OBSTETRICS

Decoding the oxidative stress hypothesis in diabetic embryopathy through proapoptotic kinase signaling

Peixin Yang, PhD; E. Albert Reece, MBA, MD, PhD; Fang Wang, PhD; Rinat Gabbay-Benziv, MD

Maternal diabetes-induced birth defects occur in 6-10% of babies born to mothers with pregestational diabetes, representing a significant maternal-fetal health problem. Currently, these congenital malformations represent a significant maternal-fetal medicine issue, but are likely to create an even greater public health threat as 3 million women of reproductive age (19-44 years) have diabetes in the United States alone, and this number is expected to double by 2030. Neural tube defects (NTDs) and congenital heart defects are the most common types of birth defects associated with maternal diabetes. Animal studies have revealed that embryos under hyperglycemic conditions exhibit high levels of oxidative stress resulting from enhanced production of reactive oxygen species and impaired antioxidant capability. Oxidative stress activates a set of proapoptotic kinase signaling intermediates leading to abnormal cell death in the embryonic neural tube, which causes NTD formation. Work in animal models also has revealed that maternal diabetes triggers a series of signaling intermediates: protein kinase C (PKC) isoforms, PKCα, βII and δ; apoptosis signal-regulating kinase 1; c-Jun-N-terminal kinase (JNK)1/2; caspase; and apoptosis. Specifically, maternal diabetes in rodent models activates the proapoptotic unfolded protein response and endoplasmic reticulum (ER) stress. A reciprocal causation between JNK1/2 activation and ER stress exists in diabetic embryopathy. Molecular studies further demonstrate that deletion of the genes for *Prkc*, *Ask1*, *Jnk1*, or *Jnk2* abolishes maternal diabetes-induced neural progenitor apoptosis and ameliorates NTD formation. Similar preventive effects are also observed when apoptosis signal-regulating kinase 1, JNK1/2, or ER stress is inhibited. Cell membrane stabilizers and antioxidant supplements are also effective in prevention of diabetes-induced birth defects. Mechanistic studies have revealed important insights into our understanding the cause of diabetic embryopathy and have provided a basis for future interventions against birth defects or other pregnancy complications associated with maternal diabetes. The knowledge of a molecular pathway map identified in animal studies has created unique opportunities to identify molecular targets for therapeutic intervention.

Key words: apoptosis signal-regulating kinase 1, c-Jun-N-terminal kinase 1/2, diabetic embryopathy, endoplasmic reticulum stress, neural tube defects, oxidative stress hypothesis, proapoptotic kinase signaling, protein kinase C

Each year in the United States, about 150,000 babies—3% of all live births—are born with at least 1 major congenital malformation.[1,2] The prevalence of birth defects is significantly worse in offspring of women who have type 1 or 2 diabetes. In these cases, 6-10% of babies are born with a major congenital malformation.[3,4] Based on the National Health and Nutrition Examination Survey, conducted from 1988 through 1994, 1.1% of women 20-39 years of age have type 1 or 2 diabetes,[5] and the incidence of diabetes among women of childbearing age has increased over the past 4 decades.[3] It is projected that the number of women of childbearing age with type 2 diabetes will double by 2030,[3] suggesting that approximately 8000 babies will be born each year in the United States with a congenital malformation in pregestational type 1 or 2 diabetic pregnancies.

Observational studies in human beings have demonstrated a strong link between the extent of a mother's glycemic control and the incidence of congenital malformations in her offspring.[6-11] The putative teratogenic effects of hyperglycemia are supported by studies demonstrating that clinical intervention targeted at achieving euglycemia can reduce the incidence of diabetes-associated birth defects.[12] When euglycemia is successfully maintained periconceptionally and during the first trimester, the prevalence of malformations is reduced to a level comparable to that of the general population.[13-15] However, even with excellent compliance and clinical care, euglycemia may be difficult to achieve and maintain. In addition, it is possible that organogenesis can be affected by short periods of hyperglycemia that are not reflected in the averaged values of glycosylated hemoglobin levels used to monitor glucose levels. A further obstacle is that most women with diabetes do not seek preconceptional care and most have unplanned pregnancies.[16]

Hence, a very important public health goal is to develop and implement new and easily accessible intervention strategies to decrease the occurrence of

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

Received Oct. 1, 2014; revised Nov. 14, 2014; accepted Nov. 24, 2014.

Supported by National Institutes of Health R01DK083243, R01DK101972, and R56 DK095380 (Dr Yang); R01DK103024 (Drs Yang and Reece); by the Office of Dietary Supplements, National Institute of Health and the Basic Science Award (1-13-BS-220), American Diabetes Association (Dr Yang).

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.2014.11.036

diabetes-induced congenital anomalies. To achieve this goal, we need a thorough understanding of the biochemical and molecular mechanisms underlying diabetic embryopathy. Although we are still far from reaching this goal, one area where we have made progress is in our understanding of the link between maternal hyperglycemia and oxidative stress. The molecular pathways involved in the cellular response to stress are potential therapeutic targets to prevent diabetes-induced embryonic malformations.

