Full Paper

Nerve growth factor (NGF) has an anti-tumor effects through perivascular innervation of neovessels in HT1080 fibrosarcoma and HepG2 hepatitis tumor in nude mice

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A R T I C L E   I N F O

A B S T R A C T

This study investigated whether NGF prevents tumor growth by promoting neuronal regulation of tumor blood flow. HT1080 fibrosarcoma cells or HepG2 hepatitis cells were subcutaneously implanted into nude mice. On Day 21 after the implantation of tumor cells, human NGF (40 or 80 ng/h for 14 days) was administered using a micro-osmotic pump. Growth rates of both tumors were significantly inhibited by the treatment of NGF, and the survival rate was also extended. Significant suppression of HT1080 tumor growth lasted after withdrawing NGF. NGF markedly increased the density of α-smooth muscle actin (α-SMA)-immunoreactive (ir) cells without changing neovessel density in HT1080 tumor tissues. Double immunostaining demonstrated protein gene product (PGP) 9.5-ir nerves around α-SMA-ir cells were found in HT1080 tumor tissue treated with NGF. The blood flow in HepG2 tumors treated with saline was significantly higher than in the non-tumor control area, but the tumor blood flow was markedly reduced by NGF treatment. In *in vitro* studies, NGF significantly accelerated migration of aortic smooth muscle cells but not endothelial cells, whereas NGF had no cytotoxic action on both cells. NGF inhibits tumor growth via indirect action, probably through innervation and maturation of tumor neovasculature, which regulates blood flow into tumor tissues.

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1. Introduction

The growth of tumor cells mainly depend on angiogenesis, or new blood vessel formation to produce blood flow into tumor tissues.[1–3] It is generally accepted that the local blood flow in normal tissues is regulated by the maintenance of vascular tone via perivascular nerves innervating terminal arterioles, precapillary arterioles, and the endothelium.[4–6] We have reported that the neovasculature in tumor tissues has no perivascular innervation.[7] Moreover, the perivascular innervation of the tumor has been shown to disappear progressively from the edge of the tumor when tumor growth advances.[8] The neovasculature in tumor tissues has been reported to consist of endothelium-like and immature vessels without a coating of smooth muscle cells.[7,8] Therefore, it is expected that lack of the neuronal regulation in tumor vasculatures may produce a sufficient blood supply into tumor tissues, thereby accelerating proliferation and growth.

Nerve growth factor (NGF) is essential for the development and innervation of peripheral nerves.[9] Our previous report demonstrated that NGF innervates perivascular nerves into the neovasculature of the mouse cornea, which were derived by basic fibroblast growth factor (bFGF),[10] as well as tumor cells, including DU145 prostate and HT1080 fibrosarcoma cells.[7] Furthermore, we

have reported that NGF facilitated the re-innervation of peri-vascular adrenergic nerves and nonadrenergic noncholinergic (NANC) nerves in rat small mesenteric arteries, which were injured by topical application of phenol onto the superior mesenteric artery.[11] Moreover, we demonstrated that NGF suppressed the growth of DU145 cells by increasing the number of vascular smooth muscle cells of the neovasculature in nude mice, suggesting that NGF facilitates the innervation of perivascular nerves and matures the neovasculature in tumor tissues.[12] NGF may have suppressive effects on tumor growth by facilitating innervation in the neo-vasculature in tumor tissues. However, we could not exhibit evidence for perivascular innervation of neovessels in tumor area after NGF therapy and anti-tumor effects of NGF on tumor types other than DU145 cells.

Therefore, the present study further investigated whether NGF has anti-tumor effects on the growth of HT1080 and HepG2 hep-atitis tumor cells. Additionally, the aim of the present study was to determine whether the effects of NGF are involved in the inner-vation of perivascular nerves to regulate blood flow into tumor tissues.

