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Inhibition of nuclear factor-kappaB suppresses peritoneal dissemination of gastric cancer by blocking cancer cell adhesion

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Currently, patients with peritoneal dissemination of gastric cancer must accept a poor prognosis because there is no standard effective therapy. To inhibit peritoneal dissemination it is important to inhibit interactions between extracellular matrices (ECM) and cell surface integrins, which are important for cancer cell adhesion. Although nuclear factor-kappa B (NF-κB) is involved in various processes in cancer progression, its involvement in the expression of integrins has not been elucidated. We used a novel NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), to study whether NF-κB blocks cancer cell adhesion via integrins in a gastric cancer dissemination model in mice and found that DHMEQ is a potent suppressor of cancer cell dissemination. Dehydroxymethylepoxyquinomicin suppressed the NF-κB activity of human gastric cancer cells NUGC-4 and 44As3Luc and blocked the adhesion of cancer cells to ECM when compared with the control. Dehydroxymethylepoxyquinomicin also inhibited expression of integrin (α2, α3, β1) in *in vitro* studies. In the *in vivo* model, we injected 44As3Luc cells pretreated with DHMEQ into the peritoneal cavity of mice and performed peritoneal lavage after the injection of cancer cells. Viable cancer cells in the peritoneal cavities were evaluated sequentially by *in vivo* imaging. In mice injected with DHMEQ-pretreated cells and lavaged, live cancer cells in the peritoneum were significantly reduced compared with the control, and these mice survived longer. These results indicate that DHMEQ could inhibit cancer cell adhesion to the peritoneum possibly by suppressing integrin expression. Nuclear factor-kappa B inhibition may be a new therapeutic option for suppressing postoperative cancer dissemination. (*Cancer Sci* 2011; 102: 1052–1058)

Peritoneal dissemination is the most frequent process through which gastric cancer recurs,[1] and patients with this condition must currently accept a very poor prognosis.[2,3] Standard chemotherapy is currently not sufficiently effective for improving the survival of patients with peritoneal dissemination of gastric cancer. To inhibit peritoneal dissemination, it may be important to control the adhesion of cancer cells to the peritoneum. During cancer cell dissemination in the abdominal cavity, cancer cells make contact with the basement membrane through gaps between mesothelial cells.[4,5] The basement membrane beneath mesothelial cells comprises extracellular matrices (ECM) consisting of type 1 and 4 collagen, fibronectin or laminin,[6] and mesothelial cells also produce ECM.[7] The interactions between these ECM and cell surface integrins play very important roles in cancer cell adhesion and, therefore, cancer progression.[8]

Integrins are membrane-bound proteins that form heterodimers of α- and β-subunits at the cell surface. The α-subunits vary between 120 and 180 kD, and are non-covalently associated with β-subunits (90–110 kD). To date, 14 α subunits and eight β subunits have been identified, and after mutual dimerization, these subunits contribute to cell adhesion or regulation of signal transduction required for cell survival by making contact with appropriate ECM.[9,10] It has been reported that integrins α2, α3 and β1 play important roles in the peritoneal dissemination of gastric cancer,[11] and that antibodies to these integrins suppress peritoneal dissemination of gastric cancer in a mouse model.[12]

Nuclear factor-kappaB (NF-κB) was first identified and reported in 1986[13] and studied in the context of immune and inflammatory responses.[14] Nuclear factor-kappaB is a generic term for dimers of NF-κB1 (p50/p105), NF-κB2 (p52/p100), c-Rel, RelA (p65/NF-κB3) and RelB.[15] To date, involvement of NF-κB in cancer-related molecules such as cyclin D1,[16] intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1),[17] the Bcl family,[18] inhibitor of apoptosis (IAP), X-linked inhibitor of apoptosis protein (XIAP),[19] p53,[20] vascular endothelial growth factor (VEGF), interleukin (IL)-8,[21] MMP[22] and multidrug resistance protein 1 (MDR1),[23] has been elucidated. However, NF-κB has not been reported to be involved in cancer cell adhesion to the peritoneum via integrins.

