Photodynamic antisense regulation of mRNA having a point mutation with psoralen-conjugated oligonucleotide

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ABSTRACT

Nucleic acid-based drugs, such as antisense oligonucleotide, ribozyme, and small interfering RNA, are specific compounds that inhibit gene expression at the post-transcriptional level. To develop more effective nucleic acid-based drugs, we focused on photo-reactive antisense oligonucleotides. We have optimized the structure of psoralen-conjugated oligonucleotide to improve their sequence selectivity and photo-cross-linking efficiency. Previously, we reported that photo-reactive oligonucleotides containing 2'-O-psoralenylmethoxyethyl adenosine (2'-Ps-eom) showed drastic photo-reactivity with a strictly sequence specific manner in vitro. In this report, we evaluated the binding ability toward intracellular target mRNA. The 2'-Ps-eom selectively photo-cross-linked to the target mRNA extracted from cells. The 2'-Ps-eom also cross-linked to target mRNA in cells. Furthermore, 2'-Ps-eom did not cross-link to mRNA having a mismatch base. These results suggest that 2'-Ps-eom is a powerful antisense molecule to inhibit the expression of mRNA having a point mutation.

INTRODUCTION

Through the identification of human genome sequence and the development of molecular biology, it has been found that various cancers were caused by the consequence of a single point mutation in the gene [1-3]. The mutation affects the cellular proliferation and induces tumorigenic properties. It has been desired to inhibit gene expression of disease-causing mutation selectively, without affecting normal genes. The antisense strategy which inhibits gene expression in a sequence specific manner might be suitable for the purpose. A considerable number of studies have been conducted on chemically modified antisense oligonucleotides to increase the nuclease resistance, binding affinity to RNA, and cellular uptake [4-8]. However, development of antisense oligonucleotides which inhibit the expression of gene having a point mutation specificity have been scarcely reported.

To improve the sequence selectivity of antisense oligonucleotides for the regulation of such a gene expression, we have developed to antisense oligonucleotides containing 2'-O-psoralen-conjugated adenosine (2'-Ps-oligo). Psoralen derivatives have an ability to photo-cross-link covalently to pyrimidine bases selectively, favorably with thymine and uracil, upon UVA irradiation (320–400 nm). To use the base specificity of psoralen, we expect that it become possible to discriminate gene having purine → pyrimidine mutation. In our previous study, we reported 2'-Ps-met, which is 2'-Ps-oligo that had an adenosine anchoring psoralen at 2'-O-position of adenosine via a methylene linkage, photo-cross-linked to complementary RNA (match-RNA) upon UVA-irradiation (365 nm) and rarely did to RNA having a mismatch base (mismatch-RNA) [9]. Furthermore, to extend the linkage connecting 4'-position of psoralen and 2'-O-position of adenosine, the photo-cross-linking efficiency was drastically enhance [10]. Particularly, oligonucleotides containing 2'-O-psoralenylmethoxyethyl adenosine (2'-Ps-eom, Fig.1) showed the highest photo-cross-linking efficiency with a strictly sequence specific manner toward oligoribonucleotide fragment. In this report, we applied the photo-reactive oligonucleotide to cultured cells and evaluated the binding ability toward intracellular target mRNA.

RESULTS AND DISCUSSION

We chose mutant-K-ras mRNA as a target mRNA of antisense oligonucleotide. The point mutation in the K-ras gene occurs at codon 12 (GGT→GGT). 2'-Ps-eom whose sequence was targeting the codon 12 region of K-ras mRNA was synthesized (15mer; d(TACGCC AAAGCAGCT CC)). K-ras codon 12 mutant NIH3T3 cells (K12V cells) were gifts from Dr. N. Wake1 and Dr. K. Kato (1Obstetrics and Gynecology, Kyushu University; 2Division of Molecular and Cell Therapeutics, Kyusyu University). K12V was established by transfecting NIH3T3 with pZIP-NeoSV(x) retrovirus vector constructs containing cDNA

Fig. 1 Structure of 2'-Ps-eom
sequences encoding [12V]K-ras 4B using lipofectamine [11]. Then control mock cells (K-ras wild type) harboring these empty vector were also gifts from Dr. N. W. and Dr. K. K.

We evaluated photo-cross-linking properties of 2'-Ps-eom with mRNAs extracted from K12V cells and mock cells. Total RNAs were extracted from each cells. Total RNA and 2'-Ps-eom were mixed and annealed. UVA irradiation were carried out at 20 °C on a transilluminator (365nm, 1.6mW/cm²). To evaluate the photo-cross-linking reaction, the irradiated mixtures were alalyzed by RT-PCR system (Fig. 2). After UVA-irradiation, these RNA were transcribed by reverse transcriptase in the presence of oligo(dT)₁₆ primer, followed by PCR reactions. Two sets of PCR primers were designed. The primer set ① amplifies the region contained codon 12 of K-ras mRNA, the other (②) is not contained the region. If psoralen of 2'-Ps-eom photo-cross-links with the Uracil of the codon 12 region of K-ras mRNA, reverse transcription is stopped at the site, thus PCR products by using the primer set ① was not appeared. In the case of photo-irradiated mixture of 2'-Ps-eom and total RNA extracted from K12V cells, PCR products by using the primer set ① product was little appeared. In contrast, on photo-irradiated mixture of 2'-Ps-eom and total RNA extracted mock cells, both PCR products were appeared with comparable level. The results showed 2'-Ps-eom photo-cross-linked to the designated site of mRNA extracted from cells with high sequence selectivity.

We also evaluated whether 2'-Ps-eom could photo-cross-link to complementary intracellular mRNA in cells. 2'-Ps-eom (100mM) was transfected to K12V cells and mock cells using lipofectamine2000. After 5 hours from transfection, UVA irradiation were carried out at 20 °C on a transilluminator at 5min (365nm, 1.6mW/cm²). Then, total RNA were extracted from these UVA-irradiated cells and by using the RT-PCR system. In the case of 2'-Ps-eom treated and UVA-irradiated K12V cells, PCR product by using the primer set ② was appeared, but the case of primer set ① was not appeared. In contrast, on UVAnirradiated K12V cells, both PCR products were appeared with comparable level. On the other hand, in the case of mock cells, both PCR products derived primer set ① and ② were appeared with comparable level with or without UVA-irradiation. These results showed 2'-Ps-eom have an ability to photo-cross-link with the designated uracil at codon 12 of K-ras mRNA in cells.

CONCLUSION

The 2'-Ps-eom selectively photo-cross-linked to the complementary mRNA which was extracted from cells and also intracellular mRNA. 2'-Ps-eom will be a powerful nucleic acid drug to inhibit the expression of mRNA having a point mutation.

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