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JunB/Cyclin-D1 imbalance in placental mesenchymal stromal cells derived from preeclamptic pregnancies with fetal-placental compromise

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ABSTRACT

Introduction: In the present study, we characterized the expression of Activating Protein 1 (AP-1) factors, key cell cycle regulators, in primary placental mesenchymal stromal cells (PDMSCs) derived from normal and preeclamptic (PE) pregnancies with fetal-placental compromise.
Methods: PDMSCs were isolated from control (n = 20) and preeclamptic (n = 24) placentae. AP-1 expression was determined by semi-quantitative RT-PCR (sqRT-PCR), Real Time PCR and Western Blot assay. PDMSCs were plated and JunB siRNA was performed. JunB and Cyclin-D1 expression were assessed by Real Time and Western Blot analyses.
Results: JunB expression was significantly increased while Cyclin-D1 expression was significantly down-regulated in PE relative to control PDMSCs. JunB siRNA was accompanied by JunB down-regulation and increased Cyclin-D1 in normal PDMSCs.
Conclusions: We described, for the first time, AP-1 expression in PDMSCs derived from physiological and PE placentae. Importantly, we demonstrated that JunB over-expression in PE-PDMSCs affects Cyclin-D1 regulation. Our data suggest a possible contribution of these pathological placental cells to the altered cell cycle regulation typical of preeclamptic placentae.

1. Introduction

Preeclampsia (PE) is a severe pregnancy-related syndrome affecting about 5–8% of all women worldwide, and is thus a leading cause of fetal–maternal mortality and morbidity [1] Preeclamptic pregnancies are characterized by abnormal placenta development with immature hyper-proliferative trophoblast phenotype and shallow invasion of maternal spiral arteries [2–4]. These defects entail placental hypoperfusion that, in the most severe cases, it is associated to fetal growth restriction (FGR) [5,6].

During the last years, the investigation of the complex PE pathogenesis has been mainly focused on the trophoblast, considered the main site of those placental aberrations responsible for PE onset. Nevertheless, we recently demonstrated the central role of placental derived mesenchymal stromal cells (PDMSC) in placental physiopathology. We reported aberrant release of pro-inflammatory cytokines by PDMSCs derived from preeclamptic placental villi (PE-PDMSCS) and their ability to induce a PE-like phenotype in term physiological villous explants [7]. Moreover, mesenchymal stromal cells are the most abundant placental cellular component and they represent the structural support for the forming primary villi during placental development, and they may drive capillary network establishment [8,9]. We described a slow PE-PDMSCs proliferation rate accompanied by increased senescence [7], thus indicating the contribution of PE-PDMSCs to the aberrant villous architecture typical of PE. Indeed, PDMSCs could directly cause or contribute to the placental anomalies typical of PE and FGR.

Activating Protein-1 (AP-1) early response proto-oncogenes are key cell cycle modulators pivotal for appropriate placentation. The inducible AP-1 transcription factors are dimeric complexes that contains members of the Jun and Fos protein families. While Fos proteins (c-Fos, FosB, Fra1, Fra2) hetero-dimerize only with members of the Jun family, Jun proteins (c-Jun, JunB, JunD) both homo- and heterodimerize with Fos members to form transcriptionally active complexes. Our group recently demonstrated an aberrant AP-1 expression pattern in the PE trophoblast [10].

Abbreviations: PE, preeclampsia; FGR, fetal growth restriction; PDMSCs, placenta-derived mesenchymal stromal cells; AP-1, activating protein 1.

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In the present study, we investigated the expression of AP-1 family members in PDMSCs derived from normal and PE placentae with fetal-placental compromise in order to determine whether AP-1 dysregulation could also be present in pathological placental mesenchymal cells as we previously demonstrated for trophoblast [10].

2. Materials and methods

2.1. Ethics statement

The study was approved by the Institutional Ethical Committee of O.I.R.M. S.Anna Hospital and "Ordine Mauriziano di Torino" (n.209; protocol 39226/C.27.1 04/08/09) (Turin, Italy). All patients provided written informed consent for samples collection and subsequent analysis.

2.2. Patients

The study population included 24 singleton pregnancies complicated by severe preeclampsia fetal-placental compromise and 20 physiological term control pregnancies (Table 1). PE diagnosis was made according to the following criteria [1]: hypertension (systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg) and proteinuria (>300 mg/24 h) after twenty week of gestational age in previously normotensive women. FGR was defined as birth weight below the fifth centile according to the Italian growth curves normalized for gestational age and sex [11,12] accompanied by pathological umbilical artery Doppler waveforms (absent or reverse end diastolic flow – A/REDF) and increased resistance to flow in maternal uterine arteries (early diastolic notch or pulsatility index – PI – more than 0.58). Control patients were healthy women with singleton term physiological normotensive pregnancies and no signs of preeclampsia or FGR. Exclusion criteria were: congenital malformation, chromosomal abnormalities (in number and/or structure), maternal and/or intrauterine infections, cardiovascular diseases and metabolic syndrome.

