18

Fetal Gene Therapy

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1. Introduction

Gene therapy can be defined as the process by which a normal functional copy of a gene is transferred into the appropriate cells of an individual with the intent to correct a disease caused by a defect within the individual's own copy of the gene in question [1-3]. Gene therapy is still considered by many to be a relatively new therapeutic modality, having only developed over the last two decades. As such, it is still under intense experimental investigation to prove its therapeutic potential and safety before it can enter the clinic as a first line of treatment. Gene therapy promises to offer a precise means of permanently curing essentially any of the over 4000 currently known so-called monogenic diseases, which are caused by an error in only a single gene. These include, but are certainly not limited to, the hemophilias, lysosomal storage diseases like Gaucher's and Hurler's, hemoglobin disorders such as the thalassemias and sickle cell disease, diseases of immune function such as deficiencies in the shared γc receptor subunit or adenosine deaminase (ADA) deficiency, and cystic fibrosis. It is also anticipated that gene therapy will one day enable the treatment of a host of inherited or acquired disorders such as cancer, AIDS, and many others for which there is currently no cure [4-9].

2. Hemophilia a as an ideal target disease for correction with gene therapy

Among the monogenic disorders, the hemophilias, and hemophilia A in particular, represent ideal diseases to attempt to treat with gene therapy [10-13] because lifelong improvement or permanent cure is theoretically possible after only a single treatment. This is in contrast to current protein replacement-based therapies which, for hemophilia A, consist of frequent intravenous infusions of short-lived FVIII protein concentrates throughout the lifetime of the patient, without ever curing the underlying disease. The severity of hemophilia A is traditionally based on plasma levels of FVIII, with persons exhibiting less than 1% normal factor (<0.01 IU/mL) being considered to have severe hemophilia, persons with 1-5% normal factor moderately severe, and persons with 5%-40% of the normal FVIII levels mild [14-16]. Thus, even with the low levels of transduction that are routinely obtained with the current viral-based gene delivery systems, a marked clinical improvement would be anticipated in patients with hemophilia A, since even low levels of FVIII would likely convert most patients with severe hemophilia to either a moderate or mild phenotype, greatly improving their quality of life. Conversely, even supraphysiologic levels of FVIII as high as 150% of normal are predicted to be well tolerated, making the therapeutic window extremely wide [16]. In the past three decades, the remarkable

progress in the understanding of the molecular basis of the disease, the identification and characterization of FVIII gene, structure, and biology has furthered the interest and feasibility of treating hemophilia A with gene therapy.

Based on the promise of lifelong cure following a single treatment and the encouraging preclinical results in murine and canine models, several clinical gene therapy trials in both hemophilia A and B have been undertaken. In each case, the treatment was well tolerated with the vectors and doses used. Despite the encouraging preliminary data from these studies [10, 13, 15-23], however, the plasma levels of FVIII achieved thus far have been insufficient to free the patients from the need of exogenous factor. In addition, expression of the FVIII transgene was often transient, and none of the trials exhibited a clear relationship between vector dose and resultant FVIII levels. Another major hurdle that has plagued the successful phenotypic correction of the hemophilias by factor replacement therapy is inhibitory antibody formation, which occurs in nearly 30% of patients with severe hemophilia A. The formation of these inhibitors greatly reduces the efficacy of subsequent FVIII infusions, and can ultimately lead to treatment failure, placing the patient at risk of life-threatening hemorrhage. Disappointingly, inhibitor formation has also observed when gene therapy has been used in an attempt to treat the hemophilias, since, as discussed in more detail below, the patient's immune system still "sees" the vector-encoded FVIII or FIX as foreign.

These clinical trials also raised the troubling possibility of inadvertent germline alteration when semen samples were found to be transiently positive for vector DNA [24, 25]. This is in contrast to prior studies conducted in experimental animals [26, 27], reinforcing the importance of the choice of animal model system employed when conducting pre-clinical studies, if one wishes to extrapolate results obtained in their model to what would likely happen in the clinic. The occurrence of insertional mutagenesis-mediated leukemogenesis in children who received murine retroviral vector-transduced hematopoietic cells as treatment for X-SCID [28-30] further supports the need to perform detailed analyses in a clinically predictive animal model prior to moving into human patients, since an adverse event of this nature was never observed in the decades of animal gene therapy experiments that led up to these clinical trials.