A low-molecular-weight NF-κB inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), was newly developed by Umezawa.[24] Dehydroxymethylepoxyquinomicin specifically inhibits the nuclear translocation of p65 and prevents it binding to DNA[25]; it also has various anti-cancer effects in mouse models without obvious side-effects. Thus far, the following anti-cancer effects of DHMEQ have been reported: G1 arrest by inhibition of cyclin D1 expression;[26] and induction of apoptosis by inhibition of cIAP and XIAP,[27] or Bcl-2 and Bcl-xL.[28] Antitumor effects of DHMEQ have also been reported in *in vivo* models such as those for thyroid cancer,[27] prostate cancer,[29] hepatic cancer,[30] breast cancer,[31] pancreas cancer,[32] multiple myeloma,[28] malignant lymphoma[33] and leukemia.[26]

In the present study, we showed that NF-κB is associated with integrin expression in gastric cancer cell lines and that NF-κB inhibition by DHMEQ suppresses cancer progression by inhibiting the adhesion of gastric cancer cells to the peritoneum in a mouse model of peritoneal dissemination of gastric cancer.

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Materials and Methods

Cell cultures. The human gastric cancer cell line NUGC4 was obtained from the Japanese Cancer Research Resources Bank (JCRB, Osaka, Japan), and 44As3Luc with luciferase activity was constructed by one of the authors (K.Y.).[34] The 44As3Luc cells were derived from 44As3 cells, which is a highly peritoneal metastatic cell line, and were stably transfected with a pEGF-PLuc plasmid with CMV promoter (Clontech, Palo Alto, CA, USA). Human breast cancer cell lines MCF7 with constitutively low NF-κB activity and MDA-MB231 with constitutively high NF-κB activity were obtained from the American Type Culture Collection (Rockville, MD, USA).[31] The NUGC4 cells were cultured at 37°C in RPMI1640 (Sigma, St Louis, MO, USA) along with 10% fetal bovine serum (FBS); the 44As3Luc cells were cultured at the same temperature with RPMI1640 containing 100 μg/mL geneticin (Sigma); and the MCF7 and MDA-MB231 cells were also cultured at 37°C in 95% air and 5% CO_2 in DMEM (Sigma) along with 10% FBS.

Dehydroxymethylepoxyquinomycin (DHMEQ). We have originally designed and developed DHMEQ (molecular weight (MW): 261), a derivative of the natural antibiotic epoxyquinomycin C, to specifically target NF-κB.[24]

DNA-binding activity of NF-κB. To evaluate the DNA-binding activity of NF-κB in the steady state, 70% confluent cultures of NUGC4, 44As3Luc, MCF7 and MDA-MB231 in 10-cm dishes were stored at −80°C. To evaluate the effect of DHMEQ, the medium in the 70% confluent cultures of NUGC4 and 44As3Luc was replaced with 10 μg/mL DHMEQ solution, incubated for an appropriate time and stored at −80°C. The following day, nuclear proteins were extracted and examined using a p65 TransAM kit (ActiveMotif, Carlsbad, CA, USA). The absorbance was determined using a plate reader (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA). Each experiment was performed in triplicate.

NF-κB reporter gene assay. A GFP reporter gene construct was transfected using Cignal Reporter Assay kits (SA Biosciences, Frederick, MD, USA). Cultured cells were trypsinized and resuspended in Opti-MEM (Invitrogen, Carlsbad, CA, USA) with non-essential amino acids (Invitrogen) without antibiotics at a concentration of 2×10^5 cells in a 96-well plate. Cells were transfected with the reporter by culturing for 16 h with Surefect (SA Biosciences). After the medium was replaced with Opti-MEM with penicillin/streptomycin, the cells were incubated for an additional 8 h. The medium was then replaced with Opti-MEM containing 10 μg/mL of DHMEQ (or 0.024% of DMSO for the controls). The intensity of fluorescence was measured at appropriate times in triplicate using Varioskan Flash (excitation, 470 nm; emission, 515 nm).

mRNA expression of integrins in DHMEQ-treated cells. Real-time PCR was used to examine mRNA expression. The 44As3Luc cells were cultured in triplicate in 0.024% DMSO solution (controls) or in 10 μg/mL DHMEQ for the appropriate times. Total RNA was isolated using an RNeasy mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. For cDNA synthesis, ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) with Oligo(dT) 20 primer (Toyobo) was used in accordance with the manufacturer's instructions. For relative quantification by PCR, each cDNA product was analyzed in a LightCycler (version 1.4) using a QuantiTect SYBR Green PCR kit (Qiagen).

