Phosphoinositide 3-kinase inhibitor (wortmannin) inhibits pancreatic cancer cell motility and migration induced by hyaluronan *in vitro* and peritoneal metastasis *in vivo*

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In order to block peritoneal metastasis of pancreatic cancer cells, we have attempted to block the signal transduction pathway involving hyaluronan (HA), Src, phosphoinositide 3-kinase (PI3K) and Akt. We examined the effects of Src, PI3K and Akt inhibitors on pancreatic cancer cell motility, invasion and metastasis. The pancreatic cancer cell line SW1990, known to cause peritoneal metastasis efficiently in nude mice, was used in this study. SW1990 cells were stimulated by HA to induce Akt phosphorylation. Then, the inhibitory effects of PI3K and Src kinase inhibitors were examined. Cell motility and cell migration assays were adopted to assess the cancer cell motility and its migration capability. We also examined the therapeutic efficacies of PI3K inhibitor wortmannin on peritoneal metastasis of SW1990 cells in the nude mouse model. Stimulation of SW1990 cells by HA markedly induced the Src-PI3K-Akt signaling, thus enhancing cancer cell motility and its migration. Significantly, we found that wortmannin could exert marked inhibition of the peritoneal metastasis of SW1990 in nude mice *in vivo*. These findings indicate that the PI3K-Akt signaling pathway plays an essential role in peritoneal metastasis and PI3K inhibitors such as wortmannin can be novel modalities to prevent peritoneal metastasis of invasive cancers such as pancreatic cancer. (*Cancer Sci* 2009; 100: 770–777)

Pancreatic cancer is characterized by peritoneal metastasis, a serious complication that leads to death. Although the clinical efficacy of gemcitabine, an anticancer agent, exhibits better responses to pancreatic cancer than other drugs and shows beneficial effects on the survival of patients with advanced, recurrent or persistent pancreatic cancer, its clinical efficacy is still limited. Thus, a novel anticancer therapy that can prevent peritoneal metastasis is needed.

Peritoneal mesothelial cells are covered by hyaluronan (HA),[1] which is a large, linear glycosaminoglycan composed of repeated disaccharides of glucuronic acid and N-acetylglucosamine and distributes ubiquitously in human tissue. HA provides a molecular environment that facilitates cell proliferation and migration, required for embryonic development and wound healing.[2,3] However, in cancer HA appears to facilitate tumor progression by enhancing cancer cell invasion, growth, angiogenesis and metastasis.[4–6] HA functions as an adhesion molecule and is actively involved in the peritoneal metastasis of cancer cells by providing a molecular environment that facilitates cell proliferation and migration.[7] It has been recently established that the signal transduction induced by HA is responsible for these cancer-progression events.[8–11] Therefore, inhibition of the HA-mediated signaling should prevent cancer progression such as peritoneal metastasis.

Recent studies have revealed that phosphoinositide 3-kinase (PI3K)-Akt signaling pathway is induced by HA.[10,11] Various growth-promoting stimuli activate PI3K, which phosphorylates the D3 portion of membrane phosphoinositols that bind Akt, also known as protein kinase B (PKB), thus changing its molecular conformation, and thus recruiting 3-phosphoinositide-dependent protein kinase 1 (PDK1) which activates Akt by direct phosphorylation.[12–15] The activated Akt and its downstream kinase molecules eventually induce integrin activation,[16] matrix metalloproteinase (MMP) production,[17] cell survival,[18] cell proliferation,[19] and angiogenesis,[20] thus promoting cancer progression. Wortmannin was reported as a potent and specific inhibitor of PI3-kinase; it contains a furan ring structure and covalently interacts with the catalytic p100 subunit of PI3 kinase.[21] In our study, we demonstrated that the PI3K inhibitor wortmannin could down-regulate HA-induced Akt phosphorylation through Src. In addition, wortmannin could also down-regulate HA-induced SW1990 cell motility. In an animal study, PI3K inhibitor wortmannin not only inhibited HA-induced peritoneal metastasis in nude mice, but also significantly reduced the total weight of tumors in a dose-dependent manner.

Materials and Methods

Cell culture. Human pancreatic cancer cell line SW1990, that causes peritoneal metastasis in nude mice,[22] was purchased from American Type Culture Collection (ATCC, Rockville, MD, US). SW1990 was grown at 37°C in 5% CO_2 in Dulbecco's modified Eagle medium (DMEM, Sigma, St. Louis, MO, US) with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma), 100 U/mL penicillin, and 100 μg/mL streptomycin.

Reagents. PI3K inhibitors, wortmannin and LY294002, were purchased from Sigma. 4-Amino-5-(4-methylphenyl)-7-(*t*-butyl) pyrazolo[3,4-*d*]-pyrimidine (PP1) (Biomol, Plymouth Meeting, PA, US) was used as a Src inhibitor. Akt inhibitor was purchased from Calbiochem (La Jolla, CA, US). We confirmed that each inhibitor had no toxicity for SW1990 cells in the concentrations used in this study by 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1) Reagent (Roche, Penzberg, Germany),[23] (data not shown).

Western blotting. In order to monitor the phosphorylation of Akt protein, SW1990 cells were preincubated without FBS for 24 h, and stimulated by hyaluronan (MP Biomedicals, Aurora, OH, US) for 1 h in the presence or absence of inhibitors. The

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Abbreviations: PI3K, phosphoinositide 3-kinase; HA, hyaluronan; PKB, protein kinase B; PDK1, 3-phosphoinositide-dependent protein kinase 1; MMP, matrix metalloproteinase; ECM, extracellular matrix.

