gestational age, and who had no pathology, and placental samples obtained after birth from patients diagnosed with PE were used. Ethics Committee approval was received from the University of South Florida for the study.

Immunohistochemistry

Formalin-fixed and paraffin-embedded samples were cut into 5-µm-thick sections that were then incubated one night at 56°C. The slides were deparaffinized in xylene (x3) for 20 min, followed by 100, 90, 80, and 70% alcohol x1 for 10 min per gradient. Subsequent to deparaffinization, slides were heated in 10 mM citrate buffer for 15 min for specific antigen–antibody binding (pH 6.0). Subsequently, the sections were dipped in 3% hydrogen peroxide (50% methanol/50% distilled water) for 15 min to block endogenous peroxidase activity. These slides were then washed in tris-buffered saline (TBS) for 3–5 min and incubated with 5% normal horse serum (Lab Vision, Fremont, CA) at room temperature for 30 min in TBS to prevent unspecific bindings other than the desired antigen–antibody binding. Excess blocking serum was removed from the lamella at the end of this stage. Serial placental sections, blocking process of which was finished, were placed in 1% blocking horse serum in TBS (R&D Systems, Minneapolis, MN) with primer antibody (rabbit polyclonal anti-human GILZ antibody) (Cell Signaling Technology, Beverly, MA) by adjusting the concentration to 1:150 to be incubated during the night at 4°C. Normal rabbit IgG antibody isotypes were used in the same primary antibody concentrations as negative controls. The next day, primary antibody serial sections incubated with primary antibody were washed with TBS for 3–5 min, and the unbound antibodies were removed from the environment. Goat anti-rabbit secondary antibody biotinylated at 1:400 concentrations for 30 min at room temperature (Vector Laboratories, Burlingame, CA) was added after this stage. Antigen–antibody complex was determined using strep–avidin–biotin–peroxidase kit (Vector Lab) at room temperature for 30 min. 3.3 Diaminobenzidine tetrahydrochloride dehydrate (DAB, Vector Lab) was used as a chromogen to visualize immunoreactivity, and the sections were contrasted with hematoxylin.

GILZ immune activity was assessed semi-quantitatively using the following density categories: 0, no staining; 1+, weak but detectable staining; 2+, medium or different staining; and 3+, intensive staining. For each tissue, histological score (HSCORE)=ΣPi (i+1) formula was used and HSCORE was obtained after the percentage of stained cells at each concentration category were added, and this value was multiplied by the intensity of the immunostaining. Here, "i" represents the intensity scores and "P" the relevant percentage of the cells as described. Five fields were randomly selected on each slide and were evaluated under a light microscope (×40 magnification). These areas were evaluated at different times by two researchers who were blind to the type and source of the tissues for each concentration in these cells. The individual and individual variability coefficients for HSCORE evaluation were 10% and 12%, respectively. The average score of the two researchers who made the evaluation was used in the results.

Results

Gestational age was calculated as 38.5±0.7 in the control group (mean±SD) and as 38.7±1.2 in the PE group. There was no statistically significant difference between the two groups for the average gestational age (p=0.850). GILZ immune activity was detected in low- and intermediate-level interstitial trophoblasts and vascular endothelial cells in both the control and PE placental samples. This immune reaction was observed to be poor in the cytoplasmic region and intensive in the nuclear region in both trophoblasts and endothelial cells. When the intensity of this immunostaining was quantified numerically, the following was found: compared with the control, trophoblastnuclear HSCORE was (mean±SEM) 106.8±12.1 vs 167.9±14.9 (p=0.006), whereas the endothelial cell nuclear HSCORE was 95.0±8.1 vs 147.2±12.3 (p=0.003) in preeclamptic placental samples. GILZ immunostaining intensity was calculated to be statistically higher in the PE group. A weak GILZ level was detected in the interstitial trophoblast cytoplasmic localization. It was 59.0± 7.6 in the control group, whereas it was 60.7±6.5 (p=0.871) in the PE group. When cytoplasmic GILZ immunostaining intensity and nuclear GILZ activity were compared, GILZ HSCORE was found to be 106.8±12.1 vs 59.0±7.6 (p=0.001) in the control placental samples. Nuclear GILZ immunoreactivityin PE patients was observed as 167.9±14.9 vs 60.7±6.5 (p=0.002) (Fig. 1).

