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New development of the yolk sac theory in diabetic embryopathy: molecular mechanism and link to structural birth defects

Daoyin Dong, PhD[1], **E. Albert Reece, MD,PhD,MBA**[1,2], **Xue Lin, MD**[1], **Yanqing Wu, PhD**[1], **Natalia AriasVillela, MD**[1], 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

[3]Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Abstract

Maternal diabetes is a significant risk factor for structural birth defects, including congenital heart defects and neural tube defects (NTDs). With the rising prevalence of type 2 diabetes and obesity in women of childbearing age, diabetes-induced birth defects have become an increasingly significant public health problem. Maternal diabetes *in vivo* and high glucose *in vitro* induce yolk sac injuries by damaging the morphology of cells and altering the dynamics of organelles. The yolk sac vascular system is the first system to develop during embryogenesis, therefore, it is the most sensitive to hyperglycemia. The consequences of yolk sac injuries include impairment of nutrient transportation due to vasculopathy. Although the functional relationship between yolk sac vasculopathy and structural birth defects has not yet been established, a recent study reveals that the quality of yolk sac vasculature is inversely related to embryonic malformation rates. Studies in animal models have uncovered key molecular intermediates of diabetic yolk sac vasculopathy, including hypoxia-inducible factor-1α (HIF-1α), apoptosis signal-regulating kinase 1 (ASK1) and its inhibitor thioredoxin-1 (Trx), c-Jun-N-terminal kinases (JNK), nitric oxide (NO) and nitric oxide synthase (NOS). Yolk sac vasculopathy is also associated with abnormalities in arachidonic acid and *myo*-inositol. Dietary supplementation with fatty acids that restore lipid levels in the yolk sac lead to reduction in diabetes-induced malformations. Although the role of the human yolk in embryogenesis is less extensive than in rodents, nevertheless, human embryonic vasculogenesis is negatively affected by maternal diabetes. Mechanistic studies have identified potential therapeutic

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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targets for future intervention against yolk sac vasculopathy, birth defects, and other complications associated with diabetic pregnancies.

Keywords

yolk sac; maternal diabetes; embryopathy; vasculopathy

Globally, 60 million women of reproductive age (18–44 year old), and about 3 million American women, have diabetes mellitus, and it has been estimated that this number will double by 2030[1,2]. Due to the large number of women affected by diabetes, embryonic anomalies stemming from maternal diabetes has become a prevalent public health issue[3–5]. In fact, maternal diabetes-induced embryonic complications have become the leading cause of infant mortality in the United States[6]. Pregestational type 1 or 2 diabetes is a significant risk factor for structural birth defects, the most common anomalies being congenital heart defects and neural tube defects (NTDs)[3–5,7]. It has been well established that the rate of birth defects increases linearly with the degree of maternal hyperglycemia, which is the major teratogenic factor in maternal diabetes[5,8–13].

The yolk sac is an extra-embryonic membrane derived from the same progenitor cells that produce the embryo[14], and it plays an important role in supporting embryonic development[14, 15]. Pregestational diabetes alters the growth and structure of the human yolk sac [16,17], and abnormalities in human yolk sac structures are associated with embryonic malformations [18,19], suggesting the importance for studying the yolk sac in diabetic embryopathy. During the most critical, vulnerable period of embryogenesis, the rodent yolk sac encompasses the embryo and serves as the primitive placenta[14,15,20,21]. After implantation and prior to the formation of the placenta, embryonic growth is essentially dependent on the proper development of the yolk sac vasculature, which includes the vitelline circulation. The vitelline circulation serves as the site for the exchange of nutrients, production of red blood cells and blood vessels, and synthesis of essential embryonic proteins[20,21]. During mouse embryonic development, the yolk sac vascular system is the first system to develop, and it is the most sensitive to hyperglycemia[15]. Hyperglycemia causes yolk sac vasculopathy that ultimately leads to embryonic malformations or lethality[15,22]. Diabetes-induced defects in the vascular system have been directly linked to NTDs[23], highlighting the importance of studying diabetic yolk sac vasculopathy. This report summarizes the mechanisms underlying maternal diabetes-induced yolk sac injuries and yolk sac vasculopathy, and explores the possible causal relationship between yolk sac vasculopathy and structural anomalies.

The development of yolk sac vasculature

Although the human yolk sac resides outside of the embryo, similar to the rodent yolk sac, it plays an important role in early embryonic vasculogenesis [24]. The murine yolk sac is derived from the same progenitor cells that produce the embryo[14]. In mice, conceptus vasculogenesis starts with the emergence of vascular endothelial growth factor receptor-2-positive (VEGFR2$^+$ or Flk$^+$) cells in the yolk sac[25]. These Flk1$^+$ progenitor endothelial cells form blood islands that fuse to generate a primary capillary plexus at embryonic day 7.5

(E7.5)[25]. In addition, extra-embryonic mesodermal cells proliferate to form angioblastic cords on E7.5[26]. At E8.0, blood islands fuse and establish the primary capillary network, which is intimately associated with mural cells[27,28]. By E9.5, the capillary plexus has remodeled into a complex hierarchy of mature small and large vessels, and functional vitelline circulation is established[29]. A critical number of $Flk1^+$ cells and blood islands are crucial for normal vasculogenesis[25].

