The Role of the Placenta in Early Programming

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Placenta Functions

The placenta functions as the sole transport mechanism between mother and fetus for all essential nutrients and substances, as well as waste products (Figure).

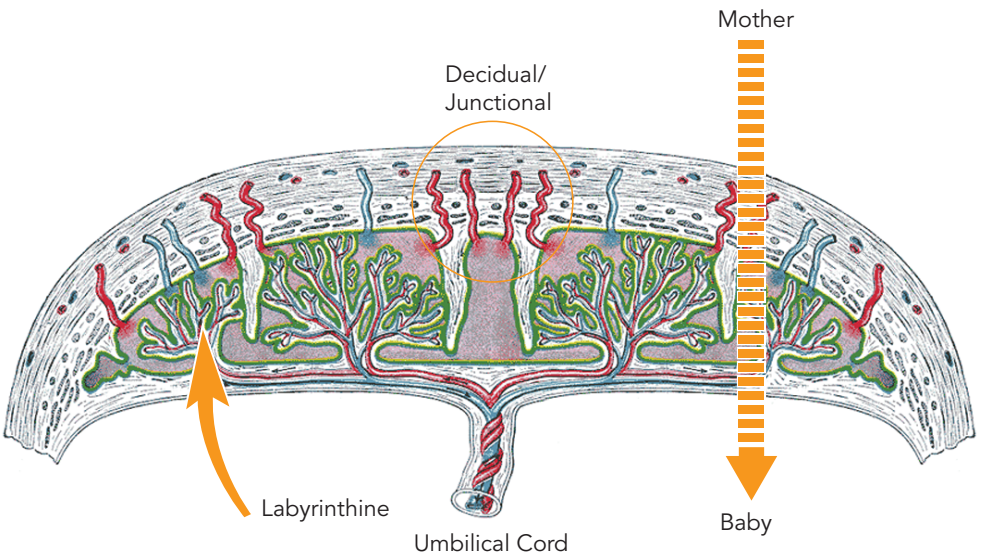

Figure. Placental structure.

The placenta is responsible for transporting oxygen, carbon dioxide, water, and any other essential nutrients. Respiratory gases pass freely from maternal to fetal blood, as do other lipophilic substances, and depend primarily on concentration differences of these substances between mother and fetus. Hydrophilic substances, such as ions, amino acids, and glucose have poor diffusion capacity across the placenta and require transport proteins and ion channels in order to become available to the fetus.[1] The amount of placenta required for adequate fetal development varies across species. The appropriate fetal-to-placental weight ratio in humans is approximately 6:1, while rats have a ratio of 10:1.[2] The rat's ability to produce more fetus per gram of placenta is thought to be due to the rodents' placental countercurrent blood exchange. In this arrangement, maternal and fetal

capillaries are in parallel to each other, and blood flows in opposing directions.[3] Humans have the less-efficient multivillous arrangement, resulting in the need for a larger placenta.[2]

The rates of gestational obesity have been increasing at the same rapid rate as the obesity epidemic in the general population.[4] These statistics are alarming not only because obese pregnancies are accompanied by various birth complications, but also because human and animal studies have shown that obese pregnancy programs offspring for a variety of adult onset diseases. The placenta regulates the transport of all nutrients between maternal and fetal circulation, and is at a marked risk for the accumulation of ectopic fat in instances of obese pregnancy. Although the connection between fetal outcomes and maternal obesity has been irrefutably established, the molecular mechanisms remain undefined. As the principal link between mother and child, the placenta has become an important factor in unraveling these mechanisms.[5,6] Poor placentation has been associated with intrauterine growth restriction (IUGR) as well as small-for-gestational-age (SGA) offspring, gestational diabetes, and possible risks of late-onset diseases.[7-9]

Signaling Pathway

Canonical Wnt signaling is transduced through Frizzled (FZD) receptors and acts through the complexing of nuclear β-catenin with the T-cell factor/lymphoid enhancer factor (TCF/LEF) family of transcription factors, Legless family docking proteins, and pygopus (PYGO) family coactivators to enhance the transcription of target genes.[10-15] Canonical Wnt signaling occurs following the binding of a Wnt molecule and the downstream phosphorylation of Dishevelled (DVL) by casein kinase I-alpha (CKIα) and its binding to Frat. The subsequent assembly of the FZD-DVL and lipoprotein receptor-related protein 5/6 (LRP5/6)-Axin-FRAT complexes releases β-catenin from CKIα and glycogen synthase kinase 3-beta (GSK3β) phosphorylation and allows it to travel to and accumulate in the nucleus.[16,17]