3. Animal models for testing gene therapy for hemophilia A

A number of animal models have been developed to evaluate new methods of not only treatment of coagulation disorders, but also the prevention and treatment of inhibitor formation. Dog models of hemophilia with congenital deficiency [31, 32] and mouse models obtained by gene targeting and knockout technology [33] are available to study FVIII function and gene therapy approaches for treating hemophilia A. Therapeutic benefit has been obtained in numerous studies using a variety of vector systems in the murine model [34-39], but phenotypic correction of hemophilia A in the dog has been much more difficult to achieve [40, 41], similar to findings in human patients, underscoring the value of large animal preclinical models for accurately predicting outcome in human patients. Transient hemophilic rabbit models induced by infusion of plasma containing inhibitors have been used to evaluate the effect of different bypass products to factor VIII [42], but this model, while valuable for inhibitor studies, does not accurately recapitulate the human disease, precluding its use for gene therapy studies.

3.1 Sheep as a preclinical model of hemophilia A

The ideal way to test gene therapy-based approaches to hemophilia A and evaluate long-term expression of clinically applicable FVIII-encoding gene therapy vectors would be to use an animal model that both closely resembles the disease process of HA and closely parallels normal human physiology. To this end, between 1979 and 1982, a number of male offspring of a single white alpine ewe at the Swiss Federal Institute of Technology all died several hours post-partum due to severe bleeding from the umbilical cord [43-45]. Daughters and granddaughters of this ewe also gave birth to lambs exhibiting the same pathology. Investigation of the affected animals showed extensive subcutaneous and intramuscular hematomas. Spontaneous hemarthroses were also frequent, leading to reduced locomotion and symptoms of pain in standing up, restricting nursing activity. Stronger injuries resulted in heavy bleeding and intensive pain. Laboratory tests showed increased PTT and FVIII levels (as assessed by aPTT) decreased to about 1% of that of the control animals. Replacement therapy with human FVIII (hFVIII) concentrate or fresh sheep plasma resulted in remission of disease and rapid clinical improvement.

Unfortunately, due to the expense and effort of maintaining these sheep, the Swiss investigators allowed the line to die out, saving only 6 straws of semen prior to allowing this valuable resource to pass into extinction. We recently used a variety of reproductive technologies to successfully re-establish this line of hemophilia A sheep and fully characterized both the clinical parameters and the precise molecular basis for their disease [46-51]. Importantly, chromogenic assays revealed undetectable FVIII activity in the circulation of these sheep, explaining their severe phenotype. In addition, we identified a frame-shift-induced premature stop codon as the molecular cause of the disease, just as occurs in a percentage of human patients with hemophilia A, making this line of sheep unique among animal hemophilia A models, since hemophilia A mice were generated through targeted gene deletion and the hemophilia A dog colonies exhibit a gene inversion.

In addition to the value of another large animal model of hemophilia A and the uniqueness of the mutation, sheep possess many characteristics that make them an ideal preclinical model for gene therapy, both postnatal and in utero. Firstly, sheep are fairly close in size to humans, weighing roughly 8lbs at birth and 150-200lbs as adults, likely obviating the need for scale-up of vector dose to move from experiments in sheep to human trials. In addition, the large size of the sheep, their long life span, and their relative ease of maintenance and breeding make it possible to conduct the long-term studies in large numbers of animals that are necessary to fully evaluate the efficacy and safety issues related to in utero gene therapy. Secondly, sheep share many important physiological and developmental characteristics with humans; for example, the pattern of fetal to adult hemoglobin switching, and the naturally occurring changes in the primary sites of hematopoiesis from yolk sac to fetal liver and finally to the bone marrow near the end of gestation. It is thus not surprising that fetal sheep have been used extensively in the study of mammalian fetal physiology, and results obtained with this model have been directly applicable to the understanding of human fetal growth and development. Thirdly, sheep are outbred, and thus represent a wide spectrum of genetic determinants of the immune response, as do humans. In addition, the development of the sheep immune system has been investigated in detail [52-58], making sheep well suited for studying the immunological aspects of gene therapy for HA. As the immune response to both the vector and the vector–encoded FVIII are likely to play a key role in FVIII inhibitor formation (or lack thereof), this represents an advantage not found in most other models, with the possible exception of the dog. For these reasons, we feel that