Vasculogenesis begins in the yolk sac prior to embryonic vasculogenesis and development of the cardiovascular system. In addition, the yolk sac and embryonic vasculatures are regulated by the same group of angiogenic and survival factors via common mechanisms[22,30, 31]. Therefore, the elucidation of the mechanism underlying hyperglycemia-induced yolk sac vasculopathy is important in the etiology of diabetic embryopathy.

Maternal diabetes induces yolk sac structure failure and dysfunction

Experimental evidence has elucidated the precise role of the yolk sac in mammalian embryonic development, as well as the relationship between yolk sac injury and embryopathy[15, 32]. The structures and prostaglandin E2 levels of human yolk sacs are altered by maternal diabetes [15,16,33]. Studies have shown that yolk sac development is morphologically impaired under hyperglycemic conditions[34]. For example, conceptuses exposed to excess glucose demonstrate decreased size and gross malformations[34]. Furthermore, exposure to excess glucose causes the visceral yolk sac capillaries and vitelline vessels to become sparse, patchy, and non-uniformly located[34]. Under high glucose conditions, the visceral yolk sac endodermal cells have reduced numbers of rough endoplasmic reticulum, ribosomes, and mitochondria[34]. These defects in yolk sac structures suggest that hyperglycemia during organogenesis has a primarily deleterious effect on yolk sac functions.

Hyperglycemic conditions also appear to affect the transport function of the yolk sac. For example, experiments using horseradish peroxidase as a tracer protein to examine the transport function of the visceral endodermal yolk sac cells have shown that the cellular uptake of peroxidase is diminished in conceptuses cultured under hyperglycemic conditions[35]. These findings indicate that hyperglycemia inhibits transport of nutrients from the yolk sac to the embryo. Coupled together with the experiments demonstrating a deleterious effect of hyperglycemia on cell morphology, these data suggest that yolk sac failure is associated with diabetic embryopathy.

Maternal diabetes induces yolk sac vasculopathy

In mice, abnormal development and arrested development of the yolk sac vasculature on E7.5 can result in congenital malformations in a wide variety of organs and tissues, as well as embryonic lethality[15,22,30,36]. The adverse effects of hyperglycemia on the yolk sac have been documented in maternal diabetic animal models and *in vitro* cultured rodent embryos[15,22,30,36]. Under hyperglycemic conditions, development of the blood vessels in the yolk sac is disrupted and the cellular structures in the vessels are altered[30,36]. Conceptuses display various, profoundly abnormal yolk sac vasculature, with some completely devoid of

vasculogenesis, and others having a branched plexus with no apparent arborization or distinction of arteries and veins[23,30,36,37].

The adverse effects of hyperglycemia on yolk sac vasculature development can be characterized by arbitrarily assigning morphological scores to individual vasculatures[23]. Using this rating system, one group showed that the yolk sac vasculature score of the hyperglycemia group was significantly lower than that of the euglycemic group[23]. Yolk sac vasculature morphologic scores were inversely correlated with embryonic malformation rates, such that the higher the score, the lower the rate of malformations, and vice versa[23].

Although the developing yolk sac contains a diverse cell population, evidence shows that vascular endothelial cells are the primary targets of hyperglycemic insults[30,37]. Platelet-derived endothelial cell adhesion molecule (PECAM-1), an endothelial cell marker, modulates endothelial cell migration, cell-cell adhesion, and *in vitro* and *in vivo* angiogenesis[38]. Under hyperglycemic conditions, the presence of yolk sac vasculopathy is associated with the failure of PECAM-1 tyrosine phosphorylation[30,37]. Thus, hyperglycemia may adversely impact vascular endothelial cell functions, including apoptosis, proliferation, and differentiation through regulation of endothelial cell specific cellular intermediates and signaling.

Molecular intermediates and signaling pathways contribute to maternal diabetes-induced yolk sac vasculopathy

Studies show that maternal diabetes induces yolk sac vasculopathy through two distinct sets of molecular events. In one set of events, hypoxia-inducible factor 1(HIF-1) and vascular endothelial growth factor (VEGF), two proteins that are typically active in normal vasculogenesis, are down-regulated by maternal diabetes[39]. In another set of events, maternal diabetes induces activation of a key apoptosis related kinase, known as apoptosis signal regulating kinase 1 (ASK1), which increases induced nitric oxide synthase (iNOS) expression and the promotion of apoptosis [40,41]. Inhibition of events downstream of ASK1 activation, such as c-Jun-N-terminal kinases (JNK1/2) signaling, abolishes maternal diabetes-induced vasculopathy[23,42]. The protective effect of thioredoxin-1, an inhibitor of ASK1, on hyperglycemia-induced vasculopathy has been demonstarted[39]. The elucidation of the mechanisms underlying hyperglycemia-induced yolk sac vasculopathy can aid in the development of preventative methods for maternal diabetes-induced cardiovascular defects in humans.

