Gene Therapy for Acute Liver Failure

Chuan-Long Zhu1,*, Yu-Wen Li2 and Ren-Tao Gao1

1Department of Infectious Disease and 2Department of Pediatrics, Affiliated Provincial Hospital, Anhui Medical University, Hefei, China, 230001

Abstract: Acute liver failure (ALF) is a life-threatening medical emergency and occurs when the liver rapidly loses its function within a short period. ALF can develop secondary to a variety of causes. Currently, the orthotopic liver transplantation is the “Gold Standard” therapy for the disease. However, due to the limited availability of donor organs and rapid progression of the disease, the mortality of ALF remains high. Therefore, it is imperative to develop novel therapeutic reagents for ALF. Gene therapy by delivering a target gene to the patients appears to be a promising approach for the treatment of ALF. Here, we review the recent advance of gene therapy for ALF, focusing on the three technical elements, animal models, vehicles for gene delivery and technique for gene delivery, which are important for the success of gene therapy as well as the potential targets of treatment for ALF.

Keywords: acute liver failure, gene therapy, viral vector, hydrodynamic gene delivery, small interfering RNA, liver transplantation

1. INTRODUCTION

Acute liver failure (ALF) is a life-threatening medical emergency and occurs when the liver rapidly loses its function within days after the first sign of liver disease, in contrast to chronic liver failure that develops over the course of years. ALF can be caused by many causes, which vary dramatically in different geographic areas and depend on socioeconomic conditions. ALF is mainly caused by viral infections, primarily HBV (hepatitis B virus), HAV (hepatitis A virus) and HEV (hepatitis E virus) in the East and developing countries, such as China and India [1, 2]. In contrast, the majority of ALF (more than 65%) is resulted from drug overdose in the West and developed countries. For example, the overdose of acetaminophen (paracetamol) is the leading cause of ALF in the United States, the United Kingdom and other European countries [3-5]. ALF can also be secondary to infection with non-hepatotropic viruses, including cytomegalovirus, Epstein-Barr virus, adenovirus and hemorrhagic fever virus, vascular diseases, such as Budd-Chiari syndrome and venoocclusive disease, metabolic diseases, such as Wilson’s syndrome, Reye’s syndrome, acute fatty liver of pregnancy and galactosemia, and idiosyncratic drug/toxin reactions [6]. The various etiologies of ALF have been reviewed previously [7-10].

Patients with ALF may display initially mild and non-specific symptoms and signs, including jaundice, nausea/vomiting, fatigue, tenderness in the upper right region of the abdomen or changes in the mental state [11]. They usually develop severe complications within hours to days, such as hepatic encephalopathy, coagulopathy, renal failure, metabolic derangement, inflammation and infection, hemodynamic and cardio-respiratory compromise, rapid leading to multiple organ failures and death [6, 12]. According to the time interval between the jaundice and encephalopathy, ALF is classified as hyper-acute with an interval within 7 days, acute with an interval from 8 to 28 days, and sub-acute with an interval from 5 to 12 weeks [13]. Histopathologically, ALF is closely associated with widespread hepatocellular death resulting from necrosis, apoptosis and both, marked parenchymal inflammation, as well as insufficiency in hepatocyte regeneration [14]. The levels of hepatic changes are proportional to the severity of ALF syndromes and affect the outcome of ALF patients. In clinic, the prognosis of ALF patients is determined by multiple factors, primarily the underlying etiologies, age of the patient, the severity of complications and the time period over which these complications evolved [8]. These factors are also essential in determining the management and therapeutic approaches in clinic. For example, patients with acetaminophen intoxication can survive longer after administration with N-acetylcysteine [15]. Lamivudine, a potent inhibitor of HBV replication, can improve the prognosis of fulminant hepatitis B in some patients [16, 17]. Therefore, the early diagnosis of the disease and etiology and treatment with special reagents can promote the survival of patients with ALF.

Despite advances in multidisciplinary therapeutic interventions developed to target the clinical signs, the natural survival of ALF is still lower than 20% without the orthotopic liver transplantation (OLT) [18], the “Gold standard” treatment for ALF, which significantly prolongs the survival of ALF patients [4, 19]. However, due to the shortage of donor organs and rapid progression of the disease, many patients die before finding the matched donor. To overcome this problem, it is imperative to develop innovative therapeutic approaches. Recent advances in the fundamental understanding of the molecular and genetic basis of ALF make gene therapy to be a promising alternative or complement to OLT. In this report, we review the experimental models of ALF, the gene delivery vehicles and techniques, and the po-
cellular inhibitor of apoptosis (c-IAP), X chromosome–
linked inhibitor of apoptosis (XIAP), TRAF1, TRAF2 and iNOS [125]. On the other hands, the activation of NFκB in Kupffer cells promotes the activation of macrophages, which secrete pro-inflammatory cytokines, augmenting the liver injury. Hence, targeting of NFκB activation in macrophages may benefit the ALF patients. Indeed, the splenic introduction of NFκB decoy double-stranded oligodeoxynucleotides (ODN) inhibited the hepatectomy-induced NFκB activation and associated inflammation and ALF[126].

