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Review

Gene Therapy for ALS: Progress and prospects

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Abstract

Amyotrophic lateral sclerosis (ALS) is a devastating disease for which there are no effective drug treatments to date. Recent advances in Gene Therapy open up the possibility of developing an effective treatment aiming at halting or delaying the degeneration of motor neurons. Viral vectors such as lentiviral vectors and adeno-associated virus can transfer genes into many different types of primary neurons from a broad range of species including man and the resulting gene expression is long-term. Numerous animal studies have now been undertaken with these vectors and correction of disease models has been obtained. These vectors have been refined to a very high level and can be produced safely for the clinic. However, we believe that there are some major issues that need to be addressed in order to see a Gene Therapy approach with viral vectors proceed to the clinic for ALS patients. This review will describe the general features of lentiviral vectors. It will then describe some key examples of gene transfer and genetic correction in animal models of motor neuron disease. The prospects for the clinical evaluation of lentiviral vectors for the treatment of human motor neuron disease will be outlined.

Keywords: Gene Therapy; ALS; Motor neuron; Viral vector; Animal model

1. Introduction

Gene Therapy approaches involving the use of viral vectors offer a promising strategy for delivery of genes to enhance motor neuron survival. Vectors based on lentiviruses and adeno-associated virus (AAV) are proving to be particularly effective for the treatment of neurological disorders [1,2]. These vectors are attractive for their simplicity and their high transduction efficiency for neurons [3]. Here we focus on lentiviral vectors which have several advantages for the treatment of neurological disorders. These vectors have been refined to a very high safety and efficiency levels [4–6]. The following properties make them suitable for Gene Therapy approaches: (i) Lentiviriral vectors efficiently transduce not only dividing but also non-dividing cells, such as neurons [7–10]. (ii) They have a large cloning capacity (8–10 kb). (iii) They integrate genes into the chromosome of the target cells, leading to stable long-term expression [4,9,11–14]. Long-term and stable expression at therapeutic levels is often essential for gene delivery to treat neurological disorders. Lentiviral vectors maintain expression for up to 16 months [15,16]. (iv) Based on rodent and monkey studies, lentiviral vectors do not give rise to immunological complications that can compromise the viability of the transduced cells [10,11,15]. However, there is no sufficient evidence about the lack of these complications in man. Human studies are therefore needed to address this issue, in particular, whose investigating the effects after repeated administration of these vectors. Finally, lentiviral vector transduction does not appear to affect electrophysiological properties of neurons [17].

2. Lentiviral vectors-mediated gene transfer to the nervous system

Transduction of neuronal cells using lentiviral vectors both in rodent and primate has been widely demonstrated [1,18]. Lentiviral vectors can be split into two groups, the primate such as those based on human immunodeficiency virus (HIV) [14], simian immunodeficiency virus (SIV) [19] and non-primate such as those derived from equine infectious anaemia virus (EIAV) [20] and Feline immunodeficiency virus (FIV) [21]. There has been a continuing evolution of the design of lentiviral vectors with the trend being to remove as much viral sequence as possible from the transfer genome. Surprisingly, it was possible to construct efficient vectors

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that lacked the lentiviral Tat gene and this has lead to the creation of very simple vector systems. This is important because the accessory and regulatory genes are biologically active and can cause detrimental effects on cells including oncogenesis (*tat*), apoptosis (*vpr*), MHC down regulation (*nef*) and differentiation (*vpu*) (for a review see [22]). Vectors lacking these genes are called minimal vector systems and they have been well developed from HIV-1 [23,24], EIAV [20], FIV [21,25] and similar strategies are being applied to other lentiviruses e.g. SIV [26] and all such vector systems share similar features.

Lentiviruses can be efficiently pseudotyped with different envelopes such as VSV-G [9,27–29], rabies-G [9,30], Mokola and Ebola [28,29] filovirus envelope protein [31] and the challenge virus standard (CVS) [32]. Currently, the HIV and EIAV-based vectors are the most attractive gene delivery systems with respect to neuronal tropism, long term expression [2,33], sustained transduction of skeletal muscles [34] and retrograde transport from muscle to spinal motor neurons [9]. The VSV-G pseudotyped lentiviral vectors expressing the reporter gene or therapeutic genes have a broad capacity to transduce a wide variety of cell types in vitro, but exhibit a preference for neuronal phenotypes in vivo. It was further demonstrated that the rabies-G pseudotyped EIAV vectors allowed entry into the central nervous system and transduction of spinal motor neurons after an intramuscular injection in rodent [9]. Thus this technology allows non-invasive peripheral administration of therapeutic genes and consequent transduction of spinal cord motor neurons from muscle delivery. These observations present novel opportunities in designing therapeutic strategies for different motor neuron disease.