The role of HIF-1 in yolk sac vasculopathy

HIF-1 is a key transcriptional regulator for hypoxia regulation of embryonic vascular development. It is an oxygen-sensitive heterodimer consisting of a constitutively expressed HIF-1β subunit, and an oxygen-regulated HIF-1α subunit[43]. Regulation of HIF-1 activity depends on the degradation of the HIF-1α subunit in normoxic conditions [43]. The molecular basis of HIF-1 α degradation is the oxygen-dependent hydroxylation of at least one of the two proline residues in its oxygen-dependent degradation domain by specific prolylhydroxylases (PHD1, PHD2 and PHD3)[44–47]. In this orm, HIF-1 α binds to the von

Hippel-Lindau tumor suppressor protein, which acts as an E3 ubiquitin ligase, and targets HIF-1α for proteasomal degradation [48,49]. During conditions of normoxia, HIF-1 β is found in the nucleus, while HIF-1 α is cytoplasmic and rapidly degraded [49]. Reduced oxygen levels during embryonic development permit the accumulation of HIF-1α protein in the cytoplasm[50]. Subsequently, HIF-1α translocates to the nucleus, engages HIF-1β, and forms the HIF-1 complex that initiates transcription[50–52].

HIF-1 functions as a master regulator of angiogenesis by controlling the expression of multiple angiogenic growth factors[52,53]. Maternal diabetes has been shown to reduce HIF-1α levels in the embryo, leading to vasculopathy[39]. Maternal diabetes reduces the embryonic hypoxic environment-induced HIF-1α. AdCA5, an adenovirus encoding a constitutively active form of HIF-1α, blocks diabetes-induced vasculopathy, demonstrating that HIF-1α reduction contributes to diabetes-induced vasculopathy [39]. Mice that lack HIF-1 activity due to HIF-1α- or HIF-1β-null mutations develop extensive vascular defects, similar to those observed in diabetic yolk sac vasculopathy, including inadequate vessel formation and aberrant vascular remodeling [54, 55]. HIF-1 deficiency also decreases cell survival, leading to abnormal vasculogenesis[56]. In our previous study, we demonstrated that a decrease in HIF-1α expression is responsible for the VEGF reduction induced by maternal diabetes[39]. This suggests that the HIF-1α-VEGF signaling pathway plays a role in maternal diabetes-induced vasculopathy (Fig. 1).

The pro-apoptotic ASK1-JNK1/2 pathway

Apoptosis has been hypothesized as a primary mechanism of diabetes-induced birth defects [57–59]. Under euglycemic conditions, very low basal levels of apoptosis are observed in the embryonic tissues during organogenesis (E7-E11)[60]. In contrast, compelling evidence demonstrates that maternal hyperglycemia enhances apoptosis in the E7-E11 embryonic tissues [31, 61–66]. However, the apoptotic mechanism in this disease process is not well understood. Evidence from clinical and experimental studies has revealed that maternal diabetes leads to an imbalance in intracellular reduction-oxidation (redox) homeostasis, resulting in intracellular oxidative stress[57–59,67–70]. Recent studies have demonstrated that oxidative stress and ER stress are the main biochemical and molecular mechanisms underlying maternal diabetes- induced apoptosis[66,71–75].

JNK1/2 are pro-apoptotic factors that belong to the mitogen-activated protein kinase (MAPK) family[76]. MAPKs are members of a complex superfamily of serine/threonine kinases that are activated in response to a variety of extracellular stimuli[76,77]. The basic assembly of the MAPK signaling pathway is a three component module[76], involving sequential activation of MAPK kinase kinase (MAP3K), MAPK kinase (MAPKK), and MAPK [78,79]. MAP3K phosphorylates and thereby activates MAPKK, and activated MAPKK in turn phosphorylates and activates MAPK[79]. Because the activation status of MAPKs largely depends on MAP3Ks, it is important to understand how MAP3Ks are regulated. Fourteen different MAP3Ks have been identified [76]. Among them, several MAP3Ks, including ASK1, TAK1 and MLK3, are known to activate the JNK pathway in response to diverse stimuli[78–80]. In our previous work, we indicated that at a concentration of 800 nM, an inhibitor of JNK1/2 (SP600125), significantly abrogated hyperglycemia-

induced yolk sac vasculopathy in both morphologic score and vasculature morphology, strongly suggesting that JNK1/2 activation plays an important role in hyperglycemia-induced yolk sac vasculopathy [23] (Fig. 2).

ASK1-mediated apoptosis is involved in the pathogenesis of several oxidative stress-related diseases such as brain ischemia[81], ischemic heart disease[82], and Alzheimer's disease[83]. ASK1 activation leads to apoptosis via the JNK or the p38MAP kinase pathways[80]. ASK1 is activated by phosphorylation of Thr-845 in its activation loop, and ASK1 is required for reactive oxygen species (ROS)- and endoplasmic reticulum (ER) stress-induced JNK activation and apoptosis[58,59,80,84–86]. Recently, it has been shown that high glucose-induced activation of ASK1 mediates hyperglycemia-induced endothelial cell senescence[87]. We have demonstrated that ASK1 is activated in diabetic yolk sac vasculopathy, and that ASK1 deletion morphologically ameliorates diabetic yolk sac vasculopathy[23]. This indicates that ASK1 mediates maternal diabetes-induced endothelial progenitor apoptosis or senescence by JNK1/2, and that activation of the ASK1-JNK1/2 pathway leads to vasculopathy (Fig. 2).

