Improvement of RecQ-siRNA ability by site-specific 2'-O-methyl modifications

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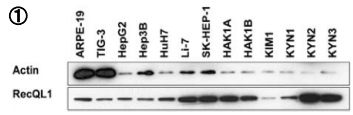
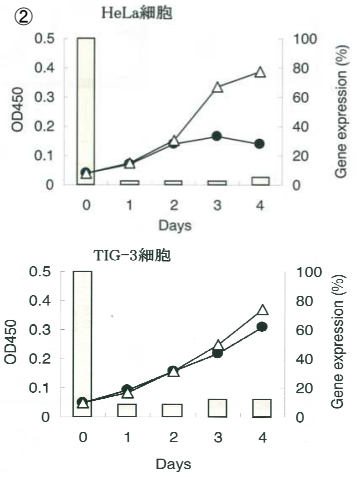
Background

①



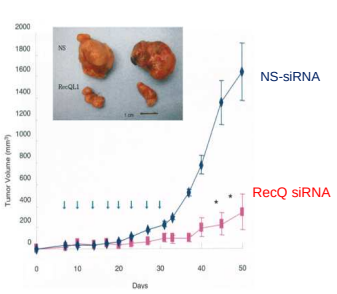
RecQ protein was high expressed in many cancer cell lines. Ref.1

②



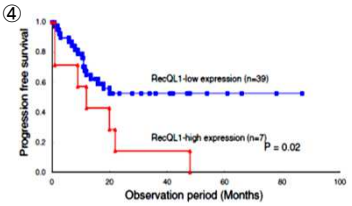
RecQ siRNA (●) suppresses proliferation of tumor cells (above), but not normal cells (bottom). Bar, RecQ expression. △, negative control siRNA. Ref.2

③



Subcutaneous injection of RecQ siRNA suppresses tumor growth in mice. Ref.3

④

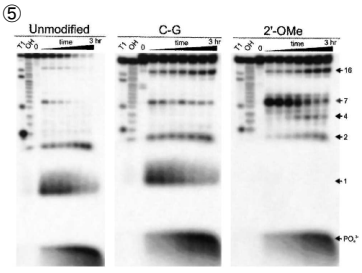


RecQ high expression relates to progression free survival in ovarian cancer patients. Ref.4

Abstract

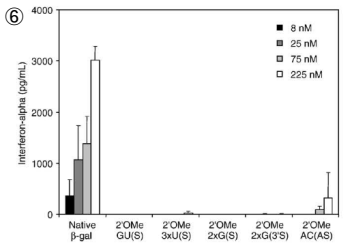
RecQL1 DNA helicase, which participates in DNA repair and recombination pathways during cell-cycle replication, mediates cell growth especially in cancer cells. We previously reported the silencing of human *RecQL1* mRNA by RNA-intereference effects induced cancer cell-specific mitotic catastrophe both *in vitro* and *in vivo*. However naked-siRNA is easily digested by several types of nucleases. Against the rapid nuclease degradation, when we added 2′-*O*-methyl nucleic modifications into the native form in order to increase stability of the siRNA molecules, we found the specific sites that the siRNA induced higher rather than similar the gene silencing of human *RecQL1* mRNA. And the modified siRNA indicated the higher ability than its native form to inhibit 2-dimensional cell proliferation in several types of human cancer cells. Also, the modified siRNA suppressed more 3-dimensional cell growth of human lung cancer cells. Our findings suggest that optimized nucleic modifications are useful and required for cancer chemotherapy by nucleic drug including siRNA.

⑤



Stabilization of the strand by Chemical modification in PBS containing 3% human serum. Ref.5

⑥



Selective 2'Ome modifications to siRNA suppresses cytokine induction in human PMBC. Ref.6

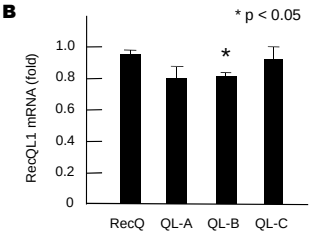
Result ① Site-specific 2′*O*Me modified RecQ siRNA enhanced the gene-silencing effect.

A

RecQ siRNA (sense)	5'-GUUCAGACCA CUUCAGCUUdTdT -3'
QL-A	5'-GuucAGAccA cuucAGcuudTdT -3'
QL-B	5'-GuucAGACCA cuucAGcuudTdT -3'
QL-C	5'-GuucAGACCA CUuCAGCUUdTdT -3'

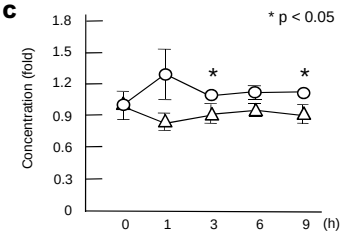
Design of sense ("passenger") sequences of *RecQL1* siRNA. Lower case indicates the nucleotide modified by 2'OMe.

