

RNA Interference Targeting Aurora Kinase A Suppresses Tumor Growth and Enhances the Taxane Chemosensitivity in Human Pancreatic Cancer Cells

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Abstract

AURKA/STK15/BTAK, the gene encoding Aurora A kinase that is involved in the regulation of centrosomes and segregation of chromosomes, is frequently amplified and overexpressed in various kinds of human cancers, including pancreatic cancer. To address its possibility as a therapeutic target for pancreatic cancer, we employed the RNA interference technique to knockdown AURKA expression and analyzed its phenotypes. We found that the specific knockdown of AURKA in cultured pancreatic cancer cells strongly suppressed *in vitro* cell growth and *in vivo* tumorigenicity. The knockdown induced the accumulation of cells in the G₂-M phase and eventual apoptosis. Furthermore, we observed a synergistic enhancement of the cytotoxicity of taxanes, a group of chemotherapeutic agents impairing G₂-M transition, by the RNA interference-mediated knockdown of AURKA. These results indicate that inhibition of AURKA expression can result in potent antitumor activity and chemosensitizing activity to taxanes in human pancreatic cancer. (Cancer Res 2005; 65(7): 2899-905)

Introduction

Pancreatic cancer is one of the most common cancers with an extremely poor prognosis around the world because of its aggressive invasion, early metastasis, resistance to existing chemotherapeutic agents and radiation therapy, and lack of specific symptoms (1). To improve the horrible prognosis, we need to find novel approaches to both diagnosis and treatment that are far more efficient than currently available techniques. Molecular studies of cancers can lead us to find new drugs for molecular target therapy such as trastuzumab in breast cancer and gefitinib in lung cancer (2, 3). Pancreatic cancer involves very complicated molecular changes (4, 5); our previous comparative genomic hybridization analysis of the pancreatic cancer genome revealed intricate genomic alterations in multiple chromosome arms, including losses of 1p, 3p, 4q, 6q, 8p, 9p, 12q, 17p, 18q, and 21q and gains of 8q and 20q (6). The increase in copy number of 20q13 is especially prominent in pancreatic cancer (6, 7). Amplification of 20q13 is also found in several other types of human cancer such as colorectal cancer, breast cancer, bladder cancer, ovarian cancer, and hepatocellular cancer, suggesting the existence of an important oncogene(s) that plays a crucial role in a

variety of human cancers in this area (8–12). *AURKA* was identified as one of the candidate oncogenes from the amplicon on 20q13 (13).

AURKA is one of three related genes (*AURKA*, *AURKB*, and *AURKC*) encoding AURORA kinases/serine-threonine kinases that play important roles in mitotic spindle formation and centrosome maturation and are physiologically essential for proper segregation of chromosomes into daughter cells (14). Since their discovery, the aurora kinases have been shown to be closely associated with carcinogenesis; an overexpression of *AURKA* transforms NIH3T3 cells and gives rise to aneuploid cells containing multiple centrosomes and multipolar spindles, indicating that *AURKA* is one of the fundamental cancer-associated genes and a potential target for diagnosis and treatment (14, 15). To further elucidate the possibility for utilization of *AURKA* in the treatment of human pancreatic cancer, we analyzed the phenotypes of cultured pancreatic cancer cells after RNA interference (RNAi)-mediated *AURKA* knockdown (16). Moreover, we tested the synergistic enhancement of the cytotoxicity of taxanes in pancreatic cancer cells by *AURKA*-RNAi.

Materials and Methods

Pancreatic cancer cell lines and cell culture. Three human pancreatic cancer cell lines, Panc-1, MIA PaCa-2, and SU.86.86, were purchased from American Type Culture Collection (Manassas, VA), and PK-1 was obtained from the original developer (17). All cells were maintained in RPMI 1640 containing 10% fetal bovine serum under atmosphere of 5% CO₂ with humidity at 37°C.

Short interference RNA transfection. Oligonucleotides of short interference double-strand RNAs (siRNA) with two thymidine residues (dTdT) overhanging at the 3' end for knock down of the expressions of *AURKA* and the *luciferase* gene (*GL2*), including 5'-AUGCCUGUCUUA-CUGUCA-3' in the sense strand corresponding to nucleotides 725 to 743 relative to its start codon for the former (18) and 5'-CGUACGCGAAUA-CUUCGA-3' in the sense strand for the latter used as a control as described previously (19), were purchased from Japan Bioservice (Asaka, Japan). The siRNAs were dissolved into 5× annealing buffer [500 nmol/L potassium acetate, 150 nmol/L HEPES-KOH (pH 7.4), and 10 nmol/L magnesium acetate] to a final concentration of 20 μmol/L, boiled for 60 seconds, and gradually cooled down to 37°C for 60 minutes to anneal them. *In vitro* transfection was done using the Oligofectamine reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Immunoblotting. A total of 3 × 10⁵ cells were plated in 6-well plates (35 mm in diameter) and allowed to adhere for 24 hours; the transfection of double-stranded siRNA oligonucleotides was done as described above. After 48 hours, cells were harvested, and protein concentrations in total cell lysates were measured using the detergent-compatible protein assay kit (Bio-Rad, Hercules, CA). A 50-μg aliquot of the protein was subjected to immunoblotting as described previously using a 10% to 20% polyacrylamide gradient gel (Bio-Rad; ref. 20). The antibodies used were anti-*AURKA*

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polyclonal antibody (Transgenic, Kumamoto, Japan), anti- β actin monoclonal antibody (Sigma, St Louis, MO), and horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin antibodies (Amersham Biosciences Co., Piscataway, NJ). For blocking conditions and concentrations of antibodies, we followed the manufacturer's recommendations. Signals were visualized by reaction with enhanced chemiluminescence Detection Reagent (Amersham Biosciences) and digitally processed using LAS 1000 Plus with a Science Lab 99 Image Gauge (Fuji Photo Film, Minamiashigara, Japan).