Flow cytometric analysis of integrin expression. p65 silencing was performed using p65 siRNA2 (BD Biosciences, Bedford, MA, USA). Next, 50% confluent cells were incubated for 24 h in medium without antibiotics in 10-cm dishes. Then, 33 nM p65 siRNA was added to each dish and transfected for 48 h. p65 silencing was confirmed by western blot analysis using primary antibodies against ×500 α-tubulin and ×1000 p65 protein (Cell Signaling, Beverly, MA, USA) and ×5000 goat anti-mouse IgG for tubulin or anti-rabbit IgG for the p65 protein. With regard to DHMEQ treatment, the medium in 70% confluent cell cultures in 10-cm dishes was replaced with 10 μg/mL DHMEQ solution (0.024% DMSO for the controls) and cultured for the appropriate times. These cells were trypsinized and analyzed using flow cytometry (FACS Caliber; Becton Dickinson, Franklin Lakes, NJ, USA). The antibodies used for these assays were integrin α2, integrin α3, integrin β1 and isotype controls for these integrins. All antibodies were obtained from R&D Systems (Minneapolis, MN, USA).

Adhesion assay. We evaluated the anti-adhesive effect of DHMEQ by using a plate pre-coated with ECM constituting the peritoneal basement membrane. The medium in 70% confluent cell cultures in 10-cm dishes was replaced with 10 μg/mL DHMEQ solution (or 0.024% DMSO for the controls), and the dishes were incubated for 24 h. These cells were trypsinized, assembled, adjusted to a concentration of 1×10^6 cells/mL with RPMI and distributed on the pre-coated plates (80 μL per plate). Next, the cells were incubated at 37°C for 1 h. Except for the non-treated plate, all plates were washed three times with 100 μL of FBS-free RPMI. After washing, 10 μL of ×50 diluted Cell Counting kit F (CCKF; Dojindo, Osaka, Japan) was added to each well, and the fluorescence intensity of the remaining live cells (adhesive cells) was evaluated using Varioskan Flash at 30 min after CCKF administration (excitation, 490 nm; emission, 515 nm). Pre-coated plates were manufactured by BD Biosciences and the ECM coated on the plates were types 1 and 4 collagen, fibronectin and laminin.

DHMEQ cytotoxicity assay. The cells were seeded into 96-well plates at 5×10^3 cells/well in 10% FBS-containing medium. Twenty-four hours later, the medium in the wells was replaced with different concentrations of DHMEQ solution or 0.048% DMSO solution, and the cells were then incubated again for 24 h. Lactate dehydrogenase (LDH) activity of the supernatant was measured using an LDH cytotoxicity detection kit (Takara Bio, Shiga, Japan).

Animal experiments. Six-week-old male BALB/c-nu/nu mice, each weighing approximately 20 g, were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were grouped as follows: (i), implantation of DMSO-treated cells; (ii) implantation of DHMEQ-treated cells; (iii) implantation of DMSO-treated cells with peritoneal lavage; and (iv) implantation of DHMEQ-treated cells with peritoneal lavage. Each group comprised four mice. Then, 2×10^6 44As3Luc cells, which had been treated with 10 μg/mL DHMEQ (or 0.024% DMSO for the controls) for 24 h, were injected intraperitoneally into the above mentioned mice. One hour after injection, laparotomy and peritoneal lavage were performed using phosphate-buffered saline (PBS). Peritoneal lavage was performed through a 1-cm incision through which 5 mL of PBS was slowly injected. Bio-imaging was performed before and after the peritoneal lavage, and on days 2, 5, 10, 15 and 20 in order to evaluate cancer progression. Luminescence was evaluated at approximately 7 min after intraperitoneal injection of 1500 μg/mouse D-luciferin potassium salt (Synchem OHG, Altenburg, Germany). *In vivo* imaging was performed using Photon Imager Hu (Biospace Lab, Paris, France) with the mice under isoflurane anesthesia (Abbott Japan, Tokyo, Japan). Images were captured using Photo Acquisition 2.6 (Biospace Lab) with 0.5 min exposure and processed using Photo Vision Plus. Signal intensity was quantified as the sum of all detected photon counts (count per minute [CPM]) within the region of interest (ROI). All procedures involving animals and their care were approved by the Ethics Committee of Hokkaido University in accordance with institutional and Japanese governmental guidelines for animal experiments.