Altered nitric oxide and nitric oxide synthase (NOS) in yolk sac vasculopathy

Nitric oxide (NO) is a small multifunctional gaseous molecule that acts as a vasoactive modulator, signaling molecule, and free radical in mammalian systems. NO is synthesized from oxidation of L-arginine by three distinct NO synthases (NOS): neuronal (nNOS), endothelial (eNOS), and inducible (iNOS), using the cofactors, NADPH, FAD, and tetrahydrobiopterin (BH_4)[88,89]. nNOS and eNOS are constitutively expressed at low levels[88]. iNOS generates very high concentrations of NO only when induced [90]. NO has been shown to be involved in cell differentiation, proliferation, and apoptosis, and the effect of NO is both physiologically essential and cytotoxic [91–93]. Upon generation, NO freely diffuses through the cell membrane into the exracellular space, and subsequently modifies protein thiols or cysteine residues. In addition, NO induces a variety of biological responses by interacting with free radicals[94–97]. NO interacts with several signaling pathways to mediate these responses, including MAPK, Janus kinase (JAK), and JNK pathways, as well as reactive oxygen depending on signaling pathways[98–100].

During blood island formation in diabetic pregnancies, the endoderm produces NO which inhibits NOS. Inhibition of NOS, L-N^G-monomethyl arginine citrate (L-NMMA), leads to developmental arrest at the primary plexus stage, and ultimately vasculopathy[22]. Administration of an NO donor reverses these adverse effects on yolk sac vasculature[22]. Additionally, it has been reported that NO derived from iNOS plays a detrimental role in human disease[101]. Moreover, iNOS and eNOS are expressed during early embryonic vasculogenesis, and the alteration of NO expression induces yolk sac vasculopathy[22]. Hyperglycemia increases iNOS protein expression and activity through ASK1[40,41]. The increase of iNOS leads to over-production of NO that causes DNA damage, ER stress, NF-kB and respiratory inhibition[102] that may play a vital role on embryonic malformation (Fig. 3).

The protective effect of the ASK1 inhibitor thioredoxin-1 in yolk sac vasculopthay

Thioredoxin-1 (Trx) is a 12-kDa protein with a redox-active dithiol in the active site (-Cys-Gly-Pro-Cys-) and constitutes a major thiol reducing system[103]. Trx is a potent antioxidant and reduces ROS through interactions with its redox-active center, which protects cells from stress-induced damage through anti-oxidative, anti-apoptotic, and anti-inflammatory effects[103]. Trx shows an anti-apoptotic function by inhibiting cell death signals[104], activating survival signaling pathways[105,106], or scavenging ROS[107]. Diabetic yolk sac vasculopathy is an oxidative stress and apoptotic disease process[39–41,58,59,71]. Therefore, Trx is able to reduce diabetic yolk sac vasculopathy via its anti-oxidative and anti-apoptotic functions (Fig. 4).

Trx is expressed ubiquitously in mammalian cells and its expression is essential for early differentiation and morphogenesis of the mouse embryo[108]. Genetic deletion of Trx leads to an early embryonic lethal phenotype[109]. Trx-deficient embryos die shortly after implantation, and the conceptuses are resorbed prior to gastrulation[109]. When preimplantation, Trx-null embryos are placed in culture, the inner mass cells of the homozygous embryos fail to proliferate[109]. This indicates that proper levels of Trx are essential for normal embryogenesis. Trx levels are reduced in embryonic tissues exposed to diabetes[39], implying that Trx reduction is involved in the pathogenesis of diabetic emrbyopathy.

Trx is expressed ubiquitously in endothelial cells[110] and protects them from ROS-induced apoptosis[111]. Trx is active in the vessel wall and functions either as an important endogenous antioxidant, or interacts directly with signaling molecules to influence cell growth, apoptosis, and inflammation[112,113]. Recent evidence implicates that Trx is involved in cardiovascular diseases associated with oxidative stress, such as atherosclerosis[110]; vascular injuries[114], ischemia reperfusion injury[115], and hypertension[116]. *In vivo* studies have shown a protective role of Trx in different cardiovascular diseases[114,115]. Thus, Trx is considered an important target for therapeutic intervention of cardiovascular disorders.

It has also been reported that Trx stimulates angiogenesis via induction of angiogenic factors[117]. For example, hyperglycemia-induced yolk sac vasculopathy in mice can be ameliorated by treating with exogenous human Trx recombinant protein[39]. Based on the profound beneficial effects of Trx on vascular functions and diabetic vasculopathy, induction or overexpression and deoxidation of Trx is able to reverse hyperglycemia-induced yolk sac vasculopathy (Fig. 4).

Therapeutic implications of targeting the yolk sac

The leading intervention strategy currently applied to prevent diabetic embryopathy is rigorous glycemic control with lifestyle modifications and various anti-diabetic agents, such as insulin, and other therapies, such as anti-hypertensives, as needed[57,71]. Unfortunately, continuous euglycemic control is difficult to achieve and maintain, and even transient exposure to hyperglycemia causes embryonic malformation[118].

Our group has shown that fatty acid supplements have some beneficial effects on the outcome of diabetic pregnancies[119]. We analyzed the fatty acid composition in major lipid groups of the yolk sac in rats[119], and found that maternal diabetes induces quantitative and qualitative abnormalities in major lipid groups of the yolk sac[119]. This implies that the teratogenic mechanism of diabetic embryopathy may be related to a deficiency in essential fatty acids in the yolk sac[119]. In addition, we used dietary supplementation of arachidonic acid and *myo*-inositol, *in vitro* and *in vivo*, and showed that these substrates can reduce the incidence of diabetes-related malformation in offspring[120].

