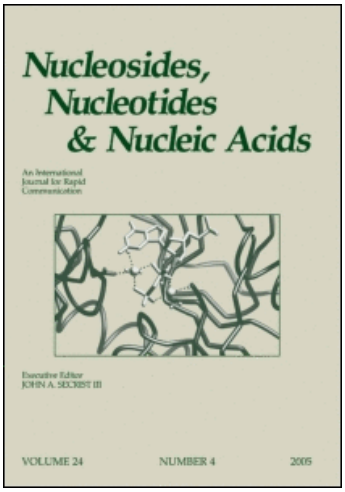

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Protein and RNA of Human Telomerase as Targets for Modified Oligonucleotides

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PROTEIN AND RNA OF HUMAN TELOMERASE AS TARGETS FOR MODIFIED OLIGONUCLEOTIDES

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ABSTRACT. Chimeric oligodeoxynucleotides containing phosphorothioate and N3′→P5′phosphoramidate linkages were synthesized. These oligomers show a high inhibitory activity against human telomerase.

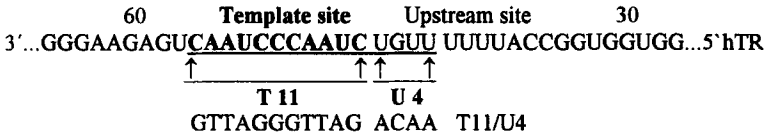
Telomerase was detected in most human tumors and is thought to be necessary for the sustained proliferation of cancer cells.[1] It is a polymerase carrying its own RNA template for the addition of multiples of TTAGGG telomeric repeats onto the ends of chromosomes.[2] Inhibition of telomerase may be considered as a potentially anticancer strategy changing the immortal state of cancer cells to a mortal one. One appropriate target might be the telomerase RNA.[3] Recently, we have found that phosphorothioate-modified oligodeoxynucleotides (PS-ODN) do not anneal sequence specifically to the RNA template but bind tightly in a sequence independent manner to a protein site of telomerase called primer binding site which produced a strong inhibition of telomerase.[4] Basing on these results we designed chimeric oligonucleotides (ODNs) combining strong protein and RNA binding properties thus retaining the high efficiency of PS-ODN but providing the required specificity by an antisense partner. Here we present the synthesis and first investigations of such chimeric oligomers.

A 15mer noncomplementary PS-ODN was extended by an antisense sequence covering T11/U4 of RNA (see below). This part of the oligomer was modified by N3′→P5′phosphoramidates as described.[5] We synthesized partially modified oligonucleotide analogs in which the 3′-oxygen of each thymidine residue was substituted with nitrogen. All oligomers were synthesized on an Applied Biosystems DNA 391

synthesizer, using 5′-cyanoethyl phosphoramidite methods. The monomer 5′-phosphoramidites and the solid supports were purchased from Glen Research.

Phosphorothioate linkages were generated by using tetraethylthiuram disulfide as the sulfurization reagent.[6] The 5′-(N,N-diisopropyl-amino-2-cyanoethyl)phosphoramidite-3′-(monomethoxytrityl)-amino-2′,3′-dideoxythymidine monomer was prepared according a published method.[5] The oligonucleotides were estimated as inhibitors of telomerase in HL-60 cell extract using TRAP-eze™ detection kit (Oncor). [7] The quantitative evaluation of the radioactive telomerase products followed by the Bioimage Analyzer BAS 2000 (Fuji).

Targeted RNA sequence of human telomerase



noncomplementary PS sequence	complementary T11/U4/Pam/PO	
5`-**TCA GAT TAG GAC TGC-** $GT^{am}T^{am}$ AGG $GT^{am}T^{am}$ AG ACAA-3′		IC_{50} = 1.1 nM
5′-**TCA GAT TAG GAC TGC-** T^{am}CA $GAT^{am}T^{am}$ AG GAC T^{am}GC-3′		IC_{50} = 4.9 nM
noncomplementary PS sequence	noncomplementary Pam/PO sequence	

T^{am} = 3′-Amino-2′,3′-dideoxythymidine

The noncomplementary 15mer PS-ODN extended at the 3′-end with the T11/U4/Pam/PO sequence displayed an IC_{50} of 1.1 nM. Replacement of the antisense sequence of the Pam-ODN part by a noncomplementary sequence gives an IC_{50} value of 4.9 nM. These data indicate that the antisense part contributes really to the effect of the chimeric ODN. Further appropriate modifications adapted to both telomerase targets might be a way to develope effective and selective inhibitors of human telomerase at the cellular level.

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