Discussion

This study provides evidence for a relationship between GILZ and PE for the first time. Studies on the role of glu-

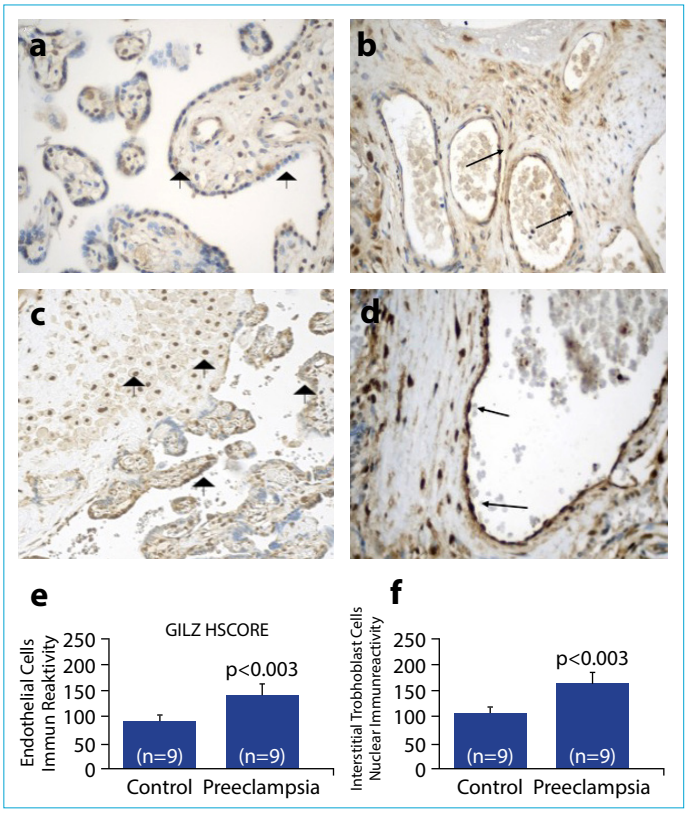

Figure 1. Increased GILZ immune reactivity in preeclamptic placental endothelium and trophoblasts.

Normal placental sample **(a, b)** is seen as weak–medium and preeclamptic placental sample **(c, d)** is seen as medium–strong in terms of GILZ immunostaining in microscopic photographs. Arrowheads indicate trophoblasts and arrows indicate placental vascular endothelial cells. Graphics show HSCORE values in endothelial cells **(e)** and trophoblasts **(f)**. Control and preeclampsia HSCORE is reflected as mean±SEM.

cocorticoids in PE pathogenesis have been previously conducted.[7, 8] However, research on the direct role of GILZ in PE still remains insufficient. Increased immunity activity in vessels and deterioration of protective barrier function due to damage to vascular endothelial cells is the most important effect on PE pathogenesis.[9-11] The immunological and molecular mechanisms that start this change in the veins and that are involved in this process have still not been understood in a clear manner, despite many studies being conducted. There are studies that have shown that glucocorticoids cause endothelial damage and function deterioration and those that use glucocorticoid in the treatment of endothelial damage.[12, 13] GILZ is a gene of the TSC-22 protein family, which is inducible by dexamethasone and characterized by the presence of TSC box and leucine.[14] GILZ's roles in mediating anti-inflammatory effects of glucocorticoids are shown invariouscell types.[15-17] GILZ gene is released in the effect of glucocorticoids.[15-17] Some studies have shown that GILZ protein suppresses intracellular NF-KB signaling pathway activation and hampers inflammation in human endothelial cells.[17] This shows that GILZ is secreted in large amounts in human tissues and it plays an important role in inflammation. Vascular endothelial cell, which has an important role in creating the blood–tissue barrier, also plays a critical role in the development of vascular inflammatory damage.[18]

