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Pancreatic Islet Hepatocyte Growth Factor and Vascular Endothelial Growth Factor A Signaling in Growth Restricted Fetuses

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Abstract

Placental insufficiency leads to intrauterine growth restriction (IUGR) and a lifelong risk of developing type 2 diabetes. Impaired islet development in the growth restricted fetus, including decreased β -cell replication, mass, and insulin secretion, is strongly implicated in the pathogenesis of later life type 2 diabetes. Currently, standard medical management of a woman with a pregnancy complicated by placental insufficiency and fetal IUGR is increased fetal surveillance and indicated preterm delivery. This leads to the dual complications of IUGR and preterm birth – both of which may increase the lifelong risk for type 2 diabetes. In order to develop therapeutic interventions in IUGR pregnancies complicated by placental insufficiency and decrease the risk of later development of type 2 diabetes in the offspring, the mechanisms responsible for impaired islet development in these cases must be determined. This review focuses on current investigations testing the hypothesis that decreased nutrient supply to the IUGR fetus inhibits an intra-islet hepatocyte growth factor – vascular endothelial growth factor A (HGF – VEGFA) feed forward signaling pathway and that this is responsible for developmental islet defects.

Keywords

Intrauterine growth restriction; insulin; hepatocyte growth factor; vascular endothelial cell growth factor; pancreatic islet; pregnancy

1. Intrauterine Growth Restriction and the Pancreatic Islet

1.1. Clinical Aspects of Intrauterine Growth Restriction (IUGR)

Placental insufficiency and intrauterine growth restriction (IUGR) complicate approximately 4-8% of pregnancies (Platz and Newman, 2008). IUGR is best defined as a failure of the fetus to reach its genetic potential size. The distinction between IUGR patients and those that are simply small for gestational age (SGA) based on their genetic make-up has

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important implications for short and long term morbidities (Von Beckerath et al., 2013). There are a variety of causes of IUGR, including maternal and placental diseases, as well as idiopathic cases. In the majority of cases, however, decreased placental nutrient and oxygen transfer to the fetus are important pathophysiological features. Current medical management includes close fetal monitoring and delivery of the fetus when the risks associated with continued development in a compromised intrauterine environment are felt to be greater than the risks of induced preterm birth (Zeitlin et al., 2000). This leads to serious complications for the baby including increased perinatal mortality as well as serious short and long term morbidities (Rosenberg, 2008). One of the most worrisome long term consequences of IUGR is an increased risk for developing type 2 diabetes later in life (Hales and Barker, 2001). The development of type 2 diabetes requires both insulin resistance and an inability of the pancreatic β -cell to compensate for this insulin resistance by increasing insulin secretion (Kasuga, 2006). Therefore, the development of the pancreatic islet and β -cell has been the subject of intense investigation (Green et al., 2010). Despite this, gaps in our understanding of the pathogenesis of IUGR limit specific therapies available for women whose pregnancies are complicated by placental insufficiency. Nutritional strategies have produced variable results, including some that actually increased the severity of fetal growth restriction and were associated with higher fetal mortality rates (Brown et al., 2011; Rush et al., 1980). Development of better therapies holds the promise of improving fetal growth, prolonging pregnancy, and decreasing the risk in the offspring of developing adult diseases like diabetes later in life. This review will highlight some of the recent work aimed at understanding the pathophysiology of impaired islet development during IUGR which might inform the design of these improved therapies.

1.2. Placental Insufficiency and Impaired Pancreatic Islet Development

In humans, severe placental insufficiency results in persistently decreased oxygen and nutrient transfer to the fetus, including amino acids and notably leucine, as early as the end of the second and beginning of the third trimester. In fact, the degree of impairment in leucine transfer to the fetus directly correlates with the severity of fetal growth restriction in cases of placental insufficiency (Jansson et al. 1998, 2002; Paolini et al., 2001; Marconi et al., 1999). Severe placental insufficiency also results in decreased fetal glucose stimulated insulin secretion when tested during percutaneous umbilical cord blood sampling (Nicolini et al., 1990), and reduced pancreatic islet vascularity and β -cell mass (Van Assche et al., 1977). This contrasts with a morphological analysis of pancreases from less severely growth restricted patients in which no difference was found in β -cell mass (Beringue et al., 2002). The differences between the Van Assche and Beringue studies highlight the important distinction between IUGR fetuses who fail to reach their genetic growth potential due to placental insufficiency from those who are simply small for gestational age based on their genotype (Von Beckerath et al., 2013). This distinction is even more critical to consider when one realizes that there are genetic mutations which result in decreased fetal insulin secretion, decreased fetal growth, and a higher risk for developing diabetes in later life. Loss of function mutations in the gene encoding glucokinase is an important example of such a mutation (Spyer et al., 2001).

