Advanced Drug Delivery Reviews 61 (2009) 760–766

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Nonviral vector-mediated RNA interference: Its gene silencing characteristics and important factors to achieve RNAi-based gene therapy ☆

Yuki Takahashi, Makiya Nishikawa, Yoshinobu Takakura *

Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8501, Japan

ARTICLE INFO

Article history:
Received 12 February 2009
Accepted 5 April 2009
Available online 20 April 2009

Keywords:
Short hairpin RNA
In vivo delivery
Duration
Side effect
Hydrodynamic delivery

ABSTRACT

RNA interference (RNAi) is a potent and specific gene silencing event in which small interfering RNA (siRNA) degrades target mRNA. Therefore, RNAi is of potential use as a therapeutic approach for the treatment of a variety of diseases in which aberrant expression of mRNA causes a problem. RNAi can be achieved by delivering siRNA or vectors that transcribe siRNA or short-hairpin RNA (shRNA). The aim of this review is to examine the potential of nonviral vector-mediated RNAi technology in treating diseases. The characteristics of plasmid DNA expressing shRNA were compared with those of siRNA, focusing on the duration of gene silencing, delivery to target cells and target specificity. Recent progresses in prolonging the RNAi effect, improving the delivery to target cells and increasing the specificity of RNAi in vivo are also reviewed.

Contents

1. Introduction

Small interfering RNA (siRNA) can degrade mRNA which has a complementary sequence to the siRNA by the mechanism called RNA interference (RNAi) [1–3]. Soon after its discovery, siRNA began to be widely used as an experimental tool to investigate the function of target genes because of its convenient, specific and potent gene silencing effect compared with conventional techniques such as antisense oligodeoxynucleotides and homologous recombination-based knockout strategy [4,5]. Moreover, therapeutic application of siRNA targeting the gene of interest has been actively investigated. In addition to siRNA, DNA vectors that transcribe siRNA or short hairpin RNA (shRNA) are also available to induce RNAi [6–8]. A number of viral and nonviral vectors have been developed, but the safety concerns of

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Towards Therapeutic Application of RNA-mediated Gene Regulation".

* Corresponding author. Tel.: +81 75 753 4615; fax: +81 75 753 4614.
E-mail address: takakura@pharm.kyoto-u.ac.jp (Y. Takakura).

0169-409X/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2009.04.006

viral vectors have not been resolved yet. Therefore, the nonviral vector-based approach using plasmid DNA (pDNA) is expected to be a safer method to induce RNAi compared with any approaches using viral vectors. Vector-based approaches and siRNA share the same RNAi pathway, but have different properties that affect the efficacy of RNAi-based therapy. In this review, we describe the current status of the development of pDNA vectors for siRNA expression, compare the profiles of gene silencing by either shRNA-expressing pDNA or siRNA and discuss the advantages and limitations of RNAi-based gene silencing in therapeutic applications at the present time.

2. Development of DNA vectors for siRNA expression

Intracellular transcription of siRNA can be achieved by introducing the vector containing a siRNA template under the control of a promoter. In this section, the current status of the development of pDNA vectors for siRNA expression in mammalian cells is summarized.

2.1. Vector construct

Two approaches have been developed for expressing siRNA inside cells: one is a tandem-type vector which transcribes a pair of sense and antisense transcripts from individual promoters; the other is a hairpin vector which transcribes a single strand RNA that forms short hairpin RNA (shRNA), which is processed into siRNA inside cells [9]. As shRNA requires only one DNA template, shRNA-expressing vectors are more convenient than those expressing two templates as far as the construction of vectors is concerned. Moreover, the knockdown efficiency of shRNA-expressing vectors is generally higher than that of tandem-type vectors [8,10,11]. Therefore, shRNA-expressing vectors have been used much more frequently. Here we will focus on shRNA-expressing vectors. In addition to shRNA, micro RNA (miRNA), an endogenous RNA molecule that regulates gene expression, has also been reported to be used for inducing RNAi. miRNA has some advantages over siRNA or shRNA, because single miRNA transcript is processed into multiple siRNAs and relatively long miRNA transcripts can be transcribed by Pol II promoter [12,13].

A pDNA vector for shRNA expression normally consists of a promoter, a shRNA template and necessary components for pDNA amplification (i.e., replication origin and selection marker genes). Gene therapy studies have demonstrated that a variety of factors affect the level and duration of transgene expression from the pDNA [14]. Of such factors, the promoter has been shown to be the most important for determining the profile of transgene expression. Therefore, the effect of the type of promoter on shRNA expression is discussed first, followed by those of other components of shRNA-expressing pDNA.