Scanning electron microscopy (SEM) of the peritoneal wall. The peritoneal walls of mice injected with cancer cells

were fixed with 10% formaldehyde for 180 min and then overnight at 4°C with 1.25% glutaraldehyde solution. The fixed samples were dehydrated in a 30–100% graded ethanol series and immersed in tert-butyl alcohol overnight at −20°C. These samples were dried using ES-2000 (Hitachi High-Technologies Co., Tokyo, Japan) for 3 h and ion-sputtered using E-1030 (Hitachi) for 120 s. The peritoneal surface was observed under a scanning electron microscope (S-3500N; Hitachi).

Statistics. The mean and SD were calculated for all variables, except the data from the flow cytometry. Between-group statistical significance was determined using the Student's *t* test. $P < 0.05$ was considered as statistically significant.

Results

DHMEQ effectively suppresses p65-DNA binding activity in gastric cancer cells. In the steady state, the p65-DNA binding activities in NUGC4 and 44As3Luc cells were as high as that in MDA-MB231 cells, a positive control cell with high binding activity. The activity in MCF7 cells is constitutively low as previously reported [31], and hence these cells were used as the negative control (Fig. 1A). The binding activities in both cells reached their lowest levels 2 h after the addition of 10 μg/mL DHMEQ (as a final concentration) and returned to initial conditions within 24 h (Fig. 1B). A GFP reporter assay showed that DHMEQ significantly suppresses transcriptional activity in both cells (Fig. 1C). On the basis of these results, we considered that DHMEQ had a similar effect in NUGC4 and 44As3Luc cells. Therefore, we used 44As3Luc cells in the following experiments. We planned to evaluate cancer progression using bioimaging.

Effect of NF-κB inhibition on integrin expression. In 44As3Luc cells, the mRNA of all integrins – α2, α3 and β1 – were significantly suppressed 2 h after the addition of 10 μg/mL DHMEQ (as a final concentration) compared with the control to which DMSO was added (Fig. 2A). The percentage reduction in the expressions of integrins α2, α3 and β1 was 27%, 31% and 8%, respectively. Flow cytometric analysis revealed that the expressions of all cell surface integrins on 44As3Luc cells were gradually suppressed after the addition of DHMEQ (Fig. 2B). Reductions in integrin expression (α2, α3 and β1) following DHMEQ addition was 68%, 83% and 45% at 24 h, respectively. Similarly, flow cytometric analysis of integrins α2, α3 and β1 revealed that the expressions of cell surface integrins in p65-deleted cells were suppressed to the same degree as in DHMEQ-treated cells (Fig. 2C). Reductions in integrin expression after p65 deletion were 34% (α2), 76% (α3) and 41% (β1). p65 silencing was confirmed by western blotting for nuclear and cytoplasmic p65 proteins (Fig. 2D).

Anti-adhesive effect of DHMEQ-treated cells in an *in vitro* assay. Significantly fewer 44As3Luc cells treated with 10 μg/mL DHMEQ (final concentration) remained alive on plates pre-coated with ECM after they were washed (ECM-adhesive cells) than 44As3Luc cells treated with DMSO (Fig. 3A). Reductions in the numbers of adhesive cells following DHMEQ addition were 18.3% (laminin), 34.8% (fibronectin), 38.2% (type 1 collagen) and 43.5% (type 4 collagen). The LDH value, which represents the cytotoxic effect, was significantly elevated in the supernatant of cells treated with DHMEQ at concentrations >17.5 μg/mL (Fig. 3B).

Effect of peritoneal lavage on implantation of DHMEQ-treated cancer cells on the abdominal wall. The number of cancer cells decreased in mice injected with DHMEQ-pretreated cells and subjected to peritoneal lavage (Fig. 4A). The intensity of bioluminescence after lavage was significantly reduced (reduction rate, 39%) in mice that were injected with DHMEQ-pretreated cells and subjected to peritoneal lavage compared with in mice injected with DMSO-pretreated control cells and subjected to

