Gene therapy: Regulations, ethics and its practicalities in liver disease

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INTRODUCTION

Gene therapy is a newly developed approach which emerged at the end of the 20th century and aims to treat human diseases based on transfer of genetic materials to cells\[1\]. It involves sets of recently developed technologies such as gene separation and purification, vector choice (viral and non-viral nature), transfer technique, etc\[2\]. This approach develops quickly and has the potential to bring a new era to the treatment of human diseases, though the fierce debate and discussion on its ethics and practicalities in human beings have never stopped since its birth.

Like conventional therapy, gene therapy is under the regulation of the Nuremberg Code (1947) and the Declaration of Helsinki (1964) which established the principal research ethics concerning the vulnerability and interest of the patient as well as the benefit of independent review. However, gene therapy also raises specific ethical issues and public concerns about the three main fronts. Firstly, there exists the risk of deliberate alteration in the human germ line, which may change the inherited nature of human beings. Secondly, various new technologies continue to propel such debates. Taking utero gene therapy for example, it is necessary and feasible in technique, to intervene in some genetic disorders during fetal development, but concerns about the issues of safety, transgenerational risks to the germ line and questions about the fetal awareness, have hampered its development. Thirdly, the impact of adverse effects has kept ethical debate going on. In September 1999, a teenager volunteer, Jesse Gelsinger, died after receiving the adenoviral gene therapy for the treatment of the inherited condition “Ornithine Transcarbamylase Deficiency” at the Penn State University. Three years later, in October and December 2002, the Necker Hospital in Paris announced two boys under gene therapy for X-SCID developed a form of leukemia\[3\]. Further evidence appeared in the same year, when Li Z et al reported that retroviral vectors can cause oncogenic transformation\[4\]. Though we still do not know to what extent the risks described by these cases can be translated to other trials, it has up-regulated the public concern and led to the stringency of safety assessments and patient monitoring in clinical gene therapy trials.

Nevertheless, the increasing problems and difficulties never put off public focus and scientists’ enthusiasm on gene therapy for it has incredible huge potential in the treatment of human diseases and what we have found is only the tip of the iceberg. In the following part, we will review the existing policy and regulations on gene therapy,
especially introduce the point of view from Chinese researchers, and then outline the general information on its application in liver disease and vector choice. Finally, we will focus on gene therapy for hepatic tumors.

**POLICY, ETHICS AND REGULATION ON GENE THERAPY**

In 1988, the European Medical Research Council first declared a formal stance against germline gene therapy, which was followed by the Council of Europe in 1991, for its violation of the basic human right to inherit a natural and unchanged genetic pattern from parents. Against this background, many individual countries have their own policies. Holland (1989) and the USA (1982) both postponed germline gene therapy due to ethical and technical barriers while Germany (1987) made it a criminal offence in a more rigorous policy. However, with the developments in biomedicine, ethical and policy analysis, the notion and practicalities of gene therapy have been under revaluation. Some scientists have pointed out that “gene therapy” may inadequately describe the process and goals of germline alteration and should be replaced by the phrase “human germline genome modification” (HGLGM).

Unlike the ethical problem of germline gene therapy, somatic gene therapy attracts public controversy on its serious and unexpected side effects, which, in its utmost extent, have taken people’s life away. Nevertheless, somatic gene therapy has been shown to be of great value in the treatment of different human diseases, which makes a big challenge for scientists to control its advantages over disadvantages. Another related issue is the moral and risk evaluation of conducting gene therapy research in healthy volunteer subjects for there can be only long term potential risk and side effects with no exact benefits for patients. Anyway, most countries support the moral legitimacy of somatic-cell gene therapy for the cure of disease.

Although gene therapy has the above mentioned risks when applied to human beings, its potential benefits could be released by our efforts. We have already done something to minimize the risks and maximize its treatment value in human diseases. Except for the legislation of general medicines, many nations have developed centralized but non-statutory device on gene therapy especially referring to the ethics. Under this condition, some specific national ethics committees and advisory boards like the USA Recombinant DNA Advisory Committee (RAC), the UK Gene Therapy Advisory committee (GTAC) and the Australian Gene Therapies Research Advisory Panel (GTRAP) were established. The aim of these regulation committees is to steer gene therapy towards the right direction.