2. Materials and methods

2.1. Animals

Five-week-old BALB/c Slc nu/nu mice (purchased from Shimizu Experimental Animals, Shizuoka, Japan) were used in this study. This study was performed in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, Japanese Government Animal Protection and Management Law No. 115 and Japanese Government Notification on Feeding and Safekeeping of Animals No. 6. Every effort was made to minimize the number of animals used and their pain.

2.2. Implantation of tumor cells into nude mice

Implantation of human fibrosarcoma HT1080 cells and human hepatitis HepG2 cells obtained from American Type Culture Collection (ATCC, Rockville, MD, USA), was carried out according to a previous report.[12] All cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated FBS (SIGMA, Tokyo, Japan), 100 U/mL penicillin, and 100 μg/mL streptomycin. Each cell (1×10^6 cell/50 μL) mixed with 50 μL of Matrigel was injected into the flanks of nude mice (100 μL/site).

2.3. NGF administration

On Day 21 after the implantation of tumor cells, a micro-osmotic pump (DURECT Corp., Cupertino, CA, USA) containing human NGF (Toyobo Co., Osaka, Japan) or sterile saline was sub-cutaneously implanted in the dorsal area of mice. NGF (40 or 80 ng/h) was administered for 14 days. The osmotic pump was surgically removed under ether anesthesia after a 2-weeks administration of NGF.

2.4. Measurement of tumor volumes

Tumor growth was determined by measuring the size of tumors from Day 7 to Day 56 after the implantation of each tumor cell. Tumor volumes were calculated according to the formula (width$^2 \times$ length)/2.

2.5. Immunohistochemistry

The immunohistochemical study on the neovasculature of tu-mor tissues was carried out according to previous reports.[7,12] On Day 35 after the saline or NGF treatment for 2 weeks, nude mice were anesthetized with pentobarbital-Na (50 mg/kg, i.p.) and sacrificed by bleeding. Thereafter, Zamboni solution (2% para-formaldehyde and 15% picric acid in 0.15 M phosphate buffer) was systemically perfused via the heart, and tumor tissues were immersion-fixed with Zamboni solution for 48 h. Five areas at the tumor periphery that contained the maximum number of discrete microvessels were identified. The density of vessels in each field was expressed as vessels per field.

To examine the effects of NGF on smooth muscle cell prolifer-ation, dehydrated and paraffined tumor tissues were sliced in 7-μm thick sections, which were incubated in Cy3-labeled anti-α-SMA mouse IgG (SIGMA, Tokyo, Japan) at 1:100 dilution for 1 h at room temperature and observed under a confocal laser scanning micro-scope (CLSM510, Carl Zeiss, Tokyo, Japan). The densities of neo-vessels and vascular smooth muscle in tumor tissues were determined using the imaging analyzer Simple PCI (View Sonic, Complex Inc., Imaging Systems, Cranberry Township, USA).

To observe perivascular innervation with double immuno-staining, the tissues were treated with 30% KHOH solution at 60 °C for 7 min. After fixation, each tissue was rinsed with PBS, immersed in 1% SDS plus 0.5% Triton-X/PBS for 48 h, and then incubated with PBS containing normal goat serum (Gibco-BRL, Gaithersburg, MD, USA) (1: 100) for 60 min. The tissues were incubated with the primary antibody of rabbit polyclonal anti-PGP 9.5 serum (1: 100) (Neo markers, Fremont, CA, USA) at 4 °C for 72 h. After the incu-bation, the tissues were washed in PBS and incubated with the secondary antibody of FITC-labeled goat anti-rabbit IgG (1: 100) (ICN Pharmaceuticals, Inc., Aurora, OH, USA) for PGP 9.5 or mono-clonal anti-α-SMA Cy3-conjugated mouse IgG (1:400) (SIGMA, Tokyo, Japan) for vascular smooth muscle at 4 °C for 60 min. The samples were washed in PBS, mounted on slides, cover-slipped with glycerol/PBS (2: 1 v/v), and observed under a confocal laser-scanning microscope (CLSM510, Carl Zeiss GmbH, Jena, Germany). Double immunostaining of perivascular nerves (PGP 9.5-ir) and vascular smooth muscle (α-SMA-ir) was performed in two fluo-rescence views in the same microscopic field.