Previous work also has indicated that arachidonic acid prevents hyperglycemia-associated yolk sac damage and embryopathy[119–122]. When rodent conceptuses were cultured in normal, arachidonic acid-supplemented normal, and arachidonic acid-supplemented hyperglycemic rat serum[122], the addition of 20 mg/ml of arachidonic acid prevented open neural tubes, increased number of lysosome-like structures in the visceral endodermal yolk sac cells, advanced neuropil formation in the neuroepithelium, significant reduction of ER, and decreased size and number of lipid droplets in embryos cultured under high glucose conditions[122].

Dietary *myo*-inositol supplements also appear to significantly decrease the incidence of NTDs in offspring of diabetic dams[123]. The results of a previous study showed that dietary therapy successfully restored *myo*-inositol levels in the yolk sac and reduced malformation[123]. These therapies hold promise for use as a dietary prophylaxis against diabetic embryopathy in humans.

Future perspectives and clinical relevance

Investigating the mechanisms underlying yolk sac vasculopathy in animal models may reveal the pathophysiology of adverse pregnancy outcomes in diabetic women, and may provide a strategy for preventing and treating diabetic embryopathy.

Pathological studies have revealed that placental vascular dysfunction and placental infarction occur in diabetic pregnancies[124–129]. While most of these studies have only reported findings after birth, we and others hypothesize that the vasculopathy actually starts as early as the yolk sac period. The primary yolk sac in humans is formed in the beginning of the second week of pregnancy (ADD BACK REF 142). Although human and murine embryonic dependence on the yolk sac differs, findings in animal models do suggest that preventing vasculopathy in the human yolk sac may influence the subsequent development of the placenta and, thus, the outcome of the pregnancy. Indeed, placental vasculopathy in humans increases the need for obstetric intervention, the rates of preterm birth, stillbirth and miscarriage[130–137].

Implementing the earliest possible interventions that can prevent aberrant embryogenesis remains a significant hurdle to improving the outcomes and reducing the healthcare costs associated with diabetic pregnancies[138–140]. Although most international guidelines recommend intensive glycemic control during diabetic pregnancy, most of the current guidelines do not stress the importance of pre-pregnancy glucose control. Unless a woman has diagnosed diabetes prior to pregnancy or a medical history of metabolic syndrome, some

women may not even be screened for diabetes until 24 to 28 weeks of gestation[141–144]. International guidelines also suggest that target glucose levels be based on glycated hemoglobin, which only represents a general blood sugar level within the past three months. However, even short spikes in glucose can be detrimental to the fetus. In reality, normalization of glucose metabolism using daily mean glucose level is preferable and desirable.

In addition, many pregnancies are unplanned[145]. Therefore, intervention strategies often miss the most important phase of organogenesis, the first weeks of the first trimester of pregnancy.. This may be a reason why there is such a high incidence of diabetes related birth defects despite modern prenatal care. Thus pregnancy education in women who currently have or who are at high-risk for diabetes should be implemented prior to pregnancy[139,146].

Because the fetuses are extremely vulnerable to hyperglycemia during the yolk sac period, it is pivotal to maintain the glucose stability very early in pregnancy. Different types of insulin are used clinically to control glucose, and insulin analogues are often used to treat type 1 or 2 diabetic patients[147]. For women whose blood glucose is poorly controlled by daily insulin injections, subcutaneous insulin pumps might be useful in such settings[148,149]. Although insulin and insulin analogues have been shown to improve HbA1c, with less risk of hypoglycaemia and with little or no adverse effects on the developing fetus[147,150–152], use of anti-diabetic therapeutics alone has not completely eliminated the incidence of hyperglycemia-induced birth defects[153].

To date, there has been no single, “best” approach to control glucose in pregnant, diabetic women. Studies in animal models have suggested that, in addition to anti-pharmaceutical interventions, dietary supplements that improve the lipid content of the yolk sac can reduce congenital malformations in offspring of diabetic dams[118]. However, only isolated clinical trials in humans have been performed to date. Large-scale, multicenter clinical trials are needed to determine if targeting the health of the yolk sac, either by using nutritional supplements or therapeutics that improve yolk sac vasculogenesis, can prevent diabetic embryopathy.