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cells were lyzed in 200 μL of ice-cold lysis buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 2 mM ethylenediamintetra-acetic acid [EDTA], 1 mM phenylmethylsulfonyl fluoride [PMSF], 1 mM dithiothreitol [DTT], 0.2% Nonidet P-40, 10 mM NaF, 1 mM Na_3VO_4, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 μg/mL pepstatin A), and cleared by centrifugation. Samples were determined for the protein concentration using Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA, US). The cell lysate was resolved by sodium dodecylsulfate – polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on nitrocellulose membrane (Amersham Biosciences, Piscataway, NJ, US). The membrane was incubated with antibody to phospho-Akt or Akt (Cell Signaling Technology, Beverly, MA, US). The immunoreactive proteins were visualized by SuperSignal West Pico Chemilluminescent Substrate (Pierce, Rockford, IL, US) as previously reported.[24,25]

Cell motility assay. Cells were assayed for their motility by a computer-assisted modification of phagokinetic assays with gold colloid-coated glass plates previously described.[26] Briefly, after 24 h incubation without FBS, cells (2×10^3 cells/well of 6-well plate) were seeded on colloidal gold particle-coated glass coverslips and incubated for 2 h to attach cells on coverslips. After 1 h of treatment with inhibitors, cells were incubated for 18 h with or without 100 μg/mL HA. Then, photographs of the coverslips were taken by a computer-assisted digital camera (model E4500, Nikon, Tokyo, Japan). The areas of particle cleared by the movement of a single cell during the incubation period were quantitatively measured by NIH image (version 1.62). For this assay, 10 cells per sample were randomly selected and examined for their motility.

Migration assay. The SW1990 cell migration was assessed by the modified Boyden chamber method.[27] After 24 h stimulation with HA (200 μg/mL) following 1 h pretreatment with inhibitors, SW1990 cells (1×10^5) suspended in serum-free medium containing inhibitors and HA were seeded into the upper part of a BD BioCoat Matrigel Invasion Chamber (separated by a Matrigel coated polyethylene terephthalate filter with 8 μm pore size; BD Biosciences, Franklin Lakes, NJ, US) in triplicates and the lower compartment was filled with the same medium as the upper part. After incubation for 18 h at 37°C, non-migrating cells on the upper surface of the filter were wiped out with a cotton swab, and migrating cells penetrated onto the lower surface of the filter were fixed with 70% ethanol and stained with Giemsa staining. Migration rate was determined by counting the number of cells in six fields randomly selected, and the extent of migration was expressed as the average number of cells per microscopic field.

Peritoneal metastasis model of nude mouse. All the experiments were performed under the approval of the Animal Experimentation Committee of Nagoya City University Graduate School of Medical Sciences. Specific pathogen-free athymic BALB/c male mice (5 weeks) were kept under sterile conditions in a laminar flow room in cages with filter bonnets and fed a sterilized mouse diet and water. In order to induce peritoneal metastasis, the mice were anesthetized by inhalation administration of sevoflurane, and SW1990 cells (1×10^6 cells/mouse) in 100 μL of PBS were injected into the peritoneal cavity using a 23-gauge needle after incubation without FBS for 24 h to inactivate Akt. To examine the effect of PI3K inhibitor, we treated mice with the intraperitoneal treatment of wortmannin at 1.0, 0.5, or 0.25 mg/kg/day or vehicle (PBS + dimethyl sulfoxide) immediately after cancer cells were inoculated. The treatment was continued for 20 consecutive days. Twenty days later, mice were sacrificed and body weight, ascites, number, and total weight of peritoneal metastatic tumors were examined. We resected tumors with mesenterium and greater omentum in a lump and weighed the samples. The weight exceeding the mean weight of mesenterium and greater omentum of normal nude mice of the same age (in weeks) was regarded as the net weight of metastatic tumors in order to minimize the interfusion of blood or tissues.

Statistical analysis. All results were analyzed by the multiple comparison authorization method of Scheffe's *F*-test, using Stat View program (Abacus Concepts Inc.). The levels of statistical significance were indicated as follows: *, $P < 0.05$; **, $P < 0.01$; n.s., not significant.

Results

HA stimulates Akt phosphorylation and cell motility in human pancreatic cancer cell line SW1990. We detected Akt phosphorylation with Western blotting. The left panel of Fig. 1(A) shows that 200 μg/mL HA stimulation induced the Akt phosphorylation 5 min after the treatment. The right panel shows that HA stimulation induced Akt phosphorylation during 30 min at the concentration of 10–200 μg/mL. This result indicated HA stimulation could induce the phosphorylation of Akt.

We investigated cancer cell motility stimulated by HA. In Fig. 1(B), the open arrowhead shows the cancer cell and the closed arrows show the extent the cell has moved. Figure 1(B)-(a) shows the cell motility in non-stimulated cells. HA significantly induced cancer cell motility as shown in Figs 1(B)-(b,c). HA induced the cell motility and its activity reached the peak at 100 μg/mL (Fig. 1C). The cells floating from coverslips were increased when they were stimulated with greater than 200 μg/mL HA. However, WST1 assay revealed that 200 μg/mL HA does not cause cytotoxicity on SW1990 (data not shown). Thus it is considered that some other effects do this, such as the increase in the three-dimensional movement of cancer cells. Nevertheless, these observations show that HA stimulated Akt phosphorylation and cell motility in human pancreatic cancer cell line SW1990.