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide assay. A total of 5×10^3 cells in 100 μ L of the medium were plated in 96-well plates, and the RNA oligonucleotides were transfected. Every 24 hours up to 7 days, the medium was replaced with 100 μ L of 0.05% 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT)/PBS (-) and incubated for 1 hour. After the incubation, the MTT solution was removed, and the cells were suspended in 100% ethanol. Absorbance was measured at 590 nm using Versamax microplate reader (Amersham Biosciences).

Flowcytometry. Cells were harvested with trypsin-EDTA, washed with PBS (-), and fixed with 70% ethanol at -20°C for a few days. The fixed cells were pelleted, resuspended in 100 μ L of hypotonic citric buffer (192 mmol/L Na_2HPO_4 and 4 mmol/L citric acid), and incubated for 30 minutes at room temperature. The cells were pelleted and suspended in PI/RNase/PBS (100 μ g/mL propidium iodide and 10 μ g/mL RNase A) overnight at 4°C . Analysis of DNA content was done on a FACSCalibur system (BD Immunocytometry Systems, San Jose, CA).

Plasmid constructions and colony formation assay. pSUPER-retro-neo+GFP (pSR) vectors (ref. 21; Oligoengine, Seattle, WA) harboring gatccccATGCCCTGTCTTACTGTCAAttcaagagaTGACAGTAAGACAGGG-CATttttta and gatccccCGTACGCGGAATACTTCGAttcaagagaTCGAAG-TATTCGCGTACGttttta at its *Bgl*II/*Hind*III sites were prepared for expressing short hairpin RNAs (shRNA), as indicated in upper cases, specific for interfering expressions of *AURKA* (pSR-shAURKA) and *luciferase* (pSR-shGL2), respectively. The fidelity of the inserts was confirmed by sequencing both strands with primers of 5'-CGA-TCTCCCTTTATCCAGC-3' for the sense strand and 5'-CAGAACACA-TAGCGACATGC-3' for the antisense strand using an ABI PRIZM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and an ABI PRIZM 310 DNA Analyzer according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). For colony formation assays, 1×10^6 cells were plated in 10-cm culture dishes and transfected with 4 μ g of either

pSR-shAURKA, pSR-shGL2, or pSR using LipofectAMINE PLUS reagent (Invitrogen) according to the manufacturer's protocol. After 24 hours, transfected cells were passaged and cultured in the appropriate culture medium containing G418 at 500 μ g/mL in concentration. After 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were manually counted.

Tumorigenicity in mice xenograft model. Four-week-old female Crj:CD-1(ICR)-nu mice were obtained from Charles River Japan, Inc. (Yokohama, Japan) and maintained under pathogen-free conditions. Each aliquot of 2×10^6 cells of MIA PaCa-2 stably transfected with either pSR-shAURKA or pSR-shGL2 was suspended into 100 μ L of PBS (-) containing 20% of Matrigel Growth Factor Reduced (Becton Dickinson Labware, Franklin, NJ). These two sets of cells were s.c. injected into both flanks of mice. The inoculations were done in six mice. Tumor diameters were measured every 3 days, and each tumor volume in mm^3 was calculated by the following formula: $V = 0.4 \times D \times d^2$ (V , volume; D , longitudinal diameter; d , latitudinal diameter). Animal experiments in this study were done in compliance with Tohoku University School of Medicine institutional guidelines.

Statistical analysis. All experiments were done in duplicate or triplicate. A two-tailed Student's t test was used for statistical analysis of comparative data using Microsoft Excel software (Microsoft Co., Tokyo, Japan). Values of $P < 0.05$ were considered as significant and indicated by asterisks in the figures.

Results

Specific knockdown of *AURKA* in pancreatic cancer cell lines. To address the question of whether *AURKA* could serve as a therapeutic target for pancreatic cancer, we employed the siRNA method in an attempt to deplete the expression of *AURKA* in cultured pancreatic cancer cells. We prepared 21-mer oligoribonucleotides targeting *AURKA* and *Photinus pyralis luciferase* (*GL2*) based on information described elsewhere (18, 19). The oligoribonucleotides were annealed to give a double-strand siRNA and were transfected at 200 nmol/L into pancreatic cancer cells, MIA PaCa-2, Panc-1, PK-1, and SU.86.86, using the Oligofectamine reagent. After 48 hours, the cells were harvested, and their total lysates were analyzed by immunoblotting to see the effects of the siRNA on *AURKA* protein levels. As shown in Fig. 1A, dramatic suppression of

