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Placental Gene Transfer: Transgene Screening in Mice for Trophic Effects on the Placenta

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Abstract

Objective—We hypothesize that gene transfer of select growth factors to the placenta may enhance placental and fetal growth. Thus, we examined the effect eight growth factor transgenes on murine placenta.

Methods—Adenoviral mediated site specific intraplacental gene transfer of eight different growth factor transgenes at e14 was performed. Transgenes included angiopoietin-1, angiopoietin-2 (Ang-2), basic fibroblast growth factor, hepatocyte growth factor, insulin-like growth factor-1 (IGF-1), placenta growth hormone, platelet-derived growth factor-B (PDGF-B), and vascular endothelial growth factor₁₂₁. Fetuses and placentas were harvested at e17 and assessed for survival, gene transfer efficiency, placenta area, and fetal and placental weights.

Results—Efficient gene transfer to the placenta was detected with minimal dissemination to the fetus. Over-expression of IGF-I, PDGF-B and Ang-2 results in an increase in placenta cross-sectional area. Only Ang-2 gene transfer results in increase fetal weight and only Ang-2 and bFGF result in a change in placental weight.

Conclusions—Site specific placental gene transfer results in efficient gene transfer with minimal dissemination to the fetus. Ad-IGF-1, Ad-PDGF-B and Ad-Ang-2 significantly increase placenta growth.

Keywords

IUGR; Placenta; Gene Therapy; Growth Factors

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Condensation:

Site specific placental gene transfer results in efficient gene transfer that can alter placental growth suggesting the role of gene therapy in diseases related secondary to placental pathology.

Introduction

Intrauterine growth restriction (IUGR), or failure to reach a genetically predetermined growth potential, is a major cause of perinatal morbidity and mortality (1,2). In addition, epidemiologic studies have suggested that growth restricted fetuses are also at higher risk for developing diabetes, obesity, hypertension, coronary artery disease and dyslipidemia later in adult life (3-4). Placental insufficiency, or abnormal placental vascular development or function, accounts for up to two-thirds of IUGR (5). Despite the relatively high prevalence of IUGR, the frequent perinatal morbidity and mortality and the potential for serious long term sequelae, there is still no effective treatment for IUGR due to placental insufficiency.

A cause of IUGR may be a placental deficiency in one or more growth factor(s). If such a deficiency could be identified, these cases may be amenable to treatment with exogenous growth factors with the goal of increasing placental size and function to correct IUGR. Gene transfer is an attractive alternative compared to exogenous protein supplementation for achieving highly efficient expression of growth factors (6). This led us to hypothesize that site-specific intraplacental gene transfer of select growth factor transgenes may result in enhanced placental growth and correction of IUGR in an animal model of utero-placental insufficiency. Before this hypothesis can be tested, it is necessary to first select the most effective growth factor for enhancing placental growth. Growth factors selected to be screened have been reported to play a role in either placental or fetal development or are suspected of being deficient in placental insufficiency. These transgenes include: angiopoietin-1 (Ang-1) (7-9), angiopoietin-2 (Ang-2) (7-9), basic fibroblast growth factor (bFGF) (10-11), hepatocyte growth factor (HGF) (12-13), insulin-like growth factor-1 (IGF-1) (14-17), placenta growth factor (PlGF) (18), platelet-derived growth factor-B (PDGF-B) (19-20), and vascular endothelial growth factor-121 (VEGF₁₂₁) (7,10,21). In order to select the most effective transgene for stimulating placental growth murine, we screened these eight candidate transgenes by over-expressing them in normal mid-gestation placenta using adenoviral-mediated site-specific gene transfer. The rationale for using normal mice was that if placental growth could be enhanced in the physiologic state of optimal growth then it would be more likely to be effective in an animal model of placental insufficiency.

