Journal of Hainan Medical University

http://hnyy.cbpt.cnki.net/

Gadd45 α expression in preeclampsia placenta and the effect of Gadd45 α on trophoblast HTR8/Svneo

Li Wang*, Lin Zhao

Maternity Branch Hospital of Panjin Central Hospital, Panjin City, Liaoning Province, 124000

ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form 13 November 2015
Accepted 21 October 2015
Available online 19 November 2015

Keywords:
Preeclampsia
Growth arrest and DNA damage 45 α
Gene knockdown
Matrix metalloproteinase

ABSTRACT

Objective: To study the expression of Gadd45 α in preeclampsia placenta and the regulating effect of Gadd45 knockdown on trophoblast HTR8/Svneo. **Methods:** Preeclampsia placenta tissue and normal placenta tissue were collected, and mRNA contents and protein contents of Gadd45 α were detected by fluorescent quantitative PCR and Western blotting respectively; trophoblast cells HTR8/Svneo were cultured and after transfection of Gadd45 α siRNA, cell invasion ability and expression of invasion-assiotiated molecules were detected. **Results:** mRNA content and protein content of Gadd45 α in preeclampsia placenta tissue were higher than those in normal placenta tissue; after transfection of Gadd45 α siRNA, mRNA content and protein content of Gadd45 α in HTR8/Svneo cells significantly decreased, and the number of invasive cells as well as expression of MMP1, MMP2, MMP3 and MMP9 significantly increased. **Conclusion:** The expression of Gadd45 α in preeclampsia placenta abnormally increases; inhibting the expression of Gadd45 α in trophoblasts HTR8/Svneo can promote invasion and increase the expression of MMPs molecules.

1. Introduction

Preeclampsia (PE) is an idiopathic disease during pregnancy characterized by hypertension and proteinuria, and it is a severe complication of maternal and neonatal deaths[1]. At present, there is still no effective means for clinical treatment of PE and its pathogenesis is not fully elucidated. In recent years, more and more studies have recognized that defect of trophoblast cell invasion and abnormality of spiral artery recasting are important links in the pathogenesis of preeclampsia[2], and exploring the regulating mechanism of trophoblast invasion defect helps to clarify the pathogenesis of PE. Growth arrest and DNA damage 45 alpha (Gadd45 α) is a newly discovered gene regulating cell function and participates in the regulation of many malignant tumor cell invasion processes[3]. In order to make clear whether abnormal expression of Gadd45 α gene was involved in the occurrence of PE, the expression of Gadd45 α in preeclampsia placenta and the regulating effect of Gadd45 α knockdown on trophoblast HTR8/Svneo were analyzed in the following research.

2. Research subjects, materials and methods

2.1. Research subjects

Pregnant women delivery in our hospital from May 2013 to June 2014 were selected for study, including 30 cases of preeclampsia pregnant women and 30 cases of normal pregnant women. Preeclampsia placenta and normal placental tissue were collected, washed with normal saline after the nature was determined through immunohistochemistry, transferred into freezing tube after absorbing the moisture completely, shortly frozen for 10-15 min in liquid nitrogen and then preserved in freezer at -80 ℃.

2.2. Materials

2.2.1. Cells and siRNA

Trophoblast HTR8/Svneo cell lines were sent by Professor Graham from Canada; Gadd45 α siRNA and negative control siRNA were

*Corresponding author: Li Wang, Maternity Branch Hospital of Panjin Central Hospital, Panjin City, Liaoning Province, 124000.
Tel: 13154273199
Fund Project: Liaoning Provincial Science and Technology Department to Start the Doctoral Project No.20111107.

built by Shanghai Genepharma Company.

2.2.2. Main reagents

Fetal bovine serum and RPMI1640 medium were purchased from Thermo Company, transfection reagents were lipofectamine 2000 from Invitrogen Company, Transwell chambers (pore diameter 8 μm) were from Millipore Company, Matrigel was from BD Company, RNA extraction kits RNO iso as well as reverse transcription kits, PCR reaction kits were from Takara Company, protein extraction and western blotting gel kits were from Beyotime Company and ECL developer was from Santa Cruz Company.