It is interesting to note that a PubMed search of the term “ethics gene therapy” provides 1097 citations, and when narrowed to different Western countries there are always still many papers. However, when narrowed to “ethics gene therapy in China”, only two papers appear—one from UK, the other from Germany but none from China. In terms of language restriction and the large population, it is meaningful to introduce the general regulations and ethics’ consideration of gene therapy in China. After search through the Chinese biomedicine web database and Chinese scientific journals database, a total of 42 reviews were retrieved focusing on ethics of gene therapy. To sum up, currently there are no specific national ethics committees and advisory boards in China at different levels and comparing with Western countries, the legislation of gene therapy is still lagging behind, though three regulations including “the management of the safety of genetic engineering”, “the clinical and research control of human somatic gene therapy”, and “the management of human genetic resource” were established and all gene therapy-related activities are under their supervision. In academic arena, gene therapy also seems to be a grey area, where germline gene therapy is forbidden but somatic gene therapy and other therapies involving genetic engineering techniques are under intensive research. To our knowledge, the researches on gene therapy of hemophilia and mediterranean anemia are supported by Chinese government and some breakthroughs have been achieved. It seems that the awareness of and discussion on gene therapy at public level are less fierce than those in Western countries and actually, people are more interested in the beneficial and potential risks of transgenic food, which is closely related to their daily lives.

**GENE THERAPY FOR LIVER DISEASE AND VECTOR CHOICE**

The liver has vital functions in metabolism of lipids, carbohydrates and proteins while liver disease damages the synthesis of plasma proteins and coagulation factors, the clearance of lipids and toxins from serum, the secretion of bile to intestine, etc. Successful gene therapy needs relevant therapeutic gene, appropriate promoter and regulatory elements, effective vector to deliver the transgene into target cells. End-stage liver failure irrespective of its cause has a high morbidity and mortality while conventional medicine has little ability to promote recovery. However, successful gene therapy for liver disease has been achieved in animal models. Introducing the therapeutic gene through adenoviral vector has corrected hyperbilirubinemia in a Gunn rat model of Crigler-Najjar syndrome type 1. Conlon TG et al found that intramuscular administration of recombinant adeno-associated virus (rAAV) vectors expressing short-chain acyl-CoA dehydrogenase (SCAD) could systemically correct the fatty acid oxidation disorder in SCAD-deficient mice. There are many kinds of liver disease which would benefit from gene therapy, though it still has a long way to go.

The successful gene therapy for liver disease largely depends on the development of gene delivery vector which could mainly be divided into viral and non-viral vectors. In the following, we will outline both sub-divisions.

Adenovirus is a double-stranded DNA virus which possesses a combination of features that make them highly suitable as vectors for expression of a heterologous gene. Adenoviral vectors are widely used in experimental gene therapy for cancers and also have a natural tropism...
for the liver and a high efficiency for transferring non-dividing cells[23]. The non-integrating feature makes it a two-edge sword, which, on the one hand, will decrease the risk of oncogenic side effects caused by gene integration and mutation, on the other hand, leads to transient expression in host cells. Recently, the so called high-capacity adenoviral (HC-Ad) vectors, lacking all the viral sequences except for the packaging signals, provides a prolonged transgene expression by escaping the host immune response[19]. Goncalves MA et al[21] have generated a hybrid gene transfer vehicle consisting of recombinant adeno-associated virus (AAV) replicative intermediates packaged in adeno virus (Ad) capsids for stable transduction of large DNA.

Retrovirus is a single stranded DNA virus which has the ability to integrate into the host cells. This characteristic increases the long term expression of the transferred gene in host cells but also results in potential side effects like leukemia caused by insertion mutagenesis. In order to decrease such risks, scientists have been exploring the molecular detail of genome packaging, retrovirus assembly and target site selection[8,19]. Retroviral vectors have another shortcoming in that they cannot easily transferring cells like hepatocytes that do not proliferate actively under physiological conditions. However, the new development of human lentiviral vectors, allowing for the transduction of non-dividing cells and stable gene expression, has shed light on the way forward[29].

