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Advances in revealing the molecular targets downstream of oxidative stress-induced pro-apoptotic kinase signaling in diabetic embryopathy

Fang Wang, PhD[1], **E. Albert Reece, MD, PhD and MBA**[1,2], and **Peixin Yang, PhD**[1,2]

[1]Department of Obstetrics, Gynecology & Reproductive Sciences, University of Maryland School of Medicine, Baltimore, MD 21201

[2]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201

Abstract

Pre-existing maternal diabetes is a high risk factor of diabetic embryopathy, such as neural tube defects (NTDs) and congenital heart defects (CHD). Maternal diabetes significantly increases the production of reactive oxygen species (ROS) resulting in oxidative stress and diabetic embryopathy. Multiple cellular and metabolic factors contribute to these processes. FoxO3a has been demonstrated as a key transcription factor in the signaling transduction pathways responsible for maternal diabetes-induced birth defects. ASK1 activated by oxidative stress stimulates nuclear translocation of FoxO3a, resulting in over-expression of TRADD, which, in turn, leads to caspase 8 activation and apoptosis. Maternal diabetes-activated JNK1/2, downstream effectors of ASK1, can be blocked by SOD1 overexpression, suggesting that oxidative stress is responsible for JNK1/2 signaling activation. Deletion of JNK1/2 significantly suppressed the activity of FoxO3a. These obersvations indicate that maternal diabetes-induced oxidative stress stimulates the activation of ASK1, JNK1/2, FoxO3a, TRADD, caspase 8 cleavage, finally, apoptosis and diabetic embryopathy.

Keywords

maternal diabetes; birth defects; oxidative stress; apoptosis; kinase signaling

Introduction

Pregnancy with pre-existing maternal diabetes significantly increases the risk of excess apoptosis occurs in target tissues of the developing embryos resulting in diabetes-induced

***Address Correspondence to and reprint request to*:** Peixin Yang, PhD, University of Maryland School of Medicine, Department of Obstetrics, Gynecology & Reproductive Sciences, BRB11-039, 655 W. Baltimore Street, Baltimore, MD 21201, pyang@fpi.umaryland.edu, Tel: 410-706-8402, Fax: 410-706-5747.

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birth defects, such as neural tube defects (NTDs) and congenital heart defects (CHDs) [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, FoxO3a, 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 PI3K/AKT pathway, in which AKT inactivates FoxO3a by phosphorylation [15]. Maternal diabetes activates FoxO3a by using several different manners: inhibited AKT function and activated ASK1-JNK1/2 pathway [3, 7].

The expression of TRADD, an apoptotic gene, is up-regulated in diabetic embryopathy and we propose that TRADD is a FoxO3a responsive gene which 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 pro-apoptotic JNK pathway, which is downstream of 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 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 crosstalk relationship of molecules downstream of oxidative stress-induced kinase signaling in diabetic embryopathy.

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, induction of oxidative stress by depletion of GSH [20, 21], by exposure to xathine/xanthine which directly generate 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 due to enhanced ROS production and weakening of the cellular antioxidant systems (Fig. 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-PGE2 [25-28] and malondialdehyde [29],

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are dramatically elevated in embryos cultured *in vitro* under hyperglycemic conditions as well as in diabetic patients (Fig. 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 above evidence 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 (Fig. 1).

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 three nitric oxide synthases (NOS). 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] (Fig. 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 production is not clear and need to be investigated further. Nitrosative stress resulting from elevated NO levels may be one of the mechanisms in the induction of diabetic embryopathy through the JNK pathway because nitrosative stress leads to JNK activation [43]. The role of JNK in diabetic embryopathy will be discussed later in this review.

Aberrant signaling pathways—The protein kinase C (PKC) family of serine/threonine protein kinases consists of 12 members, involved in a number of cellular activities, including proliferation, migration, apoptosis, differentiation, and secretion [44, 45] Each member plays its own unique role in cell physiology, although overlapping functions may exist for some isoenzymes. Dregulated PKC activity may be mechanistically involved in diabetic embryopathy.

The diacylglycerol (DAG)-PKC pathway has been implicated in diabetic embryopathy. Maternal hyperglycemia stimulates DAG production in embryonic cells, which, in turn stimulates PKC activity [46]. Some PKC isoforms (α, β2, and δ) are up-regulated, while others (ε and ξ) are down-regulated in diabetic embryopathy [47]. Inhibiting the activity of some PKC isoforms significantly decreases malformation rate [47] (Fig. 2).

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We have also found that activity of extracellular signal-regulated kinase 1/2 (ERK1/2) is down-regulated in diabetic embryopathy [48]. The activity of a pro-survival kinase, Akt, is reduced in diabetic embryopathy [49]. Akt is the key mediator in the phosphatidylinositol-3 kinase (PI3K) pathway, a central regulator of the mammalian target of rapamycin (mTOR). Downregulation of Akt by maternal diabetes results in the activation of FoxO3a and downstream TRADD and caspase 8 cleavage, contributing to diabetic embryopathy [7] (Fig. 2).

Our work has shown that ASK1-JNK1/2 signaling pathway plays important role in diabetic embryopathy [2, 3, 7, 16]. Under hyperglycemic condition, ASK1 is phosphorylated and activated through its dissociation from oxidized Trx [7, 50]. On one hand, ASK1 phosphorylation at Thr845 activates JNK1/2 kinase by phosphorylation, which initiates proapoptotic signaling pathways which play key roles in the diabetic embryopahthy [3, 7]; on the other hand, ASK1 phosphorylation initiates the UPR (unfolded protein response) and ER stress, which induces diabetic embryopathy by triggering beta-cell dysfunction and apoptosis [16, 51] (Fig. 2).

The crosstalk between the deregulated PKC and ASK1 signaling pathways with the JNK pathway seems to contribute to the induction of diabetic embryopathy.

Altered glucose metabolic pathways—Activity of the hexosamine biosynthetic pathway (HBP) is increased in embryos during diabetic pregnancy, which may contribute to hyperglycemia-induced oxidative stress [52]. Increased glycolytic flux can stimulate glucose flux through the HBP pathway, in which fructose-6-phosphate and glutamine are converted to glucosamine-6-phosphate and glutamate (Fig. 3). Experimental activation of the HBP pathway by glucosamine administration mimics the effects of maternal diabetes to inhibit the pentose shunt pathway, a main glucose metabolism pathway in early embryonic development, and to induce oxidative stress by inhibiting information of reduced glutathione [52] (Fig. 3).

We propose that all these hyperglycemia-triggered upstream events converge on the Foxo3a central transcription mechanism leading to hyperglycemia-induced apoptosis in the embryonic neural epithelium cells.

Maternal diabetes-induced apoptosis—primary mechanism of diabetic embryopathy

Compelling evidence demonstrates that maternal hyperglycemia increases apoptosis in the embryo [49, 53-55]. Apoptosis is specifically seen in neuroepithelial cells which are particularly susceptible to hyperglycemic damage [55]. Multiple studies have confirmed that excess cell death, at least in the central nervous system, contributes to the abnormal development of structures in the embryos of diabetic animals [53, 55-58]. Hyperglycemia-induced apoptosis involves altered regulation of Bcl-2 family members and caspase activation, critical events in the mitochondrial apoptotic pathway (Fig. 4). In addition, hyperglycemia-associated oxidative stress increases the Bax:Bcl-2 ratio which is associated with cytochrome c release and activation of caspase 3 in embryonic cells [59] (Fig. 4). An increase of Bax expression and release of cytochrome c and activated caspase 3 are characteristics of embryonic cells undergoing apoptosis under maternal hyperglycemic conditions [49, 55] (Fig. 4). These

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observations strongly suggest that high glucose concentrations cause damage to the neural progenitor cells, leading to excessive apoptosis and abnormal organogenesis. Moreover, our recent studies, in which we used antioxidants to neutralize oxidative stress, suggest a direct connection between hyperglycemia-induced oxidative stress and apoptosis [14].

