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Mitochondrial translocation of human telomerase reverse transcriptase in cord blood mononuclear cells of newborns with gestational diabetes mellitus mothers☆

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ABSTRACT

Aims: To better understand the role of oxidative stress in fetal programming, we assessed the hypothesis that the mitochondrial translocation of human telomerase reverse transcriptase (hTERT) could protect neonatal mitochondrial DNA (mtDNA) from oxidative damage during pregnancies complicated by gestational diabetes mellitus (GDM).

Methods: 26 GDM mothers and 47 controls and their newborns were enrolled. The plasma levels of 8-isoprostaglandin $F_{2\alpha}$ in maternal and cord blood were measured to evaluate oxidative stress. Western blotting was then used to assess the mitochondrial localization of hTERT in cord blood mononuclear cells (CBMCs). Finally, the relative mtDNA content was analyzed by real-time PCR.

Results: GDM mothers and their newborns had significantly higher levels of oxidative stress than controls. hTERT was localized in both the nuclei and mitochondria of CBMCs, and the increased CBMC mitochondrial hTERT levels were significantly correlated with elevated oxidative stress in newborns. The neonatal mtDNA content in the GDM group was comparable to controls, and was positively correlated with mitochondrial hTERT levels in CBMCs, suggesting that mitochondrial hTERT in CBMCs may have a protective effect on neonatal mtDNA in GDM pregnancies.

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Conclusions: This study is the first to suggest that the mitochondrial translocation of hTERT in CBMCs under heightened oxidative stress might protect neonatal mtDNA from oxidative damage in GDM pregnancies. This could be an *in utero* adaptive response of a fetus that is suffering from elevated oxidative stress, and could help our understanding of the roles of oxidative stress in fetal programming.

1. Introduction

Gestational diabetes mellitus (GDM) is a common complication of pregnancy that affects 5–10% of pregnancies in Asian females [1,2]. GDM pregnancy significantly increases the risk of a number of short- and long-term adverse consequences for the fetus, the most significant of which is a predisposition to the development of metabolic syndrome and type 2 diabetes [3–5]. The fetus in a GDM pregnancy is exposed to sustained higher glucose concentrations until birth. To adapt to a GDM intrauterine environment, the pattern of fetal metabolism is altered, including changes in the fetal pancreas allowing the production of increased levels of insulin and epigenetic modifications [6,7]. However, when the environmental conditions improve rapidly after birth, the *in utero* protective effects of the fetal predictive adaptive responses may have adverse effects on the long-term health of the newborns [8,9]. Although the fetal adaptive programming of metabolic disease has been proposed for a long time, its underlying mechanisms remain elusive. Notably, elevated oxidative stress (OS) is considered to be a major contributor [10–15], since not only GDM females but also their newborns exhibit a heightened level of OS [16–19]. Furthermore, increased OS was also detected in children born during compromised pregnancies, and was potentially related to insulin resistance and obesity in later life [20,21]. OS was reported to alter epigenetic modification *via* oxidant molecules that act as signaling factors, as well as induce susceptible fetal pancreas dysfunction and subsequent insulin resistance in the offspring [10,15,22]. OS plays an important role in the fetal programming of metabolic disease, although the mechanisms behind these effects remain elusive. A better understanding of the actions of OS in the newborns of GDM mothers is important to identify new ways to reinforce the programming role of OS in metabolic disease.

The mitochondrion is a critical organelle that regulates OS, and plays important roles in fetal growth and development [23–25]. MtDNA is the intrinsic genome of mitochondria, and is responsible for encoding the majority of the respiratory chain enzymes. It is also considered to be an indicator of mitochondrial function [26]. Previous studies demonstrated that mtDNA was protected by mitochondrially localized telomerase reverse transcriptase (TERT) under increased OS. TERT is the catalytic subunit of the telomerase holoenzyme, which is a ribonucleoprotein responsible for the maintenance of telomeres to alleviate replicative senescence and genetic instability. In addition to the telomere-dependent functions of nuclear TERT, studies have suggested that TERT was also localized to the mitochondria [27–30]. Importantly, endogenous mitochondrial TERT is not an artifact of the overexpression of proteins associated with cellular transformation [30–32]. A mitochondrial targeting signal (MTS) in the N-terminal sequence of hTERT and two binding regions (ND1 and ND2) in mtDNA were found to play roles in the translocation of TERT from the nucleus to the mitochondria [27,30]. Of note, increased hTERT was exported to the mitochondria and exerted protective effects on mitochondrial function under increased OS [27–30]. In addition, mitochondrial hTERT allowed cancer cells to evade death and multidrug resistance by enhancing mitochondrial function and reducing levels of cellular ROS [33,34]. Collectively, experimental data *in vitro* and *in vivo* demonstrated that the increased mitochondrial translocation of hTERT induced by OS plays a putative role in the protection of mitochondrial function. Nevertheless, little information is available on newborns of GDM pregnancies.

In this study, we investigated the mitochondrial translocation of hTERT under increased OS in newborns of GDM pregnancies, and assessed its correlation with neonatal mtDNA content. This will help us to better understand the roles of GDM-associated OS in fetal programming.

2. Materials and methods

2.1. Recruitment strategy and subjects

Twenty-six pregnant females with GDM (study group) and forty-seven healthy pregnant females without GDM (control group) as well as their newborns were finally included in the study. Pregnant females who underwent *in vitro* fertilization or with multiple pregnancies, inherited metabolic diseases, pregestational diabetes, and chronic hypertension were excluded. GDM was confirmed using the World Health Organization's diagnostic criteria. Women who had any of the following were regarded as having GDM: a 1-h 50 g glucose challenge test (GCT) $\geq$7.8 mmol/L, fasting plasma glucose of $\geq$7.0 mmol/L, 1-h oral glucose tolerance test (75 g OGTT) of $\geq$10.0 mmol/L, or 2-h OGTT $\geq$8.5 mmol/L. All patients were recruited from the outpatient clinic during their third trimester in the Obstetrical Department of Southwest Second University Hospital affiliated with Sichuan University, China from 2009–2011. All the enrolled females also delivered in this hospital. The Regional and Institutional Committee of Science and Research Ethics of the West China Second University Hospital approved the study, and a written informed consent was obtained from all participants.

2.2. Baseline data and blood sample collection

Maternal data concerning age, parity, gravidity, height, prepregnancy weight, pregnancy weight gain, family history of