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Fetal stem cell and gene therapy

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A B S T R A C T

Advances in our understanding of stem cells, gene editing, prenatal imaging and fetal interventions have opened up new opportunities for the treatment of congenital diseases either through in-utero stem cell transplantation or in-utero gene therapy. Improvements in ultrasound-guided access to the fetal vasculature have also enhanced the safety and efficacy of cell delivery. The fetal environment offers accessible stem cell niches, localized cell populations with large proliferative potential, and an immune system that is able to acquire donor-specific tolerance. In-utero therapy seeks to take advantage of these factors and has the potential to cure diseases prior to the onset of symptoms, a strategy that offers substantial social and economic benefits. In this article, we examine previous studies in animal models as well as clinical attempts at in-utero therapy. We also discuss the barriers to successful in-utero therapy and future strategies for overcoming these obstacles.

1. Background and rationale

Fetal stem cell and gene therapy have the potential to treat a number of congenital genetic, hematologic, immune, and metabolic disorders that can be prenatally diagnosed, result in significant morbidity/mortality, and lack effective postnatal treatments. Despite the clinical potential of these therapies, clinical success following in-utero hematopoietic stem cell transplantation (IUHCT) has been very limited. There have been no clinical successes following IUHCT for hemoglobinopathies, such as sickle cell disease and thalassemia [1], the two most prevalent target diseases for IUHCT. There have been no clinical attempts of in-utero gene therapy as initial studies to evaluate the safety and efficacy of postnatal gene therapy are being performed. The significant clinical potential and yet lack of success of these therapeutic interventions has led to the development of large and small animal models to study the biology of, and barriers to, successful fetal stem cell and gene therapy. The majority of work over the past 20 years has focused on IUHCT; however, much of the insight gained from these studies as well as the rationale behind IUHCT is applicable to in-utero gene therapy and in-utero cellular therapy with other stem cell populations (i.e. mesenchymal stem cells and amniotic fluid stem cells).

The concept of using the fetus as the recipient of a hematopoietic stem cell (HSC) transplant dates back to 1945 when Owen observed permanent red blood cell chimerism in dizygotic cattle twins that shared cross-placental circulation [2]. Additional studies by Billingham, Medawar, and others confirmed the ability to induce immunologic tolerance to foreign antigen by early gestational exposure to a foreign antigen [3,4]. These studies highlight one of the main rationales for in-utero cellular and gene therapy: the immunologic immaturity of the fetus allows for the introduction of an antigen (i.e. donor HSCs, viral vector, viral vector transgene product) without immunosuppressive conditioning and results in the induction of antigen-specific immune tolerance. The normal developmental ontogeny of the fetus provides for three additional rationales supporting prenatal cellular and gene therapy: (i) the small size of the fetus (30–100 g at 14–16 weeks gestation) compared to a postnatal recipient (~60 kg adult) allows the donor cell dose or viral vector titer per weight of recipient to be maximized; (ii) the fetus allows improved access to hematopoietic niches or target progenitor cells, which is required for successful HSC transplantation or gene therapy, respectively; (iii) delivering a therapeutic cell population or viral vector in utero offers the potential to treat the target disease prior to onset of symptoms.

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2. Prenatal stem cell therapy

2.1. Experimental work in IUHCT

Initial studies of IUHCT in the mouse model involved the transplacental injection of hematopoietic cells into anemic fetal mice containing a c-kit mutation [5,6]. These studies demonstrated engraftment of allogeneic fetal liver and adult bone-marrow-derived hematopoietic cells. The degree of donor erythroid engraftment was dependent on the level of underlying anemia and no donor cell engraftment was noted in nonanemic recipients. Similarly, in the mouse model of severe combined immunodeficiency disorder (SCID) in which there is a T-cell survival defect, allogeneic IUHCT reconstituted the lymphoid compartment of fetal recipients [7]. Although encouraging, early studies in non-diseased mouse models resulted in low levels of allogeneic engraftment (<1%), highlighting competition with endogenous fetal HSCs for available hematopoietic niches as a barrier to achieving high, clinically relevant levels of donor cell engraftment. Overcoming this barrier has been a focus of published and ongoing studies. Advances in injection technique, including the use of the intravascular route, have allowed for higher numbers of donor cells to be delivered with subsequent increases in donor cell chimerism (1–10%) [8]. However, these levels remain below those which are thought to be therapeutic for some target diseases. Recent studies in the mouse model have studied the ex-vivo manipulation of donor HSCs or in-vivo manipulation of endogenous fetal HSCs prior to IUHCT in an attempt to provide a donor cell competitive advantage. Specifically, the CXCR4-SDF-1α and the VCAM1-VLA4 pathways, which are instrumental in engraftment of HSCs following postnatal HSC transplantation, have been manipulated in the setting of IUHCT. Treatment of donor HSCs with Diprotin A, an inhibitor of the peptidase CD26 which breaks down SDF-1α, was shown to enhance homing and long-term engraftment of allogeneic donor cells following IUHCT [9]. Alternatively, inhibiting the above pathways in the fetal recipient was shown to mobilize fetal HSCs out of the fetal liver and allow for enhanced allogeneic donor cell homing and engraftment following IUHCT [10]. In this study, VLA4 inhibition demonstrated a more significant effect on engraftment enhancement than did the inhibition of CXCR4. Finally, fetal intrahepatic injections of anti-C-kit antibodies resulted in the depletion of fetal HSCs and allowed for enhanced congenic engraftment when followed by a neonatal bone marrow transplant [11]. These studies collectively demonstrate the feasibility of manipulating the donor or endogenous fetal HSC to provide a competitive advantage to donor cells and enhance engraftment following IUHCT.