2.6. Measurement of tumor blood flow

On Day 35 after the saline or NGF treatment for 2 weeks, the nude mice were anesthetized with pentobarbital-Na (50 mg/kg, i.p.). The blood flow of the flank surface in the HepG2 tumor and normal (non-tumor) area of the nude mouse was measured with a blood flow meter probe (OMEGAFLO FLO-C1, Omegawave Inc., Tokyo, Japan). The blood flow for one minute was repeated 3 times and the average of 3 times was calculated with following the for-mula; relative blood flow (%) = the average blood flow of tumor area/the average blood flow of normal area. The blood flow of the same area in the nude mouse without tumor implantation was taken as the control.

2.7. Cell migration assay

2.7.1. Primary culture

Primary mouse smooth muscle cell and endothelial cell cultures were established according to a modification of a previous report.[13] The BALB/c Slc mouse descending aortas were de-endothelialized via passage of an applicator. Aortic tissue was minced and frag-ments were put in Dulbecco's Modified Eagle's Medium (DMEM, Life Technologies, Inc. Tokyo, Japan) with 0.1% collagenase type II

(Life Technologies, Tokyo, Japan), 0.1% BSA (SIGMA, Tokyo, Japan), and 0.2 mM L-ascorbic acid (SIGMA, Tokyo, Japan) for 12 h at room temperature. The dispersed cells were filtered through 70-μm monofilament nylon mesh. The filtered cell suspension was centrifuged at 1000 rpm at 4 °C for 5 min. The pellet was collected and washed using DMEM. Then, primary smooth muscle cells were cultured in 6 well culture plates (Life Technologies, Inc. Tokyo, Japan) with DMEM supplemented with 10% FBS, 1% (v/v) penicillin-streptomycin (SIGMA, Tokyo, Japan), and 0.2 mM L-ascorbic acid.

To obtain endothelial cells, the C57BL/6 Slc mouse descending aorta was aseptically harvested. Aortic tissue was minced and fragments were incubated (37 °C, 5% CO_2) on Matrigel-coated 6 well culture plates in Humedia-EB2 medium (Kurabo Co., Osaka, Japan) with 2% FBS, endothelial cell growth supplements (10 ng/mL epidermal growth factor, 3 ng/mL basic fibroblast growth factor, 1 μg/mL hydrocortisone, 10 μg/mL heparin salt), 50 μL/mL genta-mycin, and 50 ng/mL amphotericin B) for seven days. Then, primary endothelial cells were cultured on gelatin-coated 6 well culture plates with supplemented Humedia-EB2 medium. Smooth muscle cells and endothelial cells were grown to 80% or greater confluence and were passaged with trypsin before being used in experiments. Only cells between passages 2 and 6 were used.

2.7.2. Assays for cell migration

The migration of endothelial cells was assayed as described previously.[13,14] In brief, confluent cells in 48 well plates were scratched with a sterile pipette tip. The cells were washed twice with Humedia-EB2 medium to remove cellular debris. Then, 300 μL of Humedia-EB2 medium with 0.5% FBS and NGF (10 and 50 ng/mL or PDGF (10 ng/mL) was added to each well. The endothelial cells were then incubated at 37 °C in 5% CO_2 for 24 h. The cell migration assays were also performed for smooth muscle cells using the same method as described above. After 24-h culture, the cells were fixed with 2% PFA. The cells that had migrated into the denuded area were photographed with a CCD camera and counted with Image J software.

2.8. Measurement of tumor cell viability

HT1080 or HepG2 cells (100 μL; 1×10^4 cells) in the logarithmic growth phase were cultured on a 96-well plate for 24 h and incubated with NGF solution (5−500 ng/mL) or saline for an additional 24 h or 48 h. After adding 10 μL of the working solution from the WST-8 kit (Kishida Chemical., Ltd, Osaka, Japan.), the absorbance was measured at 450 nm with a reference wavelength at 650 nm with the microplate reader (BioRad, Hercules, CA, USA).

