Development of cyanovinylcarbazole mediated photocrosslinking reaction toward for regulation of RNA functions in cell

Atsuo Shigeno¹, Yuichi Otaki¹, Takashi Sakamoto¹, and Kenzo Fujimoto¹,²

¹Japan Advanced Institute of Science and Technology, Department of Materials Science, Nomi, Ishikawa, 923-1292, Japan; ²Research Center for Bio-Architecture, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa, 923-1292, Japan

s1140003@jaist.ac.jp

ABSTRACT SUMMARY

In antisense method, it is required that antisense oligonucleotide (AS-ODN) have high affinity and specificity for target mRNA. Photo-regulation of the hybridization between antisense oligonucleotide and its target mRNA enables site specific induction of antisense effect without undesired side reaction. 3-Cyanovinylcarbazole nucleoside (CNV-K), which effectively photocrosslinks to the pyrimidine base in complementary RNA strands, was incorporated into antisense oligonucleotides. and we demonstrated that the reverse transcription and the translation activity of K-ras mRNA were quickly suppressed by a few seconds of photoirradiation with the addition of the photoresponsive antisense ODN.

INTRODUCTION

A lot of researchers in academia and pharmaceutical companies pay attention to the development of short nucleic acid based medicine because of its wide variety of functions. For example, ribozymes¹ which consist of tens of oligoribonucleotide (ORN) and small interfering RNAs⁵ which consist of 21~23 bp double stranded ORN can sequence specifically digest its target RNA, and aptamers⁵ and decoy DNA⁴ can specifically bind to its target proteins. AS-ODN is one of the most promising candidates of short nucleic acid based medicine. Antisense method based on specific binding between AS-ODN and complementary target RNA can regulate not only gene expression by specific binding with mRNA but also micro RNA functions by specific binding with RISC (RNA-induced silencing complex) complex. Therefore, the antisense method had been well studied as drugs for gene therapy and tools for study on micro RNAs. Until now, some functional AS-ODNs, such as highly stable, high affinity and stimuli responsible AS-ODNs have been already reported. Especially, photo-responsible AS-ODNs⁶ has a great potential for organ specific photodynamic antisense therapy. However, unexpected photodamages were also caused because its low photo-responsibility of present photo-responsible AS-ODNs. In this study, 3-cyanovinylcarbazole modified nucleoside⁵ that can effectively photo-crosslink to specific pyrimidine base in the complementary RNA strand was adopted as photo-responsible nucleotide in AS-ODN⁸. Potential of the novel photo-responsible AS-ODN was investigated from its sequence selectivity, target generality and binding ability for target mRNAs. As a target mRNA, point-mutated K-ras mRNA (codon12; GGU>GUU) that is one of the representative oncogene, was adopted. And AS-ODN containing CNV-K was designed to photocrosslink to point-mutated K-ras mRNA via photoadduct formation between CNV-K and mutated U in codon 12

EXPERIMENTAL METHODS

To prepare the endogenous target mRNA, pancreatic cancer cell lines, BxPC-3 and Capan-1, which have wild type (GGU) and G12V (GUU) mutated sequences at codon 12 of K-ras mRNA, respectively, were cultured and total RNAs were extracted from each cell line. To evaluate the photoreactivity of CNV-K-AS-ODN having CNV-K to K-ras mRNAs, the total RNAs were mixed with CNV-K-AS-ODN, photoirradiated, and then the mixtures were subjected to real-time reverse transcription polymerase chain reaction (RT-PCR). If the photocrosslinking reaction occurred, the reverse
transcription was completely inhibited by the steric hindrance caused by the formation of an irreversible photoadduct.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5’ → 3’)</th>
<th>X=≡H</th>
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<tr>
<td>CNVK-AS-ODN</td>
<td>TGCTAGGCACGCA[X]CTCCAACTA</td>
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**RESULTS AND DISCUSSION**

1 s UV irradiation caused 96% inhibition of reverse transcription, respectively, indicating that the CNVK-AS-ODN photocrosslinked to target K-ras mRNA having a point mutated sequence (GUU). Such inhibition was not observed in the case of total RNA from BxPC-3 having an unmutated K-ras sequence (Fig. 1), suggesting that the photocrosslinking reaction is quite selective to GGU to GUU point mutation.

**CONCLUSION**

In this study, photocrosslinking abilities of CNVK-AS-ODNs for its point-mutated target RNAs were evaluated. RT-PCR experiments revealed that the CNVK-AS-ODN having CNVK clearly photocrosslinks to its target mRNA in a sequence selective manner and then the function of mRNA as the substrate of reverse transcriptase is clearly regulated with only 1 s photoirradiation.

**ACKNOWLEDGMENTS**

This study was partly supported by the Grant-in-Aid for Scientific Research (B) (90293894, K.F.) of The Ministry of Education, Science, Sports and Culture of Japan.

**REFERENCES**