1.3. Animal Models to Study Impaired Pancreatic Islet Development during Placental Insufficiency

While we know some of the structural and functional islet defects that result from IUGR, in humans the mechanisms underlying impaired islet development in IUGR remain unknown. In order to determine these mechanisms, animal models must be used. One such model organism is the pregnant sheep, in which several models of placental insufficiency have been developed (Green et al., 2010; Morrison, 2008). Of these sheep models, one of the best characterized in terms of impaired islet development also is the most severe sheep placental insufficiency model of IUGR available (PI-IUGR) (Green et al., 2010). Experimental placental insufficiency and fetal growth restriction in the PI-IUGR model is established by exposing pregnant sheep to elevated ambient temperatures beginning on approximately days 35-42 of gestation (normal sheep gestation is approximately 148 days) and ending between 105-115 days of gestation. Impaired placental function is evident as early as days 95-105 (Limesand et al., 2013; de Vrijer et al., 2006). Decrements in umbilical blood flow, oxygen and nutrient transfer to the fetus, and fetal anabolic hormone concentrations become more prominent as gestation progresses and are associated with asymmetric fetal growth restriction (de Vrijer et al., 2004; Limesand et al., 2007; Brown et al., 2012; Galan et al., 2005; Regnault et al., 2007).

Pregnant sheep, and this model in particular, offer several advantages for determining the mechanism by which placental insufficiency impairs fetal islet development and function of the β -cell. These advantages include both similarities to human placental insufficiency and technical features which allow for complex *in vivo* and *in vitro* fetal studies (Green et al., 2010; Jansson et al., 1998; Paolini et al., 2001; Van Assche et al., 1977; Nicolini et al., 1990; Barry and Anthony, 2008; Barry et al., 2008; Brown et al., 2012; Limesand et al., 2005 2006; Ross et al., 1996; Rozance et al., 2015; Thorn et al., 2013). Development of the human and sheep fetal endocrine pancreas is similar. The transition of pancreatic progenitor cells into more differentiated endocrine cells with mature secretory products, formation of pancreatic islets, and replication of existing endocrine cells within the islet occur at the same gestational stages in both human and sheep fetuses (Green et al., 2010). In addition to developmental similarities, technical advantages include the ability to isolate fetal islet endothelial cells to 95% purity (Rozance et al., 2015). For the current review the most important similarities between human IUGR and our sheep PI-IUGR model are decreased nutrient supply to the fetus including amino acids and leucine, decreased fetal insulin concentrations and glucose stimulated insulin secretion, and decreased islet size and β -cell mass (Green et al., 2010; Limesand et al., 2005 2006; Ross et al., 1996; Rozance et al., 2015; Thorn et al., 2013). Interestingly, while pancreatic islets are smaller and β -cell insulin content is lower, isolated pancreatic islets from PI-IUGR fetal sheep actually secrete a higher fraction of the cellular insulin present when tested *in vitro* compared to normal fetal sheep (Limesand et al., 2005, 2006). In fact, in the *in vivo* condition hypoxemia and hypercatecholaminemia in the fetus play a major role in the suppression of insulin secretion in the PI-IUGR fetus and once the adrenergic signaling is blocked the PI-IUGR islets actually become hyper responsive to glucose stimulation (Rozance et al., 2009; Leos et al., 2010). Fetal hypoxia and hypercatecholaminemia in pregnancies complicated by fetal growth restriction (Greenough et al., 1990) might set up the formerly IUGR fetus for increased

postnatal insulin secretion leading to apparent hyperinsulinemic hypoglycemia in a subset of these patients once normoxia is established and catecholamine concentrations return to normal (Arya et al., 2013).