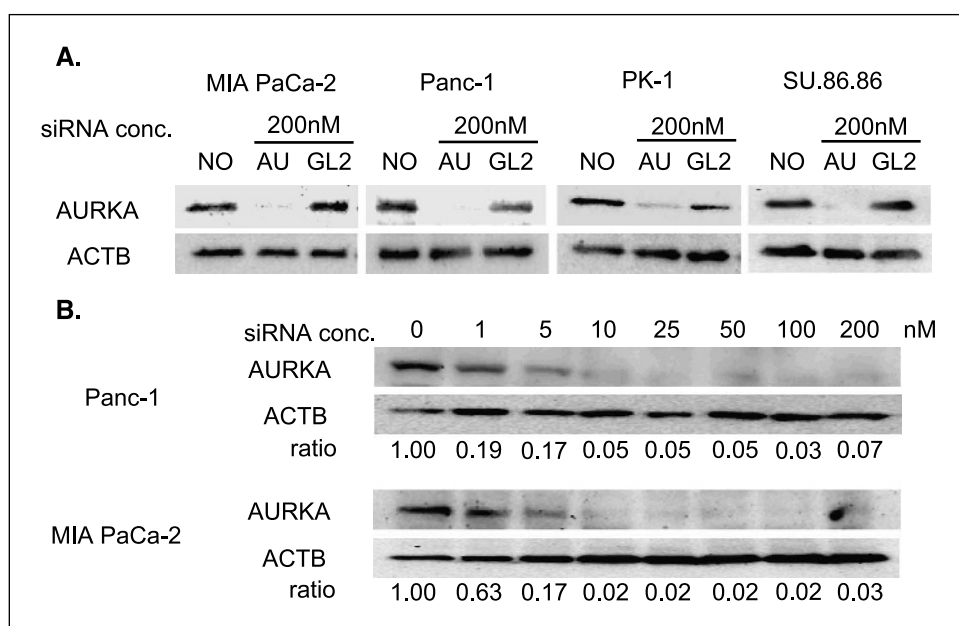


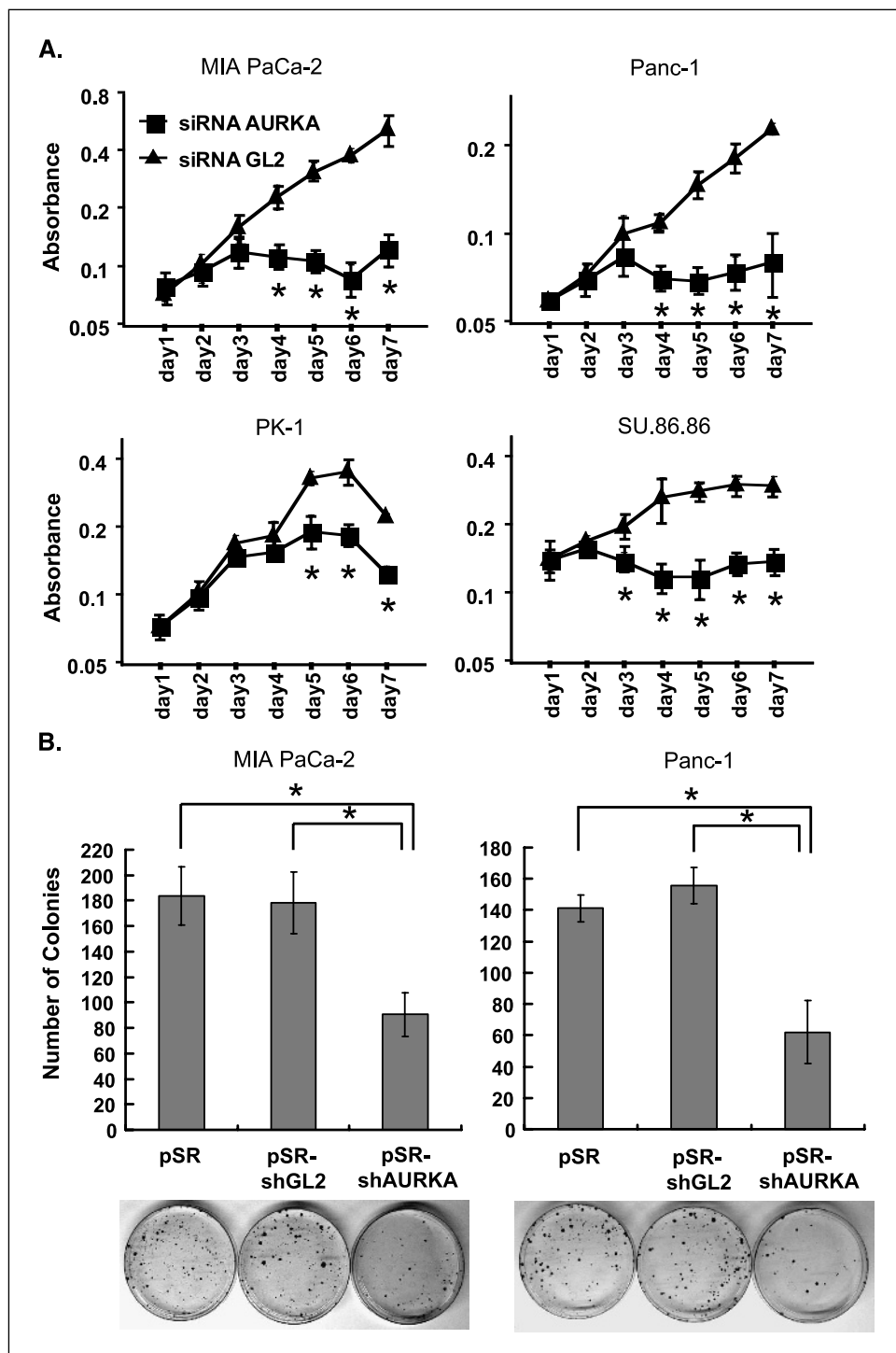
Figure 1. siRNA directed against *AURKA* specifically inhibits its expression. **A**, expression of *AURKA* 48 hours after the transfection of siRNA at 200 nmol/L directed against *AURKA* (AU) and *luciferase* (GL2) in pancreatic cancer cell lines, MIA PaCa-2, Panc-1, PK-1, and SU.86.86, detected by immunoblotting. NO, no transfection. **B**, a dose-dependent knockdown of *AURKA* expression by siRNA. Panc-1 and MIA PaCa-2 cells were transfected with the siRNA targeting *AURKA* at various concentrations ranging from 0 to 200 nmol/L. Expression of *AURKA* 48 hours after the transfection was detected by immunoblotting. β -Actin expression was monitored as the control. The ratio of *AURKA*/ β -actin was calculated by using densitometry, and values were normalized by dividing by the ratio at no treatment (0 nmol/L).

