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Different expression of placental pyruvate kinase in normal, preeclamptic and intrauterine growth restriction pregnancies

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ABSTRACT

Introduction: Preeclampsia (PE) and intrauterine growth restriction (IUGR) are two diseases that affect pregnant women and their unborn children. These diseases cause low birth weight, pre-term delivery, and neurological and cardiovascular disorders in babies. Combined they account for 20% of preterm deliveries. Pyruvate kinase M2 (PKM2) is a metabolism enzyme found in developing embryonic and cancer tissues. Our objective is to determine the expression of PKM2 in human PE and IUGR compared to normal pregnancies. Understanding expression of PKM2 in PE and IUGR could help us to better understand the mechanisms and find treatments for PE and IUGR.
Methods: Human placental tissues were obtained for PKM2 determination and analyzed by immunohistochemistry, Western blot, and a pyruvate assay. Placental samples were homogenized and cytoplasmic and nuclear proteins were extracted for Western blot analysis.
Results: Preeclampsia samples had elevated levels of p-PKM2, p-ERK, and ERK in the cytoplasm. Beta-catenin and lactose dehydrogenase (LDH) were also elevated in preeclampsia placenta samples.
Discussion and conclusion: We conclude that PKM2 is expressed in normal, PE and IUGR pregnancies. Also, that this expression is increased in the PE placenta at delivery. These results suggest placental metabolism through PKM2 could play a role in human preeclampsia.

1. Introduction

1.1. Preterm birth, intrauterine growth restriction, and preeclampsia

Preterm birth (PTB) is associated with up to 70% of neonatal deaths and leads to an increased incidence of cerebral palsy, neurological defects, and pulmonary disorders in the neonate [1]. Studies show an association with the development of intrauterine growth restriction (IUGR) and PTB, and a risk of up to 44% of PTB associated with IUGR [2,3]. IUGR defined as low birth weight (below the 10th percentile); affects up to 10% of all pregnancies and significantly increases risks of fetal and neonatal morbidity and mortality [4–6]. IUGR complications include perinatal hypoxia and asphyxia, cerebral palsy, and persistent pulmonary hypertension of the newborn [4–6]. Preeclampsia is defined as the excretion of protein (≥300 mg in 24 h) in the urine and high blood pressure after the 20th week, in pregnant women who are not typically hypertensive [7–9]. Preeclampsia accounts for about 20% of induced PTB [10]. The only “cure” for preeclampsia is to deliver the fetus and placenta, which typically results in preterm birth (PTB) [10]. Preeclampsia (PE) can also cause intrauterine fetal demise (IUFD), or death of the fetus before delivery [11]. In addition, several studies reported long-term consequences of PE and IUGR for babies; including adult hypertension, heart disease, stroke, and diabetes [12–18]. PE and IUGR placentas are characterized by a number of pathologic findings including: reduced syncytiotrophoblast surface area, decreased trophoblast invasion, and increased placental trophoblast apoptosis [19–25]. Studies have shown that aberrant trophoblast function has been associated with clinical obstetric pathologies including PE and IUGR [26]. Understanding PKM2 and the role it plays in the mechanisms of PTB, PE, and IUGR could help us to find treatments for affected mothers and babies to reduce complications and increase survivability.

Abbreviations: PE, preeclampsia; IUGR, intrauterine growth restriction; PKM2, pyruvate kinase M2; ERK, extracellular signal-regulated kinases; LDH, lactose dehydrogenase; IHC, immunohistochemistry.

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1.2. Pyruvate kinase M2 (PKM2) protein

Pyruvate kinase is a metabolic enzyme that exists in four isoforms: L, R, M1 and M2 [27–29]. PKM1 is primarily found in adult tissues, which require high levels of energy production [27,28,30]. PKM2 inhibits glycolysis, but interestingly promotes aerobic glycolysis, converting pyruvate to lactate for energy production. Inhibition of glycolysis by PKM2 allows for redistribution of glycolytic intermediates to support biosynthesis of macromolecules and cancer proliferation [30,31]. PKM2 is primarily found in cells with high levels of nucleotide synthesis such as proliferating cancer cells and developing embryonic tissues [27–30,32]. The role of PKM2 in cancer has been studied extensively since Otto Warburg's observations in the 1920's. Warburg noted cancer cells display an increased level of glucose uptake and lactate production, yet decreased overall ATP production even in the presence of sufficient oxygen [33]. This phenomenon has since been termed the Warburg effect [34,35].

Research is uncovering more about PKM2 in cancer metabolism, but we still know little about embryonic PKM2. Understanding expression of PKM2 and related proteins in placental tissues could help us to better understand placental metabolism during intrauterine growth restriction (IUGR) and preeclampsia (PE), reducing complications and mortality rates in neonates and pregnant mothers.