Induction of apoptosis by the ASK1-MKK4-JNK signaling pathway

MAP kinase (MAPK) mediates a range of cellular processes, including apoptotic cell death [60]. MAPKs are members of a superfamily of serine/threonine kinases that are activated in response to a variety of extracellular stimuli, including ROS [61, 62]. Three distinct MAPK pathways regulate extracellular-signal-regulated-kinases (ERK1 and ERK2), c-Jun NH2-terminal kinases (JNK1, JNK2 and JNK3), and p38 MAPKs (p38α, p38β, p38γ and p38δ). These pathways control a variety of cellular functions, including gene expression, mitosis, and apoptosis through the phosphorylation of specific serine and/or threonine residues of target proteins.

The basic assembly of the MAPK signaling pathway is three-component module: via sequential activation of MAPK kinase kinase (MAP3K), MAPK kinase (MAPKK), and MAPK [63, 64]. MAP3K phosphorylates and activates MAPKK, and activated MAPKK hosphorylates and activates MAPK. Because the activation status of MAPKs is largely dependent on MAP3Ks, it is important to understand how MAP3Ks are regulated. Fourteen different MAP3K have been identified. Among them, several MAP3Ks, including ASK1, TAK1 and MLK3, are known to activate the JNK pathway in response to diverse stimuli [63-65]. Those MAP3Ks phosphorylate and activate the dual specific kinases, MKK4 (SEK1) and MKK7, which in turn, phosphorylate and activate JNKs [66].

Oxidative stress is one of the most potent activators of ASK1, which is essential for oxidative stress-induced apoptosis through the activation of MKK4/MKK7-JNK cascade [18]. Oxidative stress induces phosphorylation of Thr-845 in the activation loop of ASK1, correlating with enhanced ASK1 activity and increased apoptosis [65] (Fig. 5). ASK1-mediated apoptosis is involved in pathogenesis of several oxidative stress-related diseases such as brain ischemia [67], ischemic heart disease [68] and Alzheimer's disease [69].

Most recently, it has been shown that high glucose-activated ASK1 mediates hyperglycemia-induced endothelial cell senescence [70]. These findings are consistent with our hypothesis that ASK1 functions as a mediator of diabetes-related embryo malformation. Indeed, we found that deletion of the *Ask1* gene significantly reduces maternal diabetes-induced capase cleavage, neuroepithelial cell apoptosis and NTD formation [7]. This suggests that ASK1 plays an essential role in the induction of apoptosis leading to NTD formation (Fig. 5).

The JNK pathway specifically responds to stress-induced signals that drive apoptosis [71, 72]. JNK has three isoforms (JNK1, JNK2, and JNK3) encoded by three different genes. The *Jnk1* and *Jnk2* genes are ubiquitously expressed, whereas *Jnk3* is found to be neural tissue specific [66]. The specific molecular targets of JNK include transcription factor AP-1 (mainly c-Jun, JunB, and ATF-2), Foxo factors [73, 74], as well as many other non-transcription factors, such as Bcl-2 proteins, which are closely related to apoptotic cell death factors [75].

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Both exogenous and endogenous ROS increase JNK1/2 activity [76-80]. Substantial genetic and pharmacological evidence suggests that JNK serves as a key pro-apoptotic mediator during oxidative stress [18, 81-85]. Mice having null mutations in any single JNK gene develop normally [86], as do JNK1/JNK3 or JNK2/JNK3 double mutants.[85]. Although JNK1/JNK2 null mutants die *in utero* due to abnormal apoptosis in the brain [87]. These mice are useful models for delineating apoptotic pathways involving JNKs.

The ASK1-JNK pathway appears to play mutual causation role with ER stress signaling. Maternal diabetes induces ER stress and ASK1-JNK signaling pathway (Fig. 5). ASK1-JNK is a key component of the unfolded protein response signalosome, which leads to ER stress [3, 17, 88]. We also found deletion of *Ask1, Jnk1* or *Jnk2* gene could abolish maternal diabetes-induced ER stress and subsequent apoptosis in the neuroepithelial cell [3, 16].

JNK1/2 activation-- a critical role in diabetic embryopathy

MAPK activity is altered in diabetic patients and in cells cultured in high glucose, suggesting that MAPKs may be involved in hyperglycemia-induced complications [48, 89, 90]. Less, however, is known about the activity of MAPKs in embryos under maternal diabetic conditions. An increase in JNK1/2 activity is associated with increased apoptosis in the yolk sacs of malformed embryos from diabetic mothers [19, 48]. We have reported that supplementation with antioxidants reduces JNK1/2 activity and the embryonic malformation rate in embryos [14], suggesting that hyperglycemia-induced oxidative stress is responsible for JNK1/2 activation. These observations indicate that increased JNK1/2 activity may play an important role in diabetic embryopathy (Fig. 5). In addition, an increase in phosphorylated MKK4 coincides with JNK1/2 activation in diabetic embryopathy [8].

Treatment with a JNK1/2 inhibitor, SP600125, prevents hyperglycemia-induced embryopathy [8]. Additionally, maternal diabetes-induced embryonic anomalies are significantly reduced in the JNK2 null background. It is also revealing that sorbitol, a potent JNK1/2 activator [91], mimics the teratogenic effect of hyperglycemia. This evidence strongly suggests that JNK1/2 activation is crucial for diabetic embryopathy. The neural tube and yolk sac of the conceptus are extremely vulnerable to the negative effects of maternal hyperglycemia. We have demonstrated that hyperglycemia induces yolk sac vasculopathy and embryo malformation and that blockade of JNK1/2 activation reverses these effects. Thus, pharmacologic and genetic evidences strongly suggest that JNK1/2 activation mediates the deleterious effect of hyperglycemia on embryonic development and yolk sac vasculature.

FoxO factors induces apoptosis by transcriptionally up-regulating apoptotic genes

The FoxO subfamily of Forkhead transcription factors is composed of three functionally related members, FoxO1a, FoxO3a and FoxO4 [92]. FoxO proteins are evolutionarily conserved transcriptional activators of genes involved in apoptosis [93], cell cycle inhibition and DNA repair [94]. FoxO factors interact with a core consensus DNA sequence (5′-AAAA(C/T)AAA-3′) to modulate target gene expression [95-97]. FoxOs drive apoptotic responses in stress-exposed cells [98, 99] by regulating expression of apoptosis-relevant target genes including Bim [100], TRAIL [101], Fas ligand (FasL) [102] and TRADD [95].

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The transcriptional activity of FoxO factors is controlled by subcellular localization and phosphorylation [103]. Phosphorylation of FoxO factors at Thr-24 and Thr-32 by kinases prevents nuclear translocation thereby inhibiting FoxO-dependent transcription [104]. FoxO factors mediate JNK1/2 activity-induced apoptosis [73] (Fig. 5). Phosphorylation of FoxO proteins by JNK1/2 results in FoxoO nuclear accumulation and enhanced transcription [74] (Fig. 5). The 14-3-3 protein, an adaptor protein that interacts with proteins having modifications of phosphoserine or phosphothreonine, binds to FoxO to sequester it in the cytoplasm. JNK1/2 phosphorylates the 14-3-3 chaperone protein causing the release of FoxO and its translocation to the nucleus [105] (Fig. 5).