Adeno-associated virus (AAV) is a non-pathogenic human parovirus with the deletion of all viral genes except for ITR. This vector exhibits a number of properties, making it an excellent choice of CNS gene therapy[21], for hemophilia[22], lung disease[23], retinal disease[24], etc. Despite its low immunogenicity, toxicity, long-term transgene expression and ability to transduce dividing and non-dividing cells, it has also shown a limited capacity of accommodating foreign genes and to some extent, the oncogenic trait, due to gene integrating[25,26]. There is still a lot of work to do on this specific vector.

There are also other viruses used as vectors like herpesvirus, baculovirus and the list is still expanding[27]. However, the non-viral vector has its own advantages, which could avoid the problems caused by the viral vector such as endogenous viral recombination, oncogenic effects and unexpected immune response[28]. Due to these traits, the non-viral vector has been rapidly developed and categorized into two groups: naked DNA delivery with a physical method like gene gun and electroporation or with chemical carriers such as polymer, peptide and lipid. Though various kinds of non-viral vector have appeared and new transfer techniques are emerging, it still has a long way to overcome its low transfection efficiency caused by extracellular and intracellular barriers and organ specificity[29,30].

**GENE THERAPY FOR HEPATIC TUMORS**

Hepatic tumor ranks fifth in frequency worldwide among all malignancies and causes one million deaths annually[30]. It is hard to cure when its progression precludes surgical resection and other conventional techniques, like transarterial chemoembolization and systemic chemotherapy, are of less help because of their low efficacy and high complication rate. Transfer of therapeutic genes to the tumor or peritumor tissues has opened a new door to the treatment, and great efforts have been made to promote this approach at both preclinical and clinical levels. A large number of methods can be chosen for gene therapy, including activation of tumor suppressor genes, inhibition of oncogene and tumor angiogenesis, promoting specific gene sensitivity to drugs, transfer of oncolytic virus and stimulation of anti-tumor immunity[31].

Interleukin (IL)-12 with a potent anti-tumor activity has been extensively studied during the past decades. IL-12 exerts its function as a bridge between innate and adaptive immune responses by inducing TH1 lymphocytes, inhibiting tumor angiogenesis, activating NK cells and cytotoxic T lymphocytes, and facilitating lymphocytes immigrating into the tumor tissues by up-regulating the expression of adhesion molecules on endothelial cells[32,33]. However, this cytokine is toxic when administrated systematically either as a recombinant protein or as a naked DNA (encoding IL-12)[34]. So what the scientist should do is to find the right way to express IL-12 constrained in local tumor tissue while minimize its systemic toxicity by decreasing the sera concentration.

As a reward for continuous effort, some breakthroughs have been made. Firstly, intra-tumor administration of recombinant adenovirus encoding IL-12 could eradicate neoplastic liver nodules in most of the animal models and increase long term survival. More surprisingly, treating one hepatic lesion also could lead to tumor elimination in a second non-treated hepatic lesion[35,36]. This phenomenon may be explained by the strong hepatic tropism of adenovirus as it escapes from the injected nodule to the general circulation and then infects the whole liver. Second, based on the adenoviral vectors, some changes in vector design have been made to increase the transfection efficiency and decrease the systemic toxicity. Wang et al[37] have generated a gutless adenoviral vector containing a mifepristone (RU486)-inducible system for liver-specific expression of human interleukin-12 (hIL-12) (GL-Ad/RuHIL-12), which allows a prolonged, regulatable, and tissue-specific transgene expression compared with normal adenoviral vectors. Waehler et al[38] demonstrated that intra-tumor adenoviral IL-12 immunotherapy can substantially improve its anti-tumor efficacy and safety profile when a fusion protein of two subunits of IL-12 (scIL-12) is expressed in an adenoviral vector. Dickerson et al[39] have engineered a fusion protein of IL-12 linking the vascular homing peptide CD3RGDCFC to directly target the tumor neovasculature. Significant enhancement of antiangiogenic effect, augmentation of anti-tumor activity, and decreased IL-12 toxicity were observed. Thirdly, different administration ways have been developed and tested. Except for the above mentioned intra-tumor and peri-tumor injection, adenovirus encoding IL-12 given by intra-hepatic arterial route[40] and portal vein route[41] has been also shown to significantly reduce tumor burden and prolong survival. Fourthly, though IL-12-based gene therapy has pivotal anti-tumor effects, the
toxicity caused by inducing interferon gamma production has more or less hampered its application. To solve this problem, scientists are trying to combine IL-12 with other chemokines such as a IP-10, to attract immune effector cells to tumors through IP-10 production and to activate attracted lymphocytes with IL-12[41]. We can also generate IL-12 secreting dendritic cells (DCs) by infecting them with adenovirus encoding IL-12 in vitro and then injecting these engineered DCs into the tumor[43]. This approach has proved extremely effective on liver tumor metastases from colorectal carcinoma[49]. Finally, adenovirus encoding IL-12 also has the ability to induce anti-tumor effects on liver neoplasms metastasized from other organ tumors[41,42]. This effect may be mediated by nonlymphocyte effector cells including macrophages and neutrophils and involve anti-angiogenic chemokines[46].

