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Report

Modification of the sensitivity to cisplatin with c-*myc* over-expression or down-regulation in colon cancer cells

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Human colon cancer SW480 cells express the c-*myc* gene. On the other hand, SW480DDP cell lines resistant to cisplatin exhibited decreased c-*myc* gene expression, but their cell growth rates remained similar to those of their parental cells. Antisense oligonucleotides to c-*myc* inhibited c-*myc* expression and induced increased resistance to cisplatin in SW480 cells. In contrast, SW480DDP cells showed increased c-*myc* expression and reversed sensitivity to cisplatin when these cells were transfected with c-*myc* cDNA from the pLNCX plasmid. Moreover, SW480DDP cells transfected with c-*myc* cDNA induced apoptosis when exposed to cisplatin, but not SW480 cells transfected with an antisense sequence for c-*myc*. Transfection either with a c-*myc* antisense sequence or c-*myc* cDNA to these cells did not change their growth rates. Thus enforced expression of c-*myc* in SW480 and SW620 lines sensitizes cells to cisplatin-induced apoptosis, whereas the down-regulation of c-*myc* in SW480DDP and SW620DDP increases cisplatin resistance when using antisense strategy. [© 2001 Lippincott Williams & Wilkins.]

Key words: Antisense oligonucleotides, apoptosis, cisplatin, c-*myc*, colon cancer, drug resistance.

Introduction

Cisplatin (*cis*-diamminedichloroplatinum) is one of the most widely used chemotherapeutic agents for the treatment of ovarian, testicular, head, neck and colon cancers, and sometimes even advanced cases of colon cancer.[1] Its efficacy, however, is often limited, because some patients display clinically inherent resistance to cisplatin[2] or acquire the resistance after the treatment.[3] Therefore we have undertaken to examine whether tumor cells can be made to retain their sensitivity to cisplatin, in order to improve the prognosis of many cancer cases. Along this line, we have tried to identify the genes responsible for the drug resistance to cisplatin in cancers[4] and revealed that the c-*fos* gene is implicated in cellular resistance to cisplatin in which the expression of the c-*fos* gene was increased in cisplatin-resistant cell lines.[5] Isonishi *et al.* also demonstrated cisplatin resistance in NIH 3T3 cells transfected with the mutated H-*ras* gene.[6] However, the mechanism of acquired resistance to cisplatin based on DNA damage is complex and other factors may be involved,[7] making it important to clearly identify the effectors of cisplatin resistance.

c-*myc* oncogene, a regulator of cell growth associated with cell division, is highly expressed in more than 70% of human colon cancers[8] and colon cancer is often resistant to cisplatin treatment.[2] The role of c-*myc* protein in cellular susceptibility to anticancer drugs is controversial. In fact, over-expression of c-*myc* protein has been reported to enhance tumor cell sensitivity[9–11] and to induce resistance in response to antineplastic agents.[12,13] This prompted us to investigate the role of the c-*myc* gene in cisplatin resistance in colon cancer. In this study, we used antisense oligonucleotides and transfection technology to determine whether the c-*myc* gene regulates cisplatin sensitivity in colon cancer.

Materials and methods

Reagents

Cisplatin and etoposide (VP-16) were obtained from Bristol-Myers (Tokyo, Japan). Adriamycin (ADR) was obtained from Kyowa Hakko Kogyo (Tokyo, Japan). MTT (3-[4,-5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) was obtained from Sigma (St Louis, MO).

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Genes and cells

Human c-*myc* cDNA (exons 2 and 3) was obtained from ATCC (Rockville, MD) as an insert. Human colon carcinoma cell lines SW480 and SW620 were obtained from ATCC, and maintained in Dulbecco's modified essential medium (DMEM; Gibco, Grand Island, NY), supplemented with 10% fetal bovine serum (Gibco) and penicillin–streptomycin (Gibco). SW480 and SW620 cells were grown with 2 h exposure to cisplatin every week for 6 months. SW480DDP and SW620DDP resistant sublines were obtained when the cells were transferred to fresh DMEM medium for 1 week, and colonies resistant to cisplatin were selected as described previously.[14] For cytotoxicity, the 50% inhibition concentrations for cell growth (IC_{50}) values of cisplatin in these cells were determined from doseûresponse curves from a dye reduction assay using MTT.

