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Technical note

Important aspects of placental-specific gene transfer

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ABSTRACT

The placenta is a unique and highly complex organ that develops only during pregnancy and is essential for growth and survival of the developing fetus. The placenta provides the vital exchange of gases and wastes, the necessary nutrients for fetal development, acts as immune barrier that protects against maternal rejection, and produces numerous hormones and growth factors that promote fetal maturity to regulate pregnancy until parturition. Abnormal placental development is a major underlying cause of pregnancy-associated disorders that often result in preterm birth. Defects in placental stem cell propagation, growth, and differentiation are the major factors that affect embryonic and fetal well-being and dramatically increase the risk of pregnancy complications. Understanding the processes that regulate placentation is important in determining the underlying factors behind abnormal placental development. The ability to manipulate genes in a placenta-specific manner provides a unique tool to analyze development and eliminates potentially confounding results that can occur with traditional gene knockouts. Trophoblast stem cells and mouse embryos are not overly amenable to traditional gene transfer techniques. Most viral vectors, however, have a low infection rate and often lead to mosaic transgenesis. Although the traditional method of embryo transfer is intrauterine surgical implantation, the methodology reported here, combining lentiviral blastocyst infection and nonsurgical embryo transfer, leads to highly efficient and placental-specific gene transfer. Numerous advantages of our optimized procedures include increased investigator safety, a reduction in animal stress, rapid and noninvasive embryo transfer, and higher a rate of pregnancy and live birth.

1. Introduction

Abnormal placental development has been identified as a major pathological finding associated with pregnancy-associated disorders, including preeclampsia, intrauterine growth restriction, and placental insufficiency [1–4]. Although the events associated with abnormal development remain under investigation, it is well established that pregnancy-associated disorders can result in preterm birth, a leading cause of death in woman and children [1,2,5–8].

The placenta is the defining feature of all mammals and a unique organ that is formed transiently during pregnancy. It performs numerous functions for the developing fetus including attachment; nutrient, gas, and waste exchange; hormone secretion; ion transport; vascularization; and respiration. The gross defects in placental growth or differentiation can result in embryonic lethality. Understanding the processes that control placental organogenesis is important in determining the causes responsible for improper placental development [1–12].

Developing embryos are not overly amenable to traditional gene transfer techniques. Most viral vectors have a low rate of transduction, can lead to mosaicism, and are prone to gene silencing. Embryo transfer has

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traditionally been performed by surgical implantation. Our data indicate that by combining lentiviral blastocyst infection with nonsurgical intrauterine embryo transfer, we can achieve highly efficient and placental-specific gene transfer in mice. Lentivirally transduced genes have been shown to be expressed *in vivo* and are predominantly free of silencing [13–15].

Lentiviral gene transfer at the blastocyst stage of embryonic development has recently been shown to lead to placental-specific gene transfer [9,10,12,16,17]. Lentiviral transduction specifically targets gene transfer to the trophectoderm, the future placenta, of the developing blastocyst without altering the maternal or fetal genome and provides an important advantage over traditional gene knockouts. Placental transgenesis provides an important tool to evaluate organogenesis and assess new treatments for pregnancy-associated disorders [9–11].

In this study, we have combined and optimized lentivirally induced, placental-specific transgenesis with nonsurgical embryo transfer (NSET). This method provides a rapid, highly efficient, and cost-effective method of embryo transfer to study the role of the placenta in development and disease states [18]. This method also eliminates the complications associated with the use of anesthetics and analgesics that occur during and after traditional surgical embryo transfer, while reducing maternal stress and producing a high rate of pregnancy and live birth [19].

2. Materials and methods

2.1. Materials

M2 media (#NC9460621), M2 powder (#50–582–610), KSOM powder (#NC9505463), and Polybrene (#NC9515805) were all obtained from Fisher Scientific. Nonsurgical embryo transfer devices (#60010) were purchased from Paratechs Corporation. Mouse embryo culture mineral oil (#M8410), acidic Tyrode's solution (#T1788, 100 mL), pregnant mare serum gonadotropin (G4877), and recombinant human chorionic gonadotropin (#C6322) were obtained from the Sigma-Aldrich Co., Metafectene (#T020-1.0) was obtained from Biontex. HIV type 1 p24 antigen ELISA 2.0 (#0801002) was purchased from ZeptoMetrix.

