Effect of a Novel Antibiotic, Heliquinomycin, on DNA Helicase and Cell Growth

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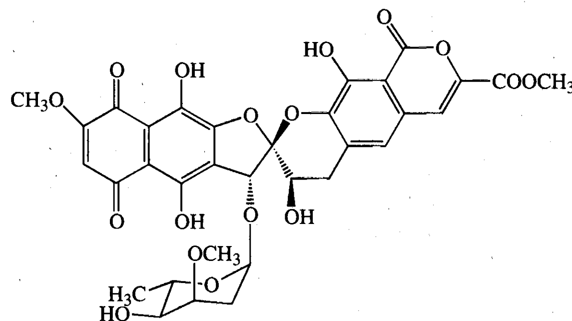
Heliquinomycin, a novel microbial product, was found to inhibit a human DNA helicase enzyme isolated from HeLa S3 cells at concentrations of 5 to 10 μg/ml. In contrast, adriamycin, etoposide and cisplatin did not inhibit this enzyme at the concentrations tested. Furthermore, the replication and repair of SV40 chromosome were not affected at heliquinomycin concentration of 50 μg/ml. The topoisomerase II and I enzymes were inhibited at 30 μg/ml and 100 μg/ml of heliquinomycin, respectively. Heliquinomycin inhibited the growth of HeLa S3, KB, LS180, K562 and HL60 human tumor cell lines at IC_{50} values of 0.96 to 2.8 μg/ml. In addition, the growth of adriamycin and cisplatin resistant P388 cell lines were inhibited at similar concentrations. Heliquinomycin inhibited both DNA and RNA synthesis in cell culture but did not inhibit protein synthesis. HeLa S3 cells were arrested at the G_2/M phase by heliquinomycin. These studies suggest that heliquinomycin is a selective inhibitor of a cellular DNA helicase and in turn, inhibits growth of tumor cell lines.

Cellular DNA replication and repair are catalyzed by a complex of enzymes which can be measured using the simian virus SV40 cell-free DNA replication and repair assay system[1~3]. Therefore, this assay can be used to identify inhibitors of DNA polymerase, proliferating cell nuclear antigen (PCNA) and the ssDNA binding proteins. One of the binding proteins, the large T antigen, is a viral protein encoded by SV40 which displays DNA helicase activity[4]. DNA helicase was first identified as a ssDNA binding protein with ATPase activity from *E. coli*[5] and is known to catalyzes the unwinding of dsDNA to single-strand template required for DNA replication and repair. Although human DNA helicase I~V[6~10], human helicase α[11], helicase ε[12] from HeLa cells and helicase B[13] from tsFT848 cells have been described, a cell specific function has not yet been fully elucidated. A selective inhibitor of human cell helicase activity could be useful in further characterize the function of helicase in cell growth and may function as a selective antitumor agent.

In this context, we identified a novel human DNA helicase inhibitor named heliquinomycin (Fig. 1). In this paper, we report the inhibitory activities of heliquinomycin on DNA helicase and on tumor cell growth.

Fig. 1. Structure of heliquinomycin.



Materials and Methods

Cells

Adriamycin-resistant P388 murine leukemia cells (P388/ADM), cisplatin-resistant cells (P388/CDDP) and their parental cells (P388/S) were kindly provided by Dr. M. INABA and Dr. T. TASHIRO, Cancer Chemotherapy Center of Japanese Foundation for Cancer Research (Tokyo, Japan). These cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 10 μM 2-mercaptoethanol at 37°C.

HeLa S3, HL-60 and LS180 cells were purchased from Dainippon Pharmaceutical Co. (Osaka, Japan), and K562 cell was supplied from RIKEN Cell Bank (Tsukuba, Japan). HeLa S3 and LS180 cells were maintained in E-MEM containing 10% fetal bovine serum, and HL-60 and K562 cells were maintained in RPMI 1640 containing 10% fetal bovine serum.