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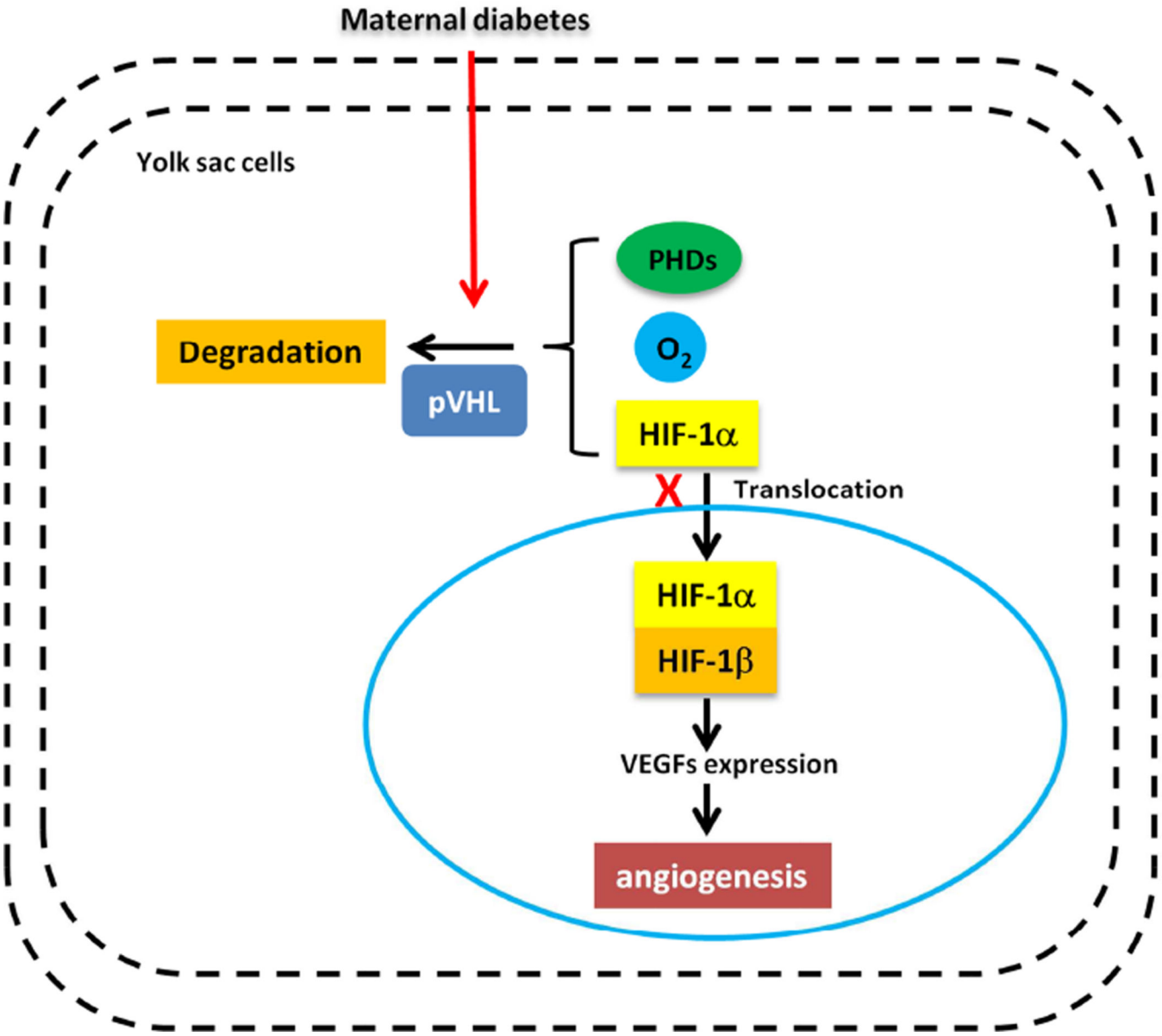

Figure 1. Maternal diabetes induces yolk sac vasculopathy via reduction of HIF-1α

Under normoxic conditions, specific prolylhydroxylases (PHDs) induce oxygen-dependent hydroxylation of HIF-1α. HIF-1α then binds to the von Hippel-Lindau tumor suppressor protein (pVHL), which acts as an E3 ubiquitin ligase and targets HIF-1α for proteasomal degradation. Under hypoxic conditions, HIF-1α translocates to the nucleus, engages HIF-1β, and forms the HIF-1 complex that initiates transcription of downstream genes, including VEGFs. Maternal diabetes reduces HIF-1α levels by enhancing its degradation. The lack of HIF-1α leads to the development of extensive vascular defects, which is similar to diabetic yolk sac vasculopathy.