Inhibition of HA induced Akt phosphorylation and cell motility in SW1990 cells. We examined the effect of inhibitors on HA-induced Akt phosphorylation as shown in Fig. 2(A). Wortmannin, one of the PI3K inhibitors, showed an inhibitory effect on HA-induced Akt phosphorylation from a very low density of 0.01 μM. LY294002, another inhibitor of PI3K, inhibited Akt phosphorylation in a dose-dependent manner. Therefore, it was clarified that HA induced Akt phosphorylation through the PI3K-Akt signaling pathway. In addition, a Src inhibitor, PP1, inhibited HA-induced Akt phosphorylation in a dose-dependent manner. This result suggests that Src is involved in the HA-PI3K-Akt signaling pathway.

In Fig. 2(B), HA induced cell motility and this cell effect was inhibited by a PI3K inhibitor, wortmannin, in a dose-dependent manner. In Fig. 2(C), to further confirm the role of PI3K signal pathway in HA-induced augmentation of cancer cell motility, another PI3K inhibitor, LY294002, was applied to block the SW1990 cell motility. The restraining effect of both chemicals is clearly shown in Fig. 2(C); wortmannin and LY294002 inhibited the cancer cell' motility in a dose-dependent manner. Since Src signal pathway was also reported to be an upstream regulator of Akt signaling, we examined the function of Src in HA-mediated cancer cell motility. PP1, a potent inhibitor of Src, inhibited the cell motility at the concentration of more than 0.1 μM. Therefore, we consider that the HA-induced motility of SW1990 cells was mediated by Akt through activation of PI3K.

HA-activated migration of SW1990 cells and the effects of inhibitors. We confirmed that HA induced the migration ability of cancer cells to extracellular matrix (ECM) by migration assay. Figure 3(A) shows that SW1990 cell migrated to the lower surface of the filter from the upper chamber, stained with Giemsa staining (the closed arrow shows the nucleus of cancer cells). We evaluated migration ability by the number of migrating cells. HA has significantly enhanced the migration of cancer cells. Figure 3(B) shows that 50–200 μg/mL HA induced the migration ability of SW1990 in a dose-dependent manner.