AURKA expression was observed in all four cell lines by the siRNA targeting *AURKA* but not *GL2*. The siRNA oligonucleotides did not cause a nonspecific inhibition of gene expression, as shown by expressions of β -actin. Furthermore, the suppression of AURKA protein levels was achieved in a dose-dependent manner as shown in Fig. 1B; partial to complete suppressions were observed along with increasing concentrations of the siRNA oligonucleotides.

Knockdown of AURKA inhibits *in vitro* growth and colony formation. In phenotypic analyses, we first investigated effects of

AURKA siRNA on the *in vitro* growth of pancreatic cancer cells. The siRNA transfection was done at 200 nmol/L to achieve complete suppression of AURKA expression, and cellular proliferations were monitored by MTT assay daily for 7 days. As shown in Fig. 2A, cell proliferation was significantly suppressed by *AURKA*-siRNA in all four pancreatic cancer cell lines as compared with *GL2*-siRNA. To observe the stable phenotypic consequences of siRNA-mediated knockdown in the cells, the 19-mer target sequences bridged by a 9-mer spacer were introduced into the

Figure 2. siRNA directed against *AURKA* suppresses *in vitro* growth of the pancreatic cancer cells. A, MTT assay of *in vitro* proliferation of the cells. These experiments were performed for four times. B, colony formation assay of G418-resistant colonies of the cells transfected either with pSUPER.retro.neo+GFP vector (pSR), pSR expressing short hairpin RNA directed against *AURKA* (pSR-shAURKA) or that directed against *luciferase* (pSR-shGL2). These experiments were performed for four times. *, $P < 0.05$.



pSUPER.retro.neo+GFP (pSR) vector to generate a short hairpin RNA targeting *AURKA* (pSR-shAURKA) or *luciferase* (pSR-shGL2), as described in Materials and Methods. For the colony formation assay using these vectors, MIA PaCa-2 and Panc-1 cells were transfected with either pSR-shAURKA, pSR-shGL2, or pSR empty vector and maintained in the selection medium containing G418 for 2 weeks. As expected from the MTT assay done in the siRNA experiment, the numbers of colonies were significantly decreased in pSR-shAURKA transfectants compared with the controls in both cell lines (see Fig. 2B). These results indicated that the RNAi-mediated specific knockdown of *AURKA* induced strong inhibition of pancreatic cancer cell growth *in vitro*.

Knockdown of *AURKA* suppresses tumorigenicity *in vivo*. We wondered whether the down-regulation of *AURKA* expression in pancreatic cancer cells would affect their ability to form tumors in nude mice. To address this question, we established stable transfectants of MIA PaCa-2 cells with treatment by either pSR-shAURKA or pSR-shGL2. These cells had modestly reduced expression of the AURKA protein (Fig. 3A). We then tested the *in vitro* growth of these cells and found that they showed rational growth retardation but not complete suppression, probably because of their modest level of knockdown of *AURKA* expression (Fig. 3B). Next, we injected the aliquot of 2×10^6 cells s.c. into six athymic nude mice and monitored their tumor growth. As shown in Fig. 3C, the pSR-shGL2 transfected cells gave rise to tumors within 4 weeks in all six mice, whereas the pSR-shAURKA transfected cells did not develop tumors in any of them. These results indicated that RNAi-mediated knockdown of *AURKA* exerted a strong antitumorigenic effect *in vivo* on pancreatic cancer cells.