In fact, unlike the fairly consistent finding of decreased fetal insulin concentrations and secretion in IUGR, studies of insulin concentrations in the early postnatal period are quite variable (Collins and Leonard 1984; Collins et al., 1990; Bazaes et al., 2003; Hoe et al., 2006; Wang et al., 2007). As formerly IUGR infants age the incidence of increased adiposity and insulin resistance increases (Hofman et al., 1997; Ong et al., 2000; Mericq et al., 2005). This complicates interpretation of insulin secretion measurements in children and young adults who experienced IUGR, because of the hyperbolic relationship between insulin secretion and insulin sensitivity (Kahn et al.; 1993). However, studies that accounted for insulin sensitivity showed impaired insulin secretion in children and young adults who had IUGR during fetal life (Li et al., 2001; Jensen et al., 2002).

2. Hepatocyte Growth Factor (HGF) and Vascular Endothelial Cell Growth Factor A (VEGFA) in the Pancreatic Islet

2.1. HGF and VEGFA in Normal Islet Development

There has been increasing interest in the role of the pancreatic vasculature and paracrine signaling between the β -cell and endothelial cells. The paracrine signals most studied in this context are hepatocyte growth factor (HGF) produced by the endothelial cell and vascular endothelial growth factor A (VEGFA) produced by the β -cell, though they are often studied in isolation from each other. Genetic manipulations that increase islet HGF or VEGFA signaling also increase adult islet vascularity, β -cell mass, and/or insulin secretion (Dai et al., 2003; Garcia-Ocana et al., 2000, 2001; Lammert et al., 2001). When islet HGF or VEGFA signaling is decreased, adult animals develop reduced islet vascularity, β -cell mass, insulin secretion, glucose intolerance, and diabetes (Dai et al., 2005; Kamba et al., 2006; Lammert et al., 2003; Brissova et al., 2006; Mellado-Gil et al., 2011; Reinert et al., 2013). Most currently available literature regarding islet HGF and VEGFA signaling does not directly address the pathogenesis of impaired islet development during placental insufficiency. Furthermore, published studies primarily used genetic and pharmacological inhibition or activation of HGF and VEGFA signaling, resulting in an “all or none” phenotype. The genetic manipulations were all performed in mice and have not been extended to models of placental insufficiency. Furthermore, very few studies have focused on islet HGF and VEGFA signaling in the pathogenesis of impaired islet development contributing to the fetal origins of diabetes following IUGR.

2.2. IUGR and Islet HGF and VEGFA

Two models of IUGR have been used to test the impact of impaired placental function and reduced nutrient and oxygen supply to the fetus on the islet vasculature and islet VEGFA, both in pregnant rats. In one model fetal growth restriction was produced by ligating the uterine arteries near the end of gestation and in the other fetal growth restriction was produced by restricting maternal protein intake during pregnancy. In both of these models, decreased fetal and/or neonatal islet vascularity and VEGFA were observed (Boujendar et

al.,2003; Ham et al., 2009). Furthermore, these decrements could be corrected or prevented. Decreased islet VEGFA and vascularity following bilateral uterine artery ligation could be corrected by treatment with a Glucagon Like Peptide-1 analog in the neonatal period (Ham et al., 2009). In the low protein diet model of fetal growth restriction, decreased islet VEGFA and vascularity could be prevented with maternal dietary taurine supplementation (Boujendar et al.,2003).

In order to first establish the importance of fetal endothelial cell secretion of HGF for stimulating isolated fetal sheep islet function, we isolated fetal sheep endothelial cells and then used these cells to condition cell culture media (Figure 1A). Isolated fetal sheep pancreatic islets were then incubated overnight in this conditioned media and the insulin content of the islets was determined. Compared to non-conditioned media, incubation in the endothelial cell-conditioned media resulted in higher islet insulin contents. This effect was blocked by the addition of a specific inhibitory anti-HGF antibody, demonstrating the necessity of HGF signaling for this islet response (Figure 1A,B). Furthermore, incubation of fetal islets in non-conditioned media with supplemental HGF also resulted in higher islet insulin contents, demonstrating that HGF also is sufficient for this islet response (Figure 1A) (Rozance et al., 2015).