the sheep are a particularly relevant model in which to examine fetal gene therapy in general and, in particular, for hemophilia A. An additional unique advantage to using sheep to study hemophilia A treatment is that in sheep, like human, a large percentage of the vWF is found within platelets rather than free in plasma. This is in contrast to dog (in which vWF circulates free in plasma [59, 60]), and may prove important given the vital role vWF plays in the stability/functionality of FVIII.

4. Rationale for performing gene therapy in utero

Importantly, many of the hurdles that have thus far prevented gene therapy from curing patients with hemophilia A, or many of the other diseases that have been investigated, could likely be circumvented by performing in utero gene therapy. At the present time, many of the diseases considered as candidates for gene therapy can be diagnosed relatively early in gestation, making it feasible to begin devising methods for performing gene therapy in utero rather than waiting until after birth. Methods for accessing both the sheep and the human fetus are well established and clinically viable. Indeed, fetal transfusions and in utero stem cell-based therapies have now been performed clinically by numerous investigators for decades, using a variety of protocols, in efforts to treat patients with a number of different diseases [61, 62]. While the stem cell trials have thus far only proven clinically successful in patients with immunodeficiencies, they have also demonstrated that accessing the early gestational human fetus multiple times poses minimal risks with modern imaging and ultrasound-guided delivery procedures[63]. Furthermore, it is important to note that experience and knowledge gained from studies performed in the fetal sheep model were used to design and perform the first curative human in utero transplantation for X-SCID [64], highlighting the value of the fetal sheep model for not only developing clinically viable methodology, but also for predicting clinical outcome. Using these established clinically applicable methodologies to perform gene therapy early in gestation would correct the disease prior to parturition, allowing the birth of a normal healthy baby who, ideally, would require no further treatments. Additionally, following prenatal diagnosis of disease, parents currently have only two options; pregnancy termination or birth of an affected child. In utero gene therapy would provide a much needed third option [65]. Although in vitro embryo screening and selection is a possible solution, this option is not widely available due to both its high cost and the lack of the required technology in developing countries. In utero gene therapy, in contrast, does not require any sophisticated equipment that would not already be in place for prenatal diagnosis. Indeed, several recent studies have now conclusively demonstrated the marked cost-effectiveness of prenatal screening for the hemophilias, even within developing third world countries [66-68].

Looking beyond the hemophilias, it is important to note that many of the diseases that could be treated with gene therapy exert a significant amount of irreversible damage to the patient prior to birth, during embryonic and fetal development. For example, irreversible neuronal damage is associated with inherited metabolic diseases such as Gaucher's, Lesch-Nyhan, and Tay Sachs. In these patients, post-natal gene therapy, while potentially capable of correcting the metabolic disorder, would be of only limited therapeutic benefit, since it could not reverse the damage which the gene defect had exerted during development. This is clearly in contrast to infants born with SCID or other genetic disorders, such as the hemophilias, who could theoretically be cured by postnatal gene therapy. Nevertheless, even in patients with diseases that can be cured postnatally, psychological and financial

benefits exist to argue for performing correction in utero, since it would allow the birth of a healthy infant, who, ideally, would require no further treatments.