Enzymes

DNA helicase was prepared from HeLa S3 cells. The exponentially growing cells were collected and stocked at −80°C. The frozen cells were thawed on ice, and a nuclear extract fraction was prepared according to the method described by DIGNAM *et al.*[16] and purified by chromatography on Bio-Rex 70 column as described by TUTEJA *et al.*[6]. SV40 large tumor antigen was prepared from insect cells (SF9) infected with a recombinant baculovirus as described by WOBBE *et al.*[17]. DNA polymerase α-primase complex[18], PCNA[19], human single-strand DNA binding protein (also designated RF-A9)[20], activator 1 (A1, also designated RF-C)[21] and DNA polymerase δ[22] were prepared from HeLa cells as described by SUGASAWA *et al.*[23]. Human topoisomerase I and II were purchased from TopoGEN, Inc. (Columbus, Ohio).

Reagents

Adriamycin and camptothecin were purchased from Sigma Co. (St. Luis, MO). Aphidicolin was purchased from Wako Pure Chemicals Industries, LTD. (Osaka, Japan). Cisplatin and etoposide were prepared by Nippon Kayaku Co. (Tokyo, Japan). [α-^{32}P]dATP, [γ-^{32}P]ATP, [*methyl*-1,2-^{3}H]thymidine, [5,6-^{3}H]uridine and [4,5-^{3}H]L-leucine were purchased from DuPont/NEN Research Products (Wilmington, DE).

DNA Helicase Assay

DNA helicase activity was assessed by the partly modified methods reported by TUTEJA *et al.*[6]. The activity was measured by means of the release of an α^{32}P-labeled DNA fragment (d(GTAAAACGACGG CCAGT)) annealed to a circular M13mp18 DNA molecule. The reaction was terminated by the addition of 0.06% SDS, 2 mM EDTA, 1% glycerol and 0.02 mg/ml bromophenol blue. After further incubation at 37°C for 5 minutes, the resulting products were detected by electrophoresis on a 12% nondenaturing polyacrylamid gel. The gel was dried under vacuum and exposed to Hyperfilm (Eastman Kodak Company, Rochester, New York) with an intensifying screen for autoradiography. The IC_{50} value was calculated for test sample by measuring 50% inhibition of enzyme activity.

Cell-free DNA Replication and Repair Assay

DNA replication assay was assessed according to the method reported by ISHIMI *et al.*[1]. The reaction mixture containing [α-^{32}P]dCTP was incubated with 1 μl of test sample at 37°C for 2 hours. The replication products were subjected to 1% agarose gel electrophoresis with Tris-acetate-EDTA buffer.

DNA repair assay was assessed using the method reported by SUGASAWA *et al.*[3]. The reaction mixture containing [α-^{32}P]dCTP was incubated with 1 μl of teat sample at 37°C for 3 hours. The resulting products were subjected to 1% agarose gel electrophoresis with Tris-acetate-EDTA buffer as desacrived previously[3].

In both test system, the incorporation of radiolabeled nucleotide into DNA was determined by Fuji BAS2000 Bio-imaging Analyzer (Fuji Photo Film Co., LTD. Tokyo, Japan).

Topoisomerase-mediated DNA Cleavage

Inhibition of topoisomerase I was assessed using the method reported by TRASK *et al.*[24]. The reaction mixture was incubated with 2 μl of test sample at 37°C for 10 minutes.

The activity on topoisomerase II was assessed using the method reported by MULLER *et al.*[25]. The reaction mixture was incubated with 2 μl of test sample at 37°C for 10 minutes and the reaction was stopped by the addition of 4 ml of 0.25 M EDTA, 0.25% BPB and 50% glycerol.

The resulting products were subjected to electrophoresis in 1% agarose gel in the presence of 0.5 μg/ml of ethidium bromide.

Macromolecular Synthesis in Cells

HeLa S3 cells were plated at 1×10^{4} cells/well in 96-well plates, incubated at 37°C for a day, and after addition

of sample reincubated for 1.5 hours, and then labeled with 67 kBq/ml [*methyl*-1,2-^{3}H]thymidine (925 Gbq/mmol), [5,6-^{3}H]uridine (1.8 TBq/mmol), or [4,5-^{3}H]L-leucine (5.25 TBq/mmol) for 1 hour. After incorporation of the radioactive precursor, the cells were harvested by MicroMate 196 cell harvester (Packard Instrument Co., Meriden, CT) and washed with H_2O five times. The incorporated radioactivity was counted using a Matrix 9600 Direct Beta Counter (Packard Instrument Co.).