2.3. Placenta-derived mesenchymal stromal cells (PDMSCs) isolation and characterization

PDMSCs were isolated by enzymatic digestion and gradient as previously described [7]. Briefly, the decidua was peeled off from the basal plate and removed in order to avoid maternal cell contamination. Next, membranes were removed and 30 g of villous tissue were sampled from the chorionic plate. Placental tissue was washed several times with Hank's Buffered Salt Solution (HBSS, Gibco, Life Technologies, Italy) in order to remove the excess of blood and next it was mechanically minced and digested for 3 h with 100 U/ml collagenase type I (Gibco, Life Technologies, Italy) plus 5 μg/ml DNAse I (Gibco, Life Technologies, Italy). Finally, cells were separated by gradient using 1.073 Ficoll Paque Premium (GE Healthcare Europe, Italy). The mononuclear cells ring was collected, washed and PDMSCs were resuspended in Dulbecco's modified Minimum Essential Medium (DMEM, Gibo, Life Technologies, Italy) supplemented with 10% Fetal Bovine Serum (FBS Australian origin, Italy) and maintained at 37 °C and 5% CO2. After passage five, physiological and PE PDMSCs were characterized by flow cytometry for the expression of the following antigens: HLA-I, HLA-DR, CD105, C166, CD90, CD34, CD73, CD133, CD20, CD326, CD31, CD45 and CD14 (Miltenyi Biotech, Italy). Normal and PE-PDMSCs were analyzed by semi-quantitative PCR to assess gene expression levels of stem cell markers Oct-4 and Nanog. Primers were designed as previously described [7]. Moreover, at the same passage, control and PE-PDMSCs were plated in 6 well plates at a density of 1×10^5 cells/ml in DMEM LG without FBS. After 72 h of culture, cells were collected and processed for mRNA and protein isolation.

2.4. RNA isolation and Real Time PCR

Total RNA was isolated from physiological and PE-PDMSCs using TRIzol reagent (Life Technologies, Italy) according to manufacturer's instructions. Genomic DNA

Table 1
Clinical features of the study population.

	Controls ($n = 20$)	Preeclampsia ($n = 24$)	P value
Nulliparae (%)	30	41.6	n.s.
Gestational age at delivery (weeks)	39.5 ± 1.08 (37–41)	33.6 ± 3.30 (28–41)	$p < 0.01$
Maternal age at delivery (years)	33.1 ± 4.54 (24–41)	33.4 ± 5.63 (20–41)	n.s
Ethnicity (%)			
Caucasian	100	100	
Prenatal medications (%)			
Albumin	–	12.5	
Antibiotics	15	16.6	
Antidepressant	–	4.1	
Diuretics	–	12.5	
Eutirox	5	–	
Folin	10	4.1	
Heparin	–	4.1	
Iron	5	4.1	
Proton Pump Inhibitors (PPIs)	5	16.6	
Ventolin	5	4.1	
Smokers (%)	10	–	
Alcohol (%)	15	–	
Previous prenatal admission (%)	10	33.3	
Systolic Blood pressure (mm Hg)	114.7 ± 14.18	150 ± 19.41	<0.01
Diastolic Blood pressure (mm Hg)	73 ± 10	95 ± 12.18	<0.01
Proteinuria (g/24 h)	Absent	2.92 ± 4.57	<0.01
A/REDF (%)	0	58.3	<0.01
Pathological Uterine Doppler (%)	0	75	<0.01
Labor (%)	60	25	$p = 0.03$
Antibiotics in labor (%)	25	100	<0.01
Delivery to processing (range in hours)	0–3	0–3	
Caesarean section (%)	55	87.5	$p = 0.02$
Anesthesia (%)	80	91.6	n.s.
Local	37.5	–	$p = 0.01$
Spinal	62.5	86.36	
Epidural	–	9.1	
General	–	4.54	
Maternal oxygen given at delivery? (%)	–	25	n.s.
Birth weight (g)	3530.5 ± 379.54	AGA ($n = 8$):2490 ± 845.3	$p = 0.017$
		FGR ($n = 16$):1245 ± 473.9	<0.01
Placental weight (g)	598 ± 95.27	349.9 ± 123.1	<0.01
Fetal sex (%)			n.s.
Male	65	45.8	
Female	35	54.2	
Magnesium sulfate (%)	–	50	<0.01

Values are expressed as mean ± SD and percentage. *$p < 0.05$. A.G.A, appropriate for gestational age; FGR, fetal growth restriction; n.s. not significant.