5. The fetus as a gene therapy recipient

In addition to the clinical and financial advantages of performing gene therapy prior to birth, numerous aspects of the fetus make it a more suitable gene therapy recipient than the adult. For example, due to their ability to integrate into the genome of the host cell, γ-retroviruses and lentiviruses have received a great deal of attention as gene delivery vectors, since transduction of a long-lived cell could provide lifelong therapy following a single administration. However, one of the main limiting factors to the successful application of these integrating vectors to in vivo gene therapy is the low level of initial transduction and the limited degree of expansion of transduced cells that occurs following gene therapy, since in the adult most cell populations in the body are relatively quiescent unless injury is used to induce cell cycling. In the case of hemophilia A, the primary site of FVIII synthesis under normal physiologic conditions is the liver [69]. Yet, in a mature animal, it is estimated that only 1 in 10,000 to 1 in 20,000 hepatocytes are actively cycling at any given time [70], making it very difficult to obtain meaningful levels of gene transfer unless the gene delivery system mediates extremely high efficiency transduction of quiescent cells, or injury such as partial hepatectomy is employed to induce cell division to enhance transduction and/or drive expansion of the limited numbers of transduced cells. In the fetus, the cells in all of the organs are actively cycling to support the continuous expansion that occurs throughout gestation. Thus, cells such as hepatocytes that are largely quiescent in the adult are far more mitotically active in the fetus. As such, these cells should be far more amenable to genetic correction with vectors requiring cell division. Furthermore, the active cycling of the cells in all of the organs to support the continuous expansion that occurs throughout gestation offers the possibility of achieving expansion of the gene-corrected cells during the remainder of gestation, such that transduction of even small numbers of target cells should lead to significant levels of gene-correction by birth.

6. Immunological advantages of in utero gene therapy

It is important to note that many patients suffer from the genetic diseases being targeted with gene therapy because they have never produced a single specific protein. As a result, their immune system has never "seen" this protein, and, following gene therapy, the cells of the immune system seek to eliminate any cells in the body that are expressing the very protein that could cure the patient of his/her disease. The low levels of gene delivery to the desired target cells and the immune response combine to yield very low levels of expression of the therapeutic protein, and even the small amounts that are produced are often only produced for a short time. In the case of hemophilia A, this cell-mediated immune response to the cells expressing the vector-encoded FVIII gene further complicates the already formidable challenge posed by the formation of inhibitory antibodies to the FVIII protein.

Remaining cognizant of the immune-aspects of hemophilia treatment, it is important to note that, in addition to the ability to target cells which are largely refractory to transduction in the adult, unique immunologic advantages also exist for performing gene therapy in utero. There is a window of time in early immunologic development, before thymic processing of mature lymphocytes, during which the fetus is largely tolerant of foreign antigens. Exposure

to foreign antigens during this period often results in sustained tolerance, which can become permanent if the presence of the antigen is maintained [71]. When one considers that most individuals with a family history of hemophilia would likely go for early prenatal screening during pregnancy to ascertain whether the fetus was affected, it should be possible to perform in utero gene therapy relatively early in gestation. Given these unique immunological advantages presented by the early fetus, one can envision that in utero gene therapy would be an ideal approach for treating hemophilia A, since lifelong tolerance could be induced to FVIII. This would thus ensure that, even if in utero gene therapy was not curative, postnatal gene therapy or protein replacement could proceed safely without the risk of inhibitor formation.

7. Experimental in utero gene therapy studies

With the knowledge that performing gene therapy in utero would provide these advantages over existing post-natal approaches, we have spent the last decade and a half using the fetal sheep model to investigate whether it is possible to exploit the highly proliferative state and relative immuno-naïveté of the early gestational fetus to achieve significant levels of gene transfer by performing a single intraperitoneal injection of a γ-retroviral vector [72-82]. This approach to in utero gene therapy is safe and technically simple, involving only a single injection into the peritoneum of the fetus, and the injection can easily be given under ultrasound guidance, greatly increasing the clinical applicability of the approach. The straightforward nature of this approach enabled us to perform the gene transfer as early as 54 days of gestation (term: 145 days), improving the chances of achieving clinical benefit in diseases with early onset, and potentially allowing induction of immune tolerance to the vector-encoded gene.