Materials and Methods

Adenoviral Constructs

As previously described (22), all constructs used in this study were first generation recombinant replication defective, serotype 5 adenovirus vectors. All adenoviral genomes had either E1 or both E1 and E3 regions deleted. All transgenes were driven by the cytomegalovirus (CMV) promoter. The parental adenovirus was either dl7001 (E1 and E3 deleted), AdEasy-1 (E1 and E3 deleted), in340 (E1 and E3 deleted) or PJM17 (E1 deleted). Viruses included in the study are listed in Table 1. Viruses were expanded in HEK 293 cells and purified by CsCl density centrifugation and desalted using a DG-10 column as previously described (23-24) (Bio-Rad Laboratories, Hercules, CA). The viral particle counts were determined by absorption at A₂₆₀ using a DU-640 spectrophotometer (Beckman Instruments, Fullerton, CA) and stored in 10% glycerol containing 10 mM Tris (pH8.0), and 0.1 mM EDTA. Vectors were tittered in 293 cells and screened as plaque-forming units (PFU) (24). Expressions of all transgenes were measured as previously described (see references in Table 1) by Western analysis, ELISA, RIA, or Northern analysis where applicable.

Animal Model

All animal procedures were preformed under protocols approved by the Institutional Animal Care and Use Committee (IACUC). Time-mated, pregnant Balb/C mice were obtained from

Jackson Laboratories (Bar Harbor, ME). Satisfactory anesthesia was achieved using methoxyflurane inhalation, and with sterile technique a midline laparotomy was made in gestation day 14 mice. The uterus was then exteriorized and supported with warmed moist gauze pads. A direct transuterine intraplacental injection of the adenovirus vector carrying the transgene or the PBS was performed with custom made micropipettes. Glass micropipettes of 100- μ l volume were pulled on a Sutter micropipette puller (Novato, CA, USA) using previously described methods (25). The tip was broken at 100- μ m diameter and sharpened on a diamond wheel (Sutter Instruments). The resulting pipettes were calibrated for 5- μ l volumes. Following placental injection (Figure 1A) the uterus was returned to the abdomen and the incision was closed in two layers. Animals were closely monitored until sternally recumbent.

Experimental Design

Eight adenoviral vectors with different growth factor transgenes were injected into the placentas of e14 pregnant Balb/C (dams: $n=21$, fetuses: $n=160$) mice to determine the effects of select growth factor transgenes on placental growth. A total of 1×10^8 plaque forming units (PFU), of Ad-Ang-1 ($n=17$), Ad-Ang-2 ($n=12$), Ad-bFGF ($n=8$), Ad-HGF ($n=9$), Ad-hIGF-1 ($n=8$), Ad-PIGF ($n=15$), Ad-PDGF-B ($n=6$), Ad-VEGF ($n=39$) or Ad-LacZ ($n=34$) reconstituted in 5 μ l of PBS was injected or 5 μ l of PBS ($n=12$) was delivered to each of the placentas via direct micropipette to the labyrinth part of the placental disc. The technique for optimal distribution was achieved by continuous injection while pulling the micropipette out of the placenta. Animals were harvested at day 17 of gestation. Dams were euthanized under methoxyflurane inhalation and the individual placentas and fetuses were carefully removed by hysterotomy. Viability of each fetus was recorded to assess effect on survival rates. Both the fetuses and placentas were weighed immediately using a Mettler AE50 scale (Hightstown, New Jersey). Fetuses and placentas were subsequently processed and transgene expression was assessed in those placentas and fetuses injected with AdLacZ. All placentas were then analyzed with computer assisted morphometric analysis. Day 14 -17 of mouse gestation are selected since this is the critical period of placental development and fetal growth. During this period imbalance of any of the growth factors mentioned above can impact fetal and placental growth.