Emerging evidence suggest that FoxO factors play a tumor suppressor role in a variety of cancers [106]. FoxO3a suppresses prostate cancer progression in mice [107]. In vivo overexpression of FoxO3a increases the expression level of p27kip1 and inhibits cell proliferation in vascular smooth muscle cells [108]. FoxO3a promotes tumor cell death. FoxO3a activator STI571 treatment in leukemia cells and breast cancer cells inhibits tumor growth. FoxO3a inhibits T cell proliferation and induces T cell apoptosis, resulting in autoimmunity prevention [109]. Genetic variation within the FoxO3a gene was strongly associated with human longevity [110]. Based on its clinical function, FoxO3a could be as a molecular target for treatment of diabetic embryopathy. The pro-apoptotic effect of FoxO3a may be implicated in diabetic embryopathy.

Indeed, FOXO3a gene deletion reduces maternal diabetes-induced apoptosis and NTD formation [7]. In previous study, the NTD rate of FoxO3a$^{-/-}$ embryos from diabetic mice was 3.1%, significantly lower than that of wild-type embryos (25.6%) from diabetic dams. Moreover, FoxO3a deletion was associated with a reduction in the maternal diabetes-increased mRNA and protein abundance for TRADD, cleavage of caspase 3 and 8 and neuroepithelial cell apoptosis [7] (Fig. 5).

TRADD--a critical mediator of apoptosis in the TNFα pathway

TRADD is a key adaptor protein in the tumor necrosis factor (TNF) signaling cascade that mediates TNF-induced apoptosis [111, 112]. TRADD contains a death domain which is a homotypic protein interaction module that signals cell death [111]. TRADD interacts with FADD via corresponding death domains, and this interaction triggers caspase 8 activation [113]. FADD is an adapter protein that was originally isolated as a transducer of apoptotic signals for death domain-containing receptors [114]. In addition to a death domain, FADD contains a death effector domain. TRADD overexpression leads to apoptosis in a FADD dependent manner [111, 112]. Dominant-negative FADD (DN-FADD), which lacks amino acids 1-79, can inhibit TRADD-induced apoptosis in both in vivo and in vitro systems [112]. In vitro study demonstrates that TRADD can be used for Glioblastoma Multiforme tumors by using its dual function of directly inducing rapid apoptosis and sensitizing Glioblastoma Multiforme cells to standard anti-neoplastic therapy [115]. Based on its clinical function, the apoptosis inducer TRADD could be as aother molecular target for treatment of diabetic embryopathy.

Maternal diabetes increases the expression level of TRADD, and deletion of *foxo3a* gene ameliorates maternal diabetes-increased TRADD abundance, demonstrating that the

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TRADD gene is responsive to the FoxO3a transcription factor, which inhibits maternal diabetes-induced NTD formation [7] (Fig. 5). Furthermore, deletion of *Ask1* gene significantly abrogates maternal diabetes-induced TRADD expression. These results suggest that maternal diabetes-induced ASK1 activation is responsible for TRADD stimulation and subsequent apoptosis induction [7].

Clinical significance and future prospective

About 1.85 million American women of reproductive age (18-44 years) have diabetes, and the number is continuing to increase due to the obesity epidemic [116]. Unfortunately euglycemic control by insulin administration is difficult to achieve as even transient exposure to maternal hyperglycemia causes embryonic malformation. Thus, maternal diabetes-induced birth defects remain a huge health problem. Development of accessible, convenient and effective prevention strategies is an urgent task. To achieve this goal, understanding the mechanism of maternal diabetes-induced malformations is an essential and key step. Towards this goal, we propose studies that will define the mechanism of diabetic embryopathy at both the cellular and transcriptional levels.

We have used animal models to delineate the mechanism of maternal diabetes-induced malformations [1-5, 7, 8, 16] and specifically focused on the developing neural tube. We have previously demonstrated that apoptosis is implicated in diabetic embryopathy [2, 3, 5, 7, 16]. Our goal is to identify molecular intermediates of the apoptotic cascade responsible for diabetic embryopathy, with ultimately to provide a mechanistic basis for development of therapeutic agents.

Observation of excessive apoptosis in neural stem cells of malformed neural tubes provides the basis for the hypothesis that maternal hyperglycemia-induced apoptosis in neural tube cells results in NTDs [2, 3, 5, 7, 16]. However, the mechanism of maternal hyperglycemia-induced apoptosis in target tissues remains elusive. Thus, we have been seeking to systemically dissect the mechanism responsible for maternal diabetes-induced NTDs from the signal transduction levels to transcriptional mechanism. Furthermore, we have revealed which apoptotic control proteins mediate maternal diabetes-induced apoptosis based on animal models. By unraveling the apoptotic mechanisms leading to diabetic embryopathy, we will provide a mechanistic basis for the use of cutting-edge, mechanism-based therapeutic strategies designed to prevent diabetes-associated birth defects.

Our animal studies have demonstrated that SOD1 overexpression in transgenic mice suppresses maternal diabetes-induced pro-apoptotic kinase signaling, endoplasmic reticulum stress and nitrosative stress in the developing embryo[1, 4, 117]. Besides structural birth defects, oxidative stress is also involved in the etiology of other pregnancy complications[118]. Studies using human maternal blood samples have revealed several important oxidative stress markers for early diagnosis of adverse pregnancy outcomes[119-121]. The use of antioxidants in preventing human birth defects produces conflict results. Folic acid supplementation reduces the incidences of NTDs[122]; however, periconceptional use of vitamins or supplements that contain folic acid does not reduce the incidence of congenital

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heart defects in pregestational diabetic pregnancies[123, 124]. Our animal studies have revealed many candidates that may be effective in prevention of human structural birth defects.

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REFERENCES

1. Li X, Weng H, Reece EA, Yang P. SOD1 overexpression in vivo blocks hyperglycemia-induced specific PKC isoforms: substrate activation and consequent lipid peroxidation in diabetic embryopathy. American journal of obstetrics and gynecology. 2011; 205:84 e1–6. [PubMed: 21529760]
2. Li X, Weng H, Xu C, Reece EA, Yang P. Oxidative stress-induced JNK1/2 activation triggers proapoptotic signaling and apoptosis that leads to diabetic embryopathy. Diabetes. 2012; 61:2084–92. [PubMed: 22688338]
3. Li X, Xu C, Yang P. c-Jun NH2-terminal kinase 1/2 and endoplasmic reticulum stress as interdependent and reciprocal causation in diabetic embryopathy. Diabetes. 2013; 62:599–608. [PubMed: 22961085]
4. Wang F, Reece EA, Yang P. Superoxide dismutase 1 overexpression in mice abolishes maternal diabetes-induced endoplasmic reticulum stress in diabetic embryopathy. American journal of obstetrics and gynecology. 2013; 209:345 e1–7. [PubMed: 23791840]
5. Xu C, Li X, Wang F, Weng H, Yang P. Trehalose prevents neural tube defects by correcting maternal diabetes-suppressed autophagy and neurogenesis. American journal of physiology Endocrinology and metabolism. 2013; 305:E667–78. [PubMed: 23880312]
6. Yang P, Cao Y, Li H. Hyperglycemia induces inducible nitric oxide synthase gene expression and consequent nitrosative stress via c-Jun N-terminal kinase activation. American journal of obstetrics and gynecology. 2010; 203:185 e5–11. [PubMed: 20541731]
7. Yang P, Li X, Xu C, et al. Maternal hyperglycemia activates an ASK1-FoxO3acaspase 8 pathway that leads to embryonic neural tube defects. Science signaling. 2013; 6:ra74. [PubMed: 23982205]
8. Yang P, Zhao Z, Reece EA. Involvement of c-Jun N-terminal kinases activation in diabetic embryopathy. Biochemical and biophysical research communications. 2007; 357:749–54. [PubMed: 17449011]
9. Feig DS, Palda VA. Type 2 diabetes in pregnancy: a growing concern. Lancet. 2002; 359:1690–2. [PubMed: 12020549]
10. Hagay ZJ, Weiss Y, Zusman I, et al. Prevention of diabetes-associated embryopathy by overexpression of the free radical scavenger copper zinc superoxide dismutase in transgenic mouse embryos. American journal of obstetrics and gynecology. 1995; 173:1036–41. [PubMed: 7485290]
11. Reece EA, Wu YK. Prevention of diabetic embryopathy in offspring of diabetic rats with use of a cocktail of deficient substrates and an antioxidant. American journal of obstetrics and gynecology. 1997; 176:790–7. discussion 97-8. [PubMed: 9125602]
12. Yang P, Li H. Epigallocatechin-3-gallate ameliorates hyperglycemia-induced embryonic vasculopathy and malformation by inhibition of Foxo3a activation. American journal of obstetrics and gynecology. 2010; 203:75 e1–6. [PubMed: 20417490]
13. Yang P, Reece EA. Role of HIF-1alpha in maternal hyperglycemia-induced embryonic vasculopathy. American journal of obstetrics and gynecology. 2011; 204:332 e1–7. [PubMed: 21345401]