Synthesis of antisense oligonucleotides

Oligonucleotides were prepared with an Applied Biosystems model 380B automated synthesizer and synthesized by Sawady (Japan). The following phosphorothioates (S-oligo) were used for the *in vitro* antisense experiments: 15mer c-*myc* antisense sequence (AACGTTGAGGGGCAT, AS), complementary to the translation initiation codon and downstream sequences of human c-*myc* mRNA;[15] 15-mer c-*myc* sense sequence (ATGCCGCACAACGTT, S), corresponding to the translation initiation codon and downstream sequences of c-*myc* mRNA. Sense oligonucleotides were constructed with two base mismatches as a control.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Oligonucleotides used for RT-PCR detection of c-*myc* expression were as follows: upstream, 5-CCA GCT TGT ACC TGC AGG ATC TG-3; downstream, 5-AGG AGC CTG CCT CTT TTC CAC AGA-3.[16] Those used for primers for the β-actin gene were as follows: upstream, 5-TGG GAA TGG GTC AGA AGG ACT-3, downstream, 5-TTT CAC GGT TGG CCT TAG GGT T-3 corresponding to nucleotides of β-actin mRNA. Total RNA was extracted from cell lines by guanidium isothiocyanate CsCl gradient centrifugation according to the protocol of Nippon Gene (Toyama, Japan). RNA from each group was reverse-transcribed to cDNA using Moloney murine leukemia virus reverse transcriptase (BRL, Gaithersburg, MD) and random hexamers (Boehringer Mannheim, Mannheim, Germany) for 1 h at 37°C. The resulting cDNA was divided into two aliquots and amplified with 5 U of Taq polymerase (Boehringer Mannheim) in the presence of the primers through 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 2 min and synthesis at 72°C for 2 min. The PCR products were electrophoresed in 2% agarose gel with ethidium bromide staining.

Transfection studies

The c-*myc* cDNA was cloned into the *Hin*dIII sites of the pLNCX plasmid, which is 6.6 kb in size, has a long tandem repeat (LTR) and cytomegalovirus (CMV) promoters, and including, for the selection as an expression vector, a neomycin resistance gene originating from a retrovirus (provided by A Dusty Miller, Fred Hutchinson Cancer Research Center, Seattle, WA). The sequence and orientation of the insert were confirmed by dideoxynucleotide sequencing of the construct using a Sequenase kit (USB, Cleveland, OH). Subconfluent cells were transfected by electroporation according to a protocol provided by BTX (San Diego, CA). Briefly, cells were trypsinized and centrifuged to 2×10^6 cells/ml in 1 ml of PBS containing 20 μg of plasmid DNA. The cell and DNA mixture was loaded into a sterile 1.0-ml cuvette. A BTX Munipulator 600 system was used to generate an exponential decay pulse with a set peak of 4.0 kV/cm and capacitance at 1100 F. After the pulse, the mixture of cells and DNA was kept at room temperature for 20 min. The cells were plated onto 60-mm tissue culture dishes, incubated for 24 h and then selected in growth medium containing 500 μg/ml geneticin (G418 sulfate; Gibco) for 4 weeks. G418-resistant colonies were selected individually, grown and screened for expression of c-*myc* by RT-PCR analysis. These cells were rechallenged with G418 8 weeks later to test for the presence of the neomycin resistance gene.

Growth studies

Titers of viable cells in culture were determined by counting Trypan blue-excluding cells in a hemacytometer. For clonogenic assay, cells were placed in 24-well culture plates (Nunc, Naperville, IL) in DMEM medium in 10% fetal calf serum with or without oligonucleotides. Cells were incubated for 5 days and then stained with MTT dye, counted and scored.

