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Trophoblast calcyclin is elevated in placental tissue from patients with early pre-eclampsia

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ABSTRACT

The aetiology of pre-eclampsia is thought to originate from aberrant spiral artery remodelling and invasion evoking cellular oxidative stress. Previously, we discovered differentially expressed proteins in trophoblast cells of pre-eclamptic pregnancies. One of these proteins is calcyclin (S100A6); a Ca^{2+}-binding protein associated with cellular stress response.

By immunohistochemistry on formalin-fixed paraffin-embedded placental tissue, calcyclin expression was compared between women with early pre-eclampsia ($n = 72$) and non-hypertensive control patients ($n = 66$) (χ^2, $p = 0.006$) blindly by two observers.

Significantly more intense staining was seen in trophoblast cells of pre-eclamptic pregnancies compared to control placentas suggesting that trophoblast calcyclin is elevated in early pregnancy.

Introduction

The aetiology of pre-eclampsia is currently unknown. The pathology is thought to originate from aberrant spiral artery remodelling [1]. The uterine spiral arteries undergo remodelling through endovascular cytotrophoblast invasion, which change from narrow, high resistance vessels into wide, low resistance vessels [1]. However in pre-eclampsia these changes occur partially or not at all, causing insufficient low-pressure spiral arteries and therefore disturbed placental perfusion which results in oxidative stress, endoplasmic reticulum stress and endothelial dysfunction [1].

We discovered previously differentially expressed proteins in trophoblast cells of pre-eclamptic pregnancies compared to trophoblast cells of healthy placental control tissue [2–4]. One of these proteins is calcyclin (S100A6); a Ca^{2+}-binding protein belonging to the S100 family associated with cellular stress response, in which calcyclin is upregulated [5,6]. In this study, we confirm by immunohistochemistry that trophoblast calcyclin is related to pre-eclampsia and discuss its role within the aetiology and its possible role as a marker for pre-eclampsia.

Methods

Formalin-fixed paraffin-embedded (FFPE) placental tissues were collected from the pathology archives at the Erasmus MC. Pre-eclampsia was defined as new onset hypertension ($\geqslant 140/\geqslant 90$ mmHg) and proteinuria ($\geqslant 0.3$ g/24 h) at or after 20 weeks of gestation. Eligible

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Table 1
Comparison of clinical characteristics of patients and control population.

		Mean (±SD)	Statistical value	dF	*p*-Value
Age	P (n = 72)	31.68 (4.964)	t = −1.496	121.281	0.137
	C (n = 66)	30.20 (6.505)			
GA	P (n = 72)	29.351 (2.371)	t = −3.632	136	0.000
	C (n = 66)	27.845 (2.499)			
Gravid	P (n = 72)	2.15 (1.307)	t = 1.509	110.384	0.134
	C (n = 66)	2.59 (2.000)			
Parity	P (n = 72)	0.61 (0.943)	t = 1.760	136	0.081
	C (n = 66)	0.94 (1.239)			
Birth weight	P (n = 72)	1075.25 (333.327)	t = 1.580	122.651	0.117
	C (n = 66)	1179.08 (428.050)			
SBP	P (n = 72)	162.69 (20.169)	t = −16.801	118.382	0.000
	C (n = 66)	114.75 (12.519)			
DBP	P (n = 72)	101.24 (11.759)	t = −18.857	130.638	0.000
	C (n = 66)	67.34 (9.138)			

(P = patients, C = controls, t = independent samples test, SD = standard deviation, dF = degrees of freedom, p-value = significance, GA = gestational age, SDB = systolic blood pressure, DBP = diastolic blood pressure) Birth weight in grams, age in years, GA in weeks, SBP and DBP in mmHg.

Table 2
Non-parametric test immunohistochemistry.

		−−	−	+/−	+	++	χ^2 value	dF	*p*-Value
Median									
Trophoblasts	P (n = 72)	0	4	14	38	16	14.292	4	0.006
	C (n = 66)	2	8	17	37	2			
Stroma	P (n = 72)	0	27	34	11	0	7.038	3	0.071
	C (n = 66)	0	13	39	12	2			
Observer 1									
Trophoblasts	P (n = 72)	2	2	18	27	23	12.599	4	0.013
	C (n = 66)	3	9	14	32	8			
Stroma	P (n = 72)	9	26	25	12	0	6.339	4	0.175
	C (n = 66)	4	18	28	13	3			
Observer 2									
Trophoblasts	P (n = 72)	0	6	36	26	4	25.728	4	0.000
	C (n = 66)	3	16	42	4	1			
Stroma	P (n = 72)	0	36	36	0	0	5.591	2	0.061
	C (n = 66)	0	22	42	2	0			

(P = patients, C = controls, χ^2 value = non-parametric test, dF = degrees of freedom, p-value = significance).

patients (n = 76) were defined as women who delivered between 20 and 34 weeks of gestation with a clinical diagnosis of pre-eclampsia. The control group (n = 75) was defined as women who delivered between 20 and 34 weeks without pre-eclampsia or any other hypertensive pregnancy disorder (Table 1). Only singleton pregnancies were included.

The FFPE tissues were cut in 4-μm sections, mounted on glass slides and were automatically incubated with anti-calcyclin mouse antibody (Sigma S5049, 1:25, Sigma–Aldrich, MO, USA) using the Ventana Bench Mark Ultra (Ventana, AZ, USA) and its amplification step according to the manufacturer's instructions (Ventana). Slides were counterstained with haematoxylin eosin.

Per patient 2 slides of mid-placental tissue were analysed. The entire slides were blindly evaluated by two observers and grouped in 5 subcategories (++/+/+−/−/−−, Table 2) at 100× and 400× magnification under the auspices of a pathologist. Slides exhibiting extreme heterogeneity in staining, indicating poor fixation by formalin were excluded. Results were averaged and in case of a difference no greater than 1 subcategory the highest given staining score was used.

Data were analysed using SPSS18. The immunohistochemistry data were analysed with a chi-square test (2-tailed). General characteristics of the study population tests were compared using a two-tailed independent t-test. Interobserver variation with paired data was determined through a Sign test. Results were considered statistically significant if $p < 0.05$.

Results

Calcyclin staining intensity (Fig. 1) in trophoblasts was significantly higher in pre-eclamptic patients than in controls (χ^2 = 14.292, p = 0.006). No significant difference was found in stromal cells (χ^2 = 7.038, p = 0.071). When results were analysed per reviewer independently,