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14. Reece EA, Wu YK, Zhao Z, Dhanasekaran D. Dietary vitamin and lipid therapy rescues aberrant signaling and apoptosis and prevents hyperglycemia-induced diabetic embryopathy in rats. American journal of obstetrics and gynecology. 2006; 194:580–5. [PubMed: 16458664]
15. Santo EE, Stroeken P, Sluis PV, Koster J, Versteeg R, Westerhout EM. FOXO3a is a major target of inactivation by PI3K/AKT signaling in aggressive neuroblastoma. Cancer research. 2013; 73:2189–98. [PubMed: 23378341]
16. Wang F, Wu Y, Gu H, et al. Ask1 gene deletion blocks maternal diabetes-induced endoplasmic reticulum stress in the developing embryo by disrupting the unfolded protein response signalosome. Diabetes. 2014
17. Nishitoh H, Saitoh M, Mochida Y, et al. ASK1 is essential for JNK/SAPK activation by TRAF2. Molecular cell. 1998; 2:389–95. [PubMed: 9774977]
18. Tobiume K, Matsuzawa A, Takahashi T, et al. ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO reports. 2001; 2:222–8. [PubMed: 11266364]
19. Reece EA, Wu YK, Wiznitzer A, et al. Dietary polyunsaturated fatty acid prevents malformations in offspring of diabetic rats. American journal of obstetrics and gynecology. 1996; 175:818–23. [PubMed: 8885728]
20. Sakamaki H, Akazawa S, Ishibashi M, et al. Significance of glutathione-dependent antioxidant system in diabetes-induced embryonic malformations. Diabetes. 1999; 48:1138–44. [PubMed: 10331421]
21. Trocino RA, Akazawa S, Ishibashi M, et al. Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. Diabetes. 1995; 44:992–8. [PubMed: 7622006]
22. Jenkinson PC, Anderson D, Gangolli SD. Malformations induced in cultured rat embryos by enzymically generated active oxygen species. Teratogenesis, carcinogenesis, and mutagenesis. 1986; 6:547–54.
23. Chang TI, Horal M, Jain SK, Wang F, Patel R, Loeken MR. Oxidant regulation of gene expression and neural tube development: Insights gained from diabetic pregnancy on molecular causes of neural tube defects. Diabetologia. 2003; 46:538–45. [PubMed: 12739027]
24. Eriksson UJ, Wentzel P, Minhas HS, Thornalley PJ. Teratogenicity of 3-deoxyglucosone and diabetic embryopathy. Diabetes. 1998; 47:1960–6. [PubMed: 9836531]
25. Wentzel P, Welsh N, Eriksson UJ. Developmental damage, increased lipid peroxidation, diminished cyclooxygenase-2 gene expression, and lowered prostaglandin E2 levels in rat embryos exposed to a diabetic environment. Diabetes. 1999; 48:813–20. [PubMed: 10102698]
26. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87:9383–7. [PubMed: 2123555]
27. Gopaul NK, Anggard EE, Mallet AI, Betteridge DJ, Wolff SP, Nourooz-Zadeh J. Plasma 8-epi-PGF2 alpha levels are elevated in individuals with non-insulin dependent diabetes mellitus. FEBS letters. 1995; 368:225–9. [PubMed: 7628610]
28. Decsi T, Minda H, Hermann R, et al. Polyunsaturated fatty acids in plasma and erythrocyte membrane lipids of diabetic children. Prostaglandins, leukotrienes, and essential fatty acids. 2002; 67:203–10.
29. Li R, Chase M, Jung SK, Smith PJ, Loeken MR. Hypoxic stress in diabetic pregnancy contributes to impaired embryo gene expression and defective development by inducing oxidative stress. American journal of physiology Endocrinology and metabolism. 2005; 289:E591–9. [PubMed: 15928021]
30. Ornoy A, Zaken V, Kohen R. Role of reactive oxygen species (ROS) in the diabetes-induced anomalies in rat embryos in vitro: reduction in antioxidant enzymes and low-molecular-weight antioxidants (LMWA) may be the causative factor for increased anomalies. Teratology. 1999; 60:376–86. [PubMed: 10590399]
31. Zaken V, Kohen R, Ornoy A. Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. Teratology. 2001; 64:33–44. [PubMed: 11410909]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

