A pregnant pause: curing placental insufficiency

Dr Helen Jones explains the groundbreaking strategies her lab is using to develop a non-viral delivery mechanism for placental gene therapy in intrauterine growth-restricted foetuses

How has your previous research led to the initiation of your current project?

My PhD and postdoctoral training were both in placental physiology, investigating placental function and regulation in pathological pregnancies, including maternal obesity and nutrient restriction. During my research associateship I was looking at placental gene therapy using adenovirus and wanted to develop non-viral delivery mechanisms for placental gene therapy.

Intrauterine growth restriction (IUGR) has higher rates in the developing world, implicating environmental factors in its initiation. Are its causes well understood?

No. In fact, a large amount of IUGR is idiopathic with no real cause identified. While environment certainly does impact foetal growth, many other factors also influence it and placental causes are thought to underlie a significant proportion of IUGR.

Could you outline some of the negative outcomes and complications of IUGR?

IUGR has both short- and long-term impacts. Low birthweight is highly associated with infant mortality and morbidity, and there is significant evidence that babies born growth-restricted are much more likely to develop adult-onset diseases such as cardiovascular disease and diabetes, a phenomenon known as foetal programming or the Barker Hypothesis.

How are the methods used in your lab unique among efforts to combat the health risks associated with IUGR?

There have not been any previous methods to target the placenta with gene therapy. Traditionally nanoparticles are used to locate and destroy unwanted cells, such as those found in tumours, or else for imaging. Both of these functions use techniques that would be dangerous to a developing foetus. My laboratory employs a biodegradable polymer that in animal studies has no negative impact on the developing placenta or foetus and is removed from the system after gene delivery.

We currently use a mouse model to identify effects on the foetus following the delivery of the nanoparticle. The foetuses are assessed for viability, their weights taken and any signs of inflammatory reaction by the placenta are investigated.

Can you explain why it is desirable to induce trophoblast-specific transgene expression in the placenta, and what the expected outcomes are for the foetus?

Trophoblast-specific gene expression is necessary as we do not want the genes we are...
delivering to the placenta to express in the other organs of either mothers or babies as this may cause problems. The goal is to improve placental function, which in turn would then improve the supply of nutrients and oxygen to enable the foetus to reach its full growth potential.

What are the relative merits of using the BeWo choriocarcinoma cell line or primary human trophoblast from normal term placenta in experiments such as these?

While it is ideal to conduct experiments in primary cells isolated from normal term placentas, the use of the BeWo choriocarcinoma cells, a well-used model of the trophoblast, allows us to study the effects of the nanoparticle in undifferentiated cytotrophoblast without spontaneous fusion of the cells after 48 hours.

What motivates you in your work, and what do you find most satisfying about medical research?

I’m very lucky, as my work has significant impacts in both the short and long term. My samples, the human placentas I use, are given to our researchers by mothers who have just given birth to healthy babies, a joy that currently kick starts the day in my lab. Satisfaction is hard to come by in medical research, as it takes many years to go from a concept through to impacting human lives, but I love taking joy in the little things: identifying a protein important in a certain placental function, and knowing that a nanoparticle can express transgenes in human placental cells keeps driving me forward.

How would you like to see this research developing in the future? How far away do you envisage translation into human clinical trials?

I would like to move the nanoparticle research into a primate model and assess the impact of transgene expression on mother, baby and placenta. I learnt a long time ago that moving basic science into human clinical trials is an endurance race whose length cannot be predicted, and in the current funding environment taking research one day at a time will build towards that goal.

Going non-viral

Research at Cincinnati Children’s Hospital Medical Center is making progress on the road to developing a treatment for a major cause of both short- and long-term morbidity and mortality in newborn babies.

INTRAUTERINE GROWTH RESTRICTION (IUGR) refers to the failure of a prenatal infant to fully achieve its growth potential in the womb, and it is the second leading cause of morbidity and mortality in foetuses and newborn babies. Indeed, current figures show that 5-10 per cent of all pregnancies in the US are complicated by this condition, with even higher rates in lower-income nations. The immediate health risks of IUGR are largely associated with prematurity and the lack of a sufficient oxygen supply, but their effects are not restricted to the short term; the babies that survive such intrauterine complications have a significantly heightened risk of obesity, diabetes, hypertension and coronary artery disease in their adult life.

It is thought that placental insufficiency may underlie up to 75 per cent of all IUGR cases. As the crucial connection between the developing foetus and the mother’s circulation, the placenta is responsible for allowing the uptake of nutrients, the removal of waste, the fight against infection and the secretion of vital hormones during pregnancy. Optimal foetal growth depends on proper placental development. Besides procedures for premature delivery, there are currently no remedies for IUGR, but at the Cincinnati Children’s Hospital Medical Center (CCHMC), exciting new research is taking gene therapy beyond its usual confines to challenge the traditional approaches to IUGR management.

There is currently no way to predict the occurrence of intrauterine growth restriction

MATERNAL CIRCULATION

Using animal models, the Fetal Therapy Group at CCHMC has successfully demonstrated that viral-mediated gene therapy is an effective strategy for correcting IUGR, but when it comes to humans, delivery using viruses and direct placental injection becomes problematic.

Whereas a direct placental injection of insulin-like growth factor 1 (IGF-1) in mice is able to maintain normal foetal development, for humans there is a heightened chance of miscarriage, as demonstrated by amniocentesis and chorionic villous sampling. However, while this method is clearly undesirable, it nevertheless indicates a critical stepping stone toward producing placental gene therapies for humans. With this in mind, Jones has begun to focus her efforts on the development of a placental targeting system that can achieve safe nanoparticle delivery from maternal circulation.