Histology

Each fetus and placenta was embedded into individual plastic molds using OCT Compound (Sakura Finetek, Torrance, CA). The fetuses were embedded on their sides and the placentas were embedded so that when sectioned included both the fetal and maternal sides of the placenta. For determination of the distribution and dissemination of transgenes, the slides of those animals injected with Ad-LacZ were stained for μ galactosidase expression. 4 μ m frozen sections were cut and transferred immediately to slides. Sections were then fixed in 0.5% glutaraldehyde/PBS for 10 min, rinsed twice in PBS, 1 mmol/l $MgCl_2$ for 10 min, and immersed in staining solution at 37 °C for 24 h. The staining solution consisted of 1 gm/ml of 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside, 5 mmol/l $K_3Fe(CN)_6$, 5 mmol/l $K_4Fe(CN)_6(X-gal)$, and 1 mmol/l $MgCl_2$ in PBS, pH 7.4. Slides were then counterstained in 0.5% neutral red and cover slipped. Only viable embryos were examined for LacZ expression. For analyses, the stained slides of both fetuses and placentas were examined by light microscopy (under 10X magnification). Distribution and dissemination was determined by the appearance and distribution of a blue color, indicating successful gene transfer and expression of the LacZ transgene.

Morphometric Analysis of Placental Area

For morphometric analyses, slide images were projected, using 10X magnification, by a video camera module into a semiautomatic image analyzer (Phase3 imaging). Placental area was measured using intercept counts of a superimposed grid on each histologic placental section,

as previously described (26). Briefly, each slide was visually divided into three sections. These sections are referred to as the maternal side, the labyrinth, and the fetal side. Within each section, 10 different areas of each section were examined by superimposing a grid of 30 points over each area (Figure 1B). A picture of the grid over the piece of placenta was taken and used in the counting. Therefore, there were 10 areas within each section of the placenta and overall there were 30 different sections examined with each placenta. This was done so that we were able to count the number of grid points within each individual section, in order to obtain a mean number of intercepts and a more accurate description of the overall placenta. On each picture a grid of 30 points was overlaid and points at which the grid points were over tissue were counted. Points where the grid point was over interstitial space were excluded. In this way, we were able to obtain a value to estimate placental area.

Statistical Analysis

An analysis of variance was used to compare differences between control and treated groups. ANOVA was used to compare the experimental groups and the controls. A p value less than 0.05 is considered to be significant. Data are expressed as mean $\pm$ SD.

Results

Survival

All dams for all groups survived to the time of harvest (e17) except in the Ad-VEGF₁₂₁ treated group, which had a 40% (2/5) survival rate. There was 100% survival rate for groups treated with PBS, Ad-IGF-I, and Ad-PDGF-B. There was an 88% survival rate for Ad-bFGF treated animals, a 67% survival rate for Ad-Ang-2 and Ad-HGF treated animals, a 47% survival rate for Ad-Ang-1 and Ad-PlGF treated animals.

Fetal and Placenta Weight Analysis

Only Ad-Ang-2 resulted in a significant increase in fetal wet weight compared to controls (Ang-2 0.88g $\pm$ 0.16, PBS 0.75g $\pm$ 0.1, LacZ 0.69g $\pm$.13; p<.01). No differences in fetal weights were observed between any of the other groups injected with a growth factor as compared to controls. In contrast to its effect on fetal weight, administration of Ad-Ang-2 (0.098g $\pm$.01, p<.05) resulted in a significant decrease in placental weight compared to controls (PBS 0.142g $\pm$.02, LacZ 0.134g $\pm$.02). A similar reduction was observed with Ad-bFGF (0.092 $\pm$.01, p<.05) as compared to control. There are no significant differences in placental weights for any of the other growth factors. Placental and fetal weights are summarized in Table 2.

Distribution and dissemination of Ad-LacZ

LacZ transgene expression, as detected by X-Gal histochemistry is observed in 9 of the 9 placentas. The extent of X-Gal staining varies between the different placentas. Some show expression up to 1/5 of cross-sectioned area of the placenta (See Figure 1C). The least amount of expression observed is a thin line along the track of the injection.

Only 1 of the 9 fetuses whose placentas received Ad-LacZ demonstrated any evidence of β -galactosidase expression, which was found exclusively in a single small cluster of cells in the liver of one mouse. Endogenous β -galactosidase expression could be detected in the intestine of all fetuses. This expression is also noted in fetuses whose placenta was injected with PBS or an adenoviral construct other than Ad-LacZ, suggesting that it is endogenous β -galactosidase activity.