Studies have been conducted using overexpression of GILZ by employing animal models of different inflammatory diseases or gene silencing strategies.[17, 19]Studies on rats have revealed that the suppression of GILZ contributes to inflammation by causing an increase in IL-1ß and IL-6 secretion in macrophages.[19] Again, a study on human umbilical vein endothelial cell culture (HUVEC) has reported that suppression of GILZ may play a role in the increase in inflammatory genes and in the occurrence of cardiovascular diseases.[17] GILZ has been demonstrated to be secreted at high levels in synovial endothelial cells in rheumatoid arthritis patients. In addition, same study has also shown that increase in GILZ level in HUVECs decreases tumor necrosis factor-alpha level, leukocyte migration, and adhesion to endothelial cells.[20] This result shows that GILZ has a central role in endothelial cell function and inflammation of cell levels. The first study on GILZ has shown that GILZ inhibits NF-kB activity and the migration and proliferation of T lymphocytes, B lymphocytes, and macrophages. Similarly, GILZ has been shown to have an anti-inflammatory effect on endothelial cells.[17, 20] In this study, we determined that GILZ immune staining was mostly nuclear in both trophoblasts and placental endothelial cells. This finding suggests that GILZ functions by regulating genes at the nuclear level. In the present study, detection of high GILZ reactivity in endothelial cells and trophoblasts in the placenta taken from preeclamptic patients suggest that this molecule has an important role in the pathogenesis of PE. Increasing immune activity medium in physiological levels is observedduring pregnancy.[21-23] However, the deterioration of this balance is associated with pregnancy complications.[21-23] It is not known whether the increase in GILZ level in trophoblasts and endothelial cellsis a protective reaction or has a pathological role in PE. Previous studies have shown that GILZ release increased with gestational age and it had a role in COX-2 andprostagland in synthesis.[24, 25] Cox-2 has an important function not only in the development of normal pregnancy but also in normal birth. COX-1 level decreases but COX-2 secretion increases during normal birth.If there is a deficiency in COX-2 level, this results in premature miscarriage.[26]

Trophoblast migration is extremely important for change of the spiral arteries in the placental tissues and circulation

low pressure in the materno–placental area.[27, 28] Trophoblasts migrate toward the decidual layer in placental developmental stage and replace the arterial smooth muscle layer in the spiral arteries and endothelial cells and create a materno–placental circulation, which facilitates the fine nutrient andoxygen exchange in the placental area. It is believed that this insufficient migration of trophoblasts may be effective in the formation of PE.[27-29] This increasing GILZ levelin trophoblasts may affect this change and have a role in PE. Previous studies support the finding that the placenta's exposure to glucocorticoids in various ways and forms during pregnancy leads to insufficient placental trophoblast migration, invasion, and proliferation in vitro and induced hypertension, fetal and placental growth restriction, renal function failure, and development of proteinuria, which are classic signs of PE in studies on rats.[7] Similarly, another study has shown that increased glucocorticoid metabolite level in the placenta increases the risk of PE.[30] Furthermore, increased stress in PE directly leads to an increase in cortisol level in the hypothalamic–pituitary–adrenal axis. This increased cortisol level is associated with hypertension and endothelial damage.[4] This increased cortisol level may also contribute to PE pathogenesis by causing placental GILZ release. One of the limitations of the study is that the study does not show which signaling pathways are activated or suppressed by the increased GILZ protein in PE. Furthermore, it is not known if the increased GILZ level reactional protective response in PE. Our in vivo findings suggest that the GILZ protein may create a step related to PE pathogenesis.

These results show that GILZ released by trophoblasts and endothelial cells play an important role in patients with PE. Elevated GILZ release may also contribute to local and/or systemic inflammation in PE. These findings may enable us to use GILZ as a new tool in the diagnosis and treatment of PE.

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Disclosures

Ethics Committee Approval: The study was approved by the Local Ethics Committee.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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