**Osteopontin (OPN)**

OPN is a multifunctional cytokine and can promote the adhesion and migration of inflammatory cells, contributing to the pathogenic process of autoimmune diseases, such as rheumatoid arthritis (RA), multiple sclerosis (MS), Crohn’s disease, and fulminant hepatitis [127]. The OPN expression was up-regulated in the proliferated bile ductules of the liver and correlated with the degree of liver inflammation in animal model of ALF [128]. The high levels of serum OPN were a strong indicator of the poor prognosis of ALF patients [129]. The up-regulated expression of OPN in the liver was also observed in a ConA-induced ALF mouse model, indicating the pathogenic role of OPN in the development of ALF. Actually, treatment with the OPN-specific siRNA inhibited the ConA-induced liver injury in mice [130].

**Genes Regulating Liver Regeneration**

Retarded liver regeneration is an important mechanism in the pathogenesis of ALF. Multiple cytokines, including TNFα, lymphotoxin β (LTβ), IL-1 and IL-6, and growth factors, such as hepatocyte growth factor (HGF), transforming growth factor α (TGFα), epidermal growth factor (EGF), hepatic stimulator substance (HSS), insulin and glucagon, are suggested to induce and promote the proliferation of hepatocytes in response to liver injury, respectively; while TGFβ inhibits liver regeneration [131]. These factors are the potential targets for the gene therapy of ALF.

**HGF**

HGF is an important mitogen for hepatocytes regeneration, which is corroborated by the clinical observation that the enhanced levels of serum HGF are a useful biomarker for the early diagnosis and prediction of prognosis for patients with ALF [132]. Adenovirus-mediated HGF expression in the remnant lobe of the liver ameliorated liver fibrosis and improved the survival of mice in a mouse model of liver failure [133]. Paradoxically, although patients with ALF have extremely high levels of serum HGF they have little hepatocyte regeneration. The failure of high levels of serum HGF to promote hepatocyte proliferation may come from the lack of HGF receptor (c-met) expression on hepatocytes and/or the existence of an inhibitor of HGF. Conceivably, genetic approach to induce the expression of c-met or directly stimulate the post-receptor signaling should overcome the pitfall and effectively induce hepatocyte regeneration [134].

**IL-6**

IL-6 is a regulator of the liver regeneration, mainly through the naturally existed soluble IL-6 receptor (sIL-6R). Galun et al. demonstrated that a chimeric fusion protein, hyper-IL-6, of the human IL-6 protein and a truncated form of its receptor promoted the regeneration of hepatocytes and enhanced the survival of ALF rats [135].

**CONCLUDING REMARKS**

Studies in animal models of ALF have provided new insights into understanding the clinical manifestations of ALF, the mechanisms underlying the pathogenesis of ALF and numerous gene targets for potential therapy. The validation and clinical translation of these gene targets depend on the selection of safe and effective gene-expression vector and gene delivery technique, followed by stepwise scrutiny from *in vitro* cell system, *in vivo* animal model to clinical patients. This review has focused on the four key elements for the successful development of a gene therapy for ALF: animal model, gene delivery vehicle, gene delivery technique and potential target genes. Obviously, other factors may be also important in the pathogenesis of ALF. In addition, we have to remember the great difference between experimental animals and human patients, which may make it difficult to translate “success” from animal models to human patients. Currently, individual animal models have their own advantages and limitations, representing the unique pathological process of ALF. The various genetic targets tested so far are classified into four categories: apoptosis, necrosis, inflammation and liver regeneration. Given that multiple factors contribute to the development of ALF, we propose the simultaneous targeting on multiple pathological factors by gene therapy may be a promising strategy for intervention of ALF. For example, targeting fgl2 for inhibiting necrosis, together with that on Fas to reduce apoptosis and on HGF to promote liver regeneration may synergistically block the progression of ALF. Of cause, we should recognize that a single gene product usually has multiple functions. When testing a potential therapeutic gene, we should observe its biological functions and monitor the gene therapy-associated side effect, ensuring the therapeutic safety. In addition, ALF is a rapidly progressing situation in clinic it is important to evaluate the therapeutic effect of gene therapy after the establishment of ALF.

Gene therapy for treating ALF is still in its infancy. Currently, there is no large-scale clinical trial to test a specific gene. However, gene therapy holds great promise in inhibition of the disease progression, benefiting the ALF patients. Potentially, gene therapy may become an alternative or at least complement to OLT for intervention of ALF patients.

**ACKNOWLEDGEMENTS**

This study was supported by the grants from the National Natural Science Foundation of China (NSFC, 30800973) and the Education Commission of Anhui Province of China (No. KJ2008B067).

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>Adeno-associated virus</td>
</tr>
<tr>
<td>ALF</td>
<td>Acute liver failure</td>
</tr>
<tr>
<td>ASGP-R</td>
<td>Asialoglycoprotein receptor</td>
</tr>
<tr>
<td>ASO</td>
<td>Antisense oligonucleotide</td>
</tr>
</tbody>
</table>