Experimental Cell Research 347 (2016) 201–211

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Research Article

Effects of oxidative stress on hyperglycaemia-induced brain malformations in a diabetes mouse model

Ya Jin [a,1], Guang Wang [b,1], Sha-Sha Han [a], Mei-Yao He [a], Xin Cheng [b], Zheng-Lai Ma [b], Xia Wu [a], Xuesong Yang [b,*], Guo-Sheng Liu [a,*]

[a] Department of Pediatrics, The First Affiliated Hospital, Jinan University, Guangzhou 510630, China
[b] Division of Histology & Embryology, Key Laboratory for Regenerative Medicine of the Ministry of Education, Medical College, Jinan University, Guangzhou 510632, China

ARTICLE INFO

Article history:
Received 28 February 2016
Received in revised form
25 June 2016
Accepted 3 August 2016
Available online 4 August 2016

Keywords:
PGDM
Neurodevelopmental defects
Oxidative stress
Nrf2 signalling

ABSTRACT

Pregestational diabetes mellitus (PGDM) enhances the risk of fetal neurodevelopmental defects. However, the mechanism of hyperglycaemia-induced neurodevelopmental defects is not fully understood. In this study, several typical neurodevelopmental defects were identified in the streptozotocin-induced diabetes mouse model. The neuron-specific class III beta-tubulin/forkhead box P1-labelled neuronal differentiation was suppressed and glial fibrillary acidic protein-labelled glial cell lineage differentiation was slightly promoted in pregestational diabetes mellitus (PGDM) mice. Various concentrations of glucose did not change the U87 cell viability, but glial cell line-derived neurotrophic factor expression was altered with varying glucose concentrations. Mouse maternal hyperglycaemia significantly increased $Tunel^{+}$ apoptosis but did not dramatically affect $PCNA^{+}$ cell proliferation in the process. To determine the cause of increased apoptosis, we determined the SOD activity, the expression of Nrf2 as well as its downstream anti-oxidative factors NQO1 and HO1, and found that all of them significantly increased in PGDM fetal brains compared with controls. However, Nrf2 expression in U87 cells was not significantly changed by different glucose concentrations. In mouse telencephalon, we observed the co-localization of Tuj-1 and Nrf2 expression in neurons, and down-regulating of Nrf2 in SH-SY5Y cells altered the viability of SH-SY5Y cells exposed to high glucose concentrations. Taken together, the data suggest that Nrf2-modulated antioxidant stress plays a crucial role in maternal hyperglycaemia-induced neurodevelopmental defects.

1. Introduction

The risk of one or more birth defects in the children of women with pregestational type 1 or type 2 diabetes mellitus was significantly higher than the children of pregnant women who never had diabetes or who developed GDM. [1]. In fact, these congenital malformations induced by hyperglycaemia predominantly manifest as congenital heart disease (3.4 times higher risk) and anomalies of the nervous system (2.7 times higher risk) [2,3]. Congenital anomalies are more common in the cardiovascular and nervous system in part because these compartments are formed at an early developmental stage, during which time the embryo and fetus are most vulnerable to harmful external factors. The precise mechanism underlying the teratogenicity associated with diabetes has not been identified. However, damage to the embryo and fetus during diabetic pregnancies may be related to alterations in fat and levels of reactive oxygen species (ROS) that have been shown to be altered in maternal pregestational diabetes [3]. In other words, maternal hyperglycaemia is considered to be the most important teratogen leading to congenital anomalies by generating excess ROS [4].

Oxidative stress influences a host of important reactions that either positively or negatively influence embryonic and fetal development. Oxidative stress results from an imbalance between ROS production and anti-oxidation. Moreover, accumulating experimental evidence indicates that excess ROS exerts a teratogenic effect on the developing embryo and fetus, including neurodevelopmental defects [5]. ROS are formed from oxidative phosphorylation and play a crucial role in controlling the redox reactions in various signalling pathways [6–8]. However, excessive ROS generation is often associated with pathological disorders because ROS act as primary and secondary messengers to promote cell

* Corresponding authors.
E-mail addresses: yang_xuesong@126.com (X. Yang), tlgs@jnu.edu.cn (G.-S. Liu).
[1] Contributed to this work equally.

http://dx.doi.org/10.1016/j.yexcr.2016.08.002
0014-4827/

growth and death [6–8]. In the body, excessive ROS accumulation interferes with normal cellular functions by damaging macromolecules such as proteins, lipids and DNA [9]. Deficiencies in glucose-6-phosphate dehydrogenase, an anti-oxidative stress enzyme, have been reported to increase DNA oxidation and fetal mortality [10]. Therefore, a delicate balance between ROS production and detoxification is essential for maintaining normal cellular functions [11,12]. Kelch-like ECH-associated protein 1 (Keap1) - Nuclear factor -like 2 (Nrf2) is an anti-oxidative pathway that plays a very important role on maintaining cellular redox homeostasis [13]. Nrf2 is a Cap'n'Collar basic leucine zipper transcription factor that protects cells from oxidative or electrophilic stress by regulating cellular redox homeostasis [14]. In the presence of oxidative stress, Nrf2 eventually activates transcription by specifically binding to the antioxidant response element (ARE) of its target gene promoters [15]. Certainly, the ARE-mediated expression of antioxidant enzymes is a vital mechanism against oxidative stress [16]. Heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase 1 (NQO1) have been identified as vital genes involved in regulating Nrf2 expression [16]. Keap1 is a principal repressor of Nrf2 via ubiquitination [17].