It is not until the last stage of pregnancy, the third trimester, that IUGR is currently identifiable. At this stage, the placental interface in contact with the mother’s circulation is made up of fused placental cells termed syncytiotrophoblasts. It is this nutrient transport layer that needs to be able to remove the nanoparticles from maternal circulation, but nanoparticles and placentas do not – or at least should not – mix. Nanoparticles are generally designed for imaging and targeting purposes, and while they come in very handy for eradicating unwanted cells, they also exhibit high levels of toxicity and accumulation tendencies that make them unfit for placental use.

Dr Helen Jones is Assistant Professor at the CCHMC’s Divisions of General and Thoracic Surgery and Reproductive Sciences. Since the start of her career, Jones’ focus has been the functional aspects of the placenta, and she has received grants from the University of Cincinnati and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) to set up an independent laboratory, the Jones Lab. Exploring the potential for the placenta as a target for in utero treatments, her innovative approach to gene therapy may herald a solution to both the short- and long-term consequences of placental insufficiency.
INTELLIGENCE

GENE TRANSFER TO TARGET INTRAUTERINE GROWTH RESTRICTION

OBJECTIVES

To develop gene therapy techniques to treat intrauterine growth restriction, making use of polymer-based biodegradable nanoparticles that do not put the developing foetus at risk.

KEY COLLABORATORS

Members of the Center for Fetal, Cellular and Molecular Therapy, Cincinnati Children’s Hospital Medical Center (CCHMC)

Dr Neil Ayres, Department of Chemistry, University of Cincinnati

Dr Giovanni Pauletti, Winkle College of Pharmacy, University of Cincinnati

FUNDING

University of Cincinnati, URC Interdisciplinary Grant 2011

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) K99 2012-2014

Eunice Kennedy Shriver NICHD R00 2014-2017

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HELEN JONES received her PhD at the Rowett Research Institute, University of Aberdeen, UK, before moving to the University of Cincinnati as a postdoctoral fellow. In 2009 she moved to CCHMC to pursue a research associateship with Dr Timothy Crombleholme in placental gene therapy. Jones was appointed as a research assistant professor at CCHMC in 2010 and as an independent assistant professor in 2013.

A solution to this problem comes in the form of polymer-based biodegradable nanoparticles, which disintegrate once taken into the cell and present a much lower risk to the developing foetus.

To transport the nanoparticle and its transgene companion to their target, certain measures must be taken to keep them on track – if not, there is a risk of aberrant gene expression as any maternal tissue is capable of taking up the nanoparticles in circulation. Jones’ initial in vitro studies used nanoparticle complexes formed from co-polymers and plasmids modified by the OryP19a or PLAC1 gene promoters; both of these genes contain placenta-specific promoters, making them ideal for reducing off-target gene expression and better at inducing trophoblast-specific gene expression. The Jones Lab is now exploring another method to ensure preferential uptake by the placental syncytiotrophoblast cells: “Currently, we are developing a peptide-targeting sequence that can be attached to the nanoparticle in order to help increase uptake from the mother’s blood supply,” Jones expands. Doing so should mean that the researchers are able to achieve the desired effects while delivering fewer nanoparticles, and initiating the expression of human IGF-1 in the mouse placentas.

RETROSPECTIVE REMEDY

There is currently no way to predict the occurrence of IUGR. Tracking the embryonic journey in the womb relies on sonography so it is only when foetal growth actually becomes impaired and damage takes place that IUGR can be identified. As such, for intraplacental gene therapy to be really effective it has to do more than restore placental function and foetal growth; it has to be capable of reversing the damage that has already taken place.

Previously, gene therapy was performed on mice immediately after IUGR was surgically induced, but to assess intraplacental gene therapy fully, IUGR needs to be well established and foetal growth impacted before the treatment is administered. In preliminary tests, mice with surgically induced IUGR were given a direct intraplacental injection on the sixteenth day of their 20-day gestation period. Of the mouse pups whose placentas were treated with the nanoparticle containing PLAC1 and the human IGF-1 transgene, birth weights were the same as the control group and markedly higher than those with surgically induced IUGR that did not receive treatment.

A LONG WAY TO GO

From having demonstrated altered placental function following viral-mediated gene therapy during initial proof-of-concept experiments, Jones can now report the first successful delivery of a human transgene using non-viral mechanisms into a murine placenta in vivo and human trophoblast cells in vitro with sufficient expression to restore normal foetal growth. Between this breakthrough and the actualisation of an intraplacental gene therapy viable for humans, however, there is a long way to go. “Of course, all projects have hurdles,” states Jones. “Our major ones will come when we try to move away from the mouse model and into something more representative of human.” Indeed, it still remains to be seen how gene expression fares in the long term after nanoparticle delivery, and this is what will set the agenda for studies in the immediate future.

Despite the long road ahead, the research being undertaken by this group shows great promise in developing a treatment for placental insufficiency where none currently exists. As well as a potential weapon to combat the serious ailments associated with IUGR, Jones anticipates the application of intraplacental therapy as a treatment for other major complications in pregnancy, such as pre-eclampsia and congenital birth defects.

Allowing for healthy foetal development, an effective therapy could benefit mothers and babies across the globe, as well as help to cut the huge strains these conditions place on healthcare systems everywhere.