2.3. Methods

2.3.1. Cell culture and processing

HTR8/Svneo cells were recovered, incubated and cultured with RIPM medium containing 10% fetal bovine serum, received trypsin digestion and passage when they grew in logarithmic phase and the density reached about 80%, seeded in 12-hole cell plate in the density of 1×10^5/hole, incubated until density reached about 70%, and then received siRNA transfection. Gadd45 α -siRNA group were transfected with Gadd45 α siRNA and negative control siRNA group were transfected with negative control siRNA.

2.3.2. Cell invasion detection

Matrigel was diluted in the ratio of 1:9 and then used to cover Transwell chambers, cell suspension diluted with 0.2 mL of serum-free medium was added to the upper chamber after Matrigel solidified, and cell transfection methods were followed to transfect Gadd45 α siRNA and negative control siRNA into cells; 0.6 mL of medium containing 10% fetal bovine serum was added to the lower chamber, after consecutive incubation for 12 h, 24 h and 48 h, cells on outer surface of filter membrane were fixed with paraformaldehyde, washed twice with PBS and then stained with crystal violet, finally 5 high power fields were observed under microscope and the number of cells within the fields was counted.

2.3.3. RNA extraction and PCR detection

RNA extraction kits and reverse transcription kits were used to extract RNA for reverse transcription into cDNA, then PCR kits were used to amplify Gadd45 α, MMP1, MMP2, MMP3 and MMP9, amplification curves were obtained and mRNA contents were semi-quantitatively analyzed.

2.3.4. Protein extraction and western blotting

Proteins in tissue samples and cell samples were extracted by protein lysate and added to sample buffer for denaturation under high temperature, denatured protein samples were added to preconfigured polyacrylamide gel for electrophoresis, transmembrane and antibody incubation, incubated first antibodies included Gadd45 α and β -actin, first antibodies were washed away, then second antibodies were incubated, finally protein bands were obtained through develop, and protein contents were semi-quantitatively analyzed according to grey values of the bands.

2.4. Statistical methods

SPSS 19.0 software was used for statistical processing of the data from experiment, comparison between two groups was by *t* test and comparison among different points in time within group was by repeated measure analysis of variance. Differences were considered to be statistically significant at a level of $P<0.05$.

3. Results

3.1. Gadd45 α contents in preeclampsia placenta tissue and normal placenta tissue

Protein contents of Gadd45 α in placenta tissue were detected by western blotting, and detailed analysis was as follows: protein content of Gadd45 α in preeclampsia placenta tissue was (256.23±31.45), which was significantly higher than that in control group (100.00±11.58).

Table 1

Protein contents of Gadd45 α in cells after transfection of Gadd45 α -siRNA.

	Gadd45 α - siRNA group	Negative control siRNA group	*T*	*P*
12 h after transfection	52.35±6.14&	100±13.28	9.352	<0.05
18 h after transfection	36.34±4.22&(1)	102.34±14.29	15.142	<0.05
24 h after transfection	21.52±2.54&(1)(2)	103.14±11.58	25.357	<0.05
F	$F_{\text{among three groups}}$=13.723	$F_{\text{among three groups}}$=0.262		
	*F*12 h *vs* 18 h=7.352	*F*12 h *vs* 18 h=0.162		
	*F*12 h *vs* 24 h=12.142	*F*12 h *vs* 24 h=0.446		
	*F*18 h *vs* 24 h=8.984	*F*18 h *vs* 24 h=0.287		
P	$P_{\text{among three groups}}$<0.05	$P_{\text{among three groups}}$ >0.05		
	*P*12 h *vs* 18 h<0.05	*P*12 h *vs* 18 h>0.05		
	*P*12 h *vs* 24 h<0.05	*P*12 h *vs* 24 h>0.05		
	*P*18 h *vs* 24 h<0.05	*P*18 h *vs* 24 h>0.05		