32. Cederberg J, Eriksson UJ. Decreased catalase activity in malformation-prone embryos of diabetic rats. Teratology. 1997; 56:350–7. [PubMed: 9485544]
33. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological reviews. 1991; 43:109–42. [PubMed: 1852778]
34. Lee QP, Juchau MR. Dysmorphogenic effects of nitric oxide (NO) and NO-synthase inhibition: studies with intra-amniotic injections of sodium nitroprusside and NG-monomethyl-L-arginine. Teratology. 1994; 49:452–64. [PubMed: 7538231]
35. Plachta N, Traister A, Weil M. Nitric oxide is involved in establishing the balance between cell cycle progression and cell death in the developing neural tube. Experimental cell research. 2003; 288:354–62. [PubMed: 12915126]
36. Feng Q, Song W, Lu X, et al. Development of heart failure and congenital septal defects in mice lacking endothelial nitric oxide synthase. Circulation. 2002; 106:873–9. [PubMed: 12176963]
37. Topel I, Stanarius A, Wolf G. Distribution of the endothelial constitutive nitric oxide synthase in the developing rat brain: an immunohistochemical study. Brain research. 1998; 788:43–8. [PubMed: 9554947]
38. Jawerbaum A, Gonzalez ET, Novaro V, Faletti A, Sinner D, Gimeno MA. Increased prostaglandin E generation and enhanced nitric oxide synthase activity in the non-insulin-dependent diabetic embryo during organogenesis. Reproduction, fertility, and development. 1998; 10:191–6.
39. Jawerbaum A, Sinner D, White V, et al. Modulation of PGE2 generation in the diabetic embryo: effect of nitric oxide and superoxide dismutase. Prostaglandins, leukotrienes, and essential fatty acids. 2001; 64:127–33.
40. Jawerbaum A, Higa R, White V, et al. Peroxynitrites and impaired modulation of nitric oxide concentrations in embryos from diabetic rats during early organogenesis. Reproduction (Cambridge, England). 2005; 130:695–703.
41. Jawerbaum A, Gonzalez E. The role of alterations in arachidonic acid metabolism and nitric oxide homeostasis in rat models of diabetes during early pregnancy. Current pharmaceutical design. 2005; 11:1327–42. [PubMed: 15853688]
42. Szabo C. Multiple pathways of peroxynitrite cytotoxicity. Toxicology letters. 2003; 140-141:105–12. [PubMed: 12676456]
43. Shen HM, Liu ZG. JNK signaling pathway is a key modulator in cell death mediated by reactive oxygen and nitrogen species. Free radical biology & medicine. 2006; 40:928–39. [PubMed: 16540388]
44. Dempsey EC, Newton AC, Mochly-Rosen D, et al. Protein kinase C isozymes and the regulation of diverse cell responses. American journal of physiology. 2000; 279:L429–38. [PubMed: 10956616]
45. Wright MM, McMaster CR. Phospholipid synthesis, diacylglycerol compartmentation, and apoptosis. Biological research. 2002; 35:223–9. [PubMed: 12415740]
46. Hiramatsu Y, Sekiguchi N, Hayashi M, et al. Diacylglycerol production and protein kinase C activity are increased in a mouse model of diabetic embryopathy. Diabetes. 2002; 51:2804–10. [PubMed: 12196474]
47. Zhiyong Z, Wu YK, Reece EA. Demonstration of the essential role of protein kinase C isoforms in hyperglycemia-induced embryonic malformations. Reproductive sciences (Thousand Oaks, Calif. 2008; 15:349–56.
48. Reece EA, Ma XD, Wu YK, Dhanasekaran D. Aberrant patterns of cellular communication in diabetes-induced embryopathy. I. Membrane signalling. J Matern Fetal Neonatal Med. 2002; 11:249–53. [PubMed: 12375679]
49. Reece EA, Ma XD, Zhao Z, Wu YK, Dhanasekaran D. Aberrant patterns of cellular communication in diabetes-induced embryopathy in rats: II, apoptotic pathways. American journal of obstetrics and gynecology. 2005; 192:967–72. [PubMed: 15746699]
50. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. The EMBO journal. 1998; 17:2596–606. [PubMed: 9564042]
51. Ozcan U, Cao Q, Yilmaz E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004; 306:457–61. [PubMed: 15486293]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

52. Horal M, Zhang Z, Stanton R, Virkamaki A, Loeken MR. Activation of the hexosamine pathway causes oxidative stress and abnormal embryo gene expression: involvement in diabetic teratogenesis. Birth defects research. 2004; 70:519–27. [PubMed: 15329829]
53. Forsberg H, Eriksson UJ, Welsh N. Apoptosis in embryos of diabetic rats. Pharmacology & toxicology. 1998; 83:104–11. [PubMed: 9783328]
54. Phelan SA, Ito M, Loeken MR. Neural tube defects in embryos of diabetic mice: role of the Pax-3 gene and apoptosis. Diabetes. 1997; 46:1189–97. [PubMed: 9200655]
55. Sun F, Kawasaki E, Akazawa S, et al. Apoptosis and its pathway in early postimplantation embryos of diabetic rats. Diabetes research and clinical practice. 2005; 67:110–8. [PubMed: 15649569]
56. Moley KH. Hyperglycemia and apoptosis: mechanisms for congenital malformations and pregnancy loss in diabetic women. Trends in endocrinology and metabolism: TEM. 2001; 12:78–82. [PubMed: 11167126]
57. Eriksson UJ, Borg LA, Cederberg J, et al. Pathogenesis of diabetes-induced congenital malformations. Upsala journal of medical sciences. 2000; 105:53–84. [PubMed: 11095105]
58. Fine EL, Horal M, Chang TI, Fortin G, Loeken MR. Evidence that elevated glucose causes altered gene expression, apoptosis, and neural tube defects in a mouse model of diabetic pregnancy. Diabetes. 1999; 48:2454–62. [PubMed: 10580436]
59. Kang BP, Frencher S, Reddy V, Kessler A, Malhotra A, Meggs LG. High glucose promotes mesangial cell apoptosis by oxidant-dependent mechanism. Am J Physiol Renal Physiol. 2003; 284:F455–66. [PubMed: 12419773]
60. Ueda S, Masutani H, Nakamura H, Tanaka T, Ueno M, Yodoi J. Redox control of cell death. Antioxidants & redox signaling. 2002; 4:405–14. [PubMed: 12215208]
61. Kyosseva SV. Mitogen-activated protein kinase signaling. International review of neurobiology. 2004; 59:201–20. [PubMed: 15006489]
62. Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. BioFactors (Oxford, England). 2003; 17:287–96.
63. Widmann C, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. Physiological reviews. 1999; 79:143–80. [PubMed: 9922370]
64. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiological reviews. 2001; 81:807–69. [PubMed: 11274345]
65. Tobiume K, Saitoh M, Ichijo H. Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer. Journal of cellular physiology. 2002; 191:95–104. [PubMed: 11920685]
66. Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell. 2000; 103:239–52. [PubMed: 11057897]
67. Zhang Q, Zhang G, Meng F, Tian H. Biphasic activation of apoptosis signal-regulating kinase 1-stress-activated protein kinase 1-c-Jun N-terminal protein kinase pathway is selectively mediated by Ca2+-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors involving oxidative stress following brain ischemia in rat hippocampus. Neuroscience letters. 2003; 337:51–5. [PubMed: 12524169]
68. Watanabe T, Otsu K, Takeda T, et al. Apoptosis signal-regulating kinase 1 is involved not only in apoptosis but also in non-apoptotic cardiomyocyte death. Biochemical and biophysical research communications. 2005; 333:562–7. [PubMed: 15953587]
69. Kadowaki H, Nishitoh H, Urano F, et al. Amyloid beta induces neuronal cell death through ROS-mediated ASK1 activation. Cell death and differentiation. 2005; 12:19–24. [PubMed: 15592360]
70. Yokoi T, Fukuo K, Yasuda O, et al. Apoptosis Signal-Regulating Kinase 1 Mediates Cellular Senescence Induced by High Glucose in Endothelial Cells. Diabetes. 2006; 55:1660–65. [PubMed: 16731828]
71. Wada T, Penninger JM. Mitogen-activated protein kinases in apoptosis regulation. Oncogene. 2004; 23:2838–49. [PubMed: 15077147]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