Placental Cross Section Analysis

Ad-IGF-I, Ad-PDGF-B, and Ad-Ang-2 intraplacental gene transfer results in a significant increase in placental cross-sectional area compared to PBS and LacZ controls (Figure 2A). No significant differences in placental grid point counts were observed in placentas treated with Ad-Ang-1, Ad-bFGF, Ad-HGF, or Ad-PlGF compared to controls (Figure 2B). Ad-VEGF₁₂₁ treated placentas were not evaluated because of the high mortality rate of the fetuses in this group.

Intraplacental Injection of Ad-IGF-1

The maternal side of the placenta shows a significantly larger number of grid point counts in animals treated with Ad-IGF-1 as compared to controls. The mean value for number of maternal side grid points for Ad-IGF-1 is 26.43 ± 1.33 compared to 23.37 ± 1.33 for PBS and 23.90 ± 1.25 for Ad-LacZ ($p < .001$). The labyrinth area of Ad-IGF-1 treated placentas again exhibit a significantly higher grid count than controls. The Ad-IGF-1 treatment group has an average number of 28.45 ± 1.42 points compared to 25.57 ± 1.98 for the PBS treated group and 26.19 ± 1.05 for the Ad-LacZ treated group ($p < .001$). Finally, the fetal side of the Ad-IGF-1 treated placentas show a significantly larger number of grid point counts when compared to controls. The Ad-IGF-1 treated group has an average number of 26.71 ± 1.62 points compared to 24.03 ± 1.06 for the PBS treated group and 24.24 ± 1.35 for the Ad-LacZ treated group ($p < .001$). Overall, Ad-IGF-1 treated placentas show a significantly larger number of grid points than either of the controls. The Ad-IGF-1 treated group has an average number of 27.2 ± 1.02 counts compared to 24.32 ± 1.44 for the PBS treated group and 24.78 ± 1.56 for the Ad-LacZ treated group ($p < .001$).

Intraplacental Injection of Ad-PDGF-B—The maternal side of the placenta shows a significantly larger number of grid point counts in animals treated with Ad-PDGF-B as compared to the controls. The mean value for number of grid points counted in this area for Ad-PDGF-B is 25.97 ± 1.64 compared to 23.37 ± 1.33 for PBS and 23.90 ± 1.25 for LacZ ($p < .001$). For the labyrinth area of the placenta, the Ad-PDGF-B treated group again exhibits a significantly higher grid count. The Ad-PDGF-B treatment group has an average number of 27.33 ± 1.83 points compared to 25.57 ± 1.98 for the PBS treated group and 26.19 ± 1.05 for the Ad-LacZ treated group ($p < .01$). The fetal side of placentas treated with Ad-PDGF-B also show a significantly larger number of grid point counts. The Ad-PDGF-B treated group has an average number of 25.63 ± 1.90 intercept points compared to 24.03 ± 1.06 for the PBS treated group and 24.24 ± 1.35 for the Ad-LacZ treated group ($p < .03$). Finally, the overall grid point count for the entire placenta is significantly higher for Ad-PDGF-B as compared to controls. The PDGF-B treated group has an average number of 26.31 ± 1.06 counts compared to 24.32 ± 1.44 for the PBS treated group and 24.78 ± 1.56 for the LacZ treated group ($p < .001$).

Intraplacental Injection of Ad-Ang-2—The maternal side of the placenta shows a significant larger number of grid point counts in animals treated with Ad-Ang-2 compared to controls. The mean value for number of maternal side grid points for Ad-Ang-2 is 26.01 ± 1.85 compared to 23.37 ± 1.33 for PBS and 23.90 ± 1.25 for Ad-LacZ ($p < .001$). The labyrinth area of Ad-Ang-2 treated placentas again exhibit a significantly higher grid count than controls. The Ad-Ang-2 treatment group has an average number of 26.78 ± 1.65 points compared to 25.57 ± 1.98 for the PBS treated group and 26.19 ± 1.05 for the Ad-LacZ treated group ($p < .03$). Finally, the fetal side of the Ad-Ang-2 placentas show a significantly larger number of grid point counts when compared to controls. The Ad-Ang-2 treated group has an average number of 25.61 ± 1.44 points compared to 24.03 ± 1.06 for the PBS treated group and 24.24 ± 1.35 for the Ad-LacZ treated group ($p < .01$). Overall, Ad-Ang-2 treated placentas show a significantly larger number of grid points than either of the controls. The Ad-Ang-2 treated group has an average number