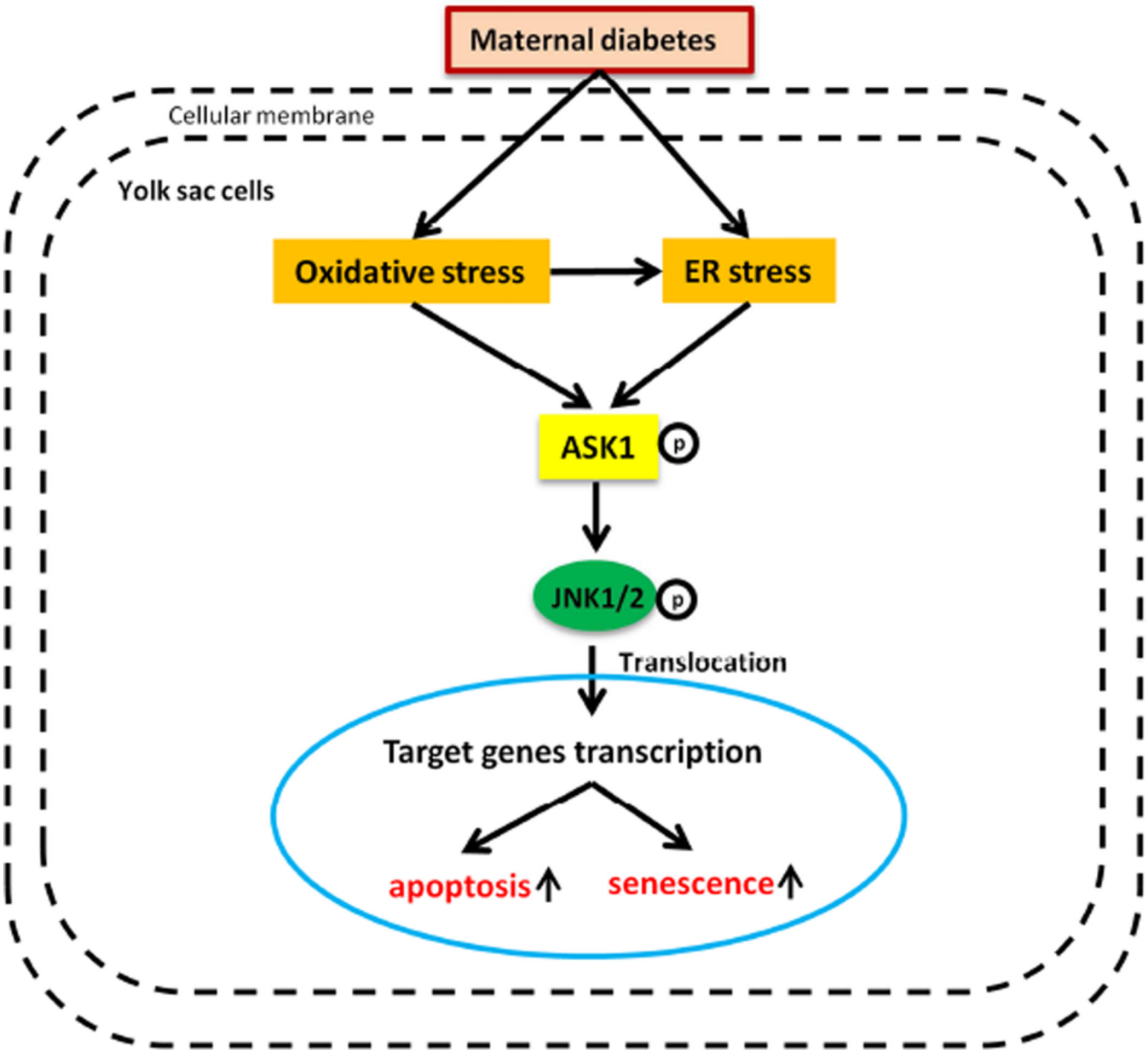

Figure 2. Maternal diabetes induces endothelial progenitor apoptosis via ASK1 activation

Maternal diabetes induces oxidative stress, which causes ER stress by aggravating unfolding protein response (UPR) events in the ER. Oxidative stress and ER stress induce phosphorylation of the Thr-845 present on the activation loop of ASK1, thereby activating ASK1. ASK1 activation then leads to the phosphorylation of JNK1/2, which activates several transcription factors. These transcription factors ultimately induce endothelial progenitor cell apoptosis and senescence.