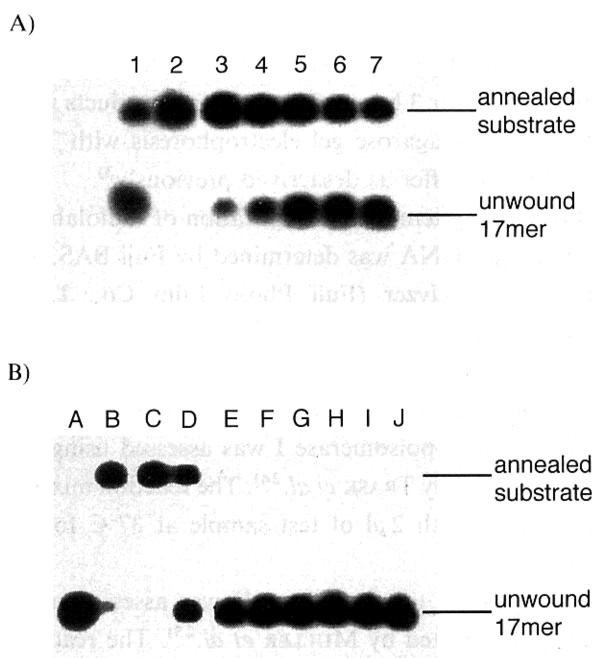
Flow Cytometry

HeLa cells were incubated in complete medium with 1.0 μg/ml aphidicolin for 16 hours to synchronize to the G_1/S-boundary. The cells were washed with PBS, and treated with heliquinomycin at 37°C. After treatment for 48 hours, cells were washed and fixed in ice-cold 50% methanol and stored at −20°C until analysis. The fixed cells were incubated with 1 mg/ml RNaseA for 1 hour at 37°C, and stained with 50 μg/ml propidium iodide for 2 hours on ice. The distribution pattern of cellular DNA content in cell cycle was analyzed by FACScan flow cytometer (Japan Spectroscopic Co., Ltd. Tokyo, Japan), counting 1×10^4 cells for each assay.

Drug Sensitivity Test

Tumor cells were incubated in 96-well plate for 24 hours. prior to the addition of test sample into culture well at varied concentrations. After 2 to 3 day incubation at 37°C, MTT reagent solution was added and incubated for further 4 hours. Growth inhibition activity was determined according to the standard MTT assay method[26] and IC_{50} was calculated.

Fig. 2. Autoradiography of DNA helicase assay mixture.



Anneald substrate DNA and unwound 17-mer substrate are separated on polyacrylamide gel electrophoresis as indicated.

A: Lane 1, control (DNA helicase alone); Lane 2, untreated DNA substrate (without enzyme); Lanes 3∼7, 10, 5, 2.5, 1.25 and 0.62 μg/ml heliquinomycin.

B: Lane A, control; Lane B, untreated DNA; Lanes C∼D, 10 and 5 μg/ml heliquinomycin; Lanes E∼F, 10 and 5 μg/ml adriamycin; Lanes G∼H, 10 and 5 μg/ml CDDP; Lanes I∼J, 10 and 5 μg/ml etoposide.

Results

Effect on DNA Helicase Prepared from HeLa S3 Cells

The effect of heliquinomycin on DNA helicase is shown in Fig. 2A. Heliquinomycin reduced the separation of 17mer primer from the annealed DNA substrate at concentrations from 5 to 10 μg/ml. Concentration of heliquinomycin below 5 μg/ml showed no significant activity. The other DNA interactive agents, adriamycin, cisplatin and etoposide tested did not inhibit DNA helicase activity at concentrations of 10 μg/ml, Fig. 2. In additional studies, heliquinomycin was demonstrated to inhibit DNA helicase in a noncompetitive manner with a *Ki* value of 6.8 μM.

