

Development of artificial nucleic acids to
selectively recognize CG inversion site for the
triplex DNA formation and its antigene
application to efficient inhibition of hTERT
gene

王, 磊

<https://hdl.handle.net/2324/2236164>

出版情報 : Kyushu University, 2018, 博士 (創薬科学) , 課程博士
バージョン :

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氏 名 : 王 磊

論文題名 : Development of artificial nucleic acids to selectively recognize CG inversion site for the triplex DNA formation and its antigene application to efficient inhibition of hTERT gene (3本鎖 DNA 形成を目指した CG 障害部位を選択的に認識する人工核酸の開発と遺伝子の発現を効率的に阻害するアンチジェン法への展開)

区 分 : 甲

論 文 内 容 の 要 旨

The antigene strategy based on site-specific recognition of duplex DNA by triplex DNA formation has been exploited in a wide range of biological activities. However, the specific triplex formation is mostly restricted to homo-purine strands within the target duplex DNA, due to the destabilizing effect of CG and TA inversion sites where there is an absence of natural nucleotides that can recognize the CG and TA base pairs. Hence, the design of artificial nucleosides, which can selectively recognize these inversion sites with high affinity, should be of great significance.

Recently, we determined that 2-amino-3-methylpyridinyl pseudo-dC ($^3\text{MeAP-}\Psi\text{dC}$, Fig. 1-1A) possessed significant affinity and selectivity toward a CG inversion site and showed effective inhibition of gene expression. It was anticipated that the 1-N position of 2-aminopyridine within $^3\text{MeAP-}\Psi\text{dC}$ was protonated to form three hydrogen bonds with the CG base pair. To affirm this hypothesis, I designed a AP- ΨdC derivative in which the 3-methyl group of the aminopyridine unit was replaced by a halogen atom (Cl, Br or I, Fig. 1-1B) that caused a reduction of the pK_a value of the 1-N position (pK_a value is ~ 4.5) and also decreased the triplex stability and selectivity to the CG inversion site. These results also supported the hypothesis of the contribution of the protonated 1-N position to the triplex formation with the AP- ΨdC derivatives.

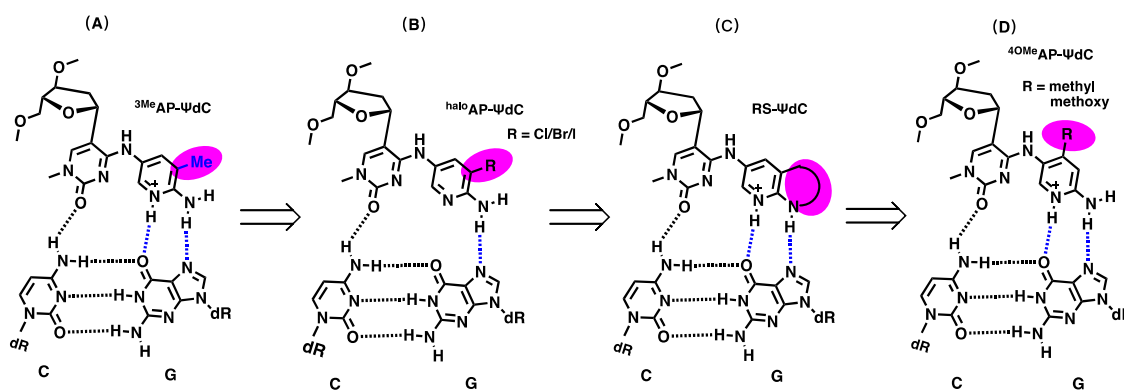


Figure 1-1 development of artificial nucleic acid for recognition of CG base pair

Therefore, the study initiated to improve the hydrogen binding affinity, by increasing the pK_a of the 1-N position of aminopyridine within AP- ΨdC derivatives, was carried out. According to the molecular design concept, I devised two types of new AP- ΨdC derivatives to confirm the hydrogen bonding effect of the 1-N

position and substituent effect of the aminopyridine unit. One series includes bicyclic compounds (Fig. 1-1C): i.e. DHAID-ΨdC, THNTD-ΨdC, and AID-ΨdC. In another series, the methyl or methoxy group was introduced at the 4-position of the 2-aminopyridine unit (Fig. 1-1D), ⁴MeAP-ΨdC and ⁴OMeAP-ΨdC, respectively. For the bicyclic derivatives (THNTD-ΨdC, DHAID-ΨdC, and AID-ΨdC), their stability is lower than the original ³MeAP-ΨdC with a sequence dependency. On the other hand, in the case of the 4-substituted derivatives, ⁴OMeAP-ΨdC showed a remarkable affinity and selectivity toward the CG base pair regardless of adjacent base pairs, which was also confirmed by DNase I footprinting assays. It was found that ⁴OMeAP-ΨdC distinguishes cytosine and 5-methylcytosine and recognizes the CG base pair in dsDNA compared to dsRNA and DNA/RNA heteroduplexes. Remarkably, ⁴OMeAP-ΨdC formed a stable triplex DNA with the sequence from the promoter region of the hTERT and the Cyclin D1 gene including multiple CG inversion sites.

In order to investigate the antigene effect on cultured cells, I used the TFOs containing ⁴OMeAP-ΨdC and their derivatives for targeting sequences including multiple CG base pairs. The Guard-oligo containing different number and position of dU base was performed to protect TFO from digestion of nuclease in the form of Guard-duplex. Once the Guard-duplex reaches the nucleus, the nuclear repair enzyme, UDG, could recognize and excise them to release the single-stranded TFO, followed by binding target gene to control the gene expression (Fig. 1-2). This hypothesis was affirmed by the assay of TFO release and triplex formation with the treatment of UDG *in vitro* and the fluorescence microscopy measurement of Hela cells. The proliferation inhibition assay by MTS method still indicated the Guard-duplex could effectively inhibit the gene expression and cell proliferation than that of single-stranded TFO.

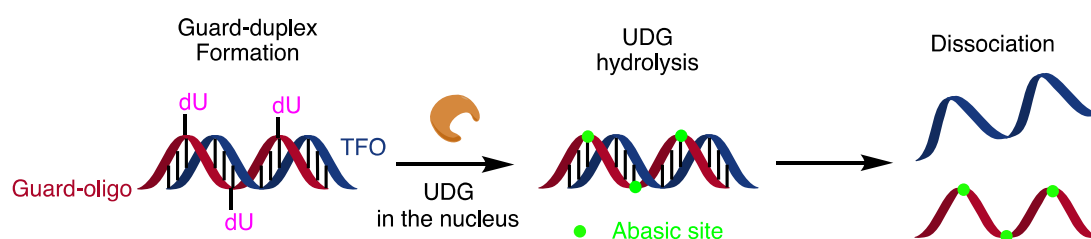


Figure 1-2 our strategy for the Guard-oligo protection and dissociation

The formation of triplex DNA has been considered to be difficult as a genomic target technology because of limited formation sequence. The AP-ΨdC derivative developed in this study is considered to be a big breakthrough in that it recognizes a CG inversion site in an arbitrary sequence and enables to form stable triplex DNA. As gene expression mechanisms are being clarified one after another, there is a growing need for techniques to recognize genomic DNA in a sequence-specific manner. From now on, we strongly hope that triplex DNA formation using AP-ΨdC derivatives will be established as an innovative genome target technology and will contribute to a wide range of fields.