2.9. Statistic analysis

All data are expressed as the mean ± S.E.M. Statistical analysis was performed using unpaired Student's t-tests or ANOVA followed by Tukey's test or Dunnet's test. P-values <0.05 were considered significant.

3. Results

3.1. Time-course changes in HT1080 tumor volume during administration and after the discontinuation of NGF

Measurable HT1080 cells developed within 14 days after the implantation. As shown in Fig. 1A, tumor volumes in the saline-treated group rapidly increased, and all mice treated with saline were sacrificed on Day 42. On contrary, the tumor volumes in NGF-treated group gradually increased during the administration, and were significantly smaller than those in saline group (Fig. 1A). Furthermore, the tumor growth inhibition in NGF-treated group was maintained even after
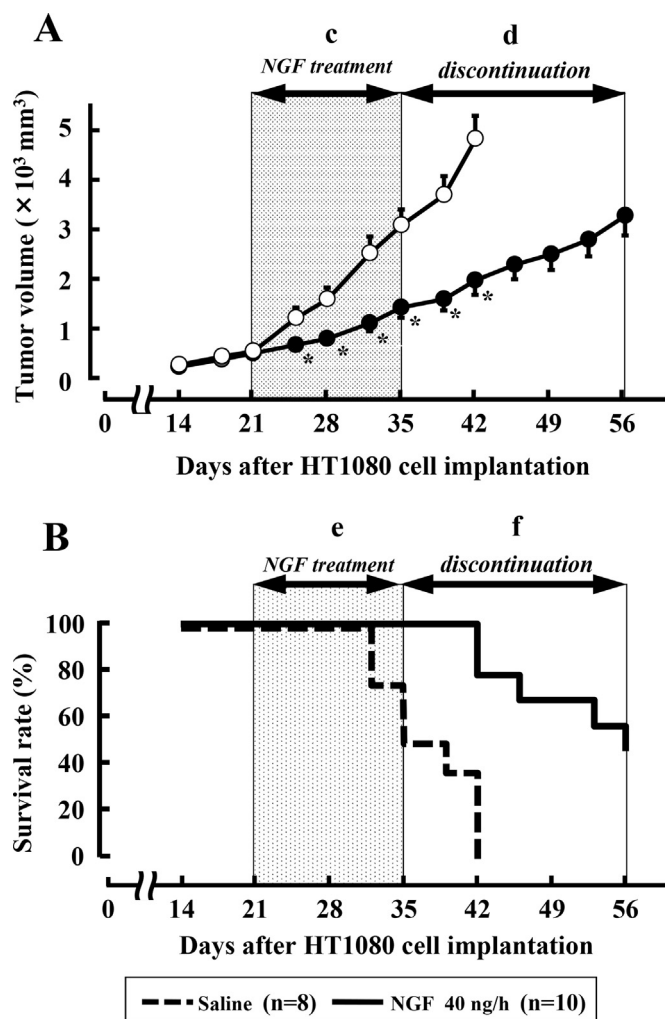

Fig. 1. Time-course changes in tumor volume (A) and survival rate (B) during administration (c, e) and discontinuation (d, f) of nerve growth factor (NGF; 40 ng/h) in nude mice implanted with HT1080 fibrosarcoma cancer cells. NGF or saline was administered from Day 21 to Day 35 with an osmotic pump. In (A), tumor volumes were calculated according to the formula (width^2 × length)/2. Each bar indicates the mean ± S.E.M. *$P < 0.05$ vs. Saline (Tukey's test). In (B), survival rate was expressed as the percentage of mice with adequate tumor control (<3000 mm³) in each group.

the discontinuation of NGF administration (Fig. 1d). The tumor volumes after NGF discontinuation were significantly smaller than those in saline-treated group until Day 42 (Fig. 1d).