2.2. Induction of superovulation and pseudopregnancy

On arrival, animals were allowed to acclimate at least 1 week before the induction of superovulation and pseudopregnancy. To induce superovulation, C57BL/6 females (4–5 weeks of age) were injected with 5 IU of pregnant mare serum gonadotropin intraperitoneally (IP) at noon and subsequently injected IP 47 hours later with 5 IU human chorionic gonadotropin. The primed females were then introduced to C57BL/6 adult males for overnight mating. C57BL/6 females were checked for a copulation plug the following morning, and embryos were designated as embryonic Day 0.5 (E0.5) (Table 1). To produce pseudopregnant females, vasectomized ICR male bedding was added to the ICR female cages 3 days before mating to help induce estrus. All cages were kept undisturbed until the day of mating when ICR female mice were introduced to the vasectomized male cage. Females were removed the following morning, checked for a copulation plug, and designated 0.5 days post-coitum (0.5 dpc) (Table 1).

2.3. Two-cell isolation and microdrop culture

Oviducts were obtained from superovulated C57BL/6 females at E1.5 for two-cell isolation. The oviducts were teased apart and embryos were collected in M2 media. M2 media was used at room temperature within 2 hours and has a shelf life of 2 weeks at 4 °C. Isolated two-cell embryos were placed in preequilibrated KSOM microdrops and cultured until E3.5. Microdrops were prepared by pipetting five individual 20 μL drops of KSOM media in a p35 tissue culture plate and covered in 2 mL of mineral oil. KSOM culture media was prepared according to the manufacturer's instructions and was preequilibrated overnight at 37.5 °C and 5.5% CO_2. KSOM culture media has a shelf life of 2 weeks at 4 °C after preparation.

2.4. Lentiviral production, concentration, and titer

293FT cells were cultured in Dulbecco's Modified Eagle's Medium/high glucose, 10% heat-inactivated fetal bovine serum (Biowest, S01520), 1% antibiotic-antimycotic (Thermo Scientific, SV30079.01), 1 mM sodium pyruvate (Mediatech, 25-000-CI), 2 mM glutaGRO supplement (Mediatech, 25-105-CI), 0.1 mM nonessential amino acids mixture (Lonza, 13-114E), and 500 μg/mL geneticin sulfate (G418, InvivoGen, ant-gn) and transfected with pLv-[GFP]-V5 (lentiviral green fluorescent protein) and ViraPower Lentiviral Packaging Mix (Invitrogen, K4975-00) using Metafectene as described by the manufacturer. Cell culture media was changed 24 hours after transfection and virus was collected at 48 to 65 hours. Virus containing media was centrifuged for 20 minutes at 3000 rpm at room temperature, and the supernatant was stored at −80 °C. To concentrate lentivirus, viral supernatant (2 mL) was mixed with 5X PEG-it viral precipitation solution (500 μL) (System Biosciences, Lv810A-1) and incubated overnight at 4 °C. The solution was centrifuged at 3000 rpm for 30 minutes at room temperature, and the lentiviral pellet was resuspended in 1XPBS and polybrene (8 μg/mL) in a volume of 20 μL. Dilutions of 1/80,000 and 1/200,000 of the concentrated virus were then used to determine the viral titer *via* use of HIV-1 p24 antigen ELISA 2.0 according to the manufacturer's instructions. Briefly, samples were added to the microplate and incubated for 24 hours at 37 °C. The samples were subsequently washed and incubated with HIV-1 detector antibody for 1 hour at 37 °C. The microplate was washed, substrate was added, and then incubated at room temperature for 30 minutes. The reaction was stopped by adding stop solution and the plate was read within 15 minutes at 450 nm on an ELISA plate reader.

2.5. Blastocyst infection and NSET

Twenty microliters of titered virus (1500 ng/mL) was placed as a microdrop covered in mineral oil and