B



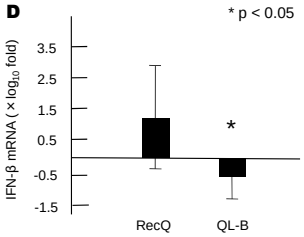
QL-B indicated higher gene silencing ability compared to RecQ.

C



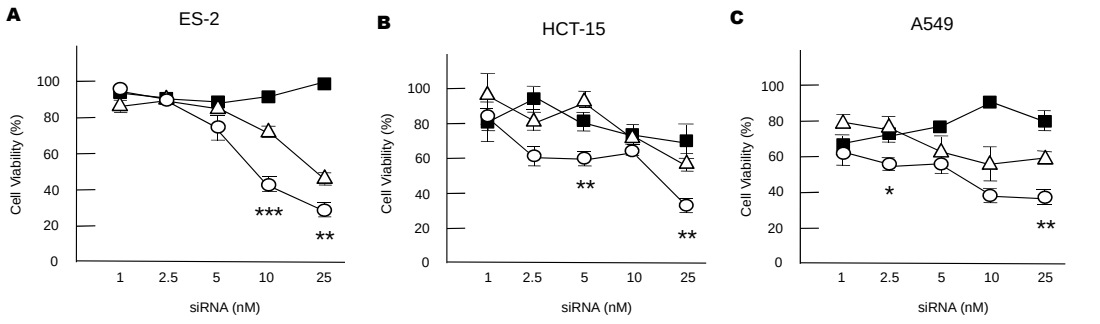
QL-B (○) stabilizes in serum to RecQ (△).

D



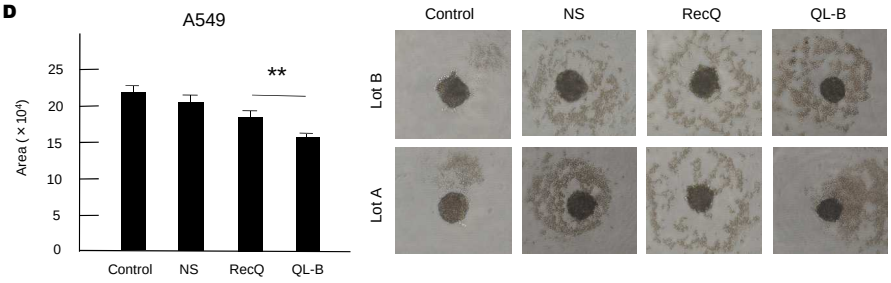
QL-B suppressed higher IFN expression.

Result ② QL-B enhanced the ability of anti-tumor effect in addition to gene-silencing effect.



QL-B (○) inhibits higher cell proliferation compared to RecQ (wild-type, △) and negative control (■) in ES-2 (*A*), HCT-15 (*B*), and A549 (*C*). Data are indicated by the mean of four or five samples. Error bars show s.e.m. * p < 0.05, ** p < 0.01, *** p < 0.001.

D



QL-B inhibits higher cell proliferation of 3-dimensional spheroids compared to RecQ and negative control (NS) in A549. Area of cells were measured by ImageJ software. ** p < 0.01.

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Materials and Methods

Cell line

HeLa S3, ES-2, HCT-15, A549 were obtained from the American Type Culture Collection (ATCC). THP-1 was provided by RIKEN BRC.

siRNA and RNA interference

siRNA targeting *RecQL1* mRNA (*RecQ* siRNA) and negative control (non-silencing; *NS*) siRNA were chemically synthesized (GeneDesign, Osaka, Japan). Cells were transfected with *RecQ* or *NS* siRNA using Lipofectamine RNAi MAX (Invitrogen).

Real-time quantitative PCR

Total RNA was extracted from cells using NucleoZOL (Macherey-Nagel, Düren, Germany), and served as a template for real-time quantitative PCR. A Roter-Gene Q 2plex system (Qiagen) and designed probes (ABI) were used for quantitative analyses of mRNA of *RecQL1*, *Ifnb*, and beta-actin (*Actb*). The level of *RecQL1* or *Ifnb* mRNA in a sample was normalized to that of *Actb* mRNA.

Serum stability test

siRNA was mixed with Phosphate-buffered saline supplemented with 10% NCS and incubated at 37◦C for 0, 1, 3, 6 and 9 h. Mixtures were taken at each of the time points and used as samples for measuring the amounts of RNA double helix by the Quant-iT RiboGreen RNA Assay Kit (ThermoFisher Scientific).

Cell proliferation assays

Cell proliferation was measured by colorimetric assays of cell viability based on cleavage of the tetrazolium salt WST-8 (Nacalai Tesque) by mitochondrial dehydrogenase at 96 h after transfection.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

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