1.3. Placental metabolism

Placental metabolism is important for a successful pregnancy. The placenta is the primary site of nutrient and gas exchange between the mother and the fetus. Trophoblasts within the placenta use large amounts of energy to mediate nutrient transfer, synthesis of hormones, and secretion of molecules [36]. Altered nutrient supply to the fetus can affect its growth and development leading to obstetric complications such as IUGR and PE [37]. IUGR and PE are associated with abnormal carbohydrate metabolism and aberrant trophoblast differentiation [36,38–40]. Placenta metabolism, which uses almost 40% of the uteroplacental oxygen uptake, is correlated with oxygen availability [37]. Like many cancers, a common feature in IUGR and PE complicated pregnancies is increased hypoxia inducible factor (HIF), suggesting the presence of hypoxia in the placenta [41–47]. Previous reports show that different tissues can alter metabolic processes to meet their ATP demands, suggesting that these tissues could promote anaerobic glycolysis to maintain ATP synthesis [48]. Thus altered placental metabolism could play a role in the placental complications of IUGR and PE.

Based on PKM2's role in cancer and embryonic tissues, along with the hypoxic environment created by PE and IUGR, we wanted to determine the expression of PKM2 and related proteins in human placental samples [27–35,41–45,49–52]. This included looking at nuclear and cytosolic extracts of the placental samples, and identifying expression of proteins in control, PE, and IUGR placentas. This could help us to determine if PKM2 and related proteins play a role in these disease states, which could lead to future treatments of these diseases.

2. Material and methods

2.1. Human placental tissues

All frozen human term placental samples and slices (Preeclampsia, IUGR, and control) were purchased from the Research Center for Women's and Infant's Health BioBank, Ontario, Canada. In total there were 10 samples analyzed for each control, PE, and IUGR group. Samples were collected from placentas delivered in conjunction with delivery of the fetus either vaginally or by C-section. IUGR placentas were confirmed by ultrasound showing placental insufficiency with uterine Doppler and absent end diastolic flow (AEDV). They also had an estimated fetal weight below the 10th percentile. Sample demographics are shown in Table 1.

2.2. Immunohistochemistry

Immunohistochemistry was performed as previously done in our lab [53]. In summary, slides were de-waxed, washed in a 1× Tris buffer solution (TBS), and blocked with Background Sniper (Biocare Medical, #BS966H, Concord, CA) for 1 h. This was followed by incubation overnight with a primary antibody (PKM2-Cell Signaling Technology, Danvers, MA, rabbit #4053, Cytokeratin 7-Dako, Carpinteria, CA, mouse #M7018 (for trophoblast localization), Beta-Catenin-Cell Signaling Technology, Danvers, MA, rabbit #9562, Lactose Dehydrogenase-B-Santa Cruz Biotechnology, Dallas, TX, #sc-100775, IgG negative control-Biocare Medical, Concord, CA). Slides were then incubated for 1 h with Mach 2 Universal HRP Polymer Detection (Biocare Medical, #M2U522H, Concord, CA), followed by color development with diaminobenzidine (DAB) for 5 min (Biocare Medical, #DS900H, #BDB900C, Concord, CA). A 5 s Hematoxylin soak was used for nuclear counter-stain. Slides were washed 3× for 5 min in TBS between each step. Slides were mounted using Permount media.

2.3. Cytoplasmic and nuclear extraction

Approximately 100 mg of each placental sample, obtained from the Research Centre for Women's and Infant's Health BioBank, was ground with mortal and pestle in liquid nitrogen. Ground tissues were homogenized with a tissue grinder in a microcentrifuge tube with 500 μL of (ice cold) CER I, and 5 μL of protease inhibitor (Thermo Scientific, Rockford, IL, #78441). The rest of the protocol was followed as distributed with the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Scientific, Rockford, IL, #78835) using 27.5 μL of (ice cold) CER II and 125 μL of ice-cold NER with 1.25 μL of protease inhibitor (Thermo Scientific, Rockford, IL, #78441). Supernatants were collected into clean, chilled, prelabeled microcentrifuge tubes and kept on ice. To determine protein concentrations, cytoplasmic and nuclear supernatants were analyzed with a Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, #23225). Briefly, 2 μL of each sample was combined with 150 μL of Reagent A and B (50:1) in a labeled 96 well plate. The plate was covered and incubated at 37 °C for 20 min, cooled to RT, and read on a SpectraMax 340 PC absorbance microplate reader (Molecular Devices, Sunnyvale, CA). Samples were run in duplicate, averaged, and compared to a bovine serum albumin standard curve. Supernatants, in microcentrifuge tubes, were stored at −80 °C for future use. Cytosolic and nuclear extract purity was determined by Western blot analysis using lamin B and beta actin antibodies.

Table 1
Demographics of placental samples. Groups were analyzed for statistical significance ($p < 0.05$) with a Kruskal–Wallis test. There was no difference in maternal age. PE and IUGR groups were statistically lower from control in gestational age ($p = 0.0003$) and fetal weight ($p < 0.0001$).

	Control	PE	IUGR	*p* Values
Maternal age	34 ± 1.7	36 ± 2.2	30 ± 1.6	0.13
Gestational age (weeks)	38 ± 0.09	32 ± 1.3	31 ± 0.7	0.0003
Fetal weight (g)	3498 ± 59	1813 ± 389	972 ± 49.3	<0.0001
% C-section/vaginal	90%/10%			