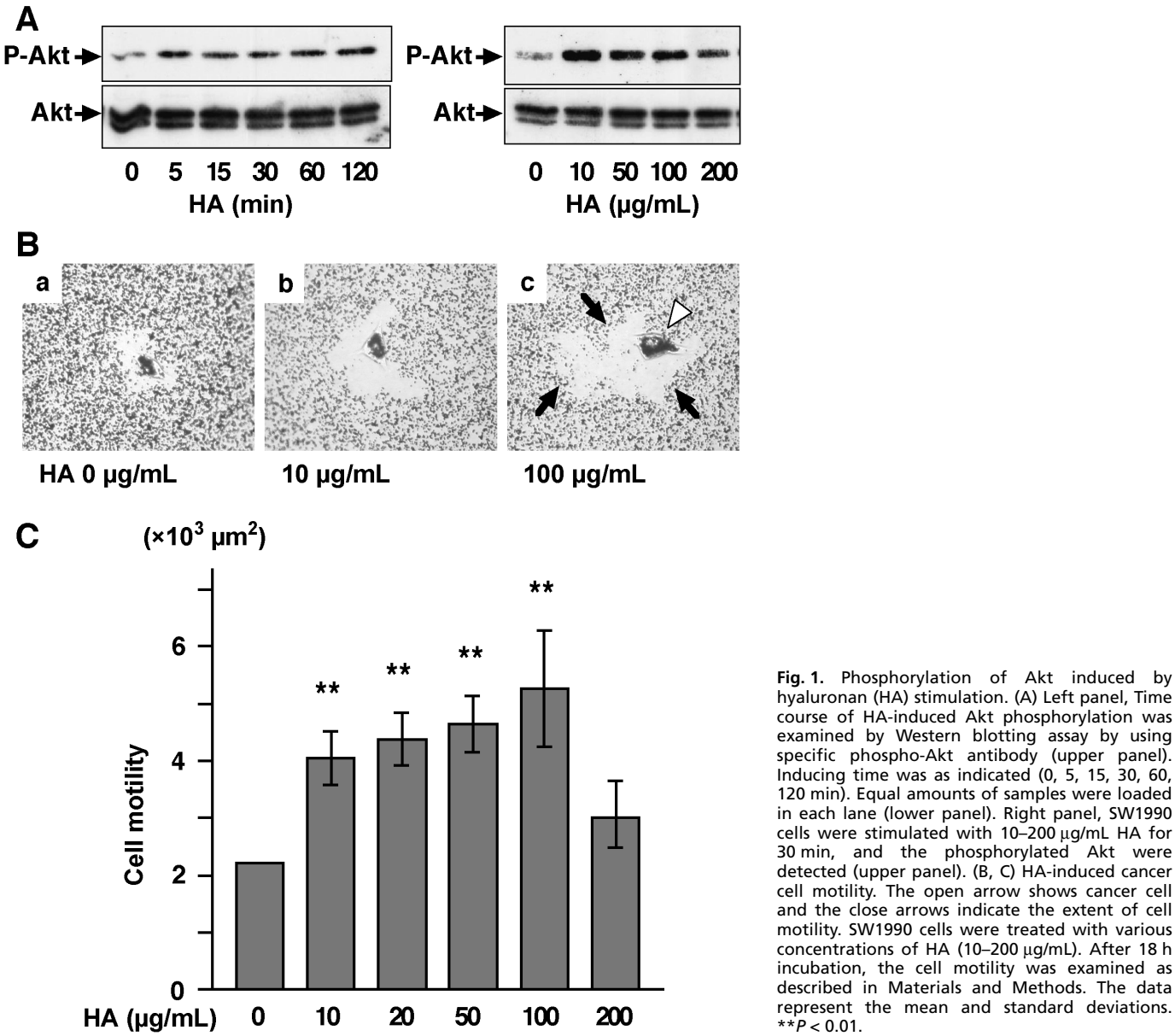

Fig. 1. Phosphorylation of Akt induced by hyaluronan (HA) stimulation. (A) Left panel, Time course of HA-induced Akt phosphorylation was examined by Western blotting assay by using specific phospho-Akt antibody (upper panel). Inducing time was as indicated (0, 5, 15, 30, 60, 120 min). Equal amounts of samples were loaded in each lane (lower panel). Right panel, SW1990 cells were stimulated with 10–200 μg/mL HA for 30 min, and the phosphorylated Akt were detected (upper panel). (B, C) HA-induced cancer cell motility. The open arrow shows cancer cell and the close arrows indicate the extent of cell motility. SW1990 cells were treated with various concentrations of HA (10–200 μg/mL). After 18 h incubation, the cell motility was examined as described in Materials and Methods. The data represent the mean and standard deviations. $^{**}P < 0.01$.

We examined some inhibitors to confirm the signaling pathway related to HA-induced migration. Figure 4(A) and (B) show that PI3K inhibitor wortmannin significantly inhibited SW1990 cell migration by HA at 0.5 μM. Therefore, we consider that HA induces cancer cell migration through HA-PI3K-Akt signaling pathway. In Fig. 4(C), Src inhibitor PP1 was also applied to block the cell migration and 0.125 μM PP1 has successfully blocked the HA-induced cell migration, which is consistent with the result in Fig. 2(C).

Suppression of peritoneal metastasis in nude mouse by PI3K inhibitor, wortmannin. The effect of PI3K inhibitor on HA induced Akt activation, the subsequent cell motility and migration, prompted us to examine its effect on peritoneal metastasis using a nude mouse model *in vivo*. Because wortmannin stably inhibited HA-induced Akt phosphorylation, cell motility and migration, even in a low density, we used it in this assay. In order to inhibit the stage that cancer cells promote motility and migration by contact with HA on the peritoneal mesothelial cells, we started the treatment just after SW1990 cell inoculation and continued it for 20 days. We injected wortmannin into abdominal cavities of nude mice once a day. All mice were sacrificed 20 days after SW1990 inoculation (Fig. 5A).

Figure 5(B) illustrates two representative mice, treated with wortmannin (mouse b) or only vehicle (mouse a). There was no significant difference in body weight among all groups, and few ascites was found in abdominal cavities. At autopsy, in mouse a, metastatic tumors were found on the mesenterium and greater omentum (Fig. 5B-c). In mouse b, tumors were very small and few (Fig. 5B-d).

Because intra-abdominal tumor growth could not be monitored directly, the number and weight of tumors were assessed at postmortem examination. We counted the number of metastatic peritoneal tumors grossly (Fig. 5C). In order to examine the total weight of tumors, we excised tumors in a lump with mesenterium and greater omentum and measured the weight of the mass, because we had thought that tumors were so small that an alien substance like blood or connective tissue caused a big error. We considered that the weight subtracted from the mean weight of mesenterium and greater omentum among healthy nude mice showed the total weight of metastatic tumors. Wortmannin

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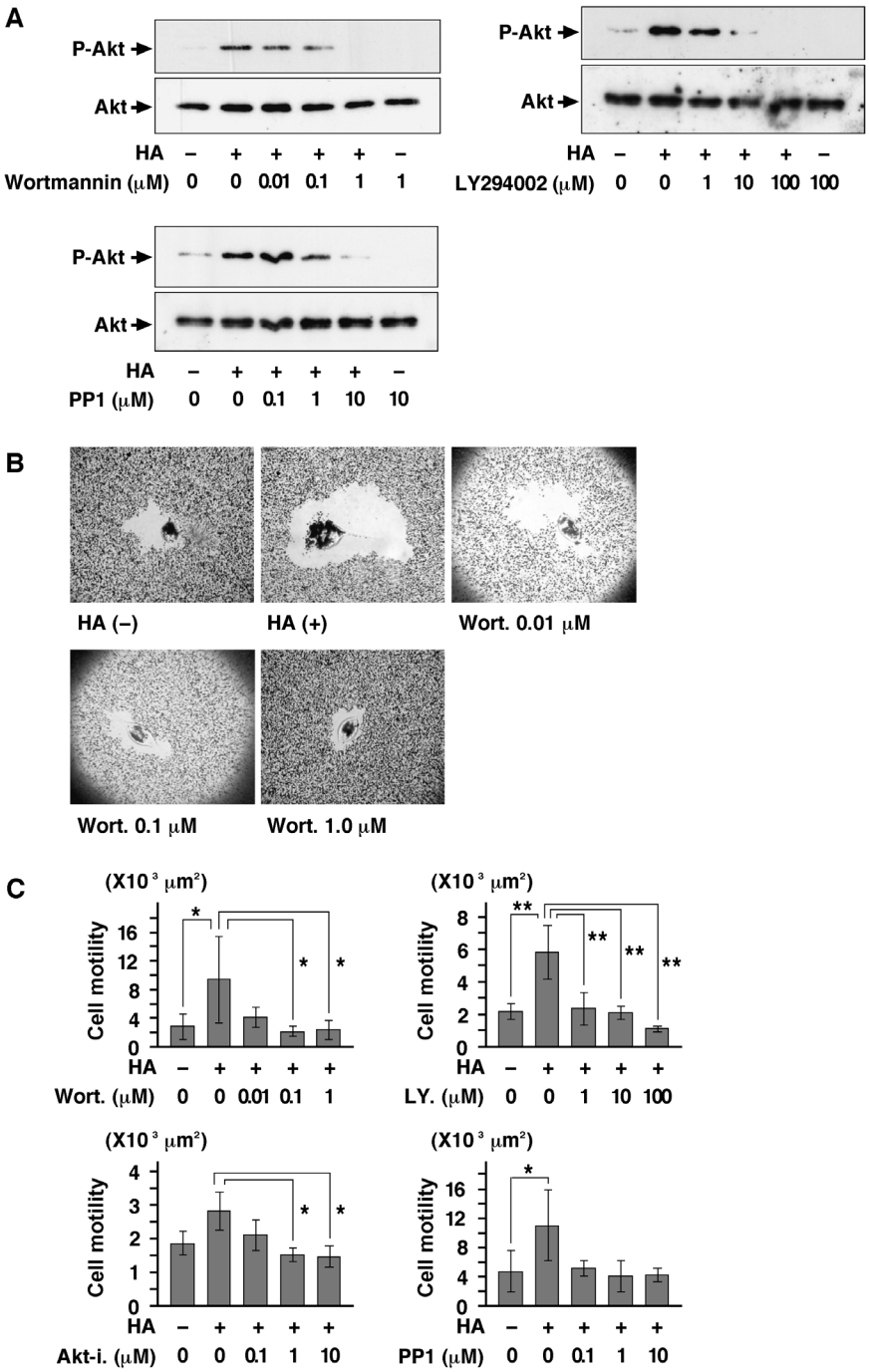