Neurulation is the morphogenetic process by which the neural tube forms in vertebrates. Neurulation is initiated by the proliferation and differentiation of neuroepithelial cells to form a neural plate. The bilateral sides of the neural plate elevate and form neural folds. These elevated folds eventually bend and fuse together at the dorsal midline to form a fluid-filled neural tube. This neural tube will finally develop into the CNS, which includes the brain and spinal cord. Rhombencephalon, mesencephalon, and prosencephalon are in the cranial region, and the spinal cord is in the trunk region. Proper neural tube formation requires a tight integration of several important cellular processes, including cell proliferation, apoptosis and differentiation Genetic mutations and external harmful factors that disrupt these morphogenetic processes during neurulation manifest as neural tube defects (NTDs) or various brain malformations in the neonate [18]. These NTDs are mainly associated with abnormal development or closure of the neural tube [19].

Using the pregestational diabetes mellitus (PGDM) mouse model induced by streptozotocin (STZ) and various cell lines, we investigated the cellular and molecular biological mechanisms underlying hyperglycaemia-associated brain malformations.

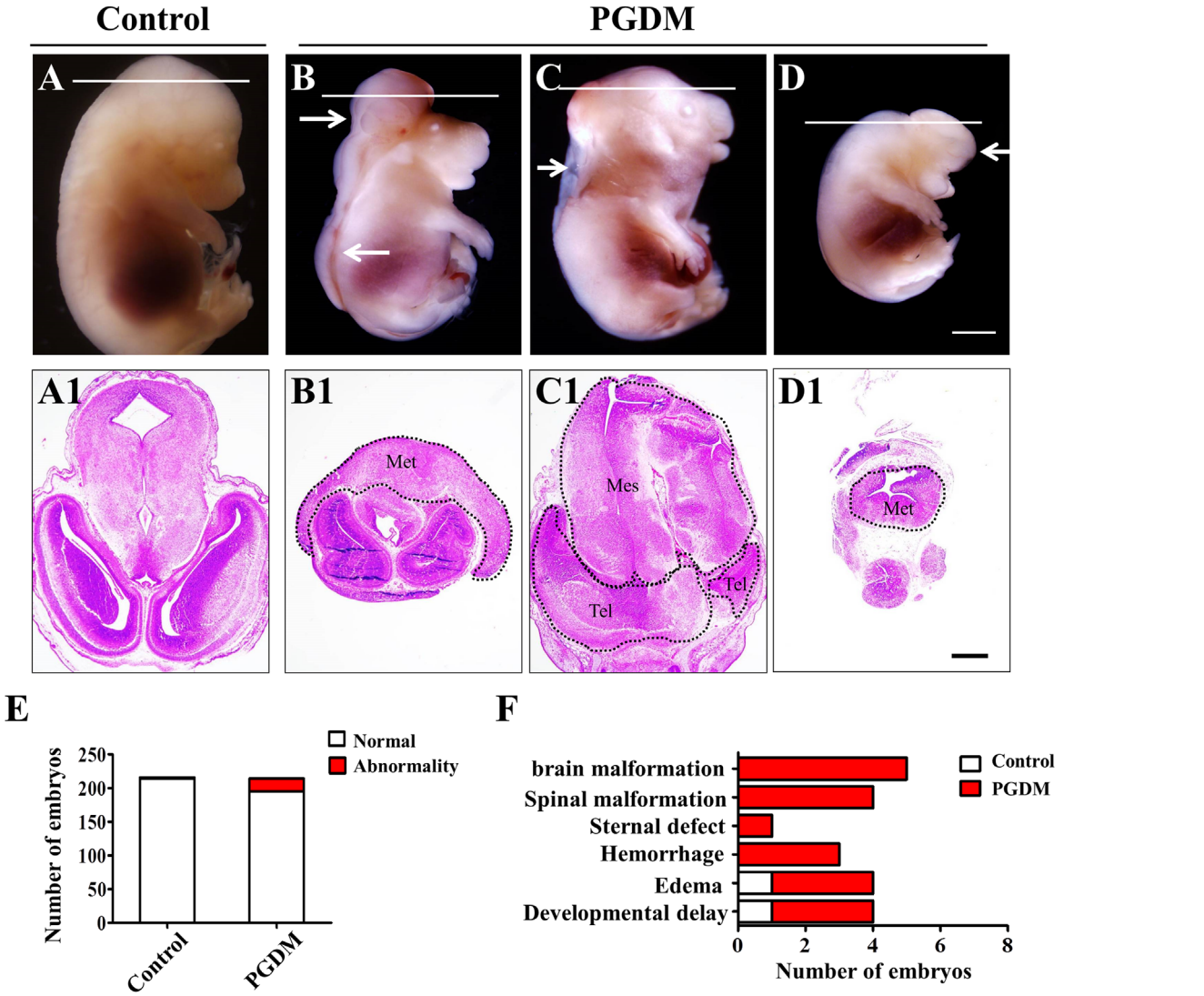

Fig. 1. *Malformations of mouse fetal brain occur in the fetuses of PGDM mice*. A–D: The representative E15.5 fetal mouse images from the control group (A) and the PGDM group (B-D), including exencephaly & spina bifida (B), oedema (C) and microencephaly (D). A1–D1: The transverse sections at the levels indicated by dotted lines in A–D. E: The bar charts showing the comparison of the number of fetal developmental anomalies between the control and PGDM groups (n=217). F: The bar charts showing the distribution of various fetal developmental anomalies between the control and PGDM groups. Abbreviation: Mes, mesencephalon; Met, metencephalon; PGDM, pregestational diabetes mellitus; Tel, telencephalon. Scale bars: 2 mm in A–B and 1 mm A1–D1.