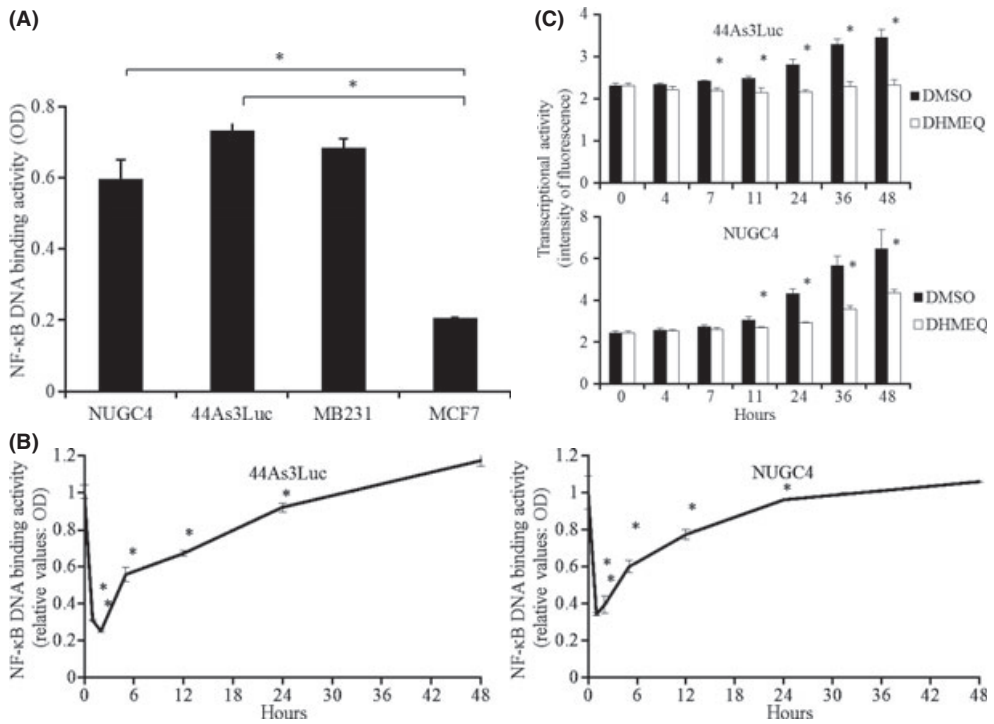

Fig. 1. Dehydroxymethylepoxyquinomicin (DHMEQ) effectively suppressed p65-DNA binding activity in gastric cancer cells. (A) Nuclear p65 protein binding activity to DNA in a steady state. MDA-MB231 cells were used as a positive control, and MCF7 cells were used as a negative one. *$P < 0.05$. (B) Time course of binding activity of nuclear p65 proteins to DNA in DHMEQ-treated cells. The binding activities of both cells were assessed at 2, 6, 12, 24 and 48 h after DHMEQ administration. *Significantly <0 h ($P < 0.05$). (C) Nuclear factor-kappa B (NF-κB) GFP reporter assay. The black bars show cells treated with DMSO, and white bars show those with DHMEQ. *Significantly more than controls ($P < 0.05$). OD, optical density.

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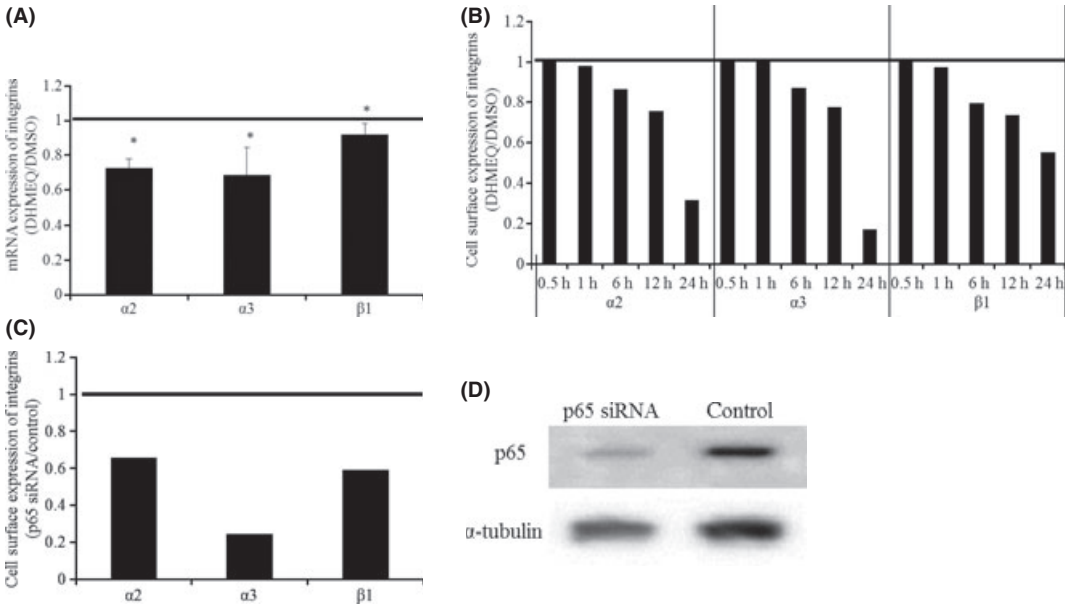

