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Research Article

SIRNA GENE THERAPY FOR ALZHEIMER'S DISEASE TARGETING *APP* GENE

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ABSTRACT

The accumulation of amyloid precursor protein (APP) products is the most prominent cause of Alzheimer's disease (AD), thus its inactivation is the most important step in the treatment of AD. The present work is to design a vector based curative therapeutic approach using Accelrys Gene software to deliver the screened siRNA for silencing the APP gene. The vector pAZLDC1 was newly designed for silencing the APP gene which may act as a foundation stone in the search of an efficient therapeutic approach for treatment of Alzheimer's disease. The animal model for the further clinical studies was identified to be as rabbit by performing the phylogenetic analysis. The siRNA candidate can be chemically optimized and used for the development of the compounds with drug -like properties and clinical effect.

Keywords: Alzheimer's disease, Acclerys Gene, Abeta peptide, RNA interference, pAZLDC1

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the brain, with no known cause or cure, characterized by the memory deterioration, behavioral disturbances, impairment of activities of daily living, and loss of independent function. Accumulation of insoluble aggregates of amyloid-beta peptide (Abeta) is thought to be the central mechanism for the pathogenesis of AD, which contribute**s** to the memory impairment[1,2]. Abeta peptides are generated from the cleavage of amyloid precursor protein (APP) by beta- and gamma-secretases and the cleavage by BACE1 (Beta Amyloid Cleaving Enzyme-1) is believed to be a prerequisite for gamma-secretase-mediated processing[3,4]. There are 18–24 million people suffering from AD worldwide, two-thirds of whom are living in developed or developing countries. This number is expected to reach 34 million by 2025[5]. It is a daunting public health threat that must be addressed through treatment and, eventually, lead to prevention[6]. AD is the most common cause of dementia[7].

No treatment or intervention options are currently available that act upon the central molecular events that constitute the pathophysiology of Alzheimer's disease[8,9]. The available pharmacological treatments are only providing more or less effective symptomatic relief[10,6,11] . Gene transfer into cells of the nervous system is one of the fastest and current growing fields in neuroscience and treatment strategies for neurodegenerative disorders[11]. In gene therapy, with the support of efficient and safe vectors under the control of a suitable promoter, therapeutic genes can be targeted to the appropriate neurons by ex vivo or in vivo techniques[12,13]. It has been proved that the siRNAs, based on the RNA interference (RNAi) pathway, can suppress the expression of a specific gene by inhibiting the translation and thus considered as potential therapeutic agents for many diseases[14,15,16,17,18]. The siRNAs have shown great promise in the laboratory though failure to deliver these novel drugs to the affected brain region has severely limited their clinical development[19,20]. Therefore, effective gene transferring methods should be developed to overcome the low siRNA stability and facilitate intracellular uptake of siRNA. The viral vectors containing expression cassettes coding for siRNA precursors have been shown to have more efficiency than others in the delivery of siRNA into mammalian cells[21,22,23,24]. The lentiviral vectors are the most efficient in delivering genes to any neuronal cell type resulting in long term gene expression with no significant immune responses or unwanted side effects [25, 26, 27].

Despite an intensive research for therapeutic intervention, no drug has proven effective in combating AD. Hence, the objective of the present work is to design a modified lentiviral expression vector for silencing the APP gene using siRNA genen therapy with the help of Accelerys Gene software which will have the potential in curative therapeutic approach for Alzheimer's disease.

MATERIALS AND MATERIALS

Gene Retrieval

The nucleotide sequence of APP gene (Accession number NT_011512) was retrieved using Accelrys Gene. The exact position of the gene of interest was obtained from NCBI Mapviewer.

Evolutionary studies

The closest homologue (animal model) for clinical experiments was identified by performing the phylogenetic analysis from Accelrys Gene. As a result the distance table showing the evolutionary distances was also obtained. The organisam showing the lowest distance with human being was selected as the animal model.