The survival rate, which was expressed as the percentage of mice with adequate tumor volume less than 3000 mm³, rapidly decreased by 50% on Day 35 in the saline-treated group and reached zero on Day 42 (Fig. 1B). However, NGF administration (40 ng/h) for 2 weeks maintained a 100% survival rate on Day 35 (Fig. 1e). Furthermore, a survival rate higher than 50% lasted until Day 56 after NGF discontinuation (Fig. 1f).

3.2. Effects of NGF on density of neovessels and smooth muscle in HT1080 tumor tissues

Tumors removed from animals administered saline and NGF had a high density of vessels, but no difference, indicating the presence of extensive angiogenesis in the tumors (Fig. 2A, B, E). NGF treatment significantly demonstrated greater α-SMA-immunoreactive (ir) cells in the tumors than with saline treatment (Fig. 2C, D, F).
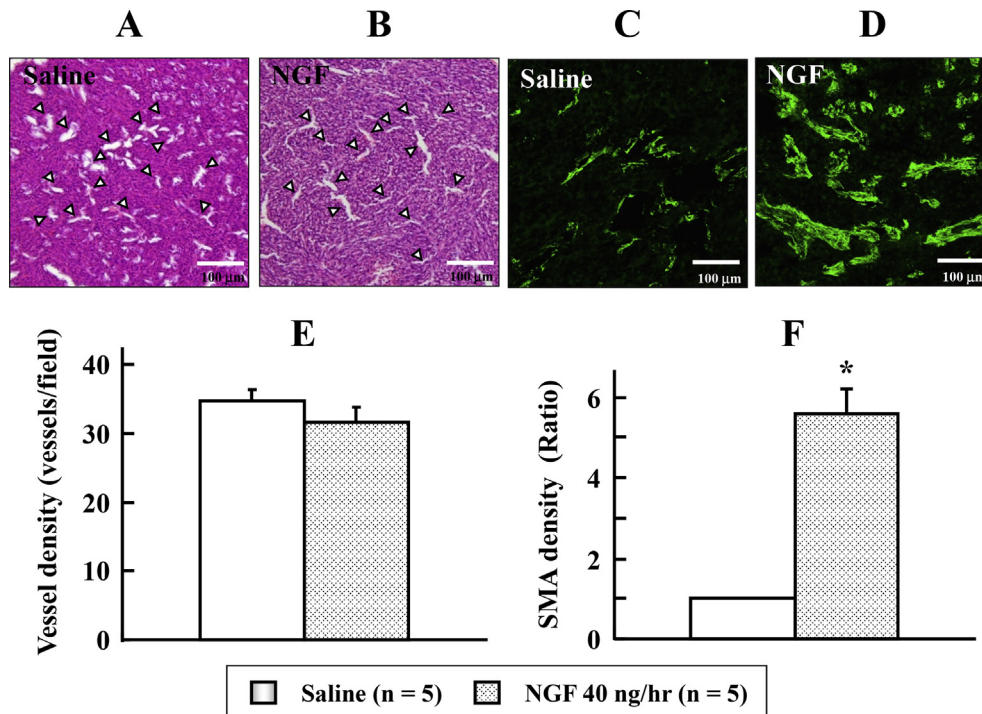
Fig. 2. Effects of nerve growth factor (NGF; 40 ng/h) on tumor angiogenesis in nude mice implanted with HT1080 cells. In the upper left panel, representative light micrographs show microvessels (open triangles) in tumors from animals that received saline (A) or NGF (B). A bar graph (E) shows quantitative analysis of microvessel density by counting the positively stained cells in five different fields (× 200). In the upper right panel, representative fluorescence micrographs show α-smooth muscle actin (α-SMA)-immunoreactive microvessels in tumors from animals that received saline (C) or NGF (D). A bar graph (F) shows quantitative analysis of vascular smooth muscle density by computer-assisted image processing in five different fields (× 200). Each bar indicates the mean ± S.E.M. *$P < 0.05$ vs. Saline (Student's t-test).
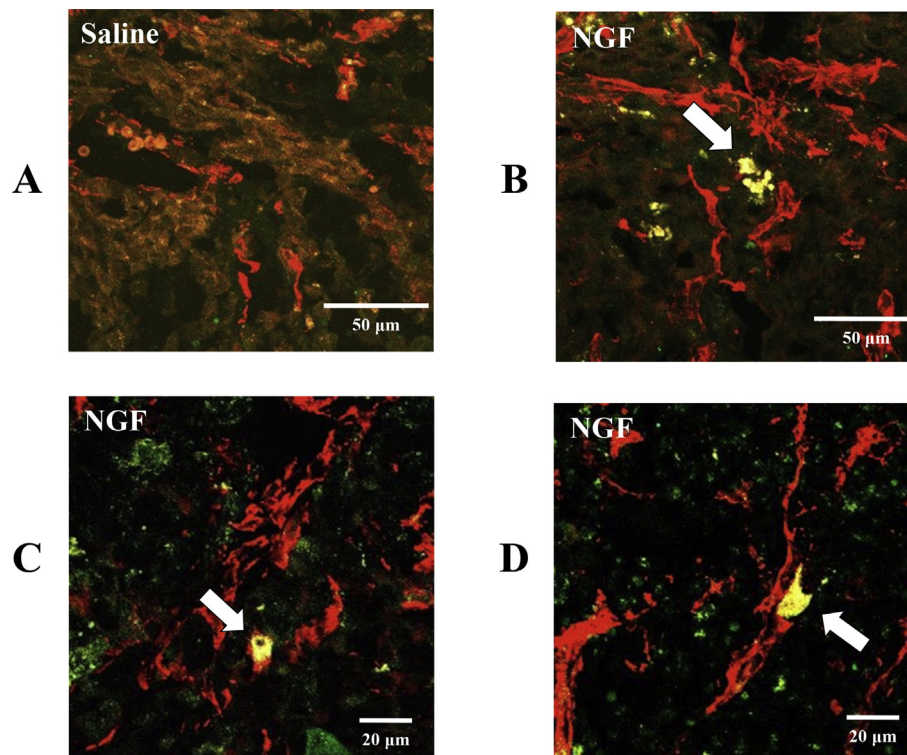
Fig. 3. Representative confocal laser micrographic images showing the distribution of protein gene product (PGP) 9.5-immunoreactive (ir) nerves (green) and α-smooth muscle actin (α-SMA)-ir neovessels (red) in the HT1080 tumor tissue of nude mice treated with saline (A) or NGF (40 ng/h) (B, C, D). White arrows indicate perivascular nerves in close contact with smooth muscles (yellow). Scale bars indicate 50 (A, B) and 20 (C, D) μm.