Analysis of cisplatin-induced apoptosis

Cells were treated with cisplatin at the IC_{50} dose. For analysis of the DNA laddering characteristics of apoptotic cell death, DNA was isolated from cells as described previously.[17] The DNA samples were

subjected to electrophoresis on a 2% agarose gel and visualized according to the RT-PCR procedure used. For flow cytometric analysis, cells (2×10^6) were briefly collected by centrifugation, fixed in 70% ethanol at 4°C for 1 h, and then resuspended in PBS containing 40 μg/ml propidium iodide and 0.1 mg/ml RNase. The fixed target cells were subjected to terminal deoxynucleotide transferase-mediated dUTP-nick end-labeling (TUNEL) using an MEBSTAIN Apoptosis kit according to the manufacture's instructions (MBL, Nagoya, Japan). The detection of apoptotic cells was performed as described previously.[18] After 30 min at 37°C, the cells were analyzed with a FACSCalibur cytofluorometer (Becton Dickinson, San Jose, CA).

Results

Expression of the c-*myc* gene was partially inhibited after incubation with c-*myc* antisense oligonucleotides at optimal concentration and time, but was not inhibited with c-*myc* sense oligonucleotides by RT-PCR analysis (Figure 1). No effects of either oligonucleotides were revealed by the altered expression of the β-actin gene. Regarding cell cytotoxicity, after incubation with c-*myc* antisense oligonucleotides, cells displayed increased resistance to cisplatin, but resistance to ADR or VP-16 was unchanged with the MTT assay (Table 1). In contrast, after incubation with c-*myc* sense oligonucleotides, cells showed no indication of cytotoxic effects.

For over-expression of c-*myc*, SW480DDP and SW620DDP cells in culture were transfected with pLNCX including c-*myc* cDNA. Four weeks after transfection, G418-resistant cells obtained from some of the resistant clones were selected and studied for their drug sensitivity by MTT assay. SW480DDP and SW620DDP cells transfected with the plasmid including c-*myc* cDNA showed increased expression of the c-*myc* gene by RT-PCR analysis (data not shown). The IC_{50} of these cells indicated reversed resistance to cisplatin but not to other agents (Table 2). Cells transfected with the vector showed no change in drug sensitivity after transfection. There were no differences in growth characteristics of the cells incubated with oligonucleotides or transfected with the plasmid (data not shown). In contrast, SW480 and SW620 cells transfected with the plasmid including c-*myc* cDNA showed increased expression of the c-*myc* gene by RT-PCR analysis (data not shown). These c-*myc* over-expressing cells also exhibited increased sensitivity to cisplatin, but not to other agents, in terms of IC_{50} (data not shown).

Cells from the SW480 and SW620 lines were treated with the same concentrations (5 nmol/ml) of the sense or antisense oligonucleotides in liquid culture for 2 days. *In vitro* colony formation of these colon cancer cell lines was not inhibited after incubation with c-*myc* antisense or sense oligonucleotides at optimal concentrations, and were counted 10 days later (Table 3). The effects of higher concentrations of oligonucleotides were not examined. c-*myc* antisense

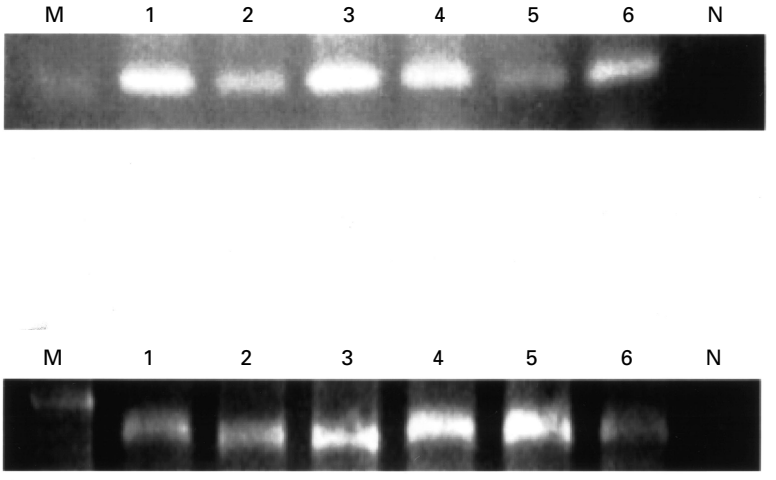

Figure 1. Effects of c-*myc* oligonucleotides on the expression of c-*myc* by RT-PCR analysis. Total RNA was isolated from cells after 48 h of incubation with or without 5 nmol/ml antisense oligonucleotides (c-*myc* antisense oligonucleotides, *myc*-AS; sense oligonucelotides, *myc*-S; no oligonucleotides, control; M, size marker; N, no template), and analyzed by RT-PCR for the expression of c-*myc* (upper) and β-actin (lower) genes. Lane 1, SW480 cells; lane 2, SW480 cells with *myc*-AS; lane 3, SW480 cells with *myc*-S; lane 4, SW620 cells; lane 5, SWSW620 cells with *myc*-AS; lane 6, SW620 cells with *myc*-S.