of 26.13 ± 8.1 counts compared to 24.32 ± 1.44 for the PBS treated group and 24.78 ± 1.56 for the Ad-LacZ treated group ($p < .001$). Data is presented in Table 3.

Discussion

This is the first demonstration of direct intra-placental adenoviral-mediated gene transfer employed as a tool to over express a growth factor transgene to stimulate placental growth and development in the mouse. Our findings suggest a significant increase in the placental cross sectional area observed in the Ad-IGF-1, Ad-PDGF-B, and Ad-Ang-2 treated groups compared to controls. It is remarkable that any differences in placental cross sectional area were observed in those normal mice as their placentas would be expected to be at maximum functioning capacity. Histologic comparison between maternal and fetal placental cross sectional areas revealed that both angiogenic growth factors, PDGF-B and Ang-2, result in a significant increase of the cross sectional area of maternal side of placenta as compared to controls. In contrast, IGF-1 had significant effects noted throughout all sections of the placenta. The contrast in which part of the placenta each growth factor stimulated growth could be due to potentially different mechanisms inducing placental growth. This is supported by several studies showing that each of these factors exert their effects by different mechanisms which is gestational age dependent (8,9,18-20). An alternative explanation for our finding of increased placental cross sectional area is that adenoviral infection elicited an inflammatory response resulting in placental edema. However, if the adenoviral vector is causing edema resulting in an increase in intercept counts, there should be a similar increase in counts in all adenoviral treated placentas, including the Ad-LacZ control, which we did not observe. In fact, there was no difference in intercept counts between Ad-LacZ and PBS treated placentas. Placental edema is also unlikely due to the lack of increase in placental weight in adenoviral treated placentas.

No significant difference in placental or fetal weight is observed in groups injected with growth factor transgenes (Ad-Ang-1, Ad-bFGF, Ad-HGF, Ad-hIGF-1, Ad-PlGF, Ad-PDGF-B, Ad-VEGF) as compared to controls. However, significant increase in fetal weight and decrease in placental weights is seen in Ad-Ang-2 as compared to control. These findings could be related to the gestational age at treatment since each of these growth factors have a specific role in fetal and placental growth and development at different time points (8,9,18-20). As for the Angiopoietin family, Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) studies from Ang-1 and Ang-2 knock out mouse showed embryonic death due to abnormal vascular placental development (27). In addition, high levels of Ang-2 mRNA have been observed localized to the endothelial cells in early gestation placentas (7) suggesting the role of Ang-2 in promoting early trophoblast growth, supporting feto-placental vascular development and remodeling the maternal vasculature consistent with our observations (28). Despite all of the above facts, there is still little information about the mechanism and function of Angiopoietin family and other growth factors in placental and fetal development.

As for survival analysis, all dams for all groups survived to the time of harvest (e17) except in the Ad-VEGF₁₂₁ treated group, which had a 40% (2/5) survival rate. This is in sharp contrast to 100% fetal survival rate for groups treated with PBS, Ad-IGF-I, and Ad-PDGF-B, 88% fetal survival rate for Ad-bFGF treated animals, a 67% survival rate for Ad-Ang-2 and Ad-HGF treated animals, and 47% survival rate for Ad-Ang-1 and Ad-PlGF treated animals. The one transgene that appeared to be toxic to both the fetus and the mother was Ad-VEGF₁₂₁. This is consistent with recent reports demonstrating that supra-physiologic expression of VEGF (7, 29-30) by the adenovirus may have induced similar findings in regard to survival. Alternatively, it may be a maternal effect, as doses of greater than 5×10^8 PFU of Ad-VEGF₁₆₅ have been reported to be toxic to non pregnant age matched mice (31). Although the individual dose in our experiment was only 1×10^8 PFU to each fetus, the cumulative dose to the mother exceeded 5×10^8 PFU.