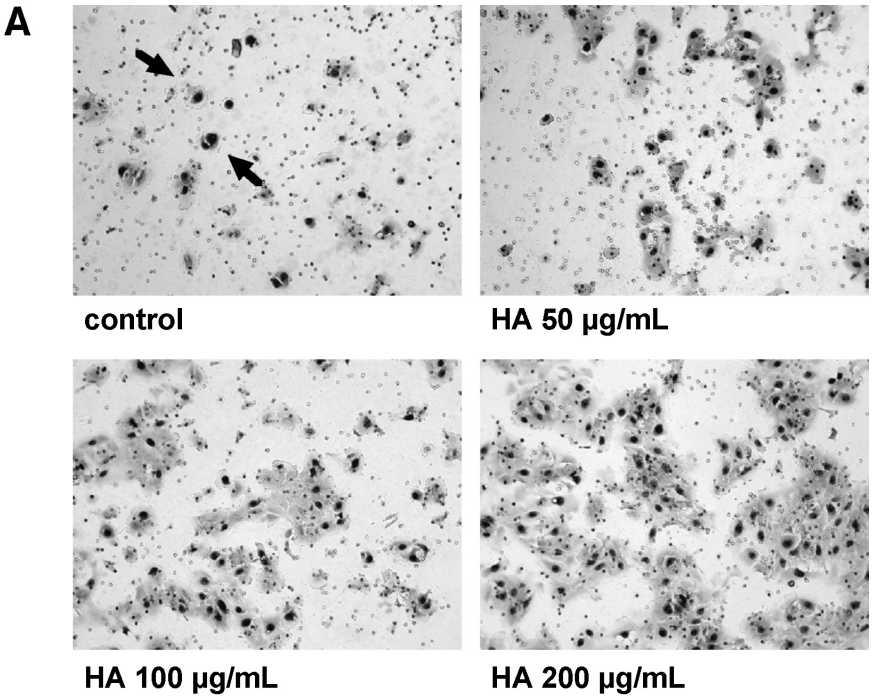
Fig. 2. The effects of PI3K inhibitor and Src inhibitor on hyaluronan (HA)-induced Akt phosphorylation. (A) SW1990 cells were treated with 100 μg/mL HA and different amounts of phosphoinositide 3-kinase (PI3K) inhibitors including wortmannin or LY294002, or a Src inhibitor 4-Amino-5-(4-methylphenyl)-7-(*t*-butyl)pyrazolo [3,4-*d*]-pyrimidine (PP1). The phosphorylated Akt was detected by Western blotting. (B) The effect of PI3K inhibitor on the HA-induced motility of SW1990 cells. The inhibitory effect of wortmannin was assessed. SW1990 cells were preincubated with wortmannin at various concentrations for 1 h. Eighteen hours after adding 100 μg/mL HA, microscopic photographs were taken (×100). (C) Effects of inhibitors on cell motility. SW1990 cells were incubated with various concentration of wortmannin (Wort.), LY294002 (LY.), Akt inhibitor (Akt-i.), or PP1 for 1 h, HA was added at 100 μg/mL, incubated for an additional 18 h, and the cell motility was measured. The data represent the mean and the standard deviations. ($n = 10$; $^{**}P < 0.01$; $^{*}P < 0.05$).

significantly reduced the number and the total weight of tumors in a dose-dependent manner (Fig. 5C,D).

Discussion

In the earliest stages of clinical peritoneal metastasis, pancreatic cancer cells leave the primary tumor, and are exposed to HA covering peritoneal mesothelial cells. HA is a polysaccharide on the abdominal mesothelial cell surface,[1,28,29] and acts as an adhesion molecule in cancer peritoneal metastasis.[7] Recent studies have revealed that HA also induces signal transduction,[8–11,29] and is involved in the invasion and metastasis of several cancers. For example, HA increases migration of breast cancer cells,[30] and invasion of colon cancer.[5] Although HA covers all peritoneal mesothelial cells, peritoneal matastasis clinically starts in the greater omentum, mesenterium or Douglas fossa that have the entrance to the lymph vessel from abdominal cavity, called milky spot or stomata.[31–34] Therefore, we consider HA acts as a stimulator of signaling pathways rather than acting as merely adhesion molecules.