Fig. 2. Effect of nuclear factor-kappa B (NF-κB) inhibition on expression of adhesion molecules. (A) Quantitative evaluation of mRNA of integrins by real-time PCR. The graph shows the average of the ratio of copies of dehydroxymethylepoxyquinomicin (DHMEQ)-treated 44As3Luc cells to DMSO-treated cells at 2 h after DHMEQ administration. When the longitudinal value is below 1 (bold line), the integrin expression of DHMEQ-treated cells is lower than that of DMSO-treated cells. *Significantly less than controls ($P < 0.05$). (B) Expression of cell surface integrins of DHMEQ-treated cells. The graph shows the expression rate of cell surface adhesion molecules of 44As3Luc cells treated with DHMEQ compared with that of DMSO-treated cells for each time point. The bold line is as described above. (C) Expression of cell surface adhesion molecules of cells knocked down by p65 siRNA. The graph shows the rate of cell surface integrins of 44As3Luc cells knocked down by p65 siRNA. The bold line is as described above. (D) p65 deletion. The p65 deletion was confirmed by western blotting.

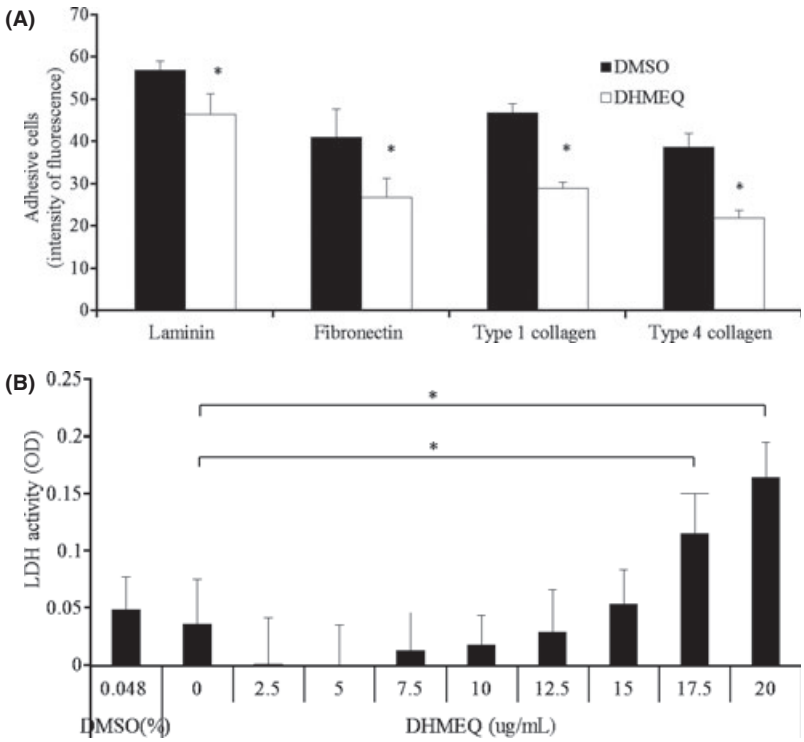

Fig. 3. Anti-adhesive effect of dehydroxymethylepoxyquinomicin (DHMEQ) pretreated cells in the *in vitro* study. (A) Adhesion assay. The bars show the fluorescence intensity of the remaining live cells on the plates. The black bars show cells pretreated with DHMEQ, and white bars show those with DMSO. *Significantly less than controls ($P < 0.05$). (B) Evaluation of cytotoxicity of DHMEQ. The graph shows lactate dehydrogenase (LDH) activity of the supernatant of the 44As3Luc cells treated with DHMEQ or DMSO. *$P < 0.05$. OD, optical density.

peritoneal lavage (Fig. 4B). The SEM revealed that cancer cells adhered less to the basement membrane of the peritoneum in mice injected with DHMEQ-pretreated cells than in those injected with DMSO-pretreated control cells (Fig. 4C).