&: compared with those transfected with negative control siRNA, there were differences; (1): compared with 12 h, there were differences; (2): compared with 18 h, there were differences;

3.2. Protein contents of Gadd45 α in cells after transfection of Gadd45 α -siRNA

After transfection of Gadd45 α siRNA, protein contents of Gadd45 α in HTR8/Svneo cells were detected by western blotting, results of statistical analysis were shown in Table 1, and details were as follows: (1) after transfection of Gadd45 α siRNA, comparison among different points in time showed that with the extending of processing time, protein content of Gadd45 α decreased; (2) comparison at same point in time between two groups showed that 12 h, 18 h and 24 h after transfection of siRNA, protein content of Gadd45 α in Gadd45 α -siRNA group was lower than that in negative control siRNA group.

3.3. Cell invasion

Cell invasion ability was detected by Transwell experiment, results of statistical analysis were shown in Table 2, and details were as follows: (1) comparison among different points in time within group showed that with the extending of processing time, the number of invasive cells increased; (2) comparison at same point in time between two groups showed that 12 h, 18 h and 24 h after transfection of siRNA, the number of invasive cells transfected with Gadd45 α -siRNA was more than that transfected with negative control siRNA.

3.4. Invasion-associated molecules

Matrix metalloproteinase is the most essential molecule involved in cell invasion and molecules including MMP1, MMP2, MMP3, MMP9, etc are all involved in the regulation of trophoblast cell invasion. Analysis of the expression of above MMP molecules in HTR8/Svneo cells showed that mRNA contents of MMP1, MMP2, MMP3 and MMP9 in Gadd45 α -siRNA group were higher than those in negative control siRNA group.

4. Discussion

Moderate invasion of trophoblast cells to uterine decidua tissue and superficial myometrium is the key to the formation of placenta and has important value for both maintenance and process of pregnancy. Trophoblast cell invasion is regulated by a variety of factors and abnormality of the process will cause the occurrence preeclampsia, fetal growth restriction, miscarriage, premature and other complications of pregnancy[4]. In recent years, more and more studies believe that the most prominent pathological characteristics of preeclampia women are weakened trophoblast invasion ability and obstacles of uterine spiral artery recasting[5]. At present, regulating mechanisms of trophoblast cell invasion are still not fully elucidated. In placenta tissue, expression products of growth arrest and DNA damage 45 α (Gadd45 α) gene are mainly located in cytoplasm and nucleus of trophoblast cells, and have regulatory effect on biological function of trophoblast cells[6]. The gene was first discovered in ovarian cells of hamsters treated with ultraviolet irradiation and methyl methanesulfonate, and subsequent studies believe that radiation, oxidative stress, serum-free starvation and other damage factors will increase Gadd45 α expression[7,8]. In recent years, oncology-associated studies believe that Gadd45 α gene shows low expression in a variety of malignant tumors and over-expression of

Table 2

Effect of transfection of siRNA on number of invasive cells.

	Gadd45 α - siRNA group	Negative control siRNA group	*T*	*P*
12 h after transfection	56.12±7.69&	40.59±5.03	6.283	<0.05
18 h after transfection	74.51±8.18&(1)	48.18±5.29(1)	7.955	<0.05
24 h after transfection	92.32±10.24&(1)(2)	60.34±6.58(1)(2)	7.103	<0.05
F	$F_{\text{among three groups}}$=12.485	$F_{\text{among three groups}}$=9.293		
	*F*12 h *vs* 18 h=8.812	*F*12 h *vs* 18 h=6.965		
	F12 h *vs* 24 h=14.832	*F*12 h *vs* 24 h=11.318		
	*F*18 h *vs* 24 h=10.304	*F*18 h *vs* 24 h=8.682		
P	$P_{\text{among three groups}}$<0.05	$P_{\text{among three groups}}$ <0.05		
	*P*12 h *vs* 18 h<0.05	*P*12 h *vs* 18 h<0.05		
	*P*12 h *vs* 24 h<0.05	*P*12 h *vs* 24 h<0.05		
	*P*18 h *vs* 24 h<0.05	*P*18 h *vs* 24 h<0.05		

&: compared with those transfected with negative control siRNA, there were differences; (1): compared with 12 h, there were differences; (2): compared with 18 h, there were differences.