Excess apoptosis is a causal event in the induction of malformations

Diabetes-associated malformations may involve ≥1 organs and frequently result in significant disability or death.[12,17] Adverse effects of maternal hyperglycemia have been documented in the yolk sac of diabetic animal models and in cultured murine embryos.[17-20] Studies with in vivo and in vitro models have determined that the stages of embryogenesis vulnerable to hyperglycemia-induced malformations comprise the critical period of organogenesis between 8.5-11.5 and 9-12 days of gestation in the mouse and rat, respectively, which is equivalent to gestational weeks 3-5 in human beings.[21,22]

Both clinical cases and animal studies have clearly demonstrated that the main characteristics of maternal hyperglycemia-associated defects are organ agenesis and underdevelopment.[17,23] The organ systems most commonly affected include the central nervous, cardiovascular, gastrointestinal, craniofacial, genitourinary, and skeletal systems.[1,23,24] Because the neural folds and the heart develop early during embryogenesis, a higher incidence of malformations is observed in these organs. In the central nervous system, abnormalities can be categorized as underdevelopment of the midbrain and hindbrain, and failure of the neural tube to close at both anterior (rostral) and posterior (caudal) ends of the neural axis.[12,17,23] The failure of posterior neural tube closure results in spina bifida, 1 of the common birth defects among offspring of diabetic mothers.[24,25]

Multiple studies have confirmed that excessive cell death, at least in the central nervous system, contributes to the abnormal development of structures in the embryos of diabetic animals.[17,26-29] These observations strongly suggest that high concentrations of glucose cause damage to the neural progenitor cells, leading to apoptosis and, ultimately, abnormal organogenesis. However, the mechanisms by which hyperglycemia triggers cell death in the embryonic cells are largely unknown.

Programmed cell death is a precisely controlled cellular event that can be triggered by extracellular signals or other stimuli under normal and pathological conditions.[30-33] In most cases, apoptosis is characterized by the condensation of chromatin, degradation (fragmentation) of DNA, and formation of apoptotic bodies.[31,34-36] The intracellular factors activated during apoptosis are the members of the Bcl-2 family,[36] notably Bax and Bim. When apoptosis initiates, Bax and Bim become activated.[37,38] Activated Bax moves to the mitochondrion to form a transmembrane channel with Bak, another Bcl-2 family member. Bim is phosphorylated and translocates to the mitochondria to help open the Bax/Bak channel, resulting in cytochrome C release into the cytosol.[39,40] Cytochrome C binds to apoptosis protease-activating factor-1, and the resulting complex activates caspase-9. Activated caspase-9 activates caspase-3, which then turns on caspase-activated DNase and other proapoptotic factors, leading to DNA fragmentation and cell death.[41,42]

Hyperglycemia-induced oxidative stress

Evidence from clinical and experimental studies demonstrates that diabetes-related hyperglycemia leads to sustained generation of reactive oxygen species (ROS) and depletion of antioxidants, resulting in intracellular oxidative stress from an imbalance in intracellular reduction-oxidation (redox) homeostasis.[43-48] Under normal physiological conditions, oxygen-free radicals, including hydroxyl radicals, superoxide anions, singlet oxygen, and hydrogen peroxide, are produced during cellular energy metabolism in mitochondria.[49-52] Physiologic levels of ROS mediate intracellular signal transduction, which, in turn, regulates a wide range of cellular functions, including proliferation, differentiation, and migration.[49-51] However, under pathological conditions, excess ROS can oxidize proteins, lipids, and DNA, causing cell injury and cell death[53] (Figure 1).

Intracellular redox homeostasis depends on the relative balance between ROS production and the thiol buffers, glutathione (GSH) and thioredoxin.[54] Normally, the intracellular environment is maintained in a highly reduced state, which is mediated by high levels of reduced GSH and thioredoxin.[54] However, ROS produced via various cellular activities converts GSH into oxidized GSH disulfide (Figure 1). If ROS production exceeds the cellular thiol-buffering capacity, the oxidizing agents build up in the cell, causing damage and promoting oxidative stress.[55]

In addition to the GSH antioxidant buffering system, cells also protect themselves by producing antioxidative enzymes that convert damaging radicals to nontoxic molecules[56-59] (Figure 1). Superoxide dismutases (SODs) convert a superoxide anion into hydrogen peroxide, which is then reduced to water by GSH peroxidase and catalase.[51,60] Two types of mammalian intracellular SODs, copper-zinc SOD (SOD1) and manganese SOD (SOD2), have been extensively studied[56,58]; SOD1 is localized in the cytoplasm, SOD2 in the mitochondrial matrix.[56,58] It has been shown that SOD1 not only controls the redox state in the cytoplasm, but also regulates mitochondrial homeostasis.[61,62] A study using transgenic mouse embryos overexpressing a human SOD1 transgene showed higher SOD activity and lower malformation rate in response to maternal diabetic conditions than wild-type embryos under the same conditions.[63] These studies strongly suggest that antioxidative enzymes play an important role in protecting embryos against hyperglycemia-augmented oxidative stress (Figure 1).