Knockdown of *AURKA* induces G₂-M accumulation and apoptosis. *AURKA* is an important regulator of bipolar spindle formation and therefore essential for accurate chromosome segregation. We hypothesized that the growth suppression of the

pancreatic cancer cells we observed by the RNAi-mediated knockdown of *AURKA* was caused by disruption of cell cycle transition with delay in mitotic entry, which has been shown in other kinds of mammalian cells (19, 22). To determine this possibility, we analyzed the DNA contents of cell populations reflecting the cell cycle distribution after knockdown of *AURKA* mediated by transfection of the siRNA in 200 nmol/L in MIA PaCa-2 and Panc-1 cells. As shown in Fig. 4A, an increase in the G₂-M population with a concomitant decrease in the G₀-G₁ population was observed after *AURKA*-siRNA treatment in both cells. Moreover, as we observed the changes in the DNA content during the time course after the transfection, we found obvious and significant increases in the sub-G₁ populations after 72 hours in MIA PaCa-2 cells and after 96 hours in Panc-1 cells (see Fig. 4B). These results indicate that the siRNA-mediated knockdown of *AURKA* led the pancreatic cancer cells to abnormal accumulation in the G₂-M phase and to eventual apoptosis.

Knockdown of *AURKA* significantly enhances cytotoxicities of taxanes. Taxanes, chemotherapeutic agents impairing the disassembly of microtubules that is crucial for the proper segregation of chromosomes during mitosis of eukaryotic cells, may synergistically enhance the effect of RNAi-mediated knockdown of *AURKA*, because it can accumulate cells in the G₂-M phase where *AURKA* plays essential roles. To determine this possibility, we investigated the synergistic effects of *AURKA* siRNA and taxanes in MIA PaCa-2 and Panc-1 cells. First, we searched for the best concentration of siRNAs in this experiment because siRNA treatment itself showed some cytotoxicities. The concentration at 10 nmol/L seemed to be the best because no significant difference in cell proliferation between *AURKA*-siRNA and *GL2*-siRNA treatments was found (data not shown). The concentration for paclitaxel and docetaxel were set by IC₅₀ previously determined by MTT assay (data not shown). Then we

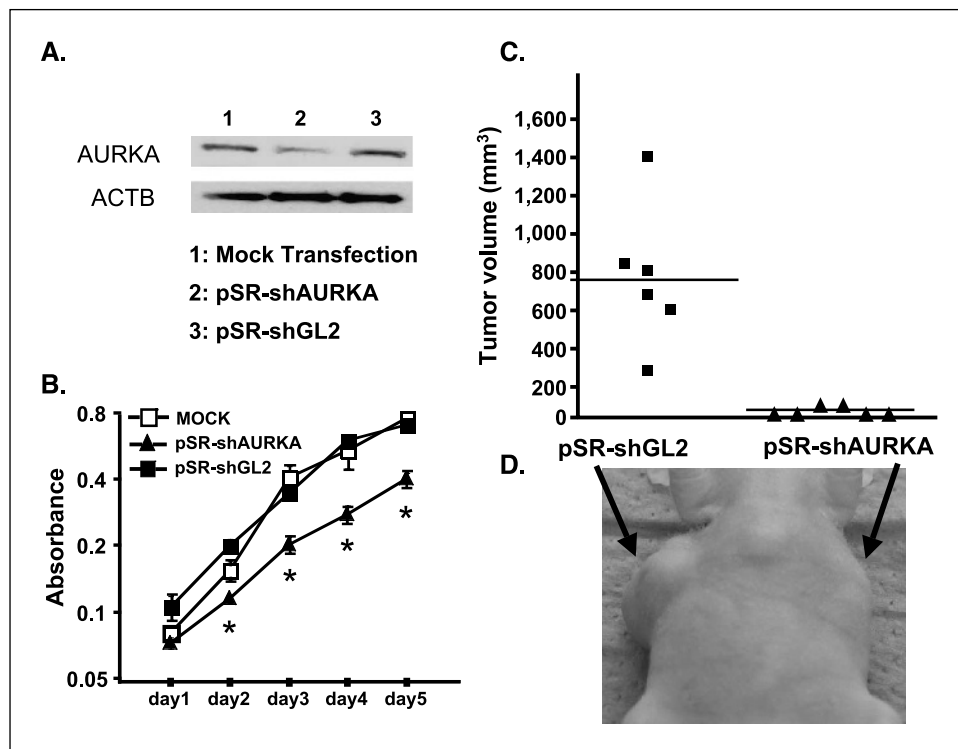
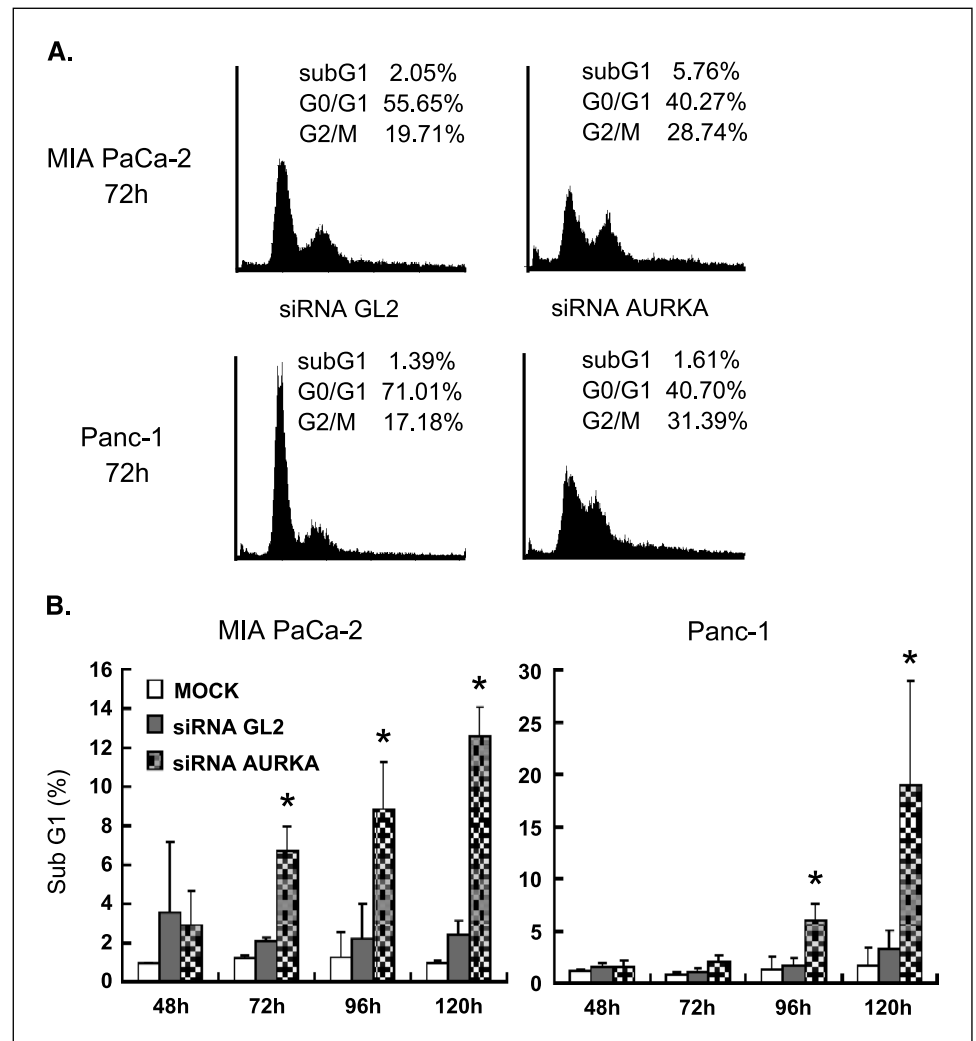