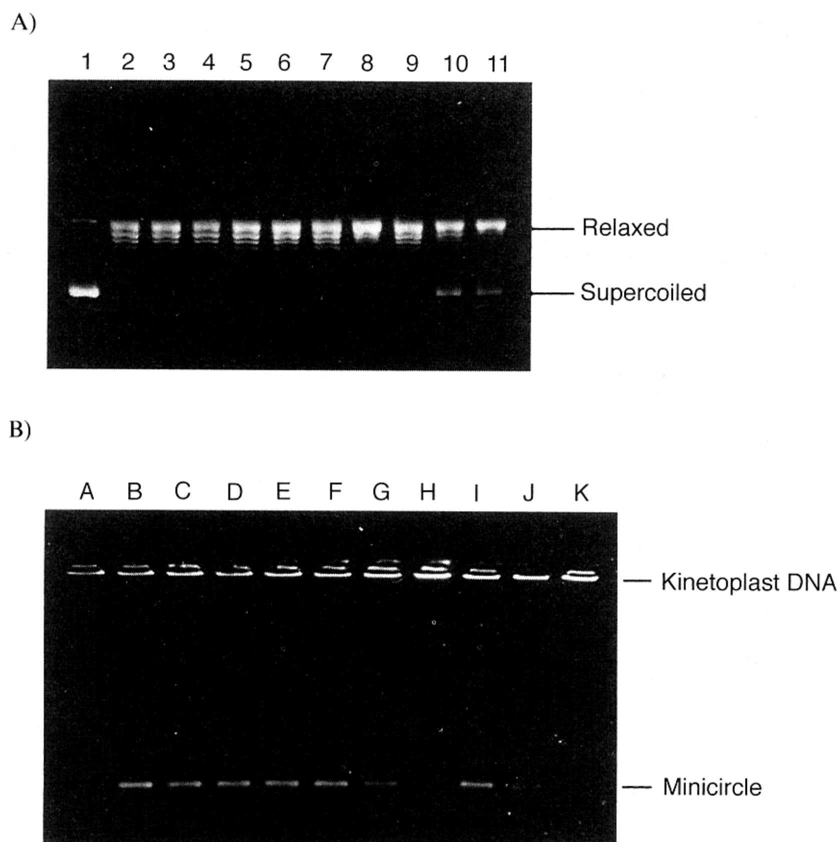
Effect on Replication and Repair of DNA in Cell-free System

Utilizing the SV40 chromosome as the template for DNA replication and the nicked SV40 DNA for DNA repair, heliquinomycin was shown to have no effect on replication and repair of DNA at concentrations of

Table 1. Effect on replication and repair of DNA in cell free system.

Compounds	IC_{50} (μg/ml)	
	DNA replication	DNA repair
Heliquinomycin	>50	>50
Aphidicolin	0.27	30
Etoposide	>50	>50

Fig. 3. Topoisomerase-mediated DNA cleavage by heliquinomycin.



A (topoisomerase I): Lane 1, supercoiled pBR 322 DNA; Lane 2, control (topoisomerase alone); Lane 3, 1% DMSO control; Lanes 4~8, 1, 3, 10, 30 and 100 μg/ml heliquinomycin; Lanes 9~11, 1, 10 and 100 μM camptothecin.

B (topoisomerase II): Lane A, kinetoplast DNA; Lane B, control (tpopisomerase II); Lane C, 1% DMSO control; Lanes D~H, 1, 3, 10, 30 and 100 μg/ml heliquinomycin; Lanes I~K, 1, 10 and 100 μg/ml etoposide.

Table 2. Inhibition of uptake of [^{3}H]thymidine, [^{3}H]uridine and [^{3}H]L-leucine into HeLa S3 cells.

Compounds	IC$_{50}$ (μg/ml)		
	[^{3}H]thymidine	[^{3}H]uridine	[^{3}H]L-leucine
Heliquinomycin	1.91	2.74	>100
Adriamycin	2.35	3.93	>10
Camptothesin	0.66	2.73	>10
Cisplatin	2.86	7.56	>10

