OBSTETRICS

Advances in revealing the molecular targets downstream of oxidative stress—induced proapoptotic kinase signaling in diabetic embryopathy

Fang Wang, PhD; E. Albert Reece, MD, PhD, MBA; Peixin Yang, PhD

Preexisting maternal diabetes is a high-risk factor of diabetic embryopathy, such as neural tube defects and congenital heart defects. Maternal diabetes significantly increases the production of reactive oxygen species, resulting in oxidative stress and diabetic embryopathy. Multiple cellular and metabolic factors contribute to these processes. Forkhead box O (FoxO)-3a has been demonstrated as a key transcription factor in the signaling transduction pathways responsible for maternal diabetes-induced birth defects. Apoptosis signal-regulating kinase 1 (ASK1) activated by oxidative stress stimulates nuclear translocation of FoxO3a, resulting in the overexpression of tumor necrosis factor receptor 1-associated death domain protein, which, in turn, leads to caspase-8 activation and apoptosis. Maternal diabetes—activated c-Jun N-terminal kinase (JNK)-1/2, downstream effectors of ASK1, can be blocked by superoxide dismutase-1 overexpression, suggesting that oxidative stress is responsible for JNK1/2 signaling activation. Deletion of JNK1/2 significantly suppressed the activity of FoxO3a. These observations indicate that maternal diabetes—induced oxidative stress stimulates the activation of ASK1, JNK1/2, FoxO3a, tumor necrosis factor receptor 1-associated death domain protein, caspase-8 cleavage, and finally, apoptosis and diabetic embryopathy.

Key words: apoptosis, birth defects, kinase signaling, maternal diabetes, oxidative stress

Pregnancy with preexisting maternal diabetes significantly increases the risk of excess apoptosis occurs in target tissues of the developing embryos resulting in diabetes-induced birth defects, such as neural tube defects (NTDs) and congenital heart defects.[1-8] Each year, 10% of babies of diabetic mothers are born with a major congenital malformation.[9] Mechanistic studies demonstrate that maternal diabetes alters multiple cellular and metabolic factors contributing to diabetic embryopathy.[1,4,10-13] We propose that these cellular and metabolic aberrations occur through a single transcriptional mechanism, a transcription factor, and its responsive gene, leading to apoptosis in embryonic cells.

We have determined that the transcription factor, Forkhead box O (FoxO)-3a, is activated in diabetic embryopathy.[7] FoxO factors are functionally diversified in the induction of apoptosis-related pathogenesis.[7,14] The transcription factor FoxO3a is a key target of phosphatidylinositol-3 kinase (PI3K)/AKT pathway, in which AKT inactivates FoxO3a by phosphorylation.[15] Maternal diabetes activates FoxO3a by using several different manners: inhibited AKT function and the activated apoptosis signal-regulating kinase 1 (ASK1)—c-Jun N-terminal kinase (JNK)-1/2 pathway.[3,7]

The expression of tumor necrosis factor receptor 1—associated death domain protein (TRADD), an apoptotic gene, is up-regulated in diabetic embryopathy, and we propose that TRADD is a FoxO3a-responsive gene that initiates caspase-dependent apoptosis in diabetic embryopathy.[7] Maternal diabetes—induced embryonic cell apoptosis is caspase dependent.[1-3,7,16]

Previous work by our group and others has suggested that the proapoptotic JNK pathway, which is downstream of the ASK1 pathway, plays a causative role in the induction of diabetic embryopathy.[2,3] Activated ASK1 stimulates JNK1/2 activation[17,18] and subsequent mitochondrial dysfunction and cell apoptosis, resulting in diabetic embryopathy. Deletion of both the *JNK1* and *JNK2* gene could inhibit nuclear translocation of FoxO3a.[3] Thus, we propose a link between the JNK pathway and FoxO3a activation.

In this review, we will discuss the general function, possible clinical application, and cross talk relationship of molecules downstream of oxidative stress—induced kinase signaling in diabetic embryopathy.

From the Departments of Obstetrics, Gynecology, and Reproductive Sciences (all authors) and Biochemistry and Molecular Biology (Drs Reece and Yang), University of Maryland School of Medicine, Baltimore, MD.

Received Nov. 21, 2014; revised Dec. 20, 2014; accepted Jan. 8, 2015.

This study is supported by National Institutes of Health grants R01DK083243, R01DK101972, and R56 DK095380 (P.Y.), and grant R01DK103024 (P.Y and E.A.R).

The authors report no conflict of interest.