Figure 3. Stable suppression of *AURKA* inhibits *in vivo* tumorigenicity. **A**, *AURKA* expressions in MIA PaCa-2 cells stably transfected with pSR-shAURKA or pSR-shGL2. **B**, MTT assay of *in vitro* growth of the stable clones. These experiments were performed for four times. *, $P < 0.05$ (significant differences between mock and pSR-shAURKA and between pSR-shGL2 and pSR-shAURKA). **C**, tumorigenicity of the stable clones in the mouse-xenografted model. The stable clones were inoculated s.c. into both flanks of six nude mice. Sizes of the tumors generated were measured at 4 weeks after the inoculation. **D**, representative features of tumors in a mouse 4 weeks after the inoculation.

Figure 4. siRNA directed against *AURKA* induces G₂/M accumulation and eventual apoptosis. **A**, cells were collected 72 hours after siRNA transfection at 200 nmol/L and subsequently assayed for their DNA content by flow cytometry. These experiments were performed for four times. Representative results are shown. **B**, time course quantification of sub-G₁ population after siRNA transfection at 200 nmol/L. These experiments were performed for four times. *, $P < 0.05$.



tested the potential enhancement of the cytotoxic effect of taxanes by *AURKA*-siRNA by treating cells either with *AURKA* or *GL2*-siRNA at 10 nmol/L in concentration followed by addition of either 10 nmol/L paclitaxel or 5 nmol/L docetaxel 4 hours later. After 72 hours of incubation, the viabilities of the cells were measured by MTT assay. Although the modest *AURKA*-siRNA at 10 nmol/L alone did not show any difference in cytotoxic effect when compared with the control treatment with *GL2*-siRNA, it enhanced the cytotoxic effects induced by taxanes significantly more strongly than the control treatment (Fig. 5A). A reciprocal set of experiments showed that taxanes can enhance the cytotoxic effect of *AURKA*-siRNA, as shown in Fig. 5B; the synergistic enhancement of the cytotoxic effect of *AURKA*-siRNA by taxanes were obvious from the treatment at 10 nmol/L siRNA and accelerated with increasing doses. These results indicate that the RNAi-mediated knockdown of *AURKA* can synergistically enhance the chemosensitivities of these pancreatic cancer cells to taxanes.

Discussion

AURKA is a commonly amplified and overexpressed gene in various types of cancers, including pancreatic cancer. In

attempting to determine the possibility of *AURKA* as a therapeutic target, we employed the RNAi technique for knockdown of its expression and analyzed its phenotype. We found that a transient knockdown of *AURKA* strikingly inhibited growth and colony formation of pancreatic cancer cells *in vitro*. Stable suppression of *AURKA* in pancreatic cancer cells revealed an almost complete abrogation of their tumorigenicity in a mouse xenograft model. The knockdown induced accumulation of the cells in the G₂-M phase and eventual apoptosis. Rojanala et al. (23) recently reported that antisense oligonucleotide mediated transient suppression of *AURKA* resulted in growth suppression, G₂-M arrest, and eventual apoptosis *in vitro*. Our results are in good agreement with theirs. We further showed that the knockdown of *AURKA* significantly enhanced the cytotoxic effect of taxanes. Our findings indicate that *AURKA* is an attractive candidate for a therapeutic target, because it can regress tumorigenicity and enhance chemosensitivity to taxanes in pancreatic cancer.