Table 3

Effect of transfection of siRNA on mRNA contents of MMPs.

Group	MMP1	MMP2	MMP3	MMP9
Negative control siRNA	100.00±15.48	100.00±16.10	100.00±16.75	100.00±14.59
Gadd45 α -siRNA	187.54±20.52	224.59±28.94	241.18±31.42	210.69±24.41
T	9.182	12.484	15.029	11.049
P	<0.05	<0.05	<0.05	<0.05

it can inhibit cancer cell migration and invasion[9,10], indicating that Gadd45 α gene has regulatory effect on biological behavior of cells. Biological characteristics of trophoblast cells in placenta have certain similarity to malignant tumor cells, and according to experimental results of Gadd45 α gene regulating cancer cell migration and invasion, it is assumed that Gadd45 α gene may be involved the regulation of biological behavior of trophoblast cells. Studies of foreign Xiong Y[11] show that expression of Gadd45 α abnormally increases in preeclampsia placenta tissue. In the research, differences of Gadd45 α expression in preeclampsia placenta tissue and normal placenta tissue were analyzed, and detection results showed that both mRNA contents and protein contents of Gadd45 α in preeclampsia placenta tissue were higher than those in normal placenta tissue, which could clarify that high Gadd45 α expression was related to the occurrence of preeclampsia.

Weakening of trophoblast invasion ability is the most important pathological feature of preeclampsia women, and in order to explore whether abnormal expression of Gadd45 α gene affected trophoblast cell invasion to be involved in the occurrence of preeclampsia, HTR8/Svneo cell lines were used as research subjects and siRNA transfection was adopted to knockdown the expression of Gadd45 α gene, thereby detecting cell invasion. The cell line derives from extravillous trophoblasts in human placenta tissue of normal early pregnancy, its biological characteristics and function are very similar to in vivo trophoblast cells, and it is an ideal tool to study biological behavior of trophoblast cells. After transfection of Gadd45 α siRNA, both protein content and mRNA content of Gadd45 α in HTR8/Svneo cell lines significantly decreased, which indicated that transfection of siRNA could effectively knockdown Gadd45 α expression.

After making clear that siRNA transfection could knockdown Gadd45 α expression, invasion of HTR8/Svneo cells was analyzed, including invasion ability and invasion-associated molecules. After cells were seeded in Transwell chamber pre-covered with Matrigel, migration of cells from inner side of the chamber to outer side needed to degrade the components of Matrigel, which could simulate in vitro invasion of cancer cells. In the research, analysis of the number of invasive cells in the chamber showed that transfection of Gadd45 α siRNA could increase the number of invasive cells and promote cell invasion. Matrix metalloproteinase is the most critical molecule involved in cell invasion and molecules including MMP1, MMP2, MMP3 and MMP9, etc are all involved in the regulation of trophoblast cell invasion[12–15]. In the research, analysis of the expression of above MMP molecules in HTR8/Svneo cells showed that transfection of Gadd45 α siRNA could increase the expression of MMP1, MMP2, MMP3 and MMP9.

Based on above discussion, it can be concluded that the expression of Gadd45 α in preeclampsia placenta abnormally increases; inhibiting the expression of Gadd45 α in trophoblasts HTR8/Svneo can promote cell invasion and increase the expression of MMPs molecules.