Corresponding author: Peixin Yang, PhD. pyang@fpi.umaryland.edu

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2015.01.016

Pathogenesis in the induction of diabetic embryopathy

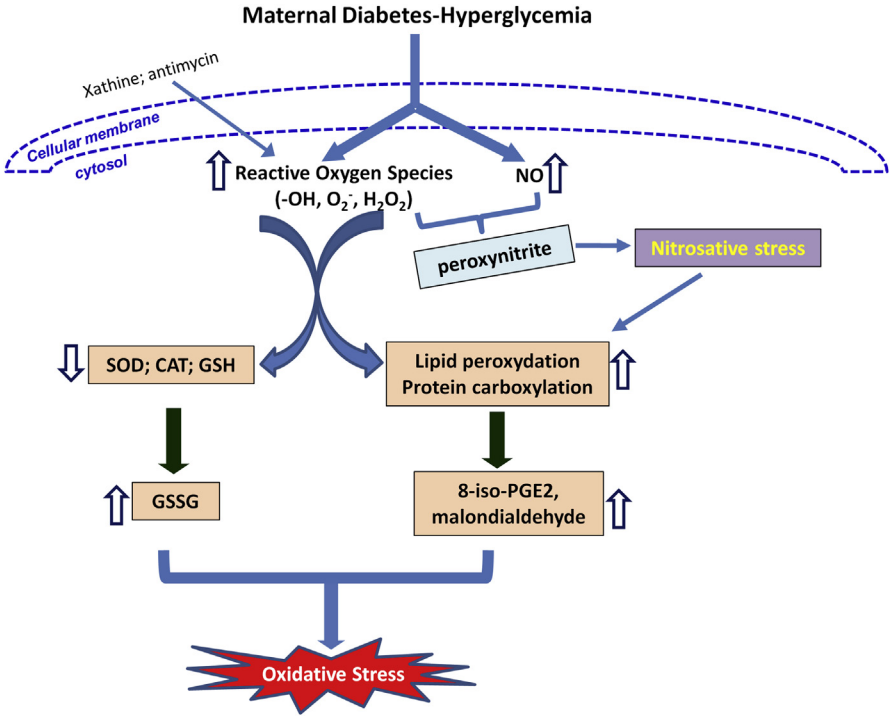
Hyperglycemia-induced oxidative stress

A variety of antioxidants have been shown to effectively suppress hyperglycemia-induced dysmorphogenesis both in vivo and in vitro.[1,4,12,14,19] Conversely, the induction of oxidative stress by the depletion of glutathione,[20,21] by exposure to xathine/xanthine, which directly generate reactive oxygen species (ROS),[22] or by treatment with antimycin,[23] a mitochondrial complex III inhibitor that stimulates superoxide production, significantly increase embryonic anomalies. Therefore, we hypothesize that oxidative stress is the primary cause of diabetic embryopathy because of the enhanced ROS production and weakening of the cellular antioxidant systems (Figure 1).

In maternal diabetes, increased levels of cellular glucose in embryonic tissues may enhance mitochondrial oxidative glucose metabolism and thus increase mitochondrial ROS production. Enhanced ROS production facilitates lipid peroxidation and protein carboxylation, contributing to overall oxidative stress in embryos under maternal diabetic conditions.[24,25] Markers of lipid peroxidation, 8-iso-prostaglandin E2[25-28] and malondialdehyde,[29] are dramatically elevated in embryos cultured in vitro under hyperglycemic conditions as well as in diabetic patients (Figure 1).

Cells possess a wide range of antioxidant systems to protect themselves from the toxic effects of excessive levels of ROS. Diabetic conditions profoundly influence cellular antioxidant potential. A significant decrease in the intracellular ROS scavenging enzyme activities of superoxide dismutase (SOD) and catalase (CAT) are seen when rat embryos and their yolk sacs are maintained under diabetic condition.[30] In addition, the levels of SOD and CAT mRNA decrease under maternal hyperglycemic conditions correlating inversely to an increase in embryonic anomalies.[31,32] The evidence cited in the previous text supports our assertion that cellular antioxidant defense systems are severely compromised in embryos and the yolk sac in response to maternal hyperglycemia, thereby contributing to cellular oxidative stress during the critical stages of organogenesis (Figure 1).

FIGURE 1
Hyperglycemia induces oxidative stress



Hyperglycemia produces oxidative stress, resulting in the induction of diabetic embryopathy. Maternal diabetes induces oxidative stress through enhanced ROS production and weakened cellular antioxidant systems. Enhanced ROS production stimulates lipid peroxidation and protein carboxylation, leading to overall oxidative stress in developing embryos under maternal diabetic conditions. Maternal diabetes elevates NO production in the embryos, which interacts with ROS to produce peroxynitrite, inducing nitrasative stress, finally resulting in diabetic embryopathy. The *downward arrow* indicates down-regulated; the *upward arrow* indicates up-regulated.

CAT, catalase; *GSH*, glutathione; *GSSG*, glutathione disulfide; *NO*, nitric oxide; *ROS*, reactive oxygen species; *SOD*, superoxide dismutase.

Wang. Elucidating molecular targets downstream of oxidative stress in diabetic embryopathy. Am J Obstet Gynecol 2015.

The role of nitric oxide

Nitric oxide (NO), a critical signaling molecule involving in many processes,[33] is produced from L-arginine by a family of 3 nitric oxide synthases. NO plays an important role in early embryonic development by regulating cell survival, apoptosis, and differentiation.[34-37] Because NO synthesis and function are critical during period of organogenesis, appropriate intracellular NO concentrations is a prerequisite for normal embryonic development and deregulated NO concentrations has been linked to abnormal embryonic outcomes. NO production that is elevated during early organogenesis in embryos from rat models of mild and severe diabetes leading to malformations.[38,39]

Elevated NO may directly interact with ROS generated under hyperglycemic conditions to form potent oxidant peroxynitrite leading to nitrosative stress[40,41] (Figure 1). The peroxynitrite anion inhibits mitochondrial electron transport, oxidizes important proteins, and initiates lipid peroxidation, thus affecting many signal transduction pathways.[42] The mechanism underlying maternal diabetes-increased NO