Follow up of gastric cancer dissemination by *in vivo* imaging. The DHMEQ-pretreated 44As3Luc cells injected in mice grew slowly compared with the DMSO-pretreated cells (Fig. 5A). The increase in the CPM/mm^2 value of the

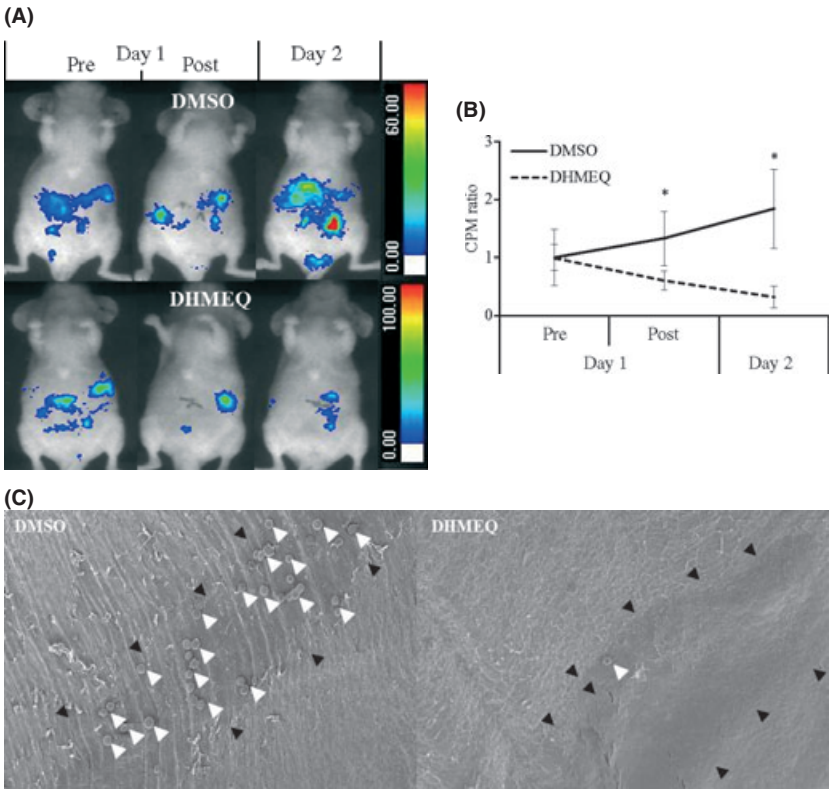

Fig. 4. Peritoneal lavage inhibited cancer cells pretreated with dehydroxymethylepoxyquinomicin (DHMEQ) from implanting into the abdominal wall. (A) *In vivo* imaging at around the time of peritoneal lavage. The luminescent value indicates the number of live cells in the abdominal wall. Pre/Post means before/after the peritoneal lavage. (B) Count per minute (CPM)/mm^2 value of pre/post peritoneal lavage. The graph shows the time course of the CPM/mm^2 value compared with the time of cancer cell injection. Initial values were adjusted to 1. *Significantly less than controls ($P < 0.05$). (C) SEM findings of the peritoneum. Left: abdominal wall injected with 44As3Luc cells pretreated with DHMEQ. Right: those with DMSO. The area indicated by black arrowheads is the area exposed to the peritoneal cavity. White arrowheads show the adhesive cancer cells.

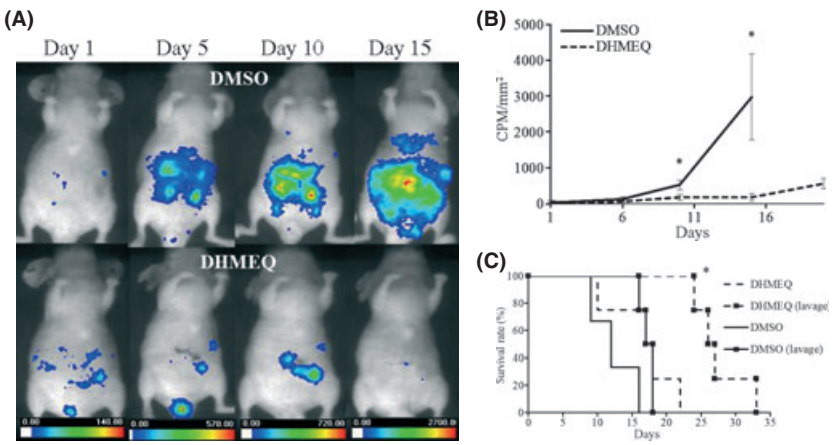

Fig. 5. Follow up after peritoneal lavage. (A) Follow-up imaging of mice subjected to peritoneal lavage. The range bars were adjusted for mice injected with DMSO pretreated cells at every evaluation day. (B) Time course of the count per minute (CPM)/mm^2 value. The black line represents the mice that were injected with DMSO-pretreated cells, and the broken line represents those injected with dehydroxymethylepoxyquinomicin (DHMEQ)-pretreated cells. *Significantly less than controls ($P < 0.05$). (C) Kaplan–Meier analysis of the survival of all groups. The line is as described above. The line with markers represents mice subjected to peritoneal lavage. *Significantly prolonged than all other groups ($P < 0.05$).