Gene therapy is rapidly progressing as a mode of treatment and prevention in several diseases (32). The rate-limiting step for successful gene therapy is the ability to transfer efficiently and safely the appropriate therapeutic gene to the target tissue with minimal expression and thus toxicity in other organs. In the current experiments, there appears to be minimal gene transfer to the fetus with intraplacental injection technique; however, this is based solely on β -galactosidase histochemistry. Although we observed only a single cluster of LacZ positive cells in the liver in one of nine animals, polymerase chain reaction to detect adenoviral genomic DNA would have been more sensitive.

A major advantage of site specific placental gene therapy is that it provides a specific tissue target where placenta is discarded once the fetus is born. This will minimize the risk of fetal gene transfer to either somatic or germ cell lines. Another advantage of this study is the choice of the mouse as the study animal model. Although the gross architecture of the human and mouse placentas differ somewhat in their details, their overall structures and the molecular mechanisms underlying placental development are thought to be quite similar(33). As a result, the mouse is increasingly used as a model for studying the essential elements of placental development especially in presence of transgenic mouse models bearing in mind the limitations in these models. Moreover, confounding variables such as gestational age for therapy, treatment position and viral dose are factors that may influence the results and will need to be determined. A limitation of this study is the use of solely histology for evaluation of gene transfer efficiency. Despite these limitations, these results provide proof of concept for efficient site-specific placental gene transfer and transgene expression.

Another potential limitation in our study is the selection of vector for gene delivery. The selection of appropriate vector in gene therapy is important since it can affect gene transfer efficiency as well as transgene expression in different tissues. It is likely that adenovirus based vectors are not the ideal platform for placental gene transfer to treat human placental insufficiency because of the inflammatory response they elicit and the relatively short duration of transgene expression (34). In addition, it has been suggested that the presence of wild type adenovirus infection in humans may be associated with spontaneous abortion (35-36). However, as proof of concept, adenoviral based vectors are ideal in short gestation animal models such as the mouse, rat and rabbit because of the relatively rapid onset of transgene expression in relation to the short gestation. In longer gestation experimental animals and in humans, alternative gene transfer vectors may include adeno-associated virus (AAV), lentivirus, or nanoparticles which elicit relatively minimal inflammatory reaction and have significantly longer duration of transgene expression (37-38).

In conclusion, these results taken together provide proof of concept that site-specific placental gene transfer can alter placental growth. While these results suggest the potential of Ad-Ang-2, Ad-IGF-1, and Ad-PDGF-B to enhance placental growth, it is unknown if these transgenes can correct related placental diseases as IUGR secondary to placental insufficiency. Further studies in a model of naturally occurring runting in the rabbit, rat and mice are planned to test this hypothesis. In-vitro studies are also important to assess the placental target of such gene therapy. Site-specific placental gene transfer is a promising technique with considerable appeal as a potential therapy for placental insufficiency for which no therapy is currently available.

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Figure 1. Mouse Placental Gene Transfer

A) Custom made beveled micropipettes were used to deliver vector to the murine placenta. B) Computer generated grids of 30 crosses were superimposed on placental images. Intercept points were assessed and used to derive differences in placental area (H&E stain 10x magnification). C) 1×10^8 PFU of an adenoviral vector with the β -galactosidase transgene injected in the murine placenta results in efficient gene transfer. (Xgal histochemistry with neutral red counter stain, 5x image)

Figure 2. Effects of Growth Factor Over Expression on Placental Growth

A) 1×10^8 PFU of adenoviral vectors encoding for eight different growth factors were delivered to the placenta. Ad-Ang-2, Ad-IGF-1 and Ad-PDGF-B resulted in significantly higher intercept counts compared to AdLacZ and PBS controls. B) In contrast, over expression of Ad-Ang-1, Ad-bFGF, Ad-HGF, and Ad-PlGF did not result in a significant increase in intercept counts. Ad-VEGF₁₂₁ treated placentas were not assessed due to a high mortality rate in this group.

