Crosstalk Between Chemical Biology and Structural Biology of RNA Interference

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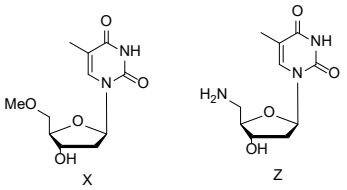
Small interfering RNA (siRNA) represents the most common and the most effective method to inhibit target gene expression in human cells. In order to optimize the chemical structure of siRNA for biological and medical applications, DDS and minimization of off-target effect are critical issues.

In the present study, we investigated RNA interference (RNAi) efficiencies of chemically modified siRNAs and the relationship with the structure of human Argonaute 2 protein[1]. Modifications include 5'-ends, major groove side of bases, and 3'-overhangs. Especially, we would like to focus on siRNAs bearing 5'-O-methylthymidine (X) and 5'-aminothymidine (Z) at 5'-end of the strands[2].

Ant-EGFP siRNAs (214-234)
sense; 5'-RACGGCAAGCUGACCCUGAag-3'
antisensne; 5'-RCAGGGUCAGCUUGCCGUAgg-3'
R = U, T, X, Z



The results showed that modification of the 5'-end of siRNA with X or Z significantly affected on the recognition of asymmetry of double stranded siRNA, namely, strand selection during RLC and RISC formation and also on the stability of RISC bearing X/Z-modified guide strand. Modification of the 5'-end of the sense strand with X or Z significantly increased the chance for the antisense strand to be selected as the guide strand. Modification of the 5'-end of the guide strand with X or Z destabilized RISC and decreased silencing efficiency of siRNA. These results strongly suggested that modification of 5'-end of the sense strand with X and Z will eliminate the off-target effect of the sense strand.

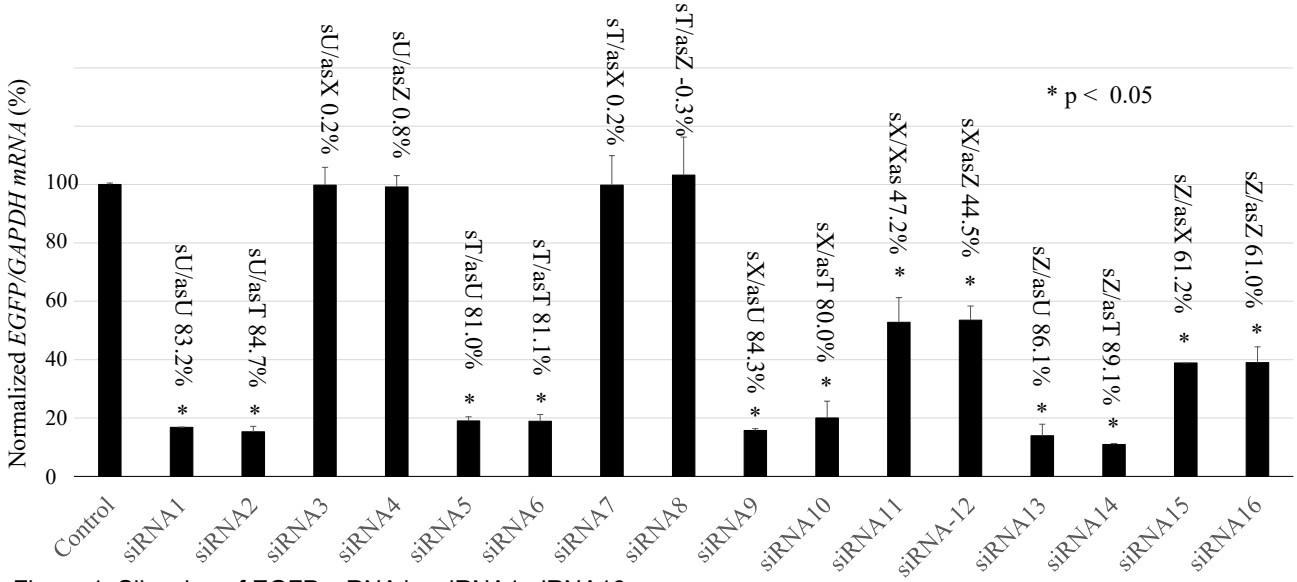


Figure 1. Silencing of EGFP mRNA by siRNA1-siRNA16.
HeLa (5 x 10^4 cells /well,10 % FBS/MEM) maintained in 5% CO_2, at 37°C, for 24h were transfected with siRNA at the final concentration of 100 nM using Lipofectamine® 2000 as described in the Materials and Methods. The values represent the mean ± SD of 3 independent experiments. The results were evaluated by Kruskal-Wallis ($p < 0.0001$) and multiple comparisons uncorrected Dunn's test. *$p < 0.05$ versus values of the negative control (scramble siRNA). Control = scramble siRNA.

References:
1. Schirle, NT. and MacRae, IJ. (2012) The crystal structure of human argonaute2. Science, 336 (6084) 1037–1040.
2. Masayuki Fujii, et al, (2022) Elimination of Off-target Effect by Chemical Modification of 5'-End of siRNA Nucleic Acid Therapeutics, 2022, 32(5):438-447.