Table 1. Drug sensitivity of cells after incubation with c-*myc* oligonucleotides

Cell line	Sensitivity		
	Cisplatin (μM)	ADR (nM)	VP-16 (nM)
SW480	13.8 ($\pm$0.8)**	0.073 ($\pm$0.019)	5.60 ($\pm$0.44)
SW480+*myc*-AS	40.8 ($\pm$2.5)*	0.054 ($\pm$0.043)	5.02 ($\pm$0.72)
SW480+*myc*-S	21.3 ($\pm$1.5)	0.092 ($\pm$0.035)	6.21 ($\pm$0.84)
SW620	15.1 ($\pm$1.7)***	0.034 ($\pm$0.024)	6.85 ($\pm$0.35)
SW620+*myc*-AS	54.2 ($\pm$3.4)***	0.029 ($\pm$0.047)	5.76 ($\pm$5.76)
SW620+*myc*-S	20.7 ($\pm$2.9)	0.044 ($\pm$0.085)	6.24 ($\pm$1.24)

myc-AS and *myc*-S: sense and antisense oligonucleotides for the c-*myc* gene. SW480 and SW620 cells were treated with 5 nmol/m *myc*-AS or *myc*-S for 2 days, then one of seven different concentrations of various drugs for 2 h. After being washed with PBS once, the cells were incubated for 5 days and cytotoxicity to drugs determined by MTT assay. The results represent mean$\pm$2SD for three separate experiments. *,***$p<0.01$; **,***$p<0.5$.

Table 2. Drug sensitivity of resistant cells with transfected c-*myc* cDNA

Cell line	Sensitivy		
	Cisplatin (μM)	ADR (nM)	VP-16 (nM)
SW480DDP	99.5 ($\pm$1.8)**	0.085 ($\pm$0.014)	7.81 ($\pm$0.30)
SW480DDP+*myc* cDNA	42.2 ($\pm$2.6)*	0.065 ($\pm$0.029)	8.42 ($\pm$0.95)
SW480DDP+vector only	88.7 ($\pm$3.5)	0.048 ($\pm$0.042)	7.27 ($\pm$1.56)
SW620DDP	90.5 ($\pm$2.4)****	0.024 ($\pm$0.016)	8.73 ($\pm$0.53)
SW620DDP+*myc* cDNA	49.0 ($\pm$3.1)***	0.017 ($\pm$0.028)	7.28 ($\pm$1.49)
SW620DDP+vector only	94.8 ($\pm$4.4)	0.019 ($\pm$0.037)	7.85 ($\pm$2.21)

SW480DDP and SW620DDP cells were obtained by exposure to cisplatin as described in Materials and methods. SW480DDP + *myc* cDNA and SW620DDP + *myc* cDNA were transfected with a vector including c-*myc* cDNA, while SW480DDP + vector only and SW620DDP + vector only were transfected with vector only. Determination of drug cytoxicity is described in Materials and methods. *,***$p<0.01$; **,***$p<0.5$.

Table 3. Effects of *myc* oligonucleotides on colony formation of colon cancer cells

	Colonies[a]
1. SW480	263$\pm$25
2. SW480+*myc*-AS	249$\pm$36
3. SW480+*myc*-S	258$\pm$32
4. SW620	237$\pm$46
5. SW620+*myc*-AS	227$\pm$28
6. SW620+*myc*-S	261$\pm$37

Colonies were counted 10 days after treatment with c-*myc* antisense (AS) or c-*myc* sense (S) oligonucleotides for 2 days. Results represent means$\pm$SD from two independent experiments. $p<0.5$ in each group.

or sense oligonucleotides had no effect on colony formation for SW480DDP or SW620DDP cells (data not shown). The effects of c-*myc* modulation transfected with c-*myc* cDNA on colony formation for SW480DDP or SW620DDP cells were similar results (data not shown).