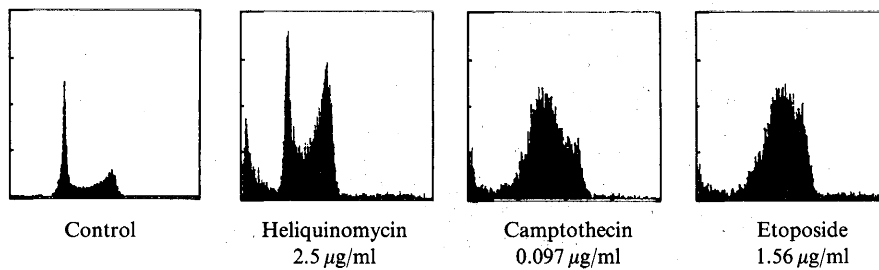
50 μg/ml (Table 1). Aphidicolin, a DNA polymerase α inhibitor, inhibited DNA replication but only partially inhibited DNA repair. In contrast etoposide, a DNA topoisomerase II inhibitor, showed no effect on replication or repair.

Effect on Topoisomerase-mediated DNA Cleavage

The topoisomerase enzymes catalyze the alteration of DNA topography and in turn, may be a target for

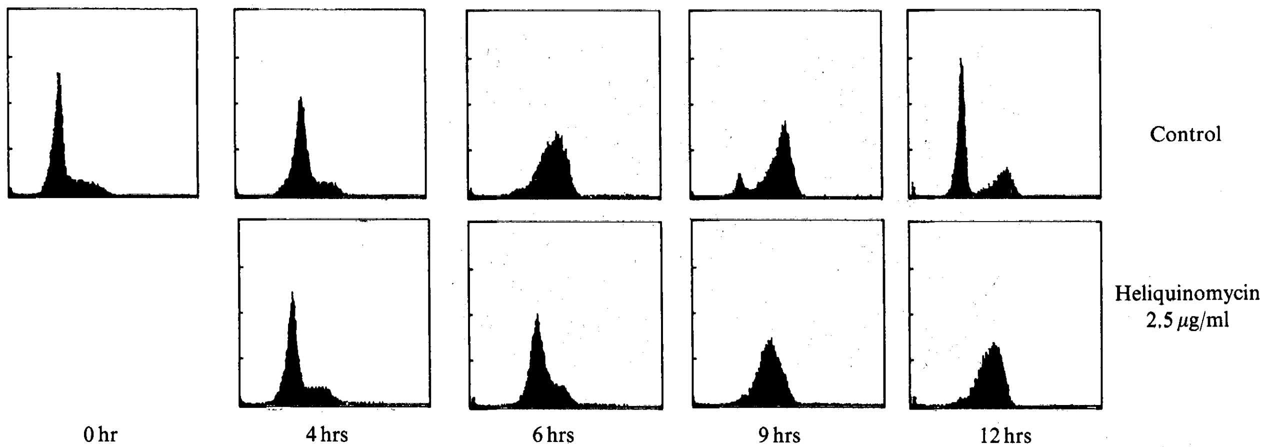
Fig. 4. Effect of heliquinomycin on the cell cycle progression of HeLa S3 cells.

A) Effect of heliquinomycin, camptothecin and etoposide on the cell cycle histogram of HeLa S3 cells.



1, Control; 2, 2.5 μg/ml heliquinomycin; 3, 0.097 μg/ml camptothecin; 4, 1.56 μg/ml etoposide.

B) Effect of heliquinomycin on the cell cycle histogram of HeLa S3 cells.



HeLa S3 cells were incubated with 2.5 μg/ml heliquinomycin.

Ordinate: frequency of cells; abscissa: DNA content per cell as represented by fluorescence intensity of propidium iodide.

Table 3. Effect of heliquinomycin against cell lines.

Cell line	Origin	IC_{50} (μg/ml)		
		Heliquinomycin	Adriamycin	Cisplatin
HL-60	Leukemia	1.00	0.04	0.37
K562	Leukemia	2.81	0.01	0.85
LS180	Colon cancer	1.18	0.24	1.46
KB	Nasophsarynx cancer	1.72	0.06	0.12
HeLa S3	Uterus cancer	1.62	0.09	0.39
Resistant cell line				
P388/S		0.36 (1)	0.06 (1)	0.24 (1)
P388/ADM		0.50 (1.3)	10.33 (164)	0.40 (1.6)
P388/CDDP		0.36 (1)	0.07 (1.1)	2.44 (10)

heliquinomycin activity. Topoisomerase I and II activities were inhibited at concentrations of heliquinomycin of 100 μg/ml (Fig. 3A) and 30 μg/ml (Fig. 3B), respectively. Camptothecin, a known topoisomerase II inhibitor, was active at 10 μg/ml in this assay system (Fig. 3B).