2.2. Promoter

The promoter that drives shRNA expression is an important factor for determining the RNAi effect produced by shRNA-expressing pDNA. RNA polymerase III (Pol III) promoters, such as small nuclear RNA U6 (U6) and human RNase P RNA H1 (H1), have been frequently used for shRNA expression because these promoters are suitable for the transcription of short RNA in large quantities and their sites of transcription initiation and termination are well defined. Lots of studies including our own have demonstrated that the knockdown efficiency of shRNA-expressing pDNA depends on the type of promoter. We found that shRNA-expressing pDNA driven by U6 promoter has more sustained effects than those driven by H1 or tRNA promoter [15]. However, the rank order of promoter strength varies among studies. For example, the results reported by Boden et al. have shown that tRNA-driven shRNA-expressing pDNA induced HIV-1-specific RNAi more efficiently than those driven by other promoters, such as U6, H1 and CMV promoters [16]. Differences in experimental conditions, such as the type of target cells, delivery methods and shRNA sequence, would explain the discrepancy among the studies, as demonstrated in a recent paper in which the effective promoter was a function of the type of cell line [17]. These results would suggest that a suitable promoter should be selected on a case-by-case basis.

Inducible expression systems provide further benefits to vectors with pol III promoters. The expression of shRNA from such vectors has been shown to be induced by chemical reagents, such as tetracycline [18–20]. This inducible system will be advantageous not only as experimental tools but also as therapeutics.

In addition to Pol III promoters, Pol II promoters are also available to transcribe shRNA [21,22]. As gene silencing in non-target cells may cause undesired effects, target cell specific gene silencing is effective in reducing possible side effects. Pol II promoters can provide cell- or tissue-specific expression of shRNA, which would be a feasible approach to achieve target cell-specific gene silencing. Cell type-specific gene silencing has already been demonstrated by using cell-specific promoters, such as telomerase reverse transcriptase promoter (tumor cells) [23], glial fibrillary acidic protein promoter (hepatic stellate cell) [24], human α_1-antitrypsin (hAAT) promoter (hepatocyte) [25] and prostate specific membrane antigen promoter/enhancer (prostate cancer) [26]. Grimm et al. reported a successful gene silencing of the envelope surface antigen (sAg) of hepatitis B virus (HBV) in the liver of HBV-transgenic mice by U6 promoter-driven shRNA targeting the gene, but they found that the ubiquitous expression of an excess amount of shRNA produced toxic effects in the mice [27]. Recently, Giering et al. have shown a solution for this toxicity by using a hepatocyte-specific promoter (hAAT promoter) to express the shRNA [25]. Hepatocyte-specific expression of shRNA was found to be effective not only in inhibiting HBV replication in HBV transgenic mice but also in avoiding shRNA-mediated toxicity.

2.3. Components of shRNA-expressing pDNA

Jenke et al. inserted a scaffold/matrix attachment region into an shRNA-expressing pDNA targeting hepatitis B virus (HBV) in order to retain the plasmid as an episome in the cells [28]. They found that the vector was effective in suppressing HBV replication for at least 8 months after the transfection of shRNA-expressing pDNA to HBV-replicating HepG2.2.15 cells. Because no in vivo results have been reported and the effect of the scaffold/matrix attachment region on the duration of the RNAi effect has not been reported, additional studies are required to confirm the importance of the insertion of the region on the duration of the knockdown effect. In addition, use of a transposon system, which has an ability to insert pDNA into genomic DNA of target cells, has been reported to be effective for long-term expression [29,30]. As the random insertion of pDNA into genome DNA carries a risk of mutagenesis, which is the same problem as that of retroviral vectors, further improvements in safety is required for the application of such transposon systems as therapeutic treatments.

In our gene therapy studies aiming to achieve sustained transgene expression, we investigated the effect of the number and position of unmethylated CpG dinucleotides (CpG motifs) in pDNA on the duration of transgene expression in vivo [31] (Mitsui et al., in press). In these studies, we have found that reducing the number of CpG motifs in pDNA is effective in prolonging the duration of transgene expression. Recently, Escoffre et al. reported the time-course of gene silencing in mice after intramuscular injection of shRNA-expressing pDNA followed by electroporation [32]. Here, one of the two types of shRNA-expressing pDNA with different numbers of CpG motifs was co-administrated with a pDNA encoding reporter gene (target for shRNA). The authors found little difference in the gene silencing effects between the two types of shRNA-expressing pDNAs. As they did not investigate the gene silencing effects on any endogenous genes, further studies are required to confirm the effect of CpG motifs in shRNA-expressing pDNA on its gene knockdown effect.