Besides IL-12 gene therapy for hepatic tumor, there are other methods of gene therapy under exploration. Since p53 is mutated in approximately 50% of human tumors and has an important role in the genesis or progression of hepatocellular cancers, we could use gene replacement therapy of p53 for tumors[47]. It was reported that adenovirus encoding CD40 ligand could induce protective and curative anti-tumor immunity[48]. There is evidence that combination of adenovirus encoding CD40 ligand and naïve dendritic cells can decrease the amount of CD40 ligand while maintaining normal anti-tumor effect levels[49]. Silencing of oncogene or other genes by RNA interference (RNAi) offers a promising approach to the treatment of hepatic tumors[40]. We can also use isolated hepatic perfusion (IHP) to increase the adenoviral vector transfection efficiency and decrease the systemic toxicity[51]. Transfer of suicide gene into tumor tissue is another way of gene therapy. Terazaki et al[52] demonstrated that optimal therapeutic expression level of a suicide gene is a novel concept and a promising method.

Although gene therapy for hepatic tumor has been proved effective in most animals, there is still a lack of information about the efficacy and safety of those treatments in humans. Therapeutic gene, dosage and route of administration, type of vector and tumor itself are all complex ingredients and need careful consideration. Gene therapy is mainly used at the moment as a supplement to conventional treatment, and many trials are carried out in patients with advanced tumors. Thus, shortage of data about early cancer may underestimate the real effect of gene therapy on tumors. However, with the booming of phase I and II clinical trials, we will get enough information for analysis and estimation of gene therapy and this treatment method has a bright future.

CONCLUSION

Gene therapy is a new and powerful tool to correct inherited disorders and treat human diseases. To some extent, this approach can be considered a revolution in the history of medicine for it deepens our vision on the nature of diseases and broadens our methods of treatment focusing on the level of genes and molecules. It can be used not only in the treatment of hepatic tumor which we have described in detail but also in other diseases once there are any changes at gene level. Though animal models and some pilot clinical trials have shown a convincing future, gene therapy is still at its beginning and there is a lot of work to be done. Referring to the techniques, we should improve the transduction efficiency of vectors, increase the duration of therapeutic gene expression, decrease the unexpected toxicity and side effects, test and polish the routes of drug administration, etc. There is also likely a challenge of ethical issues. We should remember that technique itself is innocent, whether it is an evil or a virtue depends on the users. So it is our obligation to establish consummate regulations and policies of gene therapy making it serve human beings. Another problem is how to make gene therapy affordable to ordinary patients. As a new approach, gene therapy is much more expensive than conventional therapy, which is a hurdle for its wider use. How to balance the relationship between gene therapy and conventional therapy is very important. Gene therapy has been used to complement conventional therapy for end-stage diseases that cannot be cured with the latter. With the progress in gene therapy, more and more diseases can be cured at their early stage when conventional therapy is still useful.

In conclusion, despite the difficulties and obstacles, gene therapy has the potential to become a cornerstone of modern medicine.

REFERENCES

13 Conlon TJ, Walter G, Owen R, Cossette T, Erger K, Gutierrez G, Goetzman E, Matern D, Vockley J, Flotte TR. Systemic correction of a fatty acid oxidation defect by intramuscular...