Construction of siRNA

Twenty siRNA candidate targets were obtained after Homology filtering from siRNA Target Finder. Using Blast and Multiple sequence alignment (of all siRNAs with the cDNA), the candidate targets were analyzed and the suitable siRNA was selected. The selected siRNA was modified into siRNA inserts using siRNA Construct Builder.

Selection of model vector

The suitable expression vector for the transfer of siRNA was selected from Vector – Based siRNAs of siRNA Construct Builder. The marker gene was also selected from the cloning vector pZEO(SYNLACZ).

Designing of vector

Using Accelrys Gene and NEBCUTTER 2, the restriction analysis of selected vector, gene of interest and marker gene was performed. The newly designed vector was constructed in Accelrys Gene, by the integration of siRNA using Bioedit. The molecular weight of the vector, before and after the integration of siRNA, was calculated using BiomathCalculator. The detailed map showing the position and the features of newly designed vector was obtained.

RESULTS

The nucleotide sequence of APP gene (Accession number NT_011512), used for the construction of siRNA, is directly retrieved from NCBI using Accelrys Gene. With the help of NCBI Mapviewer, the exact position of the gene of interest is obtained. Rabbit (RABAMPREO1) is the animal model selected for the further clinical studies of the newly designed vector. The distance table obtained from phylogenetic analysis shows the evolutionary distance (Table.1). Rabbit ((RABAMPREO1) shows the lowest distance of 0.1896 from man (HUMAMYB18) in the distance table.

Twenty siRNA candidates are obtained from siRNA Target Finder after the Homology filtering. From this library, the most effective siRNA sequence is screened by the analysis of all siRNAs using Blast and Multiple sequence alignment. The siRNA inserts with 76bp in length are generated using siRNA Construct Builder which includes the antisense region, sense loop, termination signal and restriction enzymes. The sequence of selected siRNA is CTAGCAACCAAAGGAGTACAA with the GC content of 42.86% and the ΔE value of 26kcal/mol.

pRNAT-in-H1.2/Lenti (Fig.1) is the model vector obtained for the integration of siRNA to silence the APP gene. It is a Lentiviral vector from Genscript siRNA technology containing 9139 bp. *LacZ* is the marker gene selected from a cloning vector pZEO (SYNLACZ) which contains 231 bp length of nucleotides. The restriction analysis of selected vector, gene of interest and marker gene is performed using Accelrys Gene and NEBCUTTER 2. Restriction Enzyme analysis, to check the compatibility of gene of interest and vector, is performed by selecting the enzyme which has minimum restriction site and blunt end region. XhoI and BamH1 are the restriction enzymes used for the insertion of siRNA to the APP targeting vector.

pAZLDC1, the newly designed vector for the transfer siRNA targeting APP gene, is constructed in Accelrys Gene by the integration of siRNA using Bioedit. pAZLDC1 contain 9139 bp length and the molecular weight of 2825073 Daltons. The molecular weight is changed from 2825063 to 2825073 in pAZLDC1. A detailed map shows the features and position of the components in the newly constructed vector is given (Table. 2). The siRNA insert is located very near to the CMV promoter, which placed between the position of 2067 to 2654 of base pair in vector. LacZ is the marker gene used in the designed vector.

Table 1: Distance Table shows the evolutionary relationship which obtained from the phylogenetic analysis.