In addition to competition from endogenous fetal HSCs for limited hematopoietic niches, studies have also raised the possibility of an immune barrier to successful allogeneic engraftment following IUHCT [12]. An assumed benefit of the fetal recipient has been the ability to induce donor-specific immune tolerance following allogeneic IUHCT. Tolerance has been shown to occur via partial deletion of donor-specific host T-cells by direct and indirect antigen presentation [13,14]. Residual donor-reactive host T-cells are maintained in a state of anergy via peripheral suppression mechanisms. Despite these findings, with improvement in injection techniques, it became clear that stable long-term engraftment was achieved in 100% of fetuses injected with a congenic donor strain in contrast to only 30% of fetal recipients of an allogeneic donor strain [12]. Detailed analysis of a potential immune barrier to in-utero transplantation demonstrated that the fetal adaptive immune system is still able to undergo tolerance induction as initially proposed by Medawar and Billingham. Instead, the immune barrier to allogeneic engraftment originates from a maternal immune response either via transfer of donor-specific maternal antibodies in the breast milk [15] or transplacental transfer of donor-specific maternal T-cells to the fetus [16]. Furthermore, the potential of an innate, NK cell-mediated immune response to allogeneic donor cells to limit engraftment following IUHCT when initial chimerism levels were low (<1.6%) has also been supported [17].

Clinical application of IUHCT will likely occur via one of two approaches. In the first approach, a single IUHCT will result in sufficiently high levels of engraftment to treat the target disease. Ongoing studies to overcome existing barriers to high levels of donor cell engraftment are being performed with this goal in mind. However, the second clinical application of IUHCT is to induce donor-specific tolerance to allow for a second same-donor non-myeloablative, non-immunosuppressive postnatal transplant to enhance or "boost" engraftment [18]. This approach has proven feasible in normal and disease mouse models, including correction of the sickle cell and thalassemia phenotype, following conditioning with low-dose total body irradiation or busulfan [13,19,20]. Finally, this approach has also demonstrated some success in the preclinical large animal canine model [21]. Future studies investigating alternative, less toxic conditioning regimens will significantly advance the clinical applicability of an approach of IUHCT combined with a postnatal "booster" transplant while also providing relevance for postnatal autologous hematopoietic stem cell (HSC) transplants for non-malignant diseases following ex-vivo HSC correction.

2.2. Large animal and preclinical models for IUHCT

One of the initial studies of IUHCT for potential therapeutic application was performed in the sheep model and demonstrated successful allogeneic engraftment in 75% of recipients of fetal-liver-derived allogeneic donor cells with levels of engraftment as high as 30% [22]. This study engendered a significant amount of enthusiasm for the clinical application of IUHCT which was quickly tempered by failed engraftment following clinical application to multiple diseases including sickle cell disease and thalassemia [1]. Although disappointing, these failures highlighted the need to study the biology of, and barriers to, engraftment in the mouse model as well as in large animal preclinical models. Studies of IUHCT demonstrated minimal or low levels of donor cell engraftment in the goat and non-human primate models [23,24]. In the pig, however, sufficiently high levels of engraftment were achieved to induce donor-specific tolerance and allow successful same-donor postnatal kidney transplants without immunosuppression [25]. More recently, evaluation of IUHCT in the canine model has been more thoroughly studied. The advantages of the canine model include: the presence of inbred and outbred strains of dogs, the presence of canine disease models, available reagents, and, most importantly, the canine model is regularly used to evaluate bone marrow transplant protocols where, among other benefits, it is a reliable model to study graft-versus-host disease (GVHD) prior to clinical application [26]. Initial studies of IUHCT in the canine model by Blakemore et al. [27] demonstrated low levels of engraftment. Subsequent studies in the canine leukocyte adhesion disorder (CLAD) model and CLAD carriers again demonstrated low levels of allogeneic donor cell engraftment following in-utero transplantation via an intraperitoneal route of injection [21]. Importantly, this disease model and the carrier status do not convey a competitive advantage to non-diseased donor cells. Low levels of engraftment were sufficient to ameliorate the disease phenotype in two CLAD dogs and induce donor-specific tolerance to allow for postnatal same-donor "booster" transplants to enhance chimerism to clinically relevant levels in a subset of dogs. Similar to the mouse model, improvements in injection technique as well as new knowledge of a potential limiting maternal immune response have led to improved levels of allogeneic donor cell engraftment in the canine model. Specifically, maternal donor cells injected intravenously (via the

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