Recent studies have demonstrated the remarkable efficacy of Lentiviral-mediated Gene Therapy approaches to neurological disorders [2,35]. Numerous animal studies have now been undertaken with these vectors and correction of disease models has been obtained. For example, in vivo lentiviral delivery of the antiapoptotic gene Bcl-xL or nerve growth factor gene has been shown to prevent degeneration of cholinergic basal forebrain neurons [36]. Furthermore, lentiviral delivery of glial-cell line derived neurotrophic factor (GDNF) efficiently prevented the loss of dopaminergic nigrostriatal neurons in rodent and monkey models of Parkinson's disease [11,15,27]. Lentiviral vectors also provide a new strategy for in vivo modeling of human diseases; for example the lentiviral-mediated overexpression of mutated human α-synuclein or huntingtin genes in basal ganglia induces neuronal pathology in animals resembling PD and HD in man [37,38]. Recent reports have demonstrated the utility of these vectors for arrayed screens and its application to biological discovery [39]. These studies provide great optimism for the future utility of lentiviral vectors in research and therapy of neurodegenerative diseases.

3. Gene Therapy for ALS

Motor neuron diseases (MNDs) are a group of rapidly fatal conditions characterized by paralysis and a variety of other motor deficits. The most well-known MND is ALS also known as Lou Gehrig's disease. It was first defined by Charcot in 1874 [40]. It typically afflicts individuals in middle adult life, leading to paralysis and death within 3 to 5 years [41]. ALS is a progressive neurodegenerative disease that mainly results from selective degeneration of motor neurons in the cortex, brain stem, and spinal cord [42]. About 10% of the ALS cases are familial, and 20% of those are associated with dominantly inherited mutations in *SOD1*, the gene that encodes human CuZn-superoxide dismutase (SOD1) [43]. Transgenic mice over-expressing a mutated form of human SOD1 develop a severe and progressive MND closely resembling the human disease [44,45] and represent therefore the best animal models for ALS. Deleterious effects of the mutant SOD1 protein arise through a novel and yet unknown process. Multiple lines of evidence from cell cultures and transgenic animal models indicate that the mutant SOD1 gene acquires toxic gain of function, but the protein still retains normal enzymatic activity and stability [45]. Several mechanisms by which expression of mutant SOD1 may lead to chronic motor neuron degeneration have been suggested. The 4 key pathogenetic hypotheses comprise genetic factors, oxidative stress, glutamatergic toxicity and protein misfolding/aggregation (for review [46,47]).

Despite worldwide efforts ALS patients have no significant treatment options, in part because ALS has complex etiology that remains insufficiently understood and methods to deliver therapeutically active neuroprotective growth factors are still inefficient. Riluzole, an inhibitor of synaptic glutamate release, is so far the only approved drug for treatment of human ALS [48,49]. In mice overexpressing the mutated G93A form of the superoxide dismutase-1 (SOD^{G93A}) gene—a widely used animal model of ALS [44], riluzole modestly (by an average of 10%) prolonged survival without affecting disease onset [50]. Several pharmacological agents, including caspase inhibitor zVAD-fmk, creatine, minocycline, celecoxib and arimoclomol, were tested in animal models of ALS and promising effects were observed [51–55]. A number of these drugs are currently being investigated in clinical trials as potential therapy for ALS [56–59].

4. Lentiviral-mediated neuroprotection for motor neurons

The isolation and cloning of a number of neurotrophic factors has created new possibilities for the treatment of neurodegenerative diseases. Neurotrophic factors are naturally occurring proteins which are essential for neuronal survival and differentiation during development, but also for the maintenance of normal function in the adult nervous system. Several neurotrophic factors including vascular endothelial growth factor (VEGF), insulin-like growth factor-I (IGF-I) and glial cell line-derived neurotrophic factor (GDNF) were evaluated in experimental models of ALS [30,60,61]. VEGF and IGF-I were found to be most efficacious [30,61,62]. However when administered either systemically or into the cerebrospinal fluid of ALS patients, trophic factors such as GDNF and IGF-1 proteins failed to prevent motor neuron death and resulted in undesirable side effects [63–65] limiting dosage options. However, the localised delivery of therapeutic molecules using Gene Therapy may circumvent some of the toxic side effects that result from the bolus administration of recombinant proteins and may increase their bioavailability.