72. Lin A, Dibling B. The true face of JNK activation in apoptosis. Aging cell. 2002; 1:112–6. [PubMed: 12882340]
73. Wang MC, Bohmann D, Jasper H. JNK extends life span and limits growth by antagonizing cellular and organism-wide responses to insulin signaling. Cell. 2005; 121:115–25. [PubMed: 15820683]
74. Essers MA, Weijzen S, de Vries-Smits AM, et al. FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. The EMBO journal. 2004; 23:4802–12. [PubMed: 15538382]
75. Lin A. Activation of the JNK signaling pathway: breaking the brake on apoptosis. Bioessays. 2003; 25:17–24. [PubMed: 12508278]
76. Torres M. Mitogen-activated protein kinase pathways in redox signaling. Front Biosci. 2003; 8:d369–91. [PubMed: 12456373]
77. Sata N, Klonowski-Stumpe H, Han B, Haussinger D, Niederau C. Menadione induces both necrosis and apoptosis in rat pancreatic acinar AR4-2J cells. Free radical biology & medicine. 1997; 23:844–50. [PubMed: 9378363]
78. Ding M, Li J, Leonard SS, et al. Differential role of hydrogen peroxide in UV-induced signal transduction. Molecular and cellular biochemistry. 2002; 234-235:81–90. [PubMed: 12162463]
79. Chan WH, Wu CC, Yu JS. Curcumin inhibits UV irradiation-induced oxidative stress and apoptotic biochemical changes in human epidermoid carcinoma A431 cells. Journal of cellular biochemistry. 2003; 90:327–38. [PubMed: 14505349]
80. Lee SA, Dritschilo A, Jung M. Role of ATM in oxidative stress-mediated c-Jun phosphorylation in response to ionizing radiation and CdCl2. The Journal of biological chemistry. 2001; 276:11783–90. [PubMed: 11278277]
81. Li Y, Arita Y, Koo HC, Davis JM, Kazzaz JA. Inhibition of c-Jun N-terminal kinase pathway improves cell viability in response to oxidant injury. American journal of respiratory cell and molecular biology. 2003; 29:779–83. [PubMed: 12842852]
82. Kim YS, Jhon DY, Lee KY. Involvement of ROS and JNK1 in selenite-induced apoptosis in Chang liver cells. Experimental & molecular medicine. 2004; 36:157–64. [PubMed: 15150444]
83. Yoon SO, Park SJ, Yoon SY, Yun CH, Chung AS. Sustained production of H(2)O(2) activates pro-matrix metalloproteinase-2 through receptor tyrosine kinases/phosphatidylinositol 3-kinase/NF-kappa B pathway. The Journal of biological chemistry. 2002; 277:30271–82. [PubMed: 12058032]
84. Yoshizumi M, Kogame T, Suzaki Y, et al. Ebselen attenuates oxidative stress-induced apoptosis via the inhibition of the c-Jun N-terminal kinase and activator protein-1 signalling pathway in PC12 cells. British journal of pharmacology. 2002; 136:1023–32. [PubMed: 12145102]
85. Dong C, Yang DD, Tournier C, et al. JNK is required for effector T-cell function but not for T-cell activation. Nature. 2000; 405:91–4. [PubMed: 10811224]
86. She QB, Chen N, Bode AM, Flavell RA, Dong Z. Deficiency of c-Jun-NH(2)-terminal kinase-1 in mice enhances skin tumor development by 12-Otetradecanoylphorbol-13-acetate. Cancer research. 2002; 62:1343–8. [PubMed: 11888903]
87. Kuan CY, Yang DD, Samanta Roy DR, Davis RJ, Rakic P, Flavell RA. The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. Neuron. 1999; 22:667–76. [PubMed: 10230788]
88. Urano F, Wang X, Bertolotti A, et al. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science. 2000; 287:664–6. [PubMed: 10650002]
89. Srivastava AK. High glucose-induced activation of protein kinase signaling pathways in vascular smooth muscle cells: a potential role in the pathogenesis of vascular dysfunction in diabetes (review). International journal of molecular medicine. 2002; 9:85–9. [PubMed: 11745003]
90. Kikkawa R, Koya D, Haneda M. Progression of diabetic nephropathy. Am J Kidney Dis. 2003; 41:S19–21. [PubMed: 12612945]
91. Bagowski CP, Xiong W, Ferrell JE Jr. c-Jun N-terminal kinase activation in Xenopus laevis eggs and embryos. A possible non-genomic role for the JNK signaling pathway. The Journal of biological chemistry. 2001; 276:1459–65. [PubMed: 11029471]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

92. Anderson MJ, Viars CS, Czekay S, Cavenee WK, Arden KC. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics. 1998; 47:187–99. [PubMed: 9479491]

93. Kajihara T, Jones M, Fusi L, et al. Differential expression of FOXO1 and FOXO3a confers resistance to oxidative cell death upon endometrial decidualization. Molecular endocrinology (Baltimore, Md. 2006; 20:2444–55.

94. Tran H, Brunet A, Grenier JM, et al. DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science. 2002; 296:530–4. [PubMed: 11964479]

95. Rokudai S, Fujita N, Kitahara O, Nakamura Y, Tsuruo T. Involvement of FKHR-dependent TRADD expression in chemotherapeutic drug-induced apoptosis. Molecular and cellular biology. 2002; 22:8695–708. [PubMed: 12446787]

96. Chong ZZ, Li F, Maiese K. Oxidative stress in the brain: novel cellular targets that govern survival during neurodegenerative disease. Progress in neurobiology. 2005; 75:207–46. [PubMed: 15882775]

97. Finkel T. Redox-dependent signal transduction. FEBS letters. 2000; 476:52–4. [PubMed: 10878249]

98. Brunet A, Sweeney LB, Sturgill JF, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science. 2004; 303:2011–5. [PubMed: 14976264]

99. Kops GJ, Dansen TB, Polderman PE, et al. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature. 2002; 419:316–21. [PubMed: 12239572]

100. Gilley J, Coffer PJ, Ham J. FOXO transcription factors directly activate bim gene expression and promote apoptosis in sympathetic neurons. The Journal of cell biology. 2003; 162:613–22. [PubMed: 12913110]

101. Modur V, Nagarajan R, Evers BM, Milbrandt J. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. The Journal of biological chemistry. 2002; 277:47928–37. [PubMed: 12351634]

102. Suhara T, Kim HS, Kirshenbaum LA, Walsh K. Suppression of Akt signaling induces Fas ligand expression: involvement of caspase and Jun kinase activation in Akt-mediated Fas ligand regulation. Molecular and cellular biology. 2002; 22:680–91. [PubMed: 11756562]

103. Lam EW, Francis RE, Petkovic M. FOXO transcription factors: key regulators of cell fate. Biochemical Society transactions. 2006; 34:722–6. [PubMed: 17052182]

104. Brunet A, Bonni A, Zigmond MJ, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999; 96:857–68. [PubMed: 10102273]

105. Sunayama J, Tsuruta F, Masuyama N, Gotoh Y. JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3. The Journal of cell biology. 2005; 170:295–304. [PubMed: 16009721]

106. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene. 2005; 24:7410–25. [PubMed: 16288288]

107. Shukla S, Bhaskaran N, Maclennan GT, Gupta S. Deregulation of FoxO3a accelerates prostate cancer progression in TRAMP mice. The Prostate. 2013; 73:1507–17. [PubMed: 23765843]

108. Abid MR, Yano K, Guo S, et al. Forkhead transcription factors inhibit vascular smooth muscle cell proliferation and neointimal hyperplasia. The Journal of biological chemistry. 2005; 280:29864–73. [PubMed: 15961397]

109. Wang Y, Zhou Y, Graves DT. FOXO transcription factors: their clinical significance and regulation. BioMed research international. 2014; 2014:925350. [PubMed: 24864265]

110. Willcox BJ, Donlon TA, He Q, et al. FOXO3A genotype is strongly associated with human longevity. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:13987–92. [PubMed: 18765803]

111. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. Cell. 1995; 81:495–504. [PubMed: 7758105]

112. Hsu H, Shu HB, Pan MG, Goeddel DV. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal transduction pathways. Cell. 1996; 84:299–308. [PubMed: 8565075]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

113. Chinnaiyan AM, Tepper CG, Seldin MF, et al. FADD/MORT1 is a common mediator of CD95 (Fas/APO-1) and tumor necrosis factor receptor-induced apoptosis. The Journal of biological chemistry. 1996; 271:4961–5. [PubMed: 8617770]

114. Boldin MP, Varfolomeev EE, Pancer Z, Mett IL, Camonis JH, Wallach D. A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain. The Journal of biological chemistry. 1995; 270:7795–8. [PubMed: 7536190]