Table 1
Adenoviral Constructs for Intraplacental Gene Therapy

Adenoviral vectors are E1⁻ or E1⁻ and E3⁻ deleted and driven by a CMV promoter. All transgene cDNA are of human origin. Ad-Ang1* is a biologically active modified version of Angiopoietin-1 and with Ang-2 were obtained from Regeneron Pharmaceuticals. Ad-PlGF was kindly provided by Peter Carmiliet (Flanders Interuniversity Institute for Biotechnology, Belgium)

Growth Factor	Abbreviation	Adenovirus	Description	Source
Angiopoietin-1	Ang-1	in340	E1-, E3-Ad5	Thurston G. (39)
Angiopoietin-2	Ang-2	AdEasy-1	E1-, E3-Ad5	Regeneron Pharmaceuticals
Basic fibroblast growth factor	bFGF	dl7001	E1-, E3-Ad5	Nesbit et al (40)
Hepatocyte growth factor	HGF	dl7001	E1-, E3-Ad5	Phaneuf et al (41)
Insulin like growth factor-1	IGF-1	AdEasy-1	E1-, E3-Ad5	Satyamoorthy et al (42)
Placenta growth factor	PlGF	pMJ17	E1-Ad5	Carmeliet Lab
Platelet derived growth factor-B	PDGF-B	dl7001	E1-, E3-Ad5	Liechty et al (43)
Vascular endothelial growth factor	VEGF	dl7001	E1-, E3-Ad5	Samadi et al (44)
β-galactosidase	LacZ	dl7001	E1-, E3-Ad5	Liechty et al (43)

Table 2
Fetal and Placental Weight After Placental Gene Transfer

Fetal and Placental weights were assessed at e17, three days after placental injection. Ad-Ang-2 results in a significant increase in fetal weight ($p < 0.01$). Both Ad-Ang-2 ($p < 0.05$) and Ad-bFGF ($p < 0.05$) result in decreases in placental weight. (p values by ANOVA)

	Fetal Weight(g)	Placental Weight (g)	P Value Fetal Weight	P Value PlacentalWeight
PBS	.750±.10	.142±.02	NA	NA
AdLacZ	.690±.10	.134±.02	NA	NA
Ad-IGF-1	.643±.11	.136±.02	.097	.563
Ad-PDGF-B	.739±.08	.163±.03	.406	.0476
Ad-Ang-2	.888±.16	.098±.01	<.01	<.05
Ad-Ang-1	.783±.08	.128±.01	>.05	>.05
Ad-FGF	.691±.07	.092±.01	.309	.000003
Ad-PGF	.630±.05	.140±.01	.035	.538
Ad-HGF	.622±.09	.133±.02	.071	.5

Table 3
Intercept Counts for Ad-IGF-1, Ad-PDGF-B, and Ad-Ang-2

Placenta were divided into 3 sections, maternal, internal, and fetal. Ad-IGF-1 results in increased intercept counts through out all 3 sections of the placenta. Similarly, Ad-Ang-2 and Ad-PDGF-B result in increased intercept counts in all three section of the placenta but have more pronounced effects in the maternal side of the placenta.

	Maternal Side	P Value	Labyrinth Space	P Value	Fetal Side	P Value	Whole Placenta	P Value
PBS	23.37±1.33	NA	25.57±98	NA	24.03±1.06	NA	24.32±1.44	NA
AdLacZ	23.90±1.25	NA	26.19±1.05	NA	24.24±1.35	NA	24.78±1.56	NA
Ad-IGF-1	26.43±.33	<.001	28.45±.42	<.001	26.71±.62	<.001	27.20±1.02	<.001
Ad-PDGF-B	25.97±.64	<.001	27.33±.83	<.01	25.63±.90	<.01	26.31±1.06	<.001
Ad-Ang-2	26.01±.85	<.001	26.78±.65	<.03	25.61±.44	<.03	26.13±.81	<.001