	DQ267686	NM_001013018	X63472	RABAMPRE01	AJ133033	AB169826	AY926583	NM_001006601	NM_019288	NM_001076796	AF030413	NM_204308
NM_001013018	0.3856											
X63472	0.2969	0.3160										
RABAMPRE01	0.1939	0.3160	0.0000									
AJ133033	0.1672	0.5099	0.1205	0.1205								
AB169826	0.5000	0.0182	0.5139	0.3302	0.5149							
AY926583	0.3964	0.0724	0.3504	0.3206	0.4920	0.0787						
NM_001006601	0.3941	0.0852	0.3413	0.3238	0.4967	0.0902	0.0666					
NM_019288	0.3799	0.1059	0.3194	0.3194	0.5050	0.1073	0.1106	0.1200				
NM_001076796	0.4883	0.0876	0.5097	0.3333	0.4868	0.0915	0.0657	0.0899	0.1248			
AF030413	0.2731	0.0625	0.2124	0.2124	0.2682	0.0792	0.0702	0.0792	0.0583	0.0792		
NM_204308	0.5107	0.1671	0.5186	0.3462	0.5099	0.1829	0.1765	0.1659	0.1657	0.1871	0.1292	
HUMAMYB18	0.2687	0.4260	0.2663	0.1896	0.3256	0.4791	0.4305	0.4456	0.4480	0.4818	0.2857	0.4918