In our severe sheep PI-IUGR model of placental insufficiency, fetal islet vessel density and β -cell mass were reduced at day 103-105 of gestation, after placental insufficiency is established (Rozance et al., 2015; Limesand et al., 2013). At day 130-135 of gestation the decrement in β -cell mass is greater (Limesand et al., 2005, 2013). However, despite significantly smaller islets, islet vascularity is decreased in proportion to islet size, such that islet vessel density is equivalent to controls (Rozance et al., 2015; Limesand et al., 2005). This finding demonstrates that reduced vessel density in the islets of PI-IUGR fetuses at day 103-105 of gestation results in the down-regulation of islet growth in order to match the vascular supply available.

In addition to determining islet morphology in the PI-IUGR sheep fetus, we also interrogated fetal islet HGF and VEGFA. Similar to the two rodent models of IUGR described above, VEGFA is significantly decreased in PI-IUGR islets (Figure 2). Thus, the primary signal for islet vascular growth is decreased in the islets of three different animal models of IUGR. We also found that both secreted and cellular HGF concentrations were lower in fetal islet endothelial cells isolated from PI-IUGR animals compared to controls (Figure 2) (Rozance et al., 2015). In order to determine the functional significance of decreased islet endothelial cell HGF production in PI-IUGR, we incubated freshly isolated normal fetal sheep islets overnight in media conditioned by isolated control and PI-IUGR islet endothelial cells. Islets incubated in control endothelial cell-conditioned media had higher insulin content than islets incubated in non-conditioned media. But islets incubated in PI-IUGR endothelial cell-conditioned media had insulin contents that were equivalent to islets incubated with non-conditioned media (Figure 3) (Rozance et al., 2015). These experimental observations reveal functional consequences of decreased HGF secretion by the PI-IUGR fetal islet endothelial cell for the adjacent fetal β -cell which limit insulin production; a hallmark of impaired islet development in the PI-IUGR fetus (Limesand et al., 2006).

What remains to be demonstrated is which phenotypic characteristic, or combination of characteristics, in PI-IUGR is responsible for islet defects in HGF and VEGFA production and if this can be reversed after onset of fetal growth restriction and islet dysfunction. It is possible that that islet HGF-VEGFA signaling is a nutrient and/or hormone sensitive feed forward signaling loop (Figure 4). This would explain how HGF-VEGFA signaling is decreased in experimental models of placental insufficiency. Insulin and IGF-1 are anabolic fetal growth factors that could act as potential stimulators of islet HGF-VEGFA signaling, because both stimulate VEGFA production in different adult organ systems and cells (Lu et al., 1999; Warren et al., 1996). IGF-1 is particularly important to investigate as a mechanism, because both circulating and pancreatic IGF-1 concentrations are lower in the PI-IUGR model of fetal growth restriction (Brown et al., 2012; Chen et al., 2012). While insulin concentrations also are low in IUGR, fetal mice with a genetic knock out of both insulin genes actually have β -cell hyperplasia and more vascularized islets than controls (Duvill et al., 2002).

A nutrient sensitive islet HGF-VEGFA feed forward signaling loop in the fetus is particularly important to test, as it would not only provide a mechanism for the matching of islet growth and vascular supply; but it also would explain how islet growth is integrated with and regulated by the fetal nutrient supply. Given insulin's role as a fetal somatic growth factor (Fowden, 1992), this also would imply that the islet endothelial cell acts in conjunction with the pancreatic β -cell as a fetal “nutrient sensor”, linking nutrient supply to signals for somatic growth.

Amino acids are likely candidate nutrient substrates for initiating or stimulating signaling through this signaling loop. Published work in hepatic cells have demonstrated that leucine stimulation of the mammalian target of rapamycin (mTOR) is essential for producing HGF secretion (Tomiya et al., 2002, 2007). mTOR directly phosphorylates and activates the transcription factor STAT3 leading to increased HGF expression (Kim et al., 2009; Laplante and Sabatini, 2013; Onda et al., 2002; Wojcik et al., 2006). The presence of a feed forward islet HGF-VEGFA signaling loop has not been definitively demonstrated. However, there is evidence for VEGFA stimulated HGF production in adult islet endothelial cells and HGF stimulated VEGFA production in numerous cell types, though not in the pancreatic β -cell (Lin et al., 2012; Matsumura et al., 2013; Moriyama et al., 1998; Van et al., 1998; Johansson et al., 2006).