3.3. Distribution of PGP 9.5-ir nerves

Double immunostaining of α-SMA- and PGP 9.5-ir cells revealed that the HT1080 tumor tissues treated with saline had no PGP 9.5-ir cells (Fig. 3A). However, the HT1080 tumor tissues treated with NGF exhibited many PGP 9.5-ir cells, most of which were in close contact with α-SMA-ir neovessels (Fig. 3B, C, D).

3.4. Time-course changes in HepG2 tumor volume after NGF administration

Measurable HepG2 tumor cells developed within 14 days after the implantation. NGF administration at 40 and 80 ng/h for 14 days dose-dependently inhibited increases in tumor volumes (Fig. 4A). In particular, there was significant difference in tumor volume between the NGF (80 ng/h)- and saline-treated groups.

The survival rate, which was expressed as the percentage of mice with adequate tumor volume less than 800 mm^3, was decreased by approximately 40% on Day 35 in the saline-treated group (Fig. 4B). However, NGF administration dose-dependently maintained an approximately 70–100% survival rate on Day 35 (Fig. 4B).

3.5. Effects of NGF on blood flow in HepG2 tumor tissues

The blood flow in HepG2 tumor tissue was significantly higher than that in non-tumor tissue (Fig. 5). The NGF treatment canceled the increased blood flow in HepG2 tumor tissue, and significantly reduced it to the control level (Fig. 5).