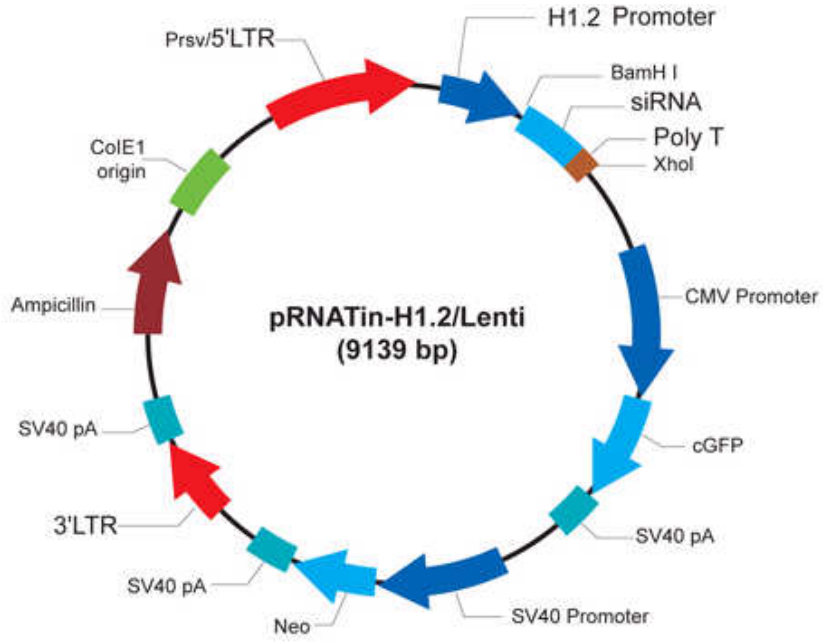

Fig. 1: Model vector for the integration of siRNA

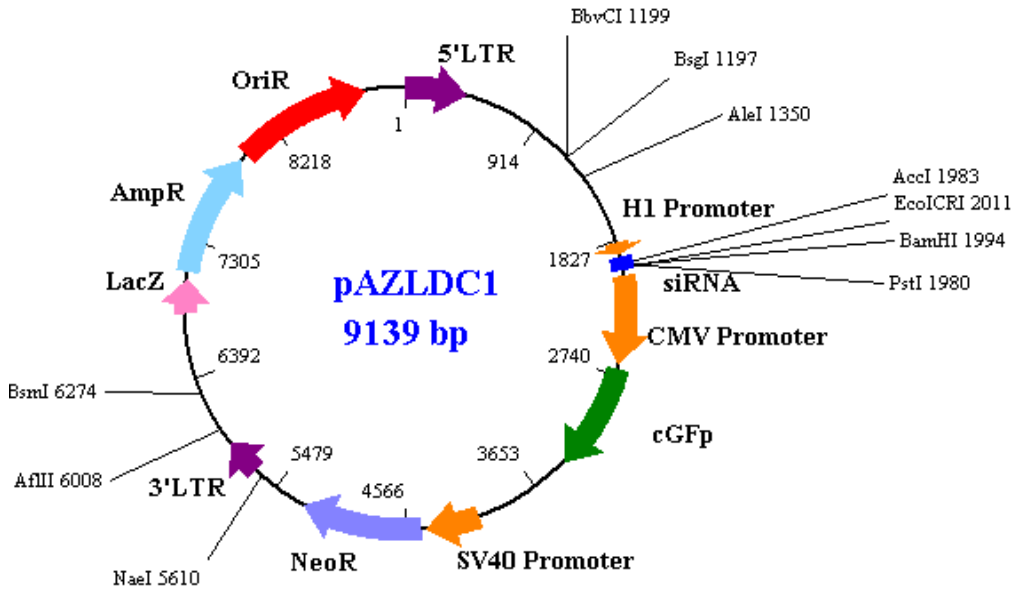

Fig. 2: pAZLDC1- Newly constructed vector targeting APP gene

Table 2: Detailed Map of Vector

Features of Vector	**Position**
5' LTR	1-410
H1 Promoter	1847-1946
Polylinker	1947-2022
CMV Promoter	2067-2654
siRNA insert	**1947 – 2022**
cGFP	2671-3390
SV40 Promoter	4079-4424
Neomycin	4465-5259
3' LTR	5660-5894
LacZ	6820 -7050
AmpR	7054-7914
OriR	7974-8860

DISCUSSION

Alzheimer's disease (AD) is the prevalent neurodegenerative disease of the elderly that places a substantial burden on patients, their families, and society. There is a lacuna in effective therapies for most of the neurodegenerative diseases especially Alzheimer's. Currently available treatments for AD offer primarily symptomatic benefits, providing temporary cognitive improvement and deferred decline with little or no evidence of slowing disease progression[9,6,10]. The production and accumulation of amyloid beta (Abeta) peptide oligomers are increasingly thought to be central to AD pathogenesis. Molecular, genetic, animal and clinical studies suggest that the generation of abeta peptide oligomers from amyloid precursor protein (APP) is the pivotal initiating event in AD[28,29,30,31]. As the accumulation of amyloid precursor protein (APP) products is the most prominent cause of Alzheimer's disease, APP has been proposed as a key therapeutic target in treating AD. Thus, the inactivation of APP can act an important step in the treatment of Alzheimer disease. Several strategies are being used experimentally for targeting the putatively toxic amyloid beta peptide, especially its inhibition is considered to be of great therapeutic interest[9,32,33,34,35].

Many studies are going on to design drug for the disorders like AD[36]. However, medical therapies have failed so far to find out a complete remedy for neurodegenerative disorders in human[10]. To achieve this ambitious goal current investigation is focused on the development of a curative therapeutic approach for Alzheimer disease. The discovery that, siRNA can specifically suppresses targeted gene expression and be delivered effectively to disease models, opens new perspectives in molecular therapeutics[5,39]. The basic principle of siRNA therapy is RNA interference (RNAi) which has been observed in plants, *C. elegans* and *Drosophila* for a number of years. In siRNA gene therapy, the small interfering RNA (siRNA) molecules interfere with the expression of a specific gene by inhibiting the translation. There are many gene carrying technologies like antibody coupling, electroporation, phosphate buffered saline method, artificial cell penetrating peptide and recently nanoparticles have been investigated for delivering the siRNAs to target cell[38,39,40,41,42] .

It is very important that the selection and design of gene-specific siRNAs have the desired silencing characteristics. In the present analysis 20 siRNAs were synthesized using Genscript siRNA target finder. From this library of siRNAs, a potentially effective siRNA sequence is selected by using blast analysis and multiple sequence alignment for silencing the APP gene. The selected siRNA can be put into a vector with an inducible promoter to study its effect. It can be delivered by using viral vector which could be further used for gene therapy purpose against AD[43,44]. They can be mimicked by chemical molecule and used for drug development. This siRNA can be chemically optimized and efficiently delivered to create therapeutically relevant compounds with drug - like properties with desired clinical effect.