We were able to achieve almost complete suppression of *AURKA* expression by using our siRNA treatment strategy in pancreatic cancer cells. The knockdown of *AURKA* induced the strong suppression of growth, accumulation in G₂-M phase, and eventual apoptosis of the cells. This result suggests that *AURKA* is an

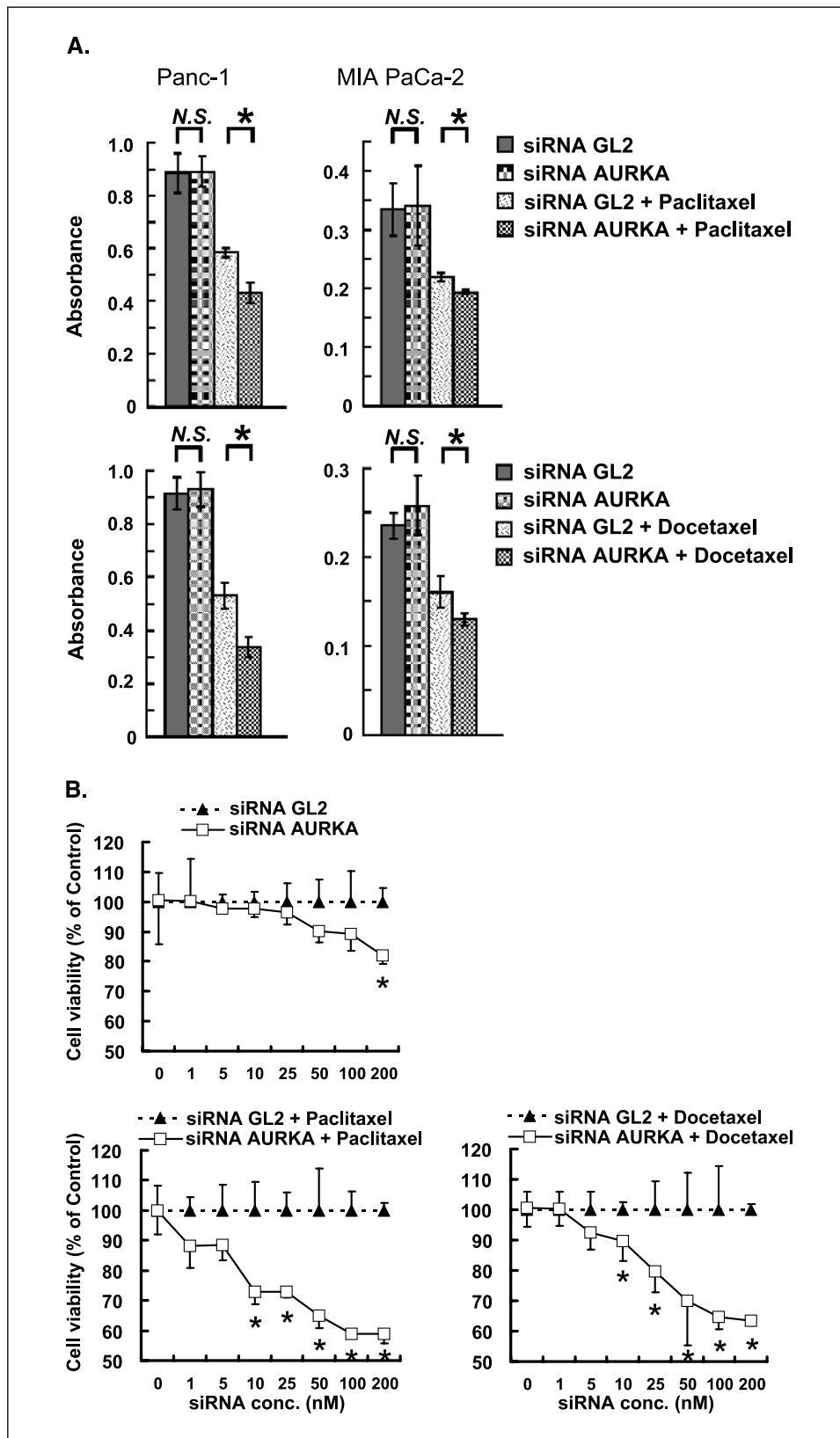


Figure 5. Synergistic enhancement of cytotoxicity between siRNA directed against *AURKA* and taxanes. **A**, survival cells quantitated by MTT assay after siRNA transfection targeting *AURKA* or *luciferase* (GL2) at 10 nmol/L only or subsequent addition of 10 nmol/L paclitaxel or 5 nmol/L docetaxel. These experiments were performed for four times. *, $P < 0.05$. *N.S.*, not significantly different. **B**, survival cells quantitated by MTT assay after various doses of siRNA transfection targeting *AURKA* or *luciferase* (GL2) only or subsequent addition of 10 nmol/L paclitaxel or 5 nmol/L docetaxel. Values were normalized by dividing them by control values of siRNA-GL2 at each concentration. These experiments were performed for four times. *, $P < 0.05$.

essential molecule for proliferation of cancer cells and a good target for halting proliferation and triggering apoptosis; this can be explained by its key roles in mitosis, as we expected. More strikingly, the knockdown of *AURKA* completely inhibited tumor-

igenesis *in vivo*, even in the modest suppression of its expression achieved by our stable vector-mediated shRNAi strategy. This result suggests that overexpression of *AURKA* is strongly associated with the *in vivo* tumorigenic ability of pancreatic cancer cells,

leading us to an interpretation of the frequent overexpression of *AURKA* in primary pancreatic cancer tissues and to an expectation that the knockdown strategy will be practical in stopping the progression of the cancer *in vivo*.