HA stimulation is known to promote certain signaling pathways in cells through the cleavage of CD44 intracellular domain,[35,36] Ras-mitogen-activated protein (MAP) kinese pathway[37] or PI3K-Akt pathway.[10,11] PI3K-Akt signaling pathway is involved in cell proliferation, cell survival,[10] cell motility,[11] activation of integrins,[16] MMP production[17] and angiogenesis,[20] thus



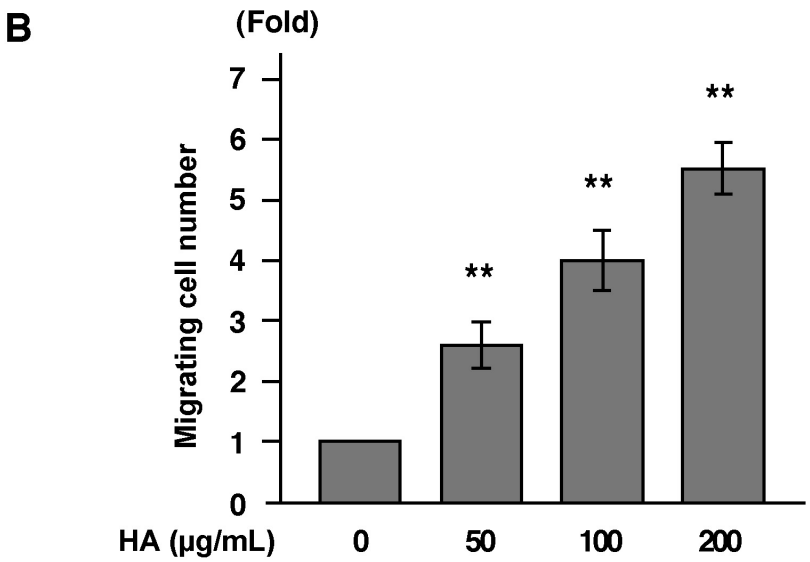


Fig. 3. Hyaluronan (HA) induced the migration of SW1990 cells through extracellular matrix (ECM). (A) Microphotographs of migrating cells on the underside of chambers. SW1990 cells were treated with different doses of HA, and migration ability was assessed by modified Boyden chamber method. After incubation with HA, migrating cells on the lower surface of the filter were fixed with 70% ethanol and stained with Giemsa staining. Photographs were taken by a phase contrast microscope (×200). Closed arrow shows the migrating cell nucleus. (B) The extent of migration is expressed as the number of cells per microscopic field. Data are shown as mean ratio to the control migration. The data represent the mean and the standard deviations. ($n = 6$; $^{**}P < 0.01$).

playing important roles in cancer invasion and metastasis. In addition, recent studies have shown that HA stimulates cell motility.[11,36]

We confirmed that HA quickly induced Akt phosphorylation in the SW1990 human pancreatic cancer cell line. We performed cell motility assay to examine the effect of HA on SW1990 cell motility. We found HA stimulation increased cell motility up to 100 μg/mL in a dose-dependent manner (Fig. 1). Thus, we consider that the signaling pathway induced by HA through Akt could be an efficient molecular target in preventing peritoneal metastasis, although the mechanism of PI3K-Akt pathway activation by HA remains unclear.

CD44 is one of the HA ligands on the cell surface and has many splicing variants. Although pancreatic cancer cell lines express them strongly, pancreatic duct cell lines express them weakly.[38] A previous study showed that SW1990 expresses CD44H.[22] We also confirmed this by fluorescent-activated cell sorter (FACS) (data not shown). It has been reported that CD44 mediates intracellular signaling through Src.[37,39] Src has been reported to directly phosphorylate Akt.[40] Lu *et al.*[41] have recently shown that activated Src inhibits the PI3K antagonist phosphatase and tensin homolog deleted on chromosome 10 (PTEN), leading to alternations in signaling through the PI3K-Akt pathway. Therefore, we presume that HA stimulates the PI3K-Akt pathway through Src.

We have demonstrated that PI3K inhibitors, wortmannin and LY294002, inhibited HA-induced Akt phosphorylation (Fig. 2). Furthermore, PP1, a Src inhibitor, inhibited HA-induced Akt phosphorylation in a dose-dependent manner. These findings indicate that Src is involved in the HA-PI3K-Akt pathway. Wortmannin and LY294002, PI3K inhibitors, remarkably inhibited HA-activated cell motility, suggesting HA activated the motility through the PI3K-Akt pathway. Moreover, Akt inhibitor (Akt specific inhibitor), which does not inhibit PI3K in the low dose