To test the role of the c-*myc* gene on the induction of apoptosis mediated by cisplatin, isolated DNA was analyzed by agarose gel electrophoresis. DNA fragmentation was observed in SW480 cells but not SW480DDP cells after cisplatin exposure (Figure 2A). In contrast, DNA fragmentation was observed in SW480DDP cells transfected with c-*myc* under the same conditions. Treatment with c-*myc* antisense oligonucleotide for SW480 cells showed inhibited apoptosis with cisplatin. Moreover, the detection of apoptotic cells with a TUNEL method by flow cytometry revealed similar results (Figure 2B). Analysis of apoptosis for SW620 and SW620DDP cells gave similar results (data not shown).

Discussion

We have represented here the potential of the c-*myc* gene to confer cisplatin resistance in human colon cancer. We used antisense oligonucleotides to c-*myc* to determine whether targeting c-*myc* modifies drug resistance to cisplatin in colon cancer cells. Antisense oligonucleotides for c-*myc* mRNA exhibited the potential to block specific c-*myc* expression. Uses of c-*myc* antisense oligonucleotides have been demon-

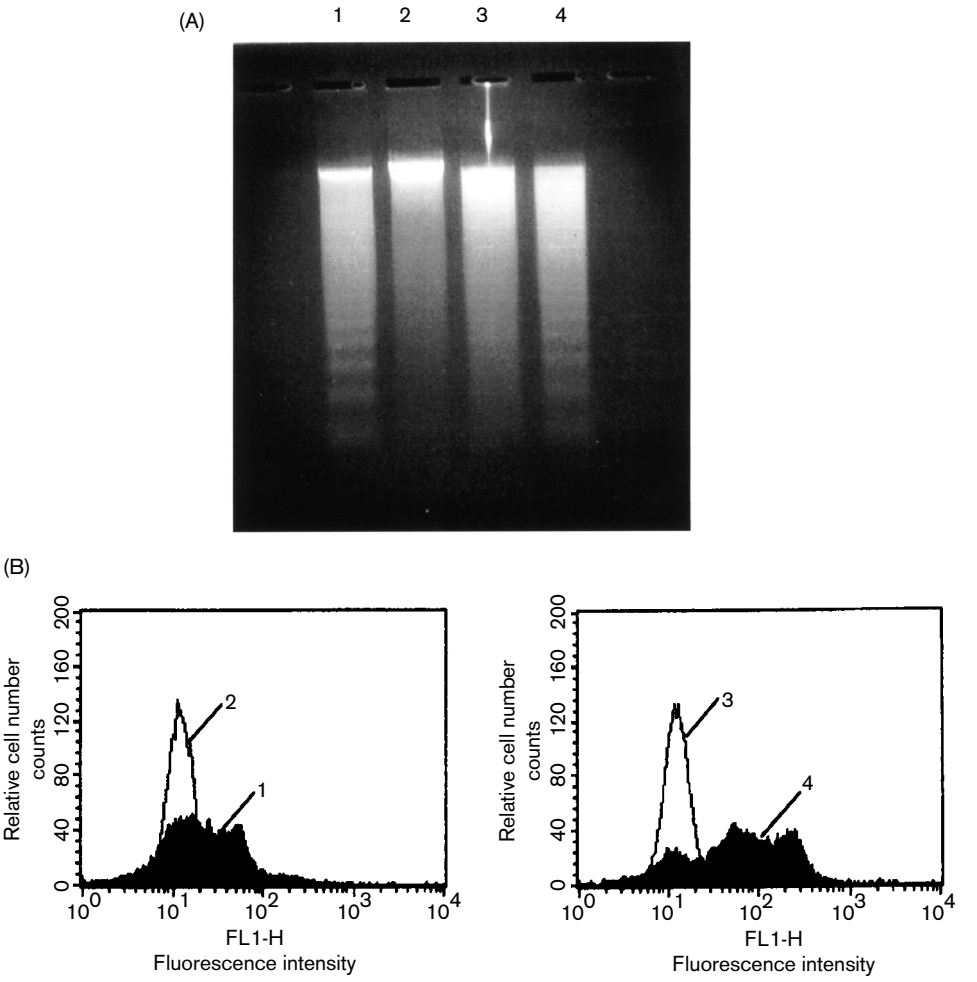

Figure 2. (A) Cisplatin-induced DNA cleavage assessed by agarose gel electrophoresis. Cells were exposed to IC_{50} dose of cisplatin for 72 h. Lane 1, SW480 cells; lane 2, SW480 cells with c-*myc*-antisense sequence; lane 3, SW480DDP cells; lane 4, SW480DDP with c-*myc*. (B) Analysis of cisplatin-induced apoptosis by TUNEL assay. Left, SW480 (1) and SW480 with c-*myc* antisense sequence (2); right, SW480DDP (3) and SW480DDP with c-*myc* (4).

strated in several reports, including investigations into the inhibitory effects of growth and proliferation modulation,[15,19,20] induced apoptosis,[21,22] and inhibition of telomerase activity[23] in cancer cells. However, previous reports relied on the use of c-*myc* antisense oligonucleotides to enhance chemosensitivity in combination with cisplatin in cases of human melanoma.