115. Yount GL, Afshar G, Ries S, et al. Transcriptional activation of TRADD mediates p53-independent radiation-induced apoptosis of glioma cells. Oncogene. 2001; 20:2826–35. [PubMed: 11420694]

116. Prevention CfDCa. CDC to indtroduce book on diabetes in women. 2001

117. Weng H, Li X, Reece EA, Yang P. SOD1 suppresses maternal hyperglycemia-increased iNOS expression and consequent nitrosative stress in diabetic embryopathy. American journal of obstetrics and gynecology. 2012; 206:448 e1–7. [PubMed: 22425406]

118. Zhou X, Zhang GY, Wang J, Lu SL, Cao J, Sun LZ. A novel bridge between oxidative stress and immunity: the interaction between hydrogen peroxide and human leukocyte antigen G in placental trophoblasts during preeclampsia. American journal of obstetrics and gynecology. 2012; 206:447 e7–16. [PubMed: 22542123]

119. Bahado-Singh RO, Akolekar R, Chelliah A, et al. Metabolomic analysis for first-trimester trisomy 18 detection. American journal of obstetrics and gynecology. 2013; 209:65 e1–9. [PubMed: 23535240]

120. Bahado-Singh RO, Akolekar R, Mandal R, et al. Metabolomic analysis for first-trimester Down syndrome prediction. American journal of obstetrics and gynecology. 2013; 208:371 e1–8. [PubMed: 23313728]

121. Bahado-Singh RO, Ertl R, Mandal R, et al. Metabolomic prediction of fetal congenital heart defect in the first trimester. American journal of obstetrics and gynecology. 2014; 211:240 e1–40 e14. [PubMed: 24704061]

122. Parker SE, Yazdy MM, Tinker SC, Mitchell AA, Werler MM. The impact of folic acid intake on the association among diabetes mellitus, obesity, and spina bifida. American journal of obstetrics and gynecology. 2013; 209:239 e1–8. [PubMed: 23711668]

123. Correa A, Gilboa SM, Botto LD, et al. Lack of periconceptional vitamins or supplements that contain folic acid and diabetes mellitus-associated birth defects. American journal of obstetrics and gynecology. 2012; 206:218 e1–13. [PubMed: 22284962]

124. Oakley GP Jr. Failing to prevent birth defects caused by maternal diabetes mellitus. American journal of obstetrics and gynecology. 2012; 206:179–80. [PubMed: 22381596]

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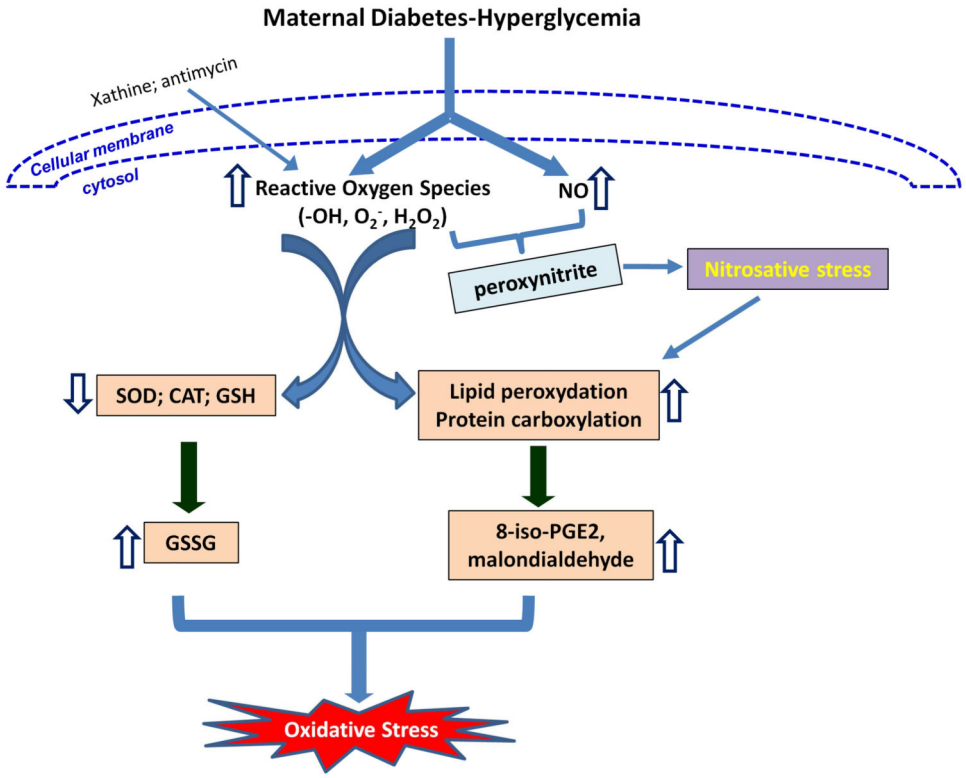

Figure 1.

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. SOD: superoxide dismutase; CAT catalase; GSH: glutathione; GSSG: glutathione disulfide; NO: nitric oxide. ⇩: downregulated; ⇧: upregulated.

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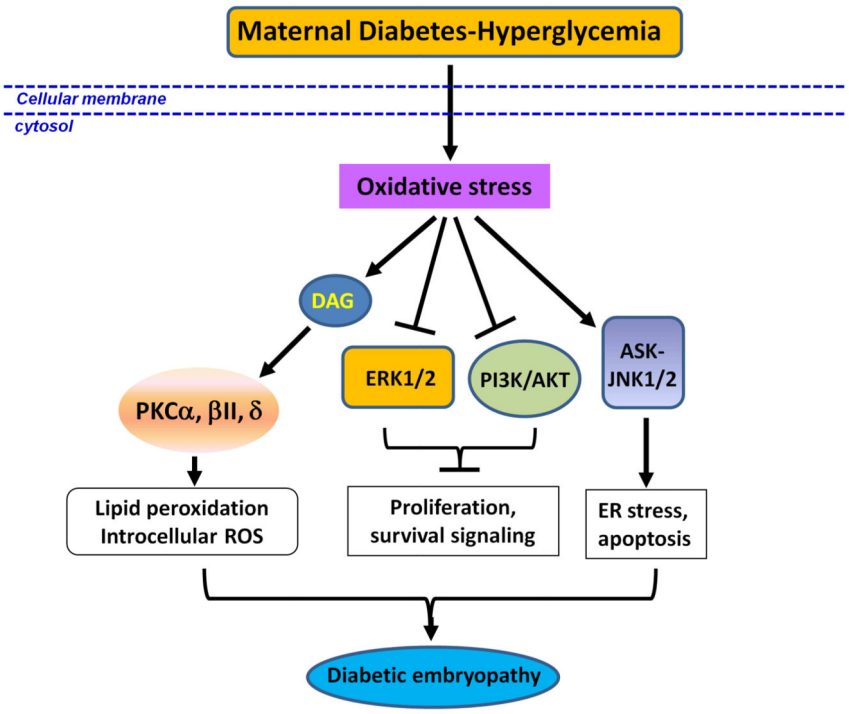

Figure 2.

Oxidative stress induces aberrant signaling pathways. Maternal diabetes-induced oxidative stress activates PKCα, -βII and -δ, stimulates lipid peroxidation, which aggravates oxidative stress; and induces apoptosis and diabetic embryopathy. The ERK1/2 and PI3K/ATK pathway is suppressed by oxidative stress induced by maternal diabetes, which further inhibits cell proliferation and survival signaling. Another pathway affected by maternal diabetes is the ASK-JNK1/2 signaling pathway, which is activated and subsequently induces ER stress and apoptosis events. These aberrant signaling pathways suppresses proliferation, which may contribute to diabetic embryopathy. DAG: diacyl glycerol; PKC: protein kinase C; ERK1/2: extracellular signal-regulated kinase 1/2.