3.6. Effects of NGF on migration of endothelial and smooth muscle cells

The control group showed no migration of endothelial and smooth muscle cells (Fig. 6A, B). PDGF, but NGF, had significant effect on the migration of endothelial cells (Fig. 6A). NGF (only 10 ng/mL) or PDGF significantly accelerated the migration of smooth muscle cells (Fig. 6B).

### 3.7. Effects of NGF on viability of HT1080 and HepG2 tumor cells

The treatment with NGF (5–500 ng/mL) for 48 h had no effect on the viability of HT1080 (Fig. 7A) or HepG2 cells (Fig. 7B).

4. Discussion

The present study demonstrated a first evidence that NGF significantly inhibits tumor growth of HT1080 fibrosarcoma cells and HepG2 liver cancer cells implanted into nude mice, which is mediated by increasing SMA density in tumor neovessels with newly distribution of perivascular nerves followed by a significant decreased blood flow in tumor tissues, resulting in prolonged the survival rate in vivo. The present findings are in good accordance with the previous study in which NGF inhibited growth of DU145 human prostate cancer cells implanted into nude mice.[12] Furthermore, the inhibitory effects of NGF on HT1080 tumor growth was maintained even after the discontinuation of NGF, suggesting that NGF causes the tumor growth inhibition by an indirect effect, probably changing environment around the tumor. This notion is supported by the present and previous findings that NGF had no direct effects on viability of HT1080, HepG2, and DU145 prostate cancer cells.[12] Our previous studies revealed that NGF has no or little effect on the development of angiogenesis derived from HT1080 or DU145 tumor cells implanted into the mouse cornea[7] or DU145 prostate cancer cells implanted into the nude mouse.[12] Furthermore, these findings were confirmed by the present study in which NGF
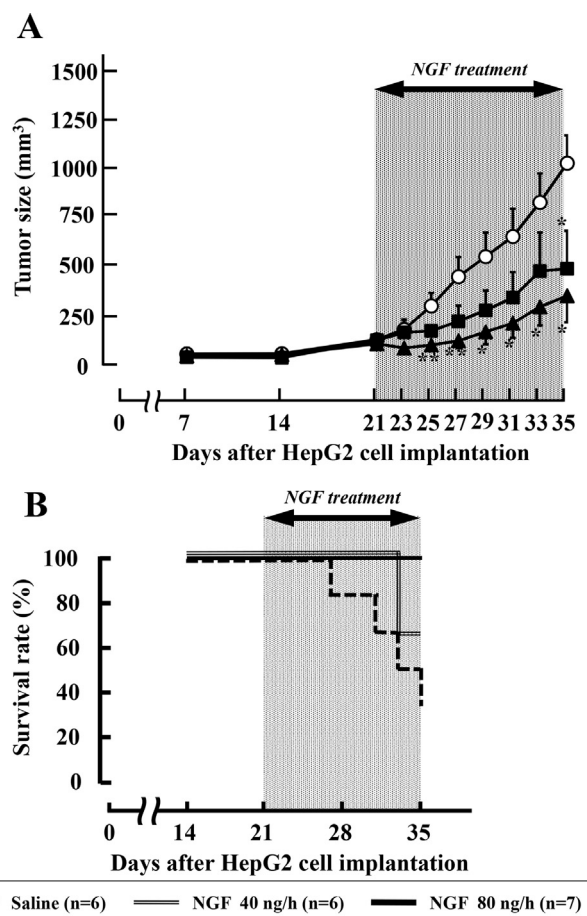
Fig. 4. Time-course changes in tumor volume (A) and survival rate (B) during administration of nerve growth factor (NGF; 40 and 80 ng/h) and saline in nude mice implanted with HepG2 hepatitis cancer cells. NGF or saline was administered from Day 21 to Day 35 with an osmotic pump. In (A), tumor volumes were calculated according to the formula (width^2 × length)/2. Each bar indicates the mean ± S.E.M. *$P < 0.05$, **$P < 0.01$ vs. Saline (Dunnet's test). In (B), survival rate was expressed as the percentage of mice with adequate tumor control (<800 mm^3) in each group.
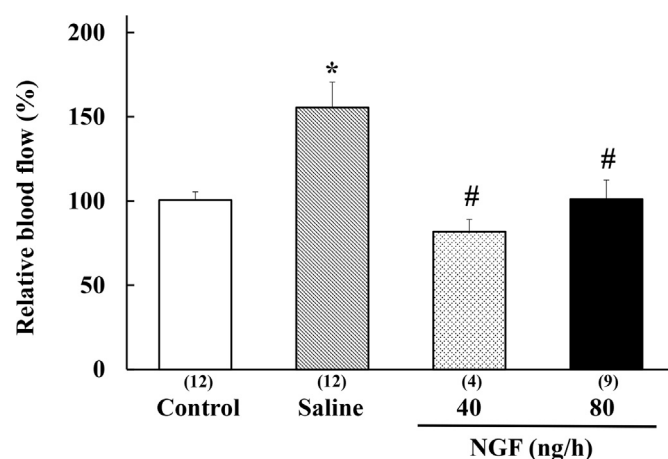
Fig. 5. Effects of nerve growth factor (NGF; 40 and 80 ng/h) on blood flow in tumor tissues of nude mice implanted with HepG2 hepatitis cancer cells. Relative blood flow was expressed as the average blood flow of tumor area/the average blood flow of normal area. *$P < 0.05$ vs. control (Dunnet's test). #$P < 0.05$ vs Saline (Dunnet's test). (n); number of mice.