Effect on Macromolecular Synthesis of HeLa S3 Cells

As shown in Table 2, heliquinomycin inhibited incorporations of thymidine and uridine into HeLa S3 cells, but did not inhibit leucine incorporation. At the concentrations tested, heliquinomycin exhibited a stronger inhibition of thymidine incorporation than uridine incorporation.

Effect of Heliquinomycin on Cell Cycle of HeLa S3 Cells

The HeLa S3 cells were arrested at G_2/M phase when cultured with 2.5 μg/ml of heliquinomycin for 48 hours (Fig. 4A). The G_2/M phase arrest was confirmed with cells synchronized at G1/S after aphidicolin treatment. Heliquinomycin delayed cell cycle traverse of HeLa S3 cells from S phase to G_2 phase in 6 hours and consequently arrested at G_2/M phase in 9 hours (Fig. 4B).

Growth Inhibition of Human Tumor Cell Lines

Heliquinomycin inhibited growth of a variety of human tumor cell lines including adriamycin and cisplatin resistant P388 cells at concentrations from 1.0 to 2.8 μg/ml (Table 3).

Discussion

DNA helicase enzymes are believed to be required for DNA replication, repair, transcription and recombination. Since DNA helicase would be required primarily during active cellular replication, it represents an attractive target for a new antitumor agent. Heliquinomycin a novel microbial product, was identified using 17 nucleotides DNA polymer hybridizes with M13mp18 as the substrate for a DNA helicase enzyme prepared from HeLa S3 cells.

In this assay system, heliquinomycin inhibits DNA helicase activity in a dose dependent manner, although adriamycin, cisplatin and etoposide did not affect the activity. The data suggests that the mechanism of action of heliquinomycin is different from the other antitumor agents tested.

Anthracycline antibiotics such as adriamycin, nogalamycin and cytoxantorone have been reported to inhibit DNA helicase[27,28]. The discrepancy between our DNA helicase and those of other investigators can be attributed to difference in the substrates used in the assays. The substrate used in this study dose not contain a 5'-AGC sequence which can bind to anthracyclines. In other assay system employing SV40 T antigen as helicase source. Heliquinomycin did not inhibit helicase activity where SV40 T antigen was used as enzyme. Since most cellular DNA helicase repair a ssDNA region adjacent to dsDNA in order to bind and unwind the dsDNA and the SV40 large T antigen, *E. coli* helicase and *E. coli* Rec do not, heliquinomycin appears to be a specific inhibitor of human DNA helicase prepared from HeLa S3 cells. Heliquinomycin also inhibits topoisomerase II at relatively high concentration but did not inhibit topoisomerase I. These results suggest that heliquinomycin is not a DNA intercalator like adriamycin[29].

Heliquinomycin delayed the traverse of some cell fraction from S phase to G_2 phase and arrested these cells at the G_2/M phase after the S phase traverse. Although it is reported that bleomycin and neocarzinostatin bind to DNA and induce non-enzymatic fragmentation of DNA and in turn arrests cells in G_2/M phase[30,31], our data suggest that the effect of heliquinomycin on cell cycle progression is due to inhibition of helicase and/or topoisomerase II rather than DNA fragmentation.

Since heliquinomycin inhibits the growth of human tumor cells, adriamycin resistant cells and cisplatin resistant cells, it can be speculated that heliquinomycin does not affect the P-glycoprotein like adriamycin and dose not make cross linkage to DNA like cisplatin[32].

In conclusion, at concentrations that heliquinomycin inhibits DNA helicase from HeLa S3 cells, it did not inhibit other DNA replication enzymes tested and therefore, appears to be a selective cellular helicase inhibitor. This unique molecular mechanism distinguishes heliquinomycin from other antitumor agents. The novel mechanism and antitumor activity of heliquinomycin support advancing the molecule into animal efficacy studies.

