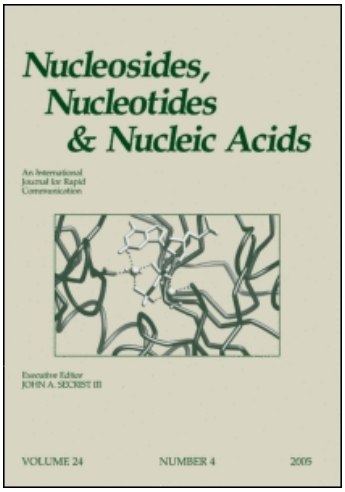

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2′-*O*,4′-*C*-Ethylene-Bridged Nucleic Acid (ENA™) for Effective Antisense Formation

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ABSTRACT

ENA™ antisense oligonucleotides for vascular endothelial growth factor (VEGF) mRNA were synthesized and evaluated in A549 lung cancer cells. It was found that the VEGF ENA-antisense inhibited not only the expression of VEGF, but also the expression of three genes, which were found in Genbank by BLAST and Clustal W search and considered likely to bind to the VEGF ENA-antisense. These results indicate that ENA-antisense oligonucleotides act in a sequence-specific manner, and could be used as effective antisense drugs.

Key Words: Oligonucleotides; Antisense; VEGF; Sequence-specific; BNA; LNA.

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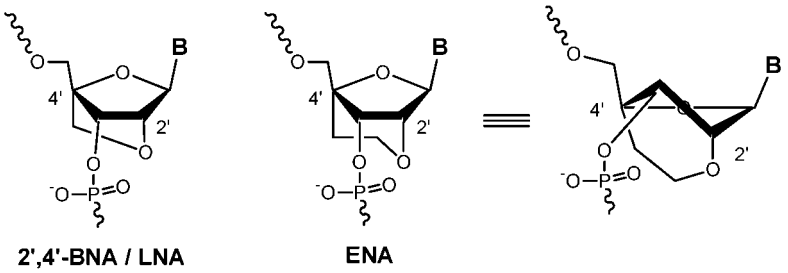

Figure 1. Structures of 2′,4′-BNA/LNA and ENA™.

INTRODUCTION

We have synthesized novel nucleosides, 2′-*O*,4′-*C*-ethylene nucleosides, and their corresponding phosphoramidites as building blocks (Fig. 1).[1,5] The ^{1}H-NMR analysis showed that the 2′-*O*,4′-*C*-ethylene linkage of these nucleosides restricts the sugar puckering to the *N*-conformation to the same extent as the linkage of 2′-*O*,4′-*C*-methylene nucleosides which are known as bridged nucleic acids (2′,4′-BNA)[2] or locked nucleic acids (LNA).[3] The ethylene-bridged nucleic acids (ENA) showed a high binding affinity for complementary RNA strands ($\Delta T_m =$ +5.2°C/modification) and were more nuclease-resistant than natural DNA and 2′,4′-BNA/LNA.[3] Here, we will describe the ENA-antisense activity for vascular endothelial growth factor (VEGF) in A549 lung cancer cells.

RESULTS AND DISCUSSION

In this study, ENA/DNA chimeric oligonucleotides with RNase H-mediated activity were used as antisense oligonucleotides. When ENA-modified antisense oligonucleotides against VEGF mRNA were introduced into the cells in the presence of a cationic polymer, more than 90% inhibition of VEGF mRNA production was observed based on RT-PCR analysis. Mismatch ENA oligonucleotides, used as controls, did not show any inhibitory activity.[4] Moreover, 13 genes that were considered likely to bind to VEGF ENA-antisense were found in Genbank by BLAST and Clustal W search, and inhibition of their expression by VEGF ENA-antisense was examined.[4] VEGF ENA-antisense inhibited the expression of three genes to almost half the expression levels of the control and the expression of the other identified genes was not affected as compared with the control. When shorter ENA-antisense oligonucleotides were used, the inhibitory activity was found to be lower. These results indicate that ENA-antisense oligonucleotides act in a sequence-specific manner, and could be used as effective antisense drugs.

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