[24] The mechanisms of acquired cisplatin resistance to c-*myc* are not yet known. Therefore, we directly assessed the efficacy of c-*myc* antisense oligonucleotides in drug sensitivity to cisplatin. The results indicate that c-*myc* antisense sequences gave drug resistance to cisplatin, but not other drugs, in drug-sensitive cell lines. Thus we postulate that c-*myc* is one of the factors related to drug sensitivity and the acquisition of cisplatin resistance.

Further, we examined if the potential of the c-*myc* gene to enhance sensitivity to cisplatin can be modified by transfected drug-resistant colon cancer cells with c-*myc* cDNA. We demonstrated that expression of the c-*myc* gene reversed drug resistance to cisplatin but not other drugs. Repeated challenges with cisplatin have established cell lines with stable resistance and elevated gene expression associated with DNA repair and signal transduction.[25] However, we did not examine c-*myc* gene involvement in the mechanisms of DNA repair or signal transduction pathway. Thus the induction of apoptosis by cisplatin is related to cisplatin sensitivity and the expression of c-*myc*, because the c-*myc* gene is a well-known factor in the induction of apoptosis.[26] High c-*myc* levels are necessary for cisplatin-induced apoptosis in resistant

cells. Thus, the present results suggest that c-*myc* confers drug sensitivity and resistance to cisplatin in human colon cancer. However, we could not analyze the effect of cisplatin and/or c-*myc* on the induction of G_1 arrest in the cell cycle. Modulation of c-*myc* activity employing antisense sequence techniques or gene transfer may provide a means to circumvent cisplatin resistance in colon cancers. Such therapeutic modulation has become a powerful tool for selectively inhibiting the expression of target genes *in vitro* and is of increasing interest in the development of *in vivo* therapeutic strategies. In fact, it is important how c-*myc* expression acts *in vivo* for more effective cisplatin therapy in colon cancer. Therefore, further investigations are needed to show why the c-*myc* gene was related to the sensitivity to cisplatin alone but not to ADR and VP-16 in other colon cancer cell lines.