References

[1] Shah DA, Khalil RA. Bioactive factors in uteroplacental and systemic circulation link placental ischemia to generalized vascular dysfunction in hypertensive pregnancy and preeclampsia. *Biochem Pharmacol* 2015; **95**(4): 211-226.

[2] Crosley EJ, Elliot MG, Christians JK, Crespi BJ. Placental invasion, preeclampsia risk and adaptive molecular evolution at the origin of the great apes: evidence from genome-wide analyses. *Placenta* 2013; **34**(2): 127-132.

[3] Zhang L, Yang Z, Liu Y. GADD45 proteins: roles in cellular senescence and tumor development. *Exp Biol Med (Maywood)* 2014; **239**(7): 773-778.

[4] Ji L, Brkic J, Liu M, Fu G, Peng C, Wang YL. Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. *Mol Aspects Med* 2013; **34**(5): 981-1023.

[5] Lyall F, Robson SC, Bulmer JN. Spiral artery remodeling and trophoblast invasion in preeclampsia and fetal growth restriction: relationship to clinical outcome. *Hypertension* 2013; **62**(6): 1046-1054.

[6] Liu XR, Mu HQ, Luo X, Qi HB. Effects of Gadd45 α on cell migration and invasion in the human trophoblast derived HTR8/SVneocells. *J Chongqing Med Univ* 2014; **39**(11): 1516-1521.

[7] Moskalev A, Plyusnina E, Shaposhnikov M, Shilova L, Kazachenok A, Zhavoronkov A. The role of D-GADD45 in oxidative, thermal and genotoxic stress resistance. *Cell Cycle* 2012; **11**(22): 4222-4241.

[8] Zhang W, Hoffman B, Liebermann DA. Ectopic expression of MyD118/Gadd45/CR6 (Gadd45beta/alpha/gamma) sensitizes neoplastic cells to genotoxic stress-induced apoptosis. *Int J Oncol* 2001; **18**(4): 749-757.

[9] Tamura RE, de Vasconcellos JF, Sarkar D, Libermann TA, Fisher PB, Zerbini LF. GADD45 proteins: central players in tumorigenesis. *Curr Mol Med* 2012; **12**(5): 634-651.

[10]Tront JS, Willis A, Huang Y, Hoffman B, Liebermann DA. Gadd45 α levels in human breast cancer are hormone receptor dependent. *J Transl Med* 2013; **24**(11): 131.

[11]Xiong Y, Liebermann DA, Holtzman EJ, Jeronis S, Hoffman B, Geifman-Holtzman O. Preeclampsia-associated stresses activate Gadd45 α signaling and sFlt-1 in placental explants. *J Cell Physiol* 2013; **228**(2): 362-370.

[12]Eleuterio NM, Palei AC, Rangel Machado JS, Tanus-Santos JE, Cavalli RC, Sandrim VC. Positive correlations between circulating adiponectin and MMP2 in preeclampsia pregnant. *Pregnancy Hypertens* 2015; **5**(2): 205-208.

[13]Deng CL, Ling ST, Liu XQ, Zhao YJ, Lv YF. Decreased expression of matrix metalloproteinase-1 in the maternal umbilical serum, trophoblasts and decidua leads to preeclampsia. *Exp Ther Med* 2015; **9**(3): 992-998.

[14]Rasstrigina IM, Milovanov AP, Fokina TV, Kadyrov M. The intensity of expression of matrix metalloproteinases type 2 and type 9 by invasive trophoblast cells in uncomplicated pregnancy and preeclampsia. *Arkh Patol* 2014, **76**(3): 24-29.

[15]Lockwood CJ, Basar M, Kayisli UA, Guzeloglu-Kayisli O, Murk W, Wang J, et al. Interferon- ɣ protects first-trimester decidual cells against aberrant matrix metalloproteinases 1, 3, and 9 expression in preeclampsia. *Am J Pathol* 2014; **184**(9): 2549-2559.