Acknowledgments

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References

1) ISHIMI, Y.; K. SUGASAWA, F. HANAOKA & A. KIKUCHI: Replication of the simian virus 40 chromosome with purified proteins. J. Biol. Chem. 266: 16141~16148, 1991

2) SUGAWARA, K.; Y. ISHIMI, T. EKI, J. HURWITZ, A. KIKUCHI & F. HANAOKA: Nonconservative segregation of parental nucleosomes durihg simian virus 40 chromosome

replication *in vitro*. Proc. Natl. Acad. Sci. USA 89: 1055～1059, 1992

3) SUGASAWA, K.; C. MASUTANI & F. HANAOKA: Cell-free repair of UV-damaged simian virus 40 chromosomes in human cell extracts. J. Biol. Chem. 268: 9098～9104, 1993
4) SCHEFFNER, M.; R. KINPPER & H. STAHL: RNA unwinding activity of SV40 large T antigen. Cell 57: 955～963, 1989
5) RUNNUON, G. T. & T. M. LOHMSN: *Escherichia coli* helicase II (UvrD)protein can completely unwind fully duplex linear and nicked circular DNA. J. Biol. Chem. 264: 17502～17512, 1989
6) NARENDRA, T.; R. TUTEJA, K. RAHMAN, L.-Y. KANG & A. FALASCHI: A DNA helicase from human cells. Nucleic Acids Res. 18: 6785～6792, 1990
7) NARENDRA, T.; R. TUTEJA, A. OCHEM, P. TANEIA, N. W. HUANG, A. SIMONCSITS, S. SUSIC, K. RAHMAN, L. MARUSIC, J. CHEN, J. ZHANG, S. WANG, S. PONGOR & A. FALASCHI: Human DNA helicase II: a novel DNA unwinding enzyme identified as the Ku autoantigen. EMBO J. 13: 4991～5001, 1994
8) NARENDRA, T.; K. RAHMAN, R. TUTEJA, A. OCHEM, D. SKOPAC & A. FALASCHI: DNA helicase III from HeLa cells: an enzyme that acts preferntially on partially unwound DNA duplexes. Nucleic Acids Res. 20: 5329～5337, 1992
9) NARENDRA, T.; K. RAHMAN, R. TUTEJA & A. FALASCHI: DNA helicase IV from HeLa cells. Nucleic Acids Res. 19: 3613～3618, 1991
10) TUTEJA, N.; K. RAHMAN, R. TUTEJA & A. FALASCHI: Human DNA helicase V, a novle DNA unwinding enzyme from HeLa cells. Nucleic Acids Res. 21: 2323～2329, 1993
11) SEO, Y.-S. & J. HURWITZ: Isolation of helicase α, a DNA helicase from HeLa cells stimulated by a fork structure and single-stranded DNA-binding proteins. J. Biol. Chem. 268: 10282～10295, 1993
12) SEO, Y.-S.; S.-H. LEE & J. HUEWITZ: Isolation of a DNA helicase from HeLa cells requiring the multisubunit human single-stranded DNA-binding protein for activity. J. Biol. Chem. 266: 13161～13170, 1991
13) SEKI, M.; T. KOUDA, T. YANO, S. TADA, J. YANAGISAWA, T. EKI, M. UI & T. ENOMOTO: Characterization of DNA synthesis and DNA-dependent ATPase activity at a restrictive temperature in temperature-sensitive tsFT848 cells with theemolabile DNA helicase B. Mol. Cell. Biol. 15: 165～172, 1995
14) CHINO, M.; K. NISHIKAWA, M. UMEKITA, C. HAYASHI, T. YAMAZAKI, T. TSUCHIDA, T. SAWA, M. HAMADA & T. TAKEUCHI: Heliquinomycin, a new inhibitor of DNA helicase, produced by *Streptomyces* sp. MJ929-SF2. I. Taxonomy, production, isolation, physico-chemical properties and biological activity. J. Antibiotics 49: 752～757, 1996
15) CHION, M.; K. NISHIKAWA, T. TSUCHIDA, R. SAWA, H. NAKAMURA, T. K. NAKAMURA, Y. MURAOKA, D. IKEDA, H. NAGANAWA, T. SAWA & T. TAKEUCHI: Heliquinomycin, a new inhibitor of DNA helicase, produce by *Streptomyces* sp. MJ929-SF2. II Structure determination of heliquinomycin. J. Antibiotics 50: 143～146, 1997