Importantly, in the PI-IUGR islet the potential for increased signaling through the HGF-VEGFA pathways exists. PI-IUGR islets have normal expression of the HGF receptor cMET and the PI-IUGR islet endothelial cells actually have increased expression of the VEGFA receptor, VEGFR2 (Figure 5) (Rozance et al., 2015). Therefore, if provided appropriate signals, including restoration of a normal fetal amino acid supply, PI-IUGR islet HGF-VEGFA signaling might increase and result in a normalization of pancreatic islet size, vascularity and function.

3. Conclusions

Strategies to treat or prevent human IUGR and the associated complications have produced variable results, including preventative strategies with high maternal protein supplementations that actually increased the severity of fetal growth restriction and were associated with higher fetal mortality rates (Brown et al., 2011). These studies, however, were performed prior to robust animal experiments. It is fundamental, therefore, to understand how the IUGR fetus responds to novel strategies in animal models and determine the mechanisms responsible for such responses prior to developing new treatment strategies to test in human pregnancies. Testing the role of these novel strategies on islet growth, development, and function will be important to determine the mechanisms responsible for improvements in fetal growth. This is because of the role that the fetal pancreatic islet plays in sensing the fetal nutrient supply and secreting appropriate amounts of the fetal anabolic growth factor, insulin. This approach will allow for the design of the most specific therapeutic interventions that will likely be safer for the IUGR fetus than previously used strategies.

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Highlights

Severely growth restricted fetuses have impaired insulin secretion, islet size, and islet vascularity.

These features of growth restricted fetuses may underlie part of their increased risk for developing type 2 diabetes as adults.

Islet HGF and VEGFA signaling represent a potential mechanism linking islet growth and vascularity.

In cases of placental insufficiency, decreased pancreatic islet HGF and VEGFA signaling are potentially responsible for impaired insulin secretion, islet size, and islet vascularity.

Figure 1. Hepatocyte Growth Factor is a necessary and sufficient component of endothelial cell conditioned media to increase islet insulin concentrations

A) Islets from 4 fetuses were divided into endothelial cell (EC) conditioned media (ECCM), ECCM + an inhibitory anti-HGF IgG antibody, and non-conditioned media + HGF (100 ng/mL). Following an overnight incubation the insulin content of the islets was determined and compared to non-conditioned media (dashed line). All conditions included non-specific IgG. * $P < 0.05$. B) Western blotting with the anti-HGF antibody on ECCM demonstrates specificity with a single band (arrow). The entire length of the membrane is shown (indicated by dashed lines). (Rozance et al., 2015)

Figure 2. VEGFA and HGF are lower in PI-IUGR islets and isolated islet endothelial cells, respectively

A) Representative western blots for VEGFA and HGF in pancreatic islet lysates and pancreatic islet endothelial cell lysates, respectively, after loading equal amounts of protein. B) Representative western blot for HGF in EC conditioned media after loading equal volumes of media. C) Quantitative analysis of islet lysate (control, n=10; PI-IUGR, n=4) and islet EC lysates and conditioned media (control, n=3; PI-IUGR, n=4). ** $P < 0.01$. (Rozance et al., 2015)

Figure 3. Media conditioned by isolated PI-IUGR islet ECs does not increase islet insulin content Islets from 4 fetuses were divided into non-conditioned media and media conditioned by ECs isolated from control (Control ECCM) or PI-IUGR (IUGR ECCM) fetal islets. Following an overnight incubation the islet insulin content was determined and compared to non-conditioned media (dashed line). ** $P < 0.01$. (Rozance et al., 2015)

Figure 4. Intra-islet HGF-VEGFA signaling

Figure 5. cMET and VEGFR2 are the same or higher in PI-IUGR islets and isolated islet ECs
A) Representative western blot for cMet and VEGFR2 in pancreatic islet lysates and pancreatic islet endothelial cell lysates, respectively. B) Quantitative analysis of islet lysate (control, n=10; PI-IUGR, n=4) and islet EC lysates (control, n=3; PI-IUGR, n=4). * $P<0.05$. (Rozance et al., 2015)