Control of siRNA expression utilizing Cre-loxP recombination system

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ABSTRACT

Vector-mediated systems for specific siRNA expression in mammalian cells using pol III promoters allowing high level of transcription activity have been developed in the past years, widening the usage of RNA interference (RNAi). In this study, we controlled the pol III promoter (U6 promoter)-driven expression of siRNA using the Cre-loxP system. Our “Cre-On” siRNA-expression vector against firefly luciferase activity could be switched on only in the presence of Cre recombinase, which, in this study, was delivered directly from the medium into the cells as TAT-NLS-Cre, a fusion protein with TAT peptide (an Arg rich peptide derived from HIV) and nuclear localizing signal (NLS). Upon the addition of TAT-NLS-Cre, complete and functional siRNAs were generated and reporter activity was suppressed.

INTRODUCTION

RNA interference (RNAi) is a post-transcriptional gene-silencing phenomenon discovered first in Caenorhabditis elegans (1, reviewed in Ref. 2), and found in evolutionary diverse organisms. In RNAi, Dicer, a member of the RNase III family, cleaves double-stranded RNAs (dsRNAs) into small interfering RNAs (siRNAs) of 21 or 22 nucleotides (nt) in length, which in turn induce the degradation of target mRNA and cause the suppression of the target gene. For mammalian cells, RNAi could be induced without causing nonspecific inhibition of protein synthesis using 21- or 22-nt RNAs with 2- or 3-nt 3’ overhangs known as small interfering RNAs (siRNAs) (3). In the past studies, several groups, including our own, have developed vector-mediated systems for inducing RNAi in mammalian cells using pol III promoters (4, reviewed in Ref. 2). Using RNAi for suppression of the expression of a gene of interest has several considerable merits: easy to design, strong site-specificity and suppressive effect, and low concentration requirement of siRNA. In this study, we aimed to establish a controllable U6 promoter-driven siRNA-expression vector based on the Cre-loxP system, a system that has been widely used in reverse genetics in various kinds of eukaryotic cells. Cre recombinase is a site-specific recombinase that catalyzes the recombination at loxP sites (5). We designed a Cre-On siRNA-expression vector, in which we inserted a region that prohibits the complete transcription of the siRNA-coding sequence between two loxP sites. In the absence of Cre recombinase, RNAi cannot be induced, as only small, incomplete RNA fragments are produced from the transcription. In the presence of Cre recombinase, as region between loxP sites is removed, complete and functional siRNAs are produced. Cre recombinase was delivered into the cells as TAT-NLS-Cre, a fusion protein with TAT (an Arg rich peptide derived from HIV) and nuclear localizing signal (NLS). Fusing Cre recombinase with TAT and NLS was reported to promote the uptake of the protein by cells from the medium, and the fusion protein retains the ability of catalyzing recombination between loxP sites efficiently (6). In this study, we showed that our Cre-On siRNA-expression vector could suppress the activity of reporter gene only when TAT-NLS-Cre was added into the medium.

RESULTS AND DISCUSSION

To achieve the purpose of establishing a Cre-controllable U6 promoter-based siRNA-expression vector, we designed a Cre-On siRNA expression vector by placing, between sense and antisense regions of siRNA encoding sequence, two loxP sites with an 813 bp insert fragment between them. The inserted fragment starts with seven thymines, and as four consecutive thymines is a terminator
In this study, we constructed and used a Cre-On siRNA expression vector, namely iGL3BCre-On, and two positive controls, namely, iGL3B and iGL3BloxP. iGL3B is an siRNA expression vector targeting firefly luciferase constructed in our laboratory as described previously (Miyagishi et al., 2003, submitted). The products of this siRNA expression vector are 21-nt hairpin-type siRNAs with a 9-nt loop each. iGL3BCre-On was designed for the Cre-On system (Fig. 1) with the same sense and antisense sequences as those of iGL3B, while iGL3BloxP was similar to iGL3B, with the exception that the loop consisted of a 34-nt loxP sequence.

To examine whether the expression of iGL3BCre-On could be controlled by Cre recombinase, which was delivered as TAT-NLS-Cre fusion protein into the cells, HeLa S3 cells were cultured in 96-well tissue culture plates to 60% confluency, and transfected with siRNA-expression vectors or a mock vector, plus firefly luciferase-expression vector (pGL3; Promega, Madison, WI) and Renilla luciferase-expression vector (5). Four hours after transfection, purified and dialyzed TAT-NLS-Cre or BSA or the dialysis medium was added into the culture medium. Twenty-four hours after transfection, luciferase activities were analyzed with the Dual Luciferase System (Promega). As shown in Figure 2, incubation with TAT-NLS-Cre of cells transfected with iGL3BCre-On (lane 2) resulted in approximately 70% suppression of firefly-luciferase activity. This value was similar to that obtained for positive controls (Fig. 2, lanes 3 and 4). These results clearly showed that the expression of iGL3BCre-On had been switched on by TAT-NLS-Cre recombinant protein.

In conclusion, we showed here that the expression U6-promoter driven siRNA expression vector could be controlled by Cre recombinase, and we believe that our work will open up new possibilities for using siRNAs in research and therapeutics.

**REFERENCES**