Because of its rapid growth and vascularization, Wnt signaling may have a vital role in the development of the placenta. In fact, of the 19 known Wnt ligands, 14 have been found in the placenta, and 8 out of the 10 FZD receptors have been detected in placental tissue.[18] Numerous studies have explored Wnt signaling in placental tissues and cell culture experiments. β-catenin, the key mediator of canonical Wnt signaling, has been implicated in trophoblast adhesion, survival, and differentiation.[19-21] Other studies have shown that Wnt7a and TCF/LEF1 are required for chorioallantoic fusion,[22,23] and that Wnt2,[24] Wnt3a,[25] Wnt5a,[26] Wnt10b,[26] Fzd5,[26] Sfrp4,[27,28] and Rspo[29,30] may be essential for proper placental vascularization and

growth. Additionally, DKK1, a potent inhibitor of Wnt signaling, has been shown to be involved in trophoblast invasion and migration.[31] Therefore, it appears that many components of the Wnt signaling pathway may have a strong link to the poor fetal outcomes associated with inadequate placental development.

Effects of Obesity on Placenta and Offspring

We examined the mechanisms behind the effect of maternal obesity on placental lipid accumulation and metabolism. Pregnant Obese Prone (OP) and Obese Resistant (OR) rat strains were fed a control diet throughout gestation. Placentas were collected on gestational d21 for analysis of fat accumulation as well as β-catenin and DKK1 localization. Additionally, DKK1 was overexpressed in JEG3 trophoblast cells, followed by treatment with nonesterified fatty acids (NEFA) and Oil Red O stain quantification and mRNA analysis to determine the relationship between placental DKK1 and lipid accumulation. Maternal plasma and placental NEFA and triglyceride (TG) were elevated in OP dams, and offspring of OP dams were smaller than those of OR dams. Placental DKK1 mRNA content was 4 fold lower in OP placentas, and β-catenin accumulation was significantly increased as well as mRNA content of fat transport and TG synthesis enzymes, including peroxisome proliferators-activated receptor-delta (PPARδ), fatty acid transport protein 1 (FATP1), fatty acid translocase CD36 (FAT/Cd36), lipin 1, and lipin 3. There was significant lipid accumulation within the decidual zones in OP but not in OR placentas, and the thickness of the decidual and junctional zones was significantly smaller in OP than in OR placentas. Overexpression of DKK1 in JEG3 cells decreased lipid accumulation and the mRNA content of PPARδ, FATP1, FAT/CD36, lipin 1, and lipin 3. Our results indicate that DKK1 may be regulating placental lipid metabolism through Wnt-mediated mechanisms.

Livers of OP offspring had increased TG content and lipid accumulation compared to offspring of OR dams. Additionally, hepatic DKK1 mRNA content was significantly decreased in OP livers compared to OR livers, and treating rat hepatocyte cells with NEFA showed that DKK1 mRNA was also decreased in NEFA-treated cells. Analysis of the DKK1 promoter in fetal livers showed a pattern of histone modifications associated with decreased gene transcription in OP offspring, which supports our gene expression data. Our results demonstrate that offspring hepatic DKK1 is epigenetically regulated via histone modification in the current model of gestational obesity, and future studies will be needed to determine whether these changes contribute to excessive hepatic lipid accumulation in offspring of obese dams.

Effects of High-Fat (HF) Diet on Placenta and Offspring

In a mouse model of gestational HF feeding, placental weight was unaffected by HF diet, accompanied by increased placental glucose and amino acid transport,[32] while another study using the same model demonstrated that a gestational HF diet led to mixed placental inflammation and oxidative stress (possibly due to increased lipid peroxidation), as well as cellular necrosis and vasculopathy.[33] A recent review of animal models of gestational HF feeding[34] concluded that despite its not being as clear as black and white, there appears to be a risk of diabetes development in offspring of HF-fed dams, independent of maternal obesity. We investigated the effect of a maternal HF diet on fetal genes in the liver that control the production of glucose, and the potential regulatory mechanisms of these genes in an OR rat model to separate the effects of gestational diet and weight. By utilizing an animal model in which dams were not sensitive to the obesigenic effects of HF intake, we intended to confirm that HF feeding alone would have a negative impact on fetal development. In our study, placental DKK1 mRNA content was 4 fold lower in OP placentas.[35] Offspring of HF-fed dams were significantly heavier and had significantly higher blood glucose levels compared to offspring of control-fed dams, which we suggest is a result of their enhanced gluconeogenic capacity in response to the gestational HF diet. While maternal gluconeogenesis and plasma glucose were not affected by the HF diet, offspring of HF-fed dams had significantly higher mRNA contents of gluconeogenic genes in addition to the elevated plasma glucose.