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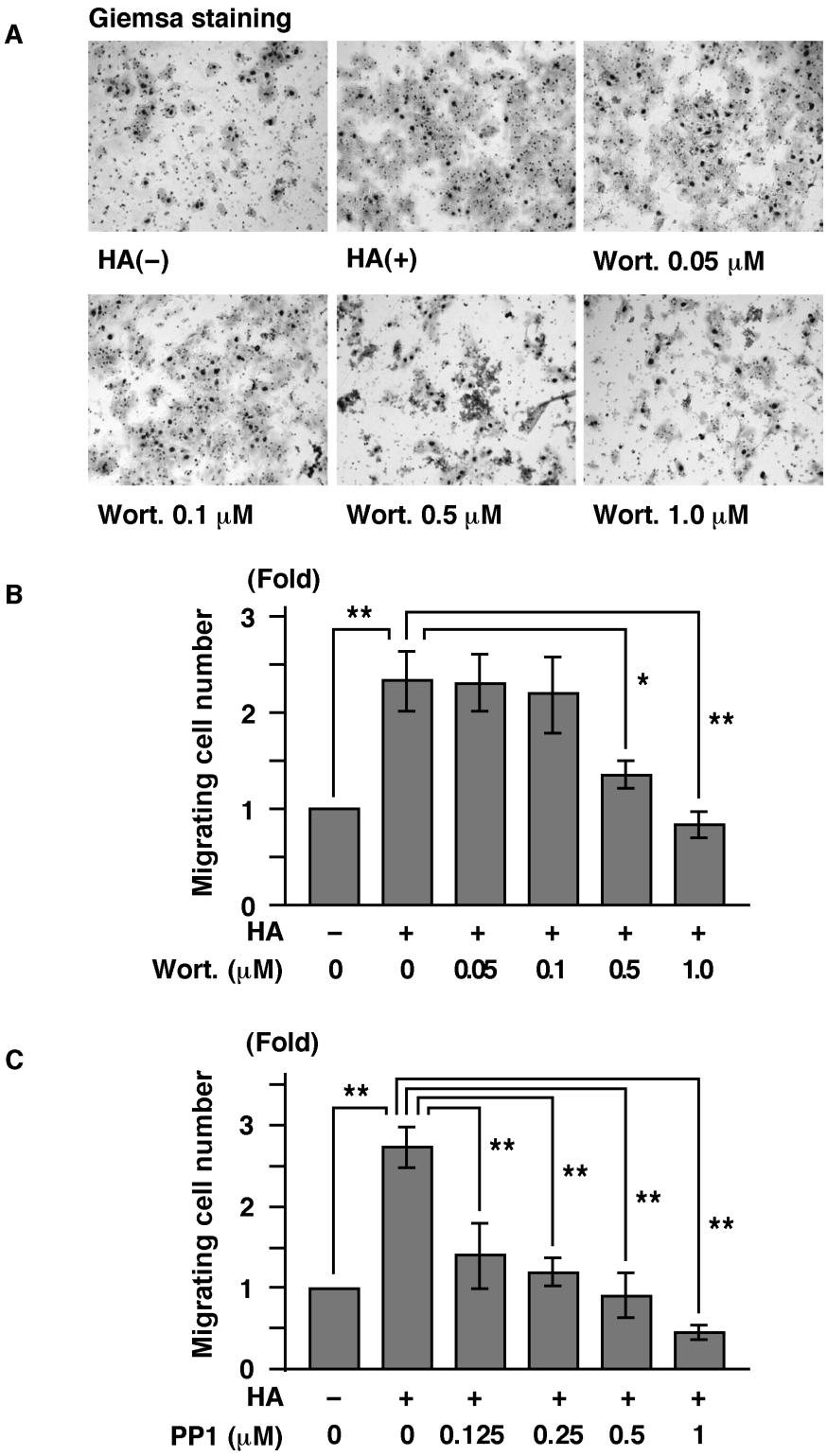

Fig. 4. The effect of wortmannin on hyaluronan (HA) induced migration of SW1990 cells. (A) The inhibitory effect of phosphoinositide 3-kinase (PI3K) inhibitor, wortmannin. SW1990 cells were preincubated with wortmannin at various concentrations for 1 h. Photographs were taken by a phase contrast microscope (×200). The black dots on photographs are the nuclei of migrating cells. (B) It also was expressed as the number of cells per microscopic field. Data are shown as mean ratio to the control migration. The data represent the mean and the standard deviations. (C) The inhibitory effect of PP1 was assessed in the same way. ($n = 6$; $^{**}P < 0.01$; $^{*}P < 0.05$).

we used in this study,[42] also inhibited HA induced cell motility. This result forcefully indicates that HA stimulates the cell motility through Akt. Although a previous report showed that Akt activates cell motility through p70S6K1 in cells expressing the activated form of Akt,[43] the signaling pathway through which Akt activates cell motility in HA-stimulated cells is still unclear.

In migration assay, HA activated SW1990 cell migration into ECM, and wortmannin inhibited it significantly (Fig. 4). These findings suggest that HA activates cancer cell migration through the PI3K-Akt signaling pathway as indicated by others.[16,17] Therefore, we considered that wortmannin might inhibit this invasive character of cancer cells.

HA is a large, non-sulfated glycosaminoglycan composed of a repeating disaccharide unit of N-acetyl-D-glucosamine and D-glucuronic acid with varying molecular weight. Previous studies have demonstrated that HA exhibits various biological effects