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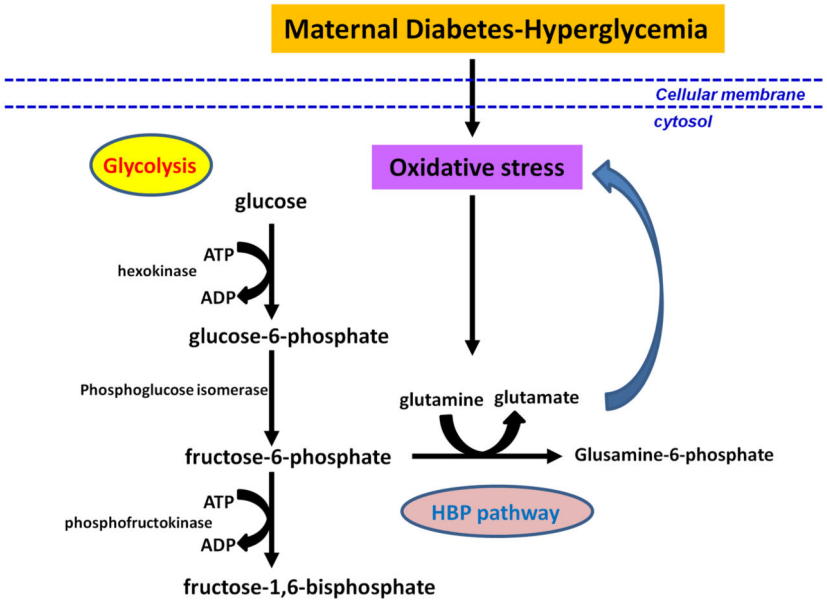

Figure 3.

Maternal diabetes-induced oxidative stress alters glucose metabolic pathways. During glycolysis, glucose is converted into fructose-6-phosphate with the help of phosphoglucose isomerase, and then further converted to fructose-1,6-biosphosphate by phosphofructolinase. Under maternal hyperglycemic condition, increased glycolytic flux in the embryo can stimulate flux through the HBP pathway. The fructose-6-phosphate will be transformed to glusamine-6-phosphte or glutamate, during which process, oxidative stress will be further enhanced. HBP: hexosamine biosynthetic pathway.

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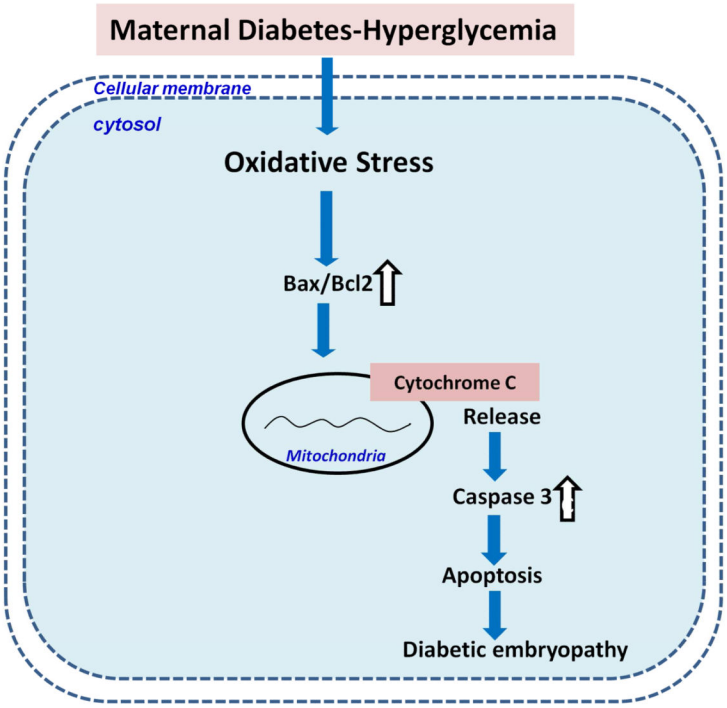

Figure 4.

Maternal diabetes induces diabetic embryopathy through apoptosis. Oxidative induces the expression of Bax, leading to the increased ratio of Bax/Bcl2. The overexpressed Bax stimulates cytochrome c releasing from mitochondria and activates downstream caspase 3, finally inducing apoptosis and abnormal development. : downregulated; ⇩: upregulated. ⇧

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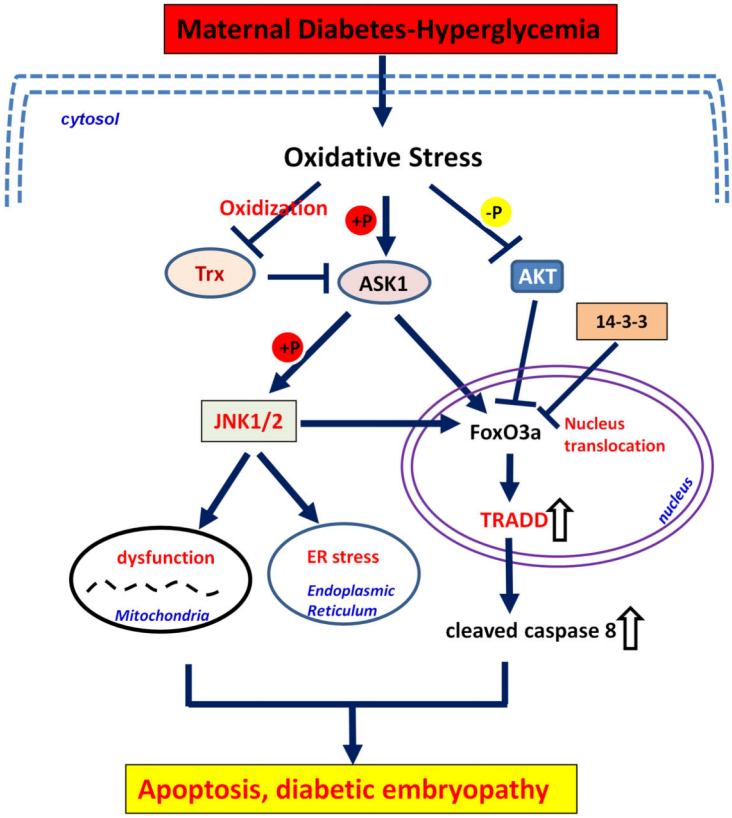

Figure 5.

The activity change and interaction of key molecular targets downstream of oxidative stress induced by maternal diabetes. Maternal diabetes-induced oxidative stress activates ASK1 signaling pathway through dissociation with Trx and then phosphorylation. Activated ASK1 further activates JNK1/2 by phosphorylation, resulting in mitochondrial dysfunction and ER stress. Activated ASK1/JNK1/2 stimulates FoxO3a nucleus translocation and subsequent TRADD expression, caspase 8 activation, which induces apoptosis and diabetic embryopathy. AKT pathway may inactivate FoxO3a function by phosphorylation. Oxidative stress inhibits AKT activity by dephosphorylation, which will increase levels of dephosphorylated and activated FoxO3a to translocate to nucleus as transcription factor inducing TRADD over-expression. Thus, the transcription factor FoxO3a is key mechanism of diabetic embryopathy induced by oxidative stress. mitochondrial dysfunction and apoptosis. : dephosphorylation; : phosphorylation; ⇩: downregulated; ⇧: upregulated.