Our results demonstrate that in utero exposure to a HF diet has the potential to program the gluconeogenic capacity of offspring through epigenetic modifications, which could potentially lead to excessive glucose production and altered insulin sensitivity in adulthood.

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Q & A

Q: I have two questions. First, do you think there is a threshold effect between when the response moved from being adaptive to being pathological? You could argue that the change is in fatty acid deposition and that the transport of fatty acid deposition in the placenta is actually adaptive in that it gets good nutrition across to the offspring. It occurs to me that some obese mothers actually have a relatively growth-retarded baby, possibly due to placental malfunction. Does your animal model mimic that in any way?

Dr Pan: Our model shows a 40% decrease of the functional zone in the placenta. The offspring of the obese mothers are actually smaller than those in the other group. If we looked at mothers before pregnancy, I am not sure we would see that what they are lacking was twice as high. We know the obese dams are heavier than obese-resistant dams but we did not do body composition tests to see how much fatter they were.

Q: Is this model pathological rather than macrosomia within the normal range?

Dr Pan: Yes.

Q: Second, in our high-fat model we showed that if we give the mother but not the offspring a statin in late pregnancy, we reverse or prevent most of the typical effects on the offspring later—for instance, changes in appetite and ability to run around and perform daily functions. If you gave a statin to one of your models, could you prevent those placental changes you see?

Dr Pan: That is a possibility. One thing that interests us about this pathway is that it is cell/cell interaction. Potentially we can give a nutrient or drug during pregnancy or earlier to modify the signaling pathway and potentially lessen the impact. For example, we believe that in obese pregnancy, it does not matter what the mother eats. As long as the diet or lifestyle does not change the obese general environment, the impact to the placenta will remain. But if we can use other things such as drugs to reverse that, then the cell/cell interaction will change and potentially we can reverse anything that happened in the placenta because of the change of the pathway.

Q: In your model, you used nonesterified fatty acids and triglycerides. Why did you not use high-density lipoprotein (HDL) particles? We know that HDL is important in vascular function. Dr Hanson mentioned statins, but HDL does the same things to reverse cholesterol.

The topic of hypertension also is important with regard to placental function. Hearing fatty acids and triglycerides discussed without glycoprotein that is related to them diminishes the effect of what you see as relevant to humans.

Dr Pan: One reason we did not use triglycerides in our study is that the placental structures of rat and human are basically permeable to fatty acids, but not to other big molecules. Triglycerides will not go through circulation to the fetus. We do not believe any other form of fatty acid will be seen by trophoblasts and other fetal tissue unless it is sent by the placenta.

Q: Several animal models of high-fat feeding show reduced birth weight. Interestingly, a subgroup of pregnant obese women has small babies, and their risks are greater than that of macrosomic babies. It is interesting to know what the difference is. In human pregnancy we are, perhaps incorrectly, assuming that a small-for-gestational birth occurs because of the inflammatory response early on and reduced placental growth. And those women are predisposed to preeclampsia. My question is about maternal glycemia. You have shown no effects of a high fat diet on maternal glucose and you are attributing the neonatal high glucose to Dickkopf-related protein 1 signaling. Have you rigorously assessed glucose homeostasis in those animals by doing, for example, an oral glucose tolerance test? Overfed mothers often have normal plasma glucose but abnormal resistance in glucose homeostasis.

Dr Pan: We do not have a metabolic lab in our facility to do those kinds of tests.

Q: We have fed rodents a high-fat diet and without the associated obesity we have not seen an effect on fasting glucose, but we do show remarkable insulin resistance and hypertension.

Dr Pan: We do not have any kind of glucose timing going on in our facility. In our model, we do not see gestational glucose increase in either obesity-prone or obesity-resistant mothers. We believe that one of the reasons the obese mothers have smaller babies is that the glucose supply is not adequate.

Q: You can look at the insulin proxy measure without doing a full glucose tolerance test.

Dr Pan: We did measure some of the markers in tissue but did not see significant change in our model.

Q: I meant in terms of plasma rather than tissues.

Dr Pan: Okay.