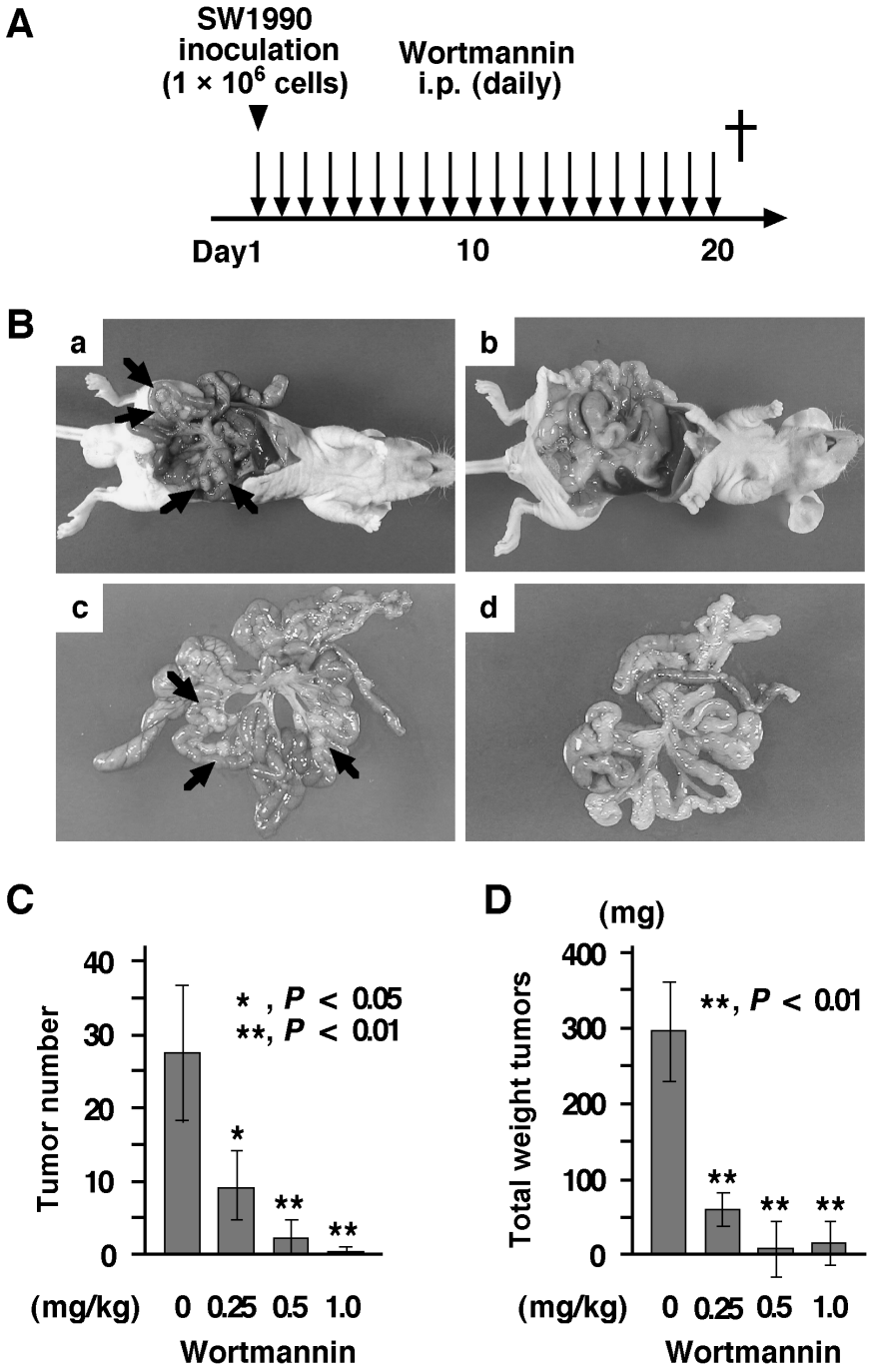

Fig. 5. Peritoneal dissemination of SW1990 cells in nude mice and the effects of wortmannin. (A) SW1990 cells (1×10^6 of cells/100 μL phosphate buffered saline [PBS]) were inoculated into the abdominal cavity of 5-week-old male nude mice. The mice were treated with 1.0, 0.5, or 0.25 mg/kg/day wortmannin or vehicle (PBS + dimethyl sulfoxide [DMSO], intraperitoneally) from immediately after the cancer cell inoculation for 20 consecutive days. Twenty days after the inoculation of SW1990 cells, mice were sacrificed, and we examined their body weights, amounts of ascites, the number of peritoneal metastatic tumors, and total weight of tumors. (B) Peritoneal appearances of the abdominal cavity of mice. Peritoneal dissemination was observed in control mice (a, c). Few metastatic tumors were evidenced in nude mice treated with 1.0 mg/kg wortmannin (b, d). (C) The number of metastatic tumors in each group. Tumors were counted with the naked eye. (D) Total weight of metastatic tumors. The data represent the mean and the standard deviation. ($n = 5$).

depending on the molecular weight.[44–47] However, the molecular weight of HA on the cell surface of peritoneal mesothelial cells is not clear. Thus, we adopted the peritoneal cancer metastasis model of nude mice by inoculating SW1990 cells into their abdominal cavity *in vivo*, and investigated the effect of PI3K-Akt pathway inhibition. We used wortmannin as a PI3K inhibitor because wortmannin could inhibit the HA-induced Akt phosphorylation, cell motility and migration excellently in this study (Figs 2 and 4).

In order to inhibit the step at which cancer cells are shedding from their primary tumors upon stimulation with HA that is present on the peritoneal mesothelial cell surface, we administered wortmannin intra-abdominally immediately after SW1990 cell inoculation. At postmortem examination on 20 days after cancer cell inoculation, we found enormous peritoneal metastasis in the control group, whereas in the treatment group wortmannin significantly reduced the number and the weight of tumors. These results indicate that the PI3K-Akt signaling pathway activated by HA plays a crucial role in the peritoneal cancer metastasis *in vivo*.

In summary, our data demonstrate that PI3K inhibitor wortmannin significantly inhibits HA-stimulated Akt phosphorylation in SW1990, and thus its cell motility, migration *in vitro* and peritoneal metastasis *in vivo*, suggesting that PI3K plays an important role in the peritoneal metastasis of pancreatic cancer. Therefore, we propose that intraperitoneal inhibition of PI3K is a potentially useful treatment modality for patients with pancreatic cancer.

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