8. Hematopoietic system

We focused our initial efforts on assessing whether this approach resulted in transduction of primitive hematopoietic stem/progenitor cells (HSC), since transduced HSC should provide a lifelong supply of gene-modified hematopoietic progeny, enabling long-term correction following a single in utero treatment. Based on the difficulty associated with transducing HSC in vitro without negatively affecting their in vivo engraftability/functionality [83], we reasoned that placing the vector directly in the fetus should conceivably expose all of the HSC present within the fetus to the vector while in their native microenvironment, potentially increasing the levels of gene transfer to the desired target cells. Indeed, in our initial studies, we observed levels of 2-3% gene-marked hematopoietic cells in the circulation [81, 82]. Furthermore, we found that by varying the age of the recipient at the time of gene transfer, we could markedly enhance the levels of hematopoietic cell transduction [75, 84].. If gene transfer was performed at only 54-57 days of gestation, gene-marking levels of 5-6% could be achieved in the peripheral blood, a level that could exert a beneficial effect in at least some genetic diseases. Importantly, these gene-marked hematopoietic cells persisted in these sheep over the course of 5 years of study [81, 82], transgene-positive CD34+ cells could be detected in the bone marrow of these animals several years post in utero gene transfer [85], and bone marrow cells isolated from these in utero gene transfer recipients successfully engrafted the hematopoietic system of secondary fetal sheep recipients upon re-transplantation. These three pieces of data provide

compelling evidence that this approach enabled us to successfully insert genes into the stem cells of the hematopoietic system, suggesting this method could provide lifelong genetic correction.

9. Non-hematopoietic tissues

While transduction of clinically significant levels of HSC within these sheep following a single injection of vector into the peritoneal cavity hinted at the therapeutic potential of this simple approach to in utero gene therapy, the retroviral vectors we employed in these studies did not possess any type of targeting moiety which would restrict transduction to cells of the hematopoietic system. It was not surprising, therefore, when we examined other tissues of the recipients, to find that gene transfer was not limited to cells of the hematopoietic system, but had occurred in essentially all of the organs we examined, including numerous cell types within the liver, lung, and brain [79, 81, 82, 86]. Concomitantly, in utero gene transfer studies performed by other investigators in sheep, rodent, and non-human primate models employing a variety of viral-based gene delivery vectors produced similar results [78-81, 87-105], raising the exciting possibility that in utero gene therapy could potentially be used to treat not only hematologic disorders, but also numerous genetic disorders that affect tissues other than the hematopoietic system. For example, in the case of the hemophilias, this method could likely be used with success to delivering genes for the missing coagulation factors to the developing liver at levels that would covert patients with severe hemophilia to a moderate or even mild phenotype [79]. Moreover, tissue-specific expression is not necessary for factor VIII (the factor deficient in hemophilia A) or factor IX (the factor deficient in hemophilia B). Thus, the transfer of either of these genes into a wide range of tissues with ready access to the circulation, followed by long-term expression, would greatly enhance the therapeutic potential of this approach for treating/curing the hemophilias. Interestingly, although the incidence of hemophilia A is 7x's that of hemophilia B, the only studies that have explored the possibility of performing in utero gene therapy for the treatment of the hemophilias have been aimed at correcting hemophilia B (factor IX deficiency), and all but one group's studies [88, 99] have been performed in mice [87, 100, 101, 103-105], making it somewhat difficult to extrapolate the results to the human clinical setting.

Despite offering many advantages in the treatment of diseases such as the hemophilias, the widespread presence of gene-modified cells throughout the body also underscored the need to carefully examine the safety of this approach to in utero gene therapy, since expression of the transferred genetic material in all tissues may not always be desirable, and, in some cases, could in fact be deleterious, if the transgene in question requires tissue-specific expression. Based on our observations in the hematopoietic system, we first examined whether the developmental stage of the recipient might impact upon which tissues were modified following in utero gene therapy. Our initial results revealed that the liver, like the hematopoietic system, is more amenable to gene transfer at earlier stages of fetal development, leading us to believe that perhaps gene transfer was always most efficient if performed earlier in gestation. However, when we examined the lungs of these same recipients, we discovered that this belief was unfounded. In the lungs we observed exactly the opposite of what we had seen in the hematopoietic system and the liver, namely, that the levels of gene-marked cells were much higher if the transfer was performed later in gestation [79, 86]. These findings thus suggest that each tissue likely possesses its own

unique developmental stage during which gene transfer is optimal. These findings also raised the intriguing possibility that it may be possible to choose, at least to some degree, which tissues will be modified following in utero gene transfer, by carefully selecting the age at which the transfer is performed.