16) DIGNAM, J. D.; R. M. LEBOVITZ & R. G. ROEDER: Accurate transcription initiation by RNA poliymarase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. 11: 1475～1489, 1983
17) WOBBE, C. R.; F. DEAN, L. WEISSBACH & J. HURWITZ: *In vitro* replication of duplex circular DNA containing the simian virus 40 DNA origin site. Proc. Natl. Acad. Sci. USA 82: 5710～5714, 1985
18) ISHIMI, Y.; A. CLAUDE, P. BULLOCK & J. HUEWITZ: Complete enzymatic synthesis of DNA containing the SV40 origin in replication. J. Biol. Chem. 263: 19723～19733, 1988
19) LEE, S.-H.; Y. ISHIMI, M. K. KENNY, P. BULLOCK, F. B. DEAN & J. HURWITZ: An inhibition of the *in vitro* elongation reaction of simian virus 40 DNA replication is overcome by proliferating-cell nuclear antigen. Proc. Natl. Acad. Sci. USA 85: 9469～9473, 1988
20) KENNY, M. K.; U. SCHLEGEL, H. FURNEAUX & J. HURWITZ: The rile of human single-stranded DNA binding protein and its individual subunits in simian virus 40 DNA replication. J. Biol. Chem. 265: 7693～7700, 1990
21) LEE, S.-H.; A. D. KOWNG, Z.-Q. PAN & J. HURWITZ: Studies on the activator 1 protein complex, an accessory factor for proliferating cell nuclear antigen-dependent DNA polymerase δ. J. Biol. Chem. 266: 594～602, 1991
22) LEE, S.-H.; T. EKI & J. HURWITZ: Synthesis of DNA containing the simian virus 40 origin of replication by the combined action of DNA polymerase α and δ. Proc. Natl. Acad. Sci. USA 86: 7361～7365, 1989
23) SUGASAWA, K.; Y. MURAKAMI, N. MIYAMOTO, F. HANAOKA & M. UI: Assembly of nascent DNA into nucleosome structures in simian virus 40 chromosomes by HeLa cell extract. J. Virol. 64: 4820～4829, 1990
24) TRASK, D. K. & M. T. MULLER: Biochemical characterization of topoisomerase I purified from avian erythrocytes. Nucleic Acids Res. 11: 2779～2800, 1983
25) TRASK, D. K.; J. A. DIDONATO & M. T. MULLER: Rapid detection and isolation of covalent DNA/protein complexes: application to topoisomerase I and II. EMBO J. 3: 671～676, 1984
26) MOSMANN, T.: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Immuno. Methods. 65: 55～63, 1983
27) BACHUR, N. R.; F. YU, R. JOHNSON, R. HICKEY, T. WU & L. MALKAS: Helicase inhibition by anthracycline anticancer angents. Mol. Pharma. 41: 993～998, 1992
28) GEORGE, J. W.; S. GHATE, S. W. MATSON & J. M. BESTERMAN: Inhibition of DNA helicase II unwinding and ATP ase activities by DNA-interacting ligands. J. Biol. Chem. 267: 10683～10689, 1992
29) WARING, M. J.: Variation of the supercoils in closed circular DNA by binding of antibiotics and drugs; evidence of molecular models involving intercalation. J. Mol. Biol. 54: 247～279, 1970
30) KIMLER, B. F.; M. H. SCHNEIDERMAN & D. B. LEEPER: Induction of concentration-dependent blockade in the G2 phase of the cell cycle by cancer chemotherapeutic agent. Cancer Res. 38: 809～814, 1978
31) EBINA, T.; K. OHTSUKI, M. SETO & N. ISHIDA: Specific G2 block in HeLa S3 cells by neocarzinostatin. Euro. Cancer Res. 11: 155～158, 1975
32) TSURUO, T.; H. IIDA, S. TSUKAGOSHI & Y. SAKURAI: Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonisits and calmodulin inhibitors. Cancer Res. 42: 4730～4733, 1982