References

1. Go RS, Adjei AA. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J Clin Oncol* 1999; **17**: 409–22.
2. Rho YS, Lee KT, Jung JC, *et al*. Efficacy of the new platinum analog DPPE in an orthotopic nude mouse model of human colon cancer. *Anticancer Res* 1999; **19**: 157–61.
3. Akiyama S, Chen ZS, Sumizawa T, *et al*. Resistance to cisplatin. *Anticancer Drug Des* 1999; **14**: 143–51.
4. Kashani-Sabet M, Rossi JJ, X.Lu X, *et al*. Detection of drug resistance in human tumors by *in vitro* enzymatic amplification. *Cancer Res* 1988; **48**: 5775–8.
5. Scanlon KJ, Kashani-Sabet M, Miyachi H, *et al*. Molecular basis of cisplatin resistant human carcinomas: model systems and patients. *Anticancer Res* 1989; **9**: 1301–12.
6. Isonishi S, Hom DK, Thiebaut FB, *et al*. Expression of the c-Ha-*ras* oncogene in mouse NIH 3T3 cells induces resistance to cisplatin. *Cancer Res* 1991; **51**: 5903–9.
7. Reed E. Platinum–DNA adduct, nucleotide excision repair and platinum based anti-cancer chemotherapy. *Cancer Treat Rev* 1998; **24**: 331–44.
8. Rigas B. Oncogenes and suppressor genes: their involvement in colon cancer. *J Clin Gastroenterol* 1990; **12**: 494–9.
9. Lotem J, Sachs L. Regulation by *bcl*-2, c-*myc*, and p53 of susceptibility to induction of apoptosis by heat shock and cancer chemotherapy compounds in differentiation-competent and -defective myeloid leukemic cells. *Cell Growth Different* 1993; **4**: 41–7.
10. Osmak M, Beketic-Oreskovic L, Matulic M, *et al*. Resistance of human laynx carcinoma cells to cisplatin, gamma-irradiation and methotrexate dose not involve overexpression of c-*myc* or c-Ki-*ras* oncogenes. *Mutat Res* 1993; **303**: 113–20.
11. Nesbit CE, Grove LE, Yin X, *et al*. Differential apoptotic behaviors of c-*myc*, N-*myc*, and L-*myc*. *Cell Growth Different* 1998; **9**: 731–41.
12. Sklar MD, Prochownik EV. Modulation of *cis*-platinum resistance in Friend erythroleukemia cells by c-*myc*. *Cancer Res* 1991; **51**: 2118–23.
13. Prados J, Melguizo C, Fernandez A, *et al*. Inverse expression of *mdr*1 and c-*myc* genes in a rhabdomyosarcoma cell line resistant to actinomycin D. *J Pathol* 1996; **180**: 85–9.
14. Funato T, Yoshida E, Jiao L, *et al*. The utility of an anti-*fos* ribozyme in reversing cisplatin resistance in human carcinomas. *Adv Enz Reg* 1992; **32**: 195–209.
15. Skorski T, Nieborowska-Skorska M, Wlodarski P, *et al*. Antisense oligonucleotide combination therapy of primary chronic myelogenous leukemia blast crisis in SCID mice. *Blood* 1996; **88**: 1005–12.
16. Watt R, Stanton LW, Marcu KB, *et al*. Nucleotide sequence of cloned cDNA of human c-*myc* oncogene. *Nature* 1983; **303**: 725–8.
17. Gavrieli Y, Sherman Y, BenSasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentaion. *J Cell Biol* 1992; **119**: 493–501.
18. Wyllie AH. Apoptosis. *Br J Cancer* 1993; **67**: 205–8.
19. Williams SA, Chang L, Buzby JS. Cationic lipids reduce time and dose of c-*myc* antisense oligodeoxynucleotides required to specifically inhibit Burkitt's lymphoma cell growth. *Leukemia* 1996; **10**: 1980–9.
20. Cerutti J, Trapasso F, Battaglia C, *et al*. Block of c-*myc* expression by antisense oligonucleotides inhibits proliferation of human thyroid carcinoma cell lines. *Clin Cancer Res* 1996; **2**: 19–26.
21. Sun SY, Yue P, Shroot B, *et al*. Implication of c-*myc* in apoptosis induced by the retinoid CD437 in human lung carcinoma cells. *Oncogene* 1999; **18**: 3894–901.
22. Hashiramoto A, Sano H, Maekawa T, *et al*. C-*myc* antisense oligonucleotides can induce apoptosis and down-regulate Fas expression in rheumatoid synoviocytes. *Arthrithis Rheum* 1999; **42**: 954–62.
23. Fujimoto K, Takahashi M. Telomerase activity in human leukemia cell lines is inhibited by antisense pentadecadeoxynucleotides targeted against c-*myc* mRNA. *Biochem Biophys Res Commun* 1997; **241**: 775–81.
24. Citro G, D'Agnano I, Leonetti C, *et al*. C-*myc* antisense oligodeoxynucleotides enhance the efficacy of cisplatin in melanoma chemotherapy *in vitro* and in nude mice. *Cancer Res* 1998; **58**: 283–9.
25. Fink D, Nebel S, Aebi S, *et al*. The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* 1996; **56**: 4881–6.
26. Thompson EB. The many roles of c-Myc in apoptosis. *Annu Rev Physiol* 1998; **60**: 575–600.

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