We found that the RNAi-mediated knockdown of *AURKA* synergistically enhanced the cytotoxicity of taxanes. Taxanes bind to free tubulin and promote the assembly of tubulin into stable microtubules by interfering with their disassembly. They inhibit cell cycle progression by accumulating cells in M phase at the metaphase-anaphase transition and subsequently lead them to apoptosis. Knockdown of *AURKA* also induced accumulation of cells in the G₂-M phase and led to eventual apoptosis. As we noted, *AURKA* is essential for the proper arrangement of centrosomes and microtubules. Our results suggest that the combination of *AURKA* knockdown and taxanes results in strong impairment of M phase progression and the synergistic induction of apoptosis. This is consistent with the recent report indicating that HeLa cells with overexpression of *AURKA* gained a resistance to paclitaxel by decreasing spindle checkpoint activity (24). In that report, Anand et al. noted that overexpression of *AURKA* may decrease spindle checkpoint activity. In our experiments, *AURKA* knockdown may have recovered spindle checkpoint activity and thus increased the sensitivity of taxanes. The mechanism that triggers apoptosis by *AURKA* knockdown remains to be clarified. Taxanes have cytotoxic activity against various types of cancers

including pancreatic cancer. Docetaxel is used for pancreatic cancers as first-line chemotherapy or a second-line combination with gemcitabine in phase II clinical trials (25, 26). Paclitaxel has been used as a radiation sensitizer (27). These taxane-mediated chemotherapies could be more effective in combination with knockdown of *AURKA*.

RNAi is becoming a conventional application for *in vivo* cancer therapy (28, 29). An efficient delivery system of siRNA into solid tumors has been developed (30). Our results suggest that RNAi-mediated knockdown of *AURKA* can be used as a specific gene-targeting therapy to suppress progression of pancreatic cancer. Interestingly, in a recent report, Harrington et al. developed a selective small-molecule inhibitor of Aurora kinases, VX-680, and showed a potent antitumor activity (31). We can assume that this different type of approach is also promising for *in vivo* abrogation of progression in pancreatic cancer.

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Announcements

MEETING OF THE RADIATION RESEARCH SOCIETY

The annual meeting of the Radiation Research Society will be held at the State University of Iowa, Iowa City, on June 22-24, 1953. The Society will be the guest of the University, and all meetings will be held on the campus. The program will consist of: (1) Two symposia, one on "The Effects of Radiation on Aqueous Solutions," which includes the following speakers: E. S. G. Barron, Edwin J. Hart, Warren Garrison, J. L. Magee, and A. O. Allen. The second is "Physical Measurements for Radiobiology" and companion talks by Ugo Fano, Burton J. Moyer, G. Failla, L. D. Marinelli, and Payne

S. Harris. (2) On Monday night, June 22, a lecture by Dr. L. W. Alvarez on meson physics has been tentatively scheduled. On Tuesday night, June 23, Dr. L. H. Gray of the Hammersmith Hospital, London, will speak on a topic to be announced. Dr. Gray's lecture is sponsored by the Iowa Branch of the American Cancer Society. Those desiring to report original research in radiation effects, or interested in attending or desiring additional information, please contact the Secretary of the Society, Dr. A. Edelman, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

ERRATUM

The following correction should be made in the article by Beck and Valentine, "The Aerobic Carbohydrate Metabolism of Leukocytes in Health and Leukemia. I. Glycolysis and Respiration," November, 1952, page 821; substitute for the last paragraph:

The data in Table 3 permit several interesting calculations. If one compares the amount of glucose actually disappearing with the sum of the amount equivalent to lactic acid produced plus that equivalent to O₂ consumption, it is seen that the amount of glucose "cleavage products" exceeds the amount of glucose utilized by 12 per cent in N and 27 per cent in CML and is exceeded

by the glucose utilized by 16 per cent in CLL. If the assumption is made that, *in this respect*, the myeloid and lymphoid cells of leukemia are similar to those of normal blood, it may be that the computed normal figure represents a summation of the myeloid (M) and lymphoid (L) cells that make up the normal leukocyte population. Thus, if M = +0.27 and L = -0.16 and the normal differential is 65 per cent M and 35 per cent L, then

$$0.65 (+0.27) + 0.35 (-0.16) = +0.12 ,$$

a figure identical to the observed +0.12 for normal leukocytes.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

RNA Interference Targeting Aurora Kinase A Suppresses Tumor Growth and Enhances the Taxane Chemosensitivity in Human Pancreatic Cancer Cells

Tatsuo Hata, Toru Furukawa, Makoto Sunamura, et al.

Cancer Res 2005;65:2899-2905.

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