Gene Therapy approaches expand the therapeutic options for the treatment of motor neuron disease. This is because potentially any gene product can be delivered without the need to manufacture

recombinant proteins. Potentially therapeutic genes include those encoding neurotrophic factors, growth factors or anti-apoptotic genes. Gene Therapy can be used to restore beneficial protein levels by gene replacement or to deliver proteins or nucleic acids that can inhibit deleterious gene expression.

Several studies have shown the remarkable ability of lentiviral vectors to transduce motor neurons both in vitro and in vivo [30,66,67]. Recently, lentiviral vectors have been extensively used as a means to perform therapeutic strategies in animal models of motor neuron diseases such as ALS and SMA [30,68–70]. For example, when using the lentiviral gene transfer system to deliver vascular endothelial growth factor (VEGF) to motor neurons in the well established $SOD1^{G93A}$ mouse ALS model, VEGF treatment prevented cell death and prolonged survival of $SOD1^{G93A}$ mice [30]. Most importantly, lentiviral-mediated Gene Therapy achieved one of the highest therapeutic effects reported in the field to date. We have also reported that lentiviral vectors encoding for human survival motor neuron (*SMN*) was successfully used to restore SMN protein levels in SMA type 1 fibroblasts [68]. The same vector system was used to treat SMA type 1 mouse model. The treatment restored SMN to motor neurons, reduced motor neuron death and increased the life expectancy compared to control groups [68]. On the other hand, Hottinger and colleagues showed that using the facial nerve lesion model in adult Balb/C mice, expression of GDNF close to motor neuron cell bodies of the facial nucleus using a lentiviral vector system leads to a complete protection against lesion-induced death [67]. Taken together these data reveal that Gene Therapy approaches have been successful in animal models of motor neuron diseases including ALS.

5. Interfering RNA as a therapy for familial ALS

RNA interference (RNAi) is a biological process based on naturally occurring phenomenon in organisms such as plants, *Caenorhabditis elegans*, *Drosophila* and mammals. RNAi mediate highly specific gene silencing and provides an exciting prospect as a novel therapeutic strategy for a wide range of disorders. Several research groups can now create small inhibitory RNA molecules to silence the expression of genes involved in genetic disorders. Although small interfering RNA (siRNA) has now been validated as a powerful research tool, advancing such an approach to therapeutic application presents a number of problems. Selection of an efficient siRNA target site is not as simple as choosing any 21 nucleotide sequence along the length of a gene. Different siRNA sequences have different efficiencies at mediating target gene silencing and indeed may have no effect whatsoever. Fortunately, key parameters that are necessary to design an efficient siRNA molecule are becoming clear and these include G/C content, RNA stability at the 5′-antisense end, lack of inverted terminal repeats and the position of particular bases at critical sites along the siRNA sequence [71,72].

Probably the most significant problem to the advancement of siRNA from a research tool to a therapeutic agent is the identification of efficient delivery methods to enable efficient and controlled gene silencing within the relevant target cell populations. Systemic administration of relatively large volumes of siRNA using high-pressure tail vein injection was previously demonstrated to mediate silencing of target gene expression in the liver and to a lesser extent in the lung, kidney, spleen and pancreas [73,74]. However, gene silencing was transient and uncontrolled. More recently chemical modification of siRNA was demonstrated to increase stability of siRNA following systemic delivery and mediate a significantly higher efficacy of target gene silencing compared with delivery of 'naked' siRNA molecules [75]. The development of viral vectors to express short hairpin RNA (shRNA) molecules which are processed into functional siRNAs molecules in the host cell provides a promising approach for therapeutic delivery of siRNAs [76]. Such vectors provide the potential for long-term expression of functional siRNA molecules and incorporation of regulatable elements has allowed regulated expression of siRNAs and subsequent gene silencing to be achieved [77,78].