10. Induction of immune tolerance following in utero gene transfer

As discussed previously, one of the major hurdles hindering treatment of the hemophilias by factor replacement therapy is the formation of inhibitory antibodies that can occur with repeated administration of these exogenous factors over time. While analyzing the tissues from the sheep that received in utero gene transfer, we noted that the thymus frequently exhibited transgene-positivity by PCR [81, 82]. Given the pivotal role of the thymus during the development of the fetal immune system's ability to distinguish self from non-self, we undertook studies to ascertain the immunologic significance of the presence of these transgene-positive cells within the thymus. In our first set of studies, [106] we demonstrated that in utero gene transfer successfully induced durable immune tolerance to the vector-encoded β-galactosidase. This tolerance induction appeared to involve both cellular and humoral mechanisms, since both antibody responses and cellular responses were blunted in these animals even several years after in utero gene transfer, providing strong evidence that IUGT induces immune tolerance to the protein product of the transgene. We next conducted studies to begin elucidating the mechanisms responsible for this observed tolerance and to assess whether the recipient gestational age had an impact upon transgene immunity/tolerance induction [107]. Immunohistochemistry revealed that thymic tissue is in fact transduced in the majority of animals following IUGT regardless of the age at which in utero gene transfer is performed. Importantly, however, we only observed transduction of thymic epithelial cells that are crucial for presentation of self-antigen during T cell thymic selection if gene transfer was performed prior to 72 days of gestation [term: 145 days], while after that point in gestation, predominantly CD45+ thymocytes were transduced. These analyses also revealed that, if in utero gene transfer is performed early in gestation, epithelial-like cells comprising the Hassall's corpuscles, as evidenced by their morphology, their CK-positivity, and their expression of thymic stromal lymphopoietin are also transduced. Flow cytometric analysis on the animals that received in utero gene transfer at varying gestational ages revealed that animals that received gene transfer early in gestation had significantly higher percentages of CD4+CD25+ Tregs within their periphery than did control animals or animals transduced later in gestation. These studies thus demonstrate that performing in utero gene transfer early in gestation takes advantage of multiple tolerogenic avenues present in the fetus, since it results in the transduction of both thymic epithelial cells, which may promote induction of central immune tolerance, and cells of Hassall's corpuscles, which can instruct dendritic cells to induce Tregs that can help maintain peripheral immune tolerance to the transgene products. These findings thus suggest that, even if not curative, in utero gene therapy would be ideal for a disease like hemophilia A, since lifelong tolerance could be induced to FVIII, thus overcoming the immune-related hurdles that currently hinder post-natal treatment of this disease. As discussed previously, however, in utero gene therapy studies to date have focused on hemophilia B [87, 88, 99-101, 103-105], rather than hemophilia A, which is intriguing, given the 7-fold higher incidence of hemophilia A, and the fact that patients with hemophilia A are more than 10x's as likely to develop inhibitory antibodies to the exogenous coagulation

factor than patients with hemophilia B [108, 109]. While the choice to focus on hemophilia B is likely due to difficulties encountered in initial attempts to express FVIII as a transgene in the context of viral vectors [110], it nevertheless makes it unclear whether the ability to induce immune tolerance to marker gene products and FIX in utero will ultimately translate into the ability to induce tolerance to FVIII, given FVIII's apparent higher degree of immunogenicity.