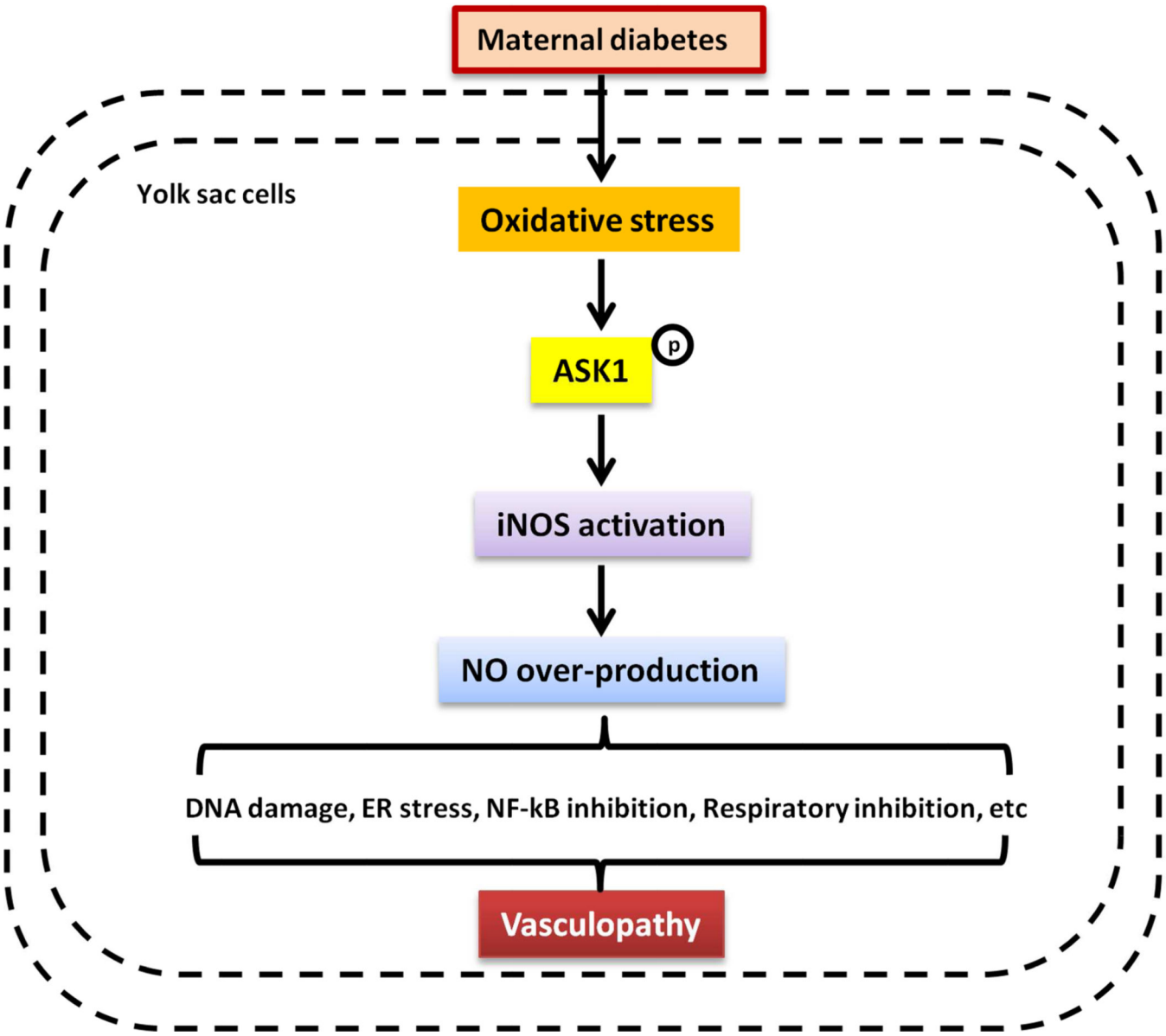

Figure 3. Overproduction of NO mediates maternal diabetes-induced yolk sac vasculopathy

Maternal diabetes-induced oxidative stress activates ASK1. The phosphorylation of ASK1 stimulates iNOS gene expression, which generates very high concentrations of NO. The detrimental role of NO derived from iNOS includes DNA damage, ER stress, NF-kB inhibition, and respiratory inhibition, all of which contribute to yolk sac vasculopathy.

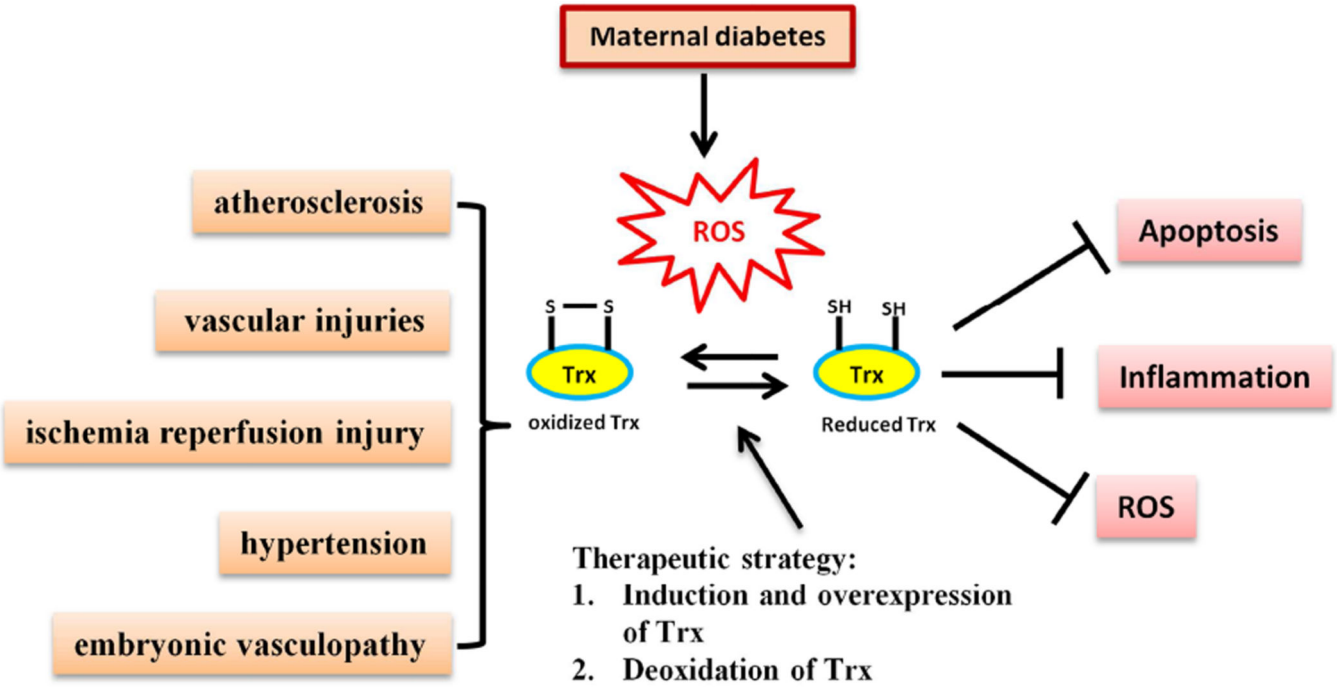

Figure 4. Thioredoxin-1 (Trx) reduces diabetic yolk sac vasculopathy by scavenging ROS

Reduced Trx is a potent antioxidant that decreases ROS levels through the function of its redox-active center. Trx ultimately protects cells from stress-induced damage by anti-oxidative, anti-apoptosis, and anti-inflammation processes. Maternal diabetes-induced oxidative stress disturbs the redox balance of Trx, leading to a disproportionate increase in oxidized Trx. High levels of oxidized Trx are associated with several cardiovascular diseases, including atherosclerosis, vascular injuries, ischemia reperfusion injury, hypertension, and yolk sac vasculopathy. The therapeutic strategy for maternal diabetes-associated embryopathy may be through induction or overexpression, and deoxidation of Trx.