DHMEQ-treated cells was significantly delayed. The error bar of the CPM/mm^2 value of the DMSO-pretreated group ranged widely, because malignant ascites possibly obscured luminescent emission at the terminal stage (Fig. 5B). Survival was only significantly prolonged in mice injected with DHMEQ-treated cells and subjected to peritoneal lavage (Fig. 5C).

Discussion

NF-κB is undoubtedly involved in various biological properties of cancer cells.[35] However, its involvement in the expression of integrins, which are associated with cancer cell adhesion to the peritoneum, has not been reported. In the present study, we investigated whether NF-κB is involved in cell adhesion to the peritoneum via regulation of integrin expression, and whether DHMEQ, as a novel NF-κB inhibitor, suppresses the dissemination of gastric cancer in a mouse model.

Several investigators reported that NF-κB activity is associated with peritoneal dissemination of cancer cells.[36–38] Sasaki *et al.*[39] evaluated human gastric cancer tissues by immunohistochemical analysis, where NF-κB activation was significantly correlated with peritoneal metastases and survival. Our results in the present study support the previously reported data that NF-κB activity of gastric cancer cell lines was markedly activated and with highly metastatic behavior, and that DHMEQ sufficiently inhibited NF-κB activity and eventually suppressed the peritoneal dissemination.

Integrins are also associated with malignant potential.[40–42] Integrins play an important role in cancer cell adhesion to the peritoneum by enabling contact with appropriate ECM. Oosterling *et al.*[43] showed that anti-β1 integrin antibody reduces surgery-induced adhesion of colon carcinoma cells to traumatized peritoneal surfaces. Fishman *et al.*[44] showed similar findings using ovarian cancer cell lines in the *in vitro* analysis. With

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regard to gastric cancer, integrins α2, α3 and β1 are key molecules in animal models and humans.[11,12,45,46] The ligands of integrin α2β1 are collagens and laminin, and those of α3β1 are fibronectin, laminin,and collagens.[10] In our *in vitro* study, DHMEQ suppressed cancer cell adhesion to the peritoneum via p65-mediated suppression of integrin expression. Also, Takatsuki *et al.*[12] reported that anti-α3 antibody strongly suppressed the adhesion of gastric cancer cells to mice peritoneum. This integrin α3 was suppressed most by DHMEQ in this study. Therefore, DHMEQ may suppress cancer cell adhesion mainly via integrin α3, while DHMEQ may associate with other adhesion molecules that are not examined in this study.

In our *in vivo* study, viable cells in mice injected with DHMEQ-treated cells and subjected to peritoneal lavage still decreased on day 2 and only this group survived significantly longer. This finding might suggest that DHMEQ exerts another effect via the anti-adhesive effect. Jiang *et al.*[47] reported that NF-κB inhibition by IκBβ reduces anchorage-independent growth in a lung cancer cell line. Scaife *et al.*[48] showed that NF-κB inhibitor causes anoikis in a human colon cancer cell line. It might be possible that DHMEQ is associated with a pro-anoikis effect in gastric cancer dissemination.

In the present study, we first demonstrated that NF-κB could play a pivotal role in the progression of gastric cancer via the regulation of integrin expression and promotion of adhesion of cancer cells to the peritoneal wall. In our *in vivo* study, a specific deletion of NF-κB (p65) by siRNA was not performed, because we considered that transient deletion of p65 protein does not reflect the same result of DHMEQ-administered cells. Additionally, we could not clarify whether the DHMEQ effect on integrins is unique to the integrin pathway or concomitant with other phenomenon such as apoptosis. Further studies are required to clarify the involvement of integrins or other molecules in the anti-adhesive effect of DHMEQ against cancer cells. We believe that NF-κB inhibitors such as DHMEQ may be potential therapeutic options to prevent gastric cancer progression during peri-operative periods.

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Disclosure Statement

The authors have no conflict of interest.

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