11. Potential risk to fetal germline

While gene transfer to the vast majority of the fetal tissues would be desirable for correcting diseases, such as the hemophilias, that would benefit from widespread systemic release of a secreted transgene product, our analyses also revealed that the fetal reproductive tissues often contained the gene therapy vector sequences, raising the troubling possibility that the developing germline might have been modified as a result of in utero gene therapy. Since prior studies had demonstrated that both the embryonic germline [111-114] and isolated primordial germ cells (PGC) [115] can readily be infected with murine retroviral vectors and pass the vector genetic material to subsequent generations in a Mendelian fashion as part of the permanent genome, we used three approaches to examine this important issue in detail: 1) We performed immunohistochemical staining on tissue sections prepared from the in utero treated animals; 2) we performed genetic analysis on the sperm cells from the treated males; and 3) we performed breeding experiments in a limited number of animals [72, 80, 91, 116, 117]. These studies indicated that although the fetal ovaries appeared to be largely unaffected by this approach to in utero gene transfer, numerous cells within the developing fetal testes were in fact modified including interstitial cells, Sertoli cells, and small numbers of both immature germ cells within the forming sex cords and the resultant sperm cells. Importantly, however, gene-modified germ cells were only observed in 2 of the 6 animals examined in our studies, and, in these two animals, the incidence of germ cell modification was roughly 1 in 6250, a frequency that is well below the theoretical level of spontaneous mutation within the human genome [118]. This low frequency of modification coupled with observations that genetic alterations to the germ cells may produce deleterious effects, placing them at a disadvantage during fertilization suggest that the likelihood that any genetic alterations present would be passed to subsequent offspring would be extremely unlikely. In agreement with this supposition, we did not observe transfer of the vector sequences in any of the 10 offspring we studied, even when both the parents had received gene transfer in utero. This is clearly an issue that will need to be addressed in greater detail, nevertheless, prior to moving in utero gene therapy into clinical trials. This need for further investigation is underscored by the fact that, in other studies employing lentiviral vectors in non-human primates, the authors observed modification of the female germline, but no effect upon the male germ cells [90]. Thus, the issue of germline safety will likely have to be investigated in more than one preclinical model, and the specific vector being considered for clinical use will have to be employed, in order to obtain an accurate assessment of the risk posed by the procedure.

12. Conclusions

In conclusion, our findings in the sheep model and those of other groups exploring fetal gene delivery in sheep, mouse, and non-human primate substantial evidence now exists that

in utero gene therapy possesses many advantages over postnatal gene therapy, both from a scientific standpoint and from a socioeconomic/psychological point of view, since it is one of the only therapies that could promise the birth of a normal healthy infant following prenatal diagnosis of disease. Importantly, in our in utero studies, none of the sheep that received murine MoLV-based vector preparations exhibited any type of pathology upon examination, even at time points of greater than 5 years post-transduction. Given the highly proliferative state of the fetus at the time of injection, the transduction of repopulating HSC, and the chance of insertional mutagenesis as a result of MoLV genomic integration, the lack of pathology within these animals is a finding of significance, and suggests that although not ideal, MoLV-based vectors may be relatively safe, at least in this context. Equally importantly, the risk to the fetus appears to be minimal, at least when administering the vector via the peritoneal cavity. Following in utero gene transfer, multiple tissues of the developing fetus were transduced and transgene expression persisted long-term (over 5 years), suggesting that this approach may one day be a viable therapeutic option for diseases affecting any of the major organ systems. Moreover, even if not curative, in utero gene therapy would be ideal for a disease like hemophilia A, since lifelong immunologic tolerance could be induced to FVIII, thus overcoming the immune-related hurdles that currently hinder post-natal treatment of this disease. Despite its great potential, however, it is important to realize that in utero gene therapy is still in the experimental stages and many issues need to be clarified before it can become a clinically viable treatment option for hemophilia A or any of the host of other monogenic diseases. Nevertheless, having recently re-established an extinct line of sheep with hemophilia A [43-45, 47-51] that accurately recapitulate the genetics and clinical symptoms of human patients with severe hemophilia A, we are now in an ideal position to apply our experience with in utero gene delivery and this clinically predictive large animal model to begin developing safe and effective approaches to treat hemophilia A with in utero gene therapy.

13. References

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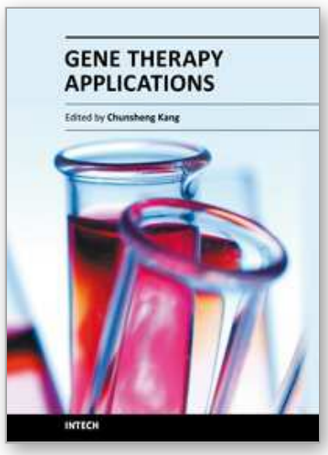

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The aim of our book is to provide a detailed discussion of gene therapy application in human diseases. The book brings together major approaches: (1) Gene therapy in blood and vascular system, (2) Gene therapy in orthopedics, (3) Gene therapy in genitourinary system, (4) Gene therapy in other diseases. This source will make clinicians and researchers comfortable with the potential and problems of gene therapy application.

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