One of the fastest growing fields in neuroscience is the research pertaining to the gene transfer into cells of the nervous system[11]. In gene therapy, with the support of efficient and safe vectors under the control of a suitable promoter, therapeutic genes can be targeted to the appropriate neurons by ex vivo or in vivo techniques[12,13]. Gene delivery to the nervous system represents the ultimate challenge of gene therapy in view of the complexity of this system and the need to deliver the gene to non-dividing cells[45,46].The intracellular delivery of the siRNA into the target cells is necessary to exert their silencing effects on target genes. The therapeutic applications of siRNA have been hampered by their instability, poor cellular uptake, and mainly the lack of efficient delivery methods. Therefore, development of the carriers with the capacity to stabilize siRNAs and facilitate their uptake by target cells is important[47, 48].

In the present study, the vector selected from Genscript siRNA technology for the transfer of siRNA to the APP gene is a Lentiviral vector (pRNAT-in-H1.2/Lenti). Lac Z, from the cloning vector pZEO (SYNLACZ), is used as the marker gene for the expression vector. This gene contains 231 bp lengths of nucleotides. After the delivery into the nervous system, a Lentiviral vector induces no significant immune responses as well as no unwanted side effects. Ralph et al[27]Showed that viral vectors based on lentiviruses provide particularly attractive vehicles for delivery of therapeutic genes to treat neurological diseases. Given this host of advantages, lentiviral vectors have been widely used to express many siRNAs, which bring about stable down regulation of many genes There is no need for ex vivo treatment with lentiviral vectors, and the target cells are simply recognised through their cell membrane receptor proteins [49, 50, 51, 52]. These vectors thus offer a valuable tool for testing gene function in neuronal cells in vivo. Restriction enzyme analysis is very significant in vector construction as it allows the compatibility testing of the gene of interest and vector. The compatibility analysis is performed by selecting the enzyme which has minimum restriction site and blunt end region. Xhol and BamH1 are the compatible enzymes obtained for the insertion of siRNA to the APP targeting vector.

The expression vector pAZLDC1 (Fig. 2) is constructed using Accelrys Gene and Bioedit. pAZLDC1 contain 9139 bp and it is designed for the transfer of siRNA to the APP gene. Its molecular weight is 2825073 Daltons. After the integration of marker gene and siRNA insert, molecular weight was found to be approximately same. The molecular weight is changed slightly from 2825063 to 2825073 in pAZLDC1. The detailed map (Table 2) shows the features and position of newly constructed vector. The CMV promoter is placed between the position of 2067 to 2654 of base pair in the vector. siRNA insert of 76 base pair length is introduced very near to this promoter. The marker gene Lac Z is placed between 6820 to 7050 position in the vector.

Experimental animal models are needed for testing the efficacy of therapeutic approach. Homologue search is very important in gene therapy as further clinical experiments cannot be directly performs in human beings. Rabbit (RABAMPREO1) shows the lowest distance of 0.1896 from man (HUMAMYB18) in the distance table obtained from the phylogenetic analysis. Thus in the present study, rabbit is identified as the closest homologue to man for the experimental study of the newly constructed vector targeting APP gene.

The construction of an expression vector in wet lab will consume more time and cost. Thus the study using bioinformatics tools can provide a basic framework for the wet lab analysis. Efficacy of the present vector can be evaluated only after the clinical experiments. The present investigation is an initial attempt for the future therapeutic study in Alzheimer disease. The newly designed vector pAZLDC1 can act as a building block in the search of an efficient therapeutic approach leading to treatment of Alzheimer's disease.

CONCLUSION

siRNA therapy is a new tool in the battle against Alzheimer's disease based on the principle of RNA interference through which double-stranded RNAs silence cognate genes. The present study designed a new expression vector for Alzheimer's disease by taking APP gene as the key target using various Bioinformatics tools. The undertaken study shows promise for the determining new strategies in Alzheimer disease treatment.

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