Several dominantly inherited diseases provide particularly good candidate disorders for investigating siRNA-based therapies. Critically, some of these diseases have particularly relevant animal models, which provide a good platform for determining potential therapeutic efficacy. Some impressive efficacy data are emerging, in particular the use of viral vectors for RNAi delivery to treat the dominantly inherited neurological disorders, including Huntington's disease and related polyglutamine disorders, dystonia, and familial Alzheimer's disease [79–82]. Approximately 20% of familial cases of ALS are caused by dominantly inherited mutations in the gene encoding cytosolic superoxide dismutase (SOD1) conferring a toxic gain of function to this protein [43,44]. Silencing of mutant SOD1 expression provides a possible therapeutic strategy for treating such cases of FALS. Recent studies have investigated the therapeutic potential of using siRNA in mouse models of ALS [69,70,83]. Only one study showed limited benefits for treating ALS symptoms in ALS model [83]. Treatment of $SOD1^{G93A}$ mice with EIAV-based lentiviral vector system resulted in an efficient and specific reduction of hSOD1 expression and improved survival in vulnerable motor neuron populations of the brain stem and spinal cord. Furthermore, hSOD1 silencing mediated an improved motor performance in these animals resulting in a significant delay in the onset of ALS symptoms by over 100% and an extension in survival by nearly 80% of their normal life span [69]. The retrograde transport capacity of EIAV-based lentiviral vectors pseudotyped with particular envelope glycoproteins was used to specifically deliver shRNAs targeted against human mutant SOD1 to vulnerable motor neuron populations by simple intramuscular injection. A similar approach was adopted to express functional RNAi molecules specifically targeted against the human SOD1 (hSOD1) gene using AAV vectors and some limited success has been reported [83]. Alternatively, direct injections of lentiviral vectors expressing inhibitory siRNA into the spinal cord in animal model of ALS has demonstrated the capacity of RNAi to silence mutant SOD1 [70]. These studies highlight the therapeutic potential of siRNA in vivo for a dominantly inherited disease. Since SOD-1 linked FALS is caused by a stable mutation in the SOD-1 gene an effective siRNA-based therapy would require sustained delivery of the active molecule throughout the lifetime of the patient. Lentiviral vectors such as those used in the studies

described above have the advantage that the viral DNA is stably integrated into the host cell genome following transduction. Therefore, transgene expression from these vectors is long-term and may provide an ideal delivery vector for siRNA-mediated therapy of dominantly inherited diseases such as FALS.

For some dominantly inherited diseases, the translation to therapy of RNAi may require an allele-specific strategy. RNAi would therefore ideally silence the mutant allele but not its normal counterpart. Allele-specific approach has already been reported for various neurological disorders genes in vitro, including *APP* in familial Alzheimer's disease, *MJD1* in Machado–Joseph disease, and *SOD1* in ALS. Studies are now required to determine whether the allele-specific silencing could be extended to animal models of ALS. The targeted region can be the mutation itself in *SOD1* gene. However, the design of RNAi for clinical applications in SOD1-linked ALS cases can be limited by the presence of over a hundred of different mutations in *SOD1* gene. In this case, combination of RNAi with a viral vector expressing wild type SOD1 may provide alternative strategy for allele-specific approach for ALS. However, siRNA treatment of FALS is relevant to only a minority of ALS patients.

6. Clinical prospects for lentiviral vectors

Gene Therapy approaches involving the use of viral vectors offer a promising strategy for the delivery of genes to enhance motor neuron survival and repair. Lentiviral vectors are powerful tools to gene transfer into the nervous system. Various research groups are investigating multiple gene transfer strategies using lentiviral vectors to improve motor neuron survival in animal models of ALS. In addition, Lentiviral vector-mediated gene transfer has been validated in several animal models of neurological disorders including Parkinson's disease and Alzheimer's disease (for review, [2]). The safety testing and manufacturing protocols for these vectors are also being vigorously developed (reviewed in [84,85]). A number of clinical protocols are now being prepared and we anticipate seeing these move through the regulatory process. There seems to be a good rationale for testing lentiviral vectors quite soon in late stage neurological disease where there are no other reasonable treatment options. We believe that there are two major issues that need to be addressed in order to see a Gene Therapy approach with lentiviral vectors proceed to the clinic for ALS patients. First, making a stable producer line that will produce clinical grade vector is critical for ALS clinical trial, where a lot of virus will be required per patient (several injections in several muscle groups). Second, careful assessment of delivery, biodistribution and toxicity issues of the lentiviral vector systems in primates is needed.

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