did not accelerate neovessel formation in HT1080 tumors in nude mice *in vivo* and had no significant effects on migration of endothelial cells *in vitro*. Thus, it appears that NGF does not promote angiogenesis under pathological conditions such as tumors.

The neovessels in tumor tissues are mainly constituted from an endothelium without a cover of vascular smooth muscle cells and become frangible blood vessels.[15] Our previous study demonstrated that severe bleeding and/or blood leakage appeared in the HT1080 and DU145 tumor area of the mouse cornea, suggesting that DU145- and HT1080-derived neovessels are immature and fragile vessels with less vascular smooth muscle.[7]

On the other hand, in the present study, it should be noted that NGF-treated HT1080 tumor tissues had a marked increase in α-SMA immunoreactive (ir) cells. NGF has been reported to enhance migration of human lung fibroblasts as well as human vascular smooth muscle cells.[16,17] Furthermore, our previous studies demonstrated that NGF caused a significant increase in α-SMA-ir cells in the mouse cornea implanted with HT1080 or DU145 cells, and in the DU145 tumors in nude mice.[7,12] These effects of NGF were supported by the present study in which NGF significantly enhanced the migration of smooth muscle cells, similar with PDGF.

This effect of NGF on the migration appeared to be specific to smooth muscle cells because NGF had no effect on endothelium migration, whereas PDGF accelerated the migration of both cell types. Taken together, it is strongly suggested that NGF facilitates the migration of vascular smooth muscle cells to tumor neovessels, directly or indirectly, probably via perivascular nerves. Furthermore, it seems likely that NGF accelerates the maturation of tumor neovessels by smooth muscle cell migration, and leads to regulation of blood flow toward the tumor tissues, resulting in the significant suppression of tumor growth. As the peripheral nerves provide a template that determines the organotypic pattern of blood vessel branching and arterial differentiation in the skin,[18] it is highly possible that the migration of smooth muscles and maturation of neovessels resulted from perivascular innervation by NGF.

In the present double immunostaining study using the neuroaxonal marker PGP 9.5 and smooth muscle marker α-SMA, PGP9.5-ir nerves were not observed in the HT1080 tumor tissue treated with saline. This result is supported by the previous findings that blood vessels and neovessels within tumor tissues lacked a neuronal apparatus,[19] and PGP9.5-ir nerves in the HT1080 tumor area of the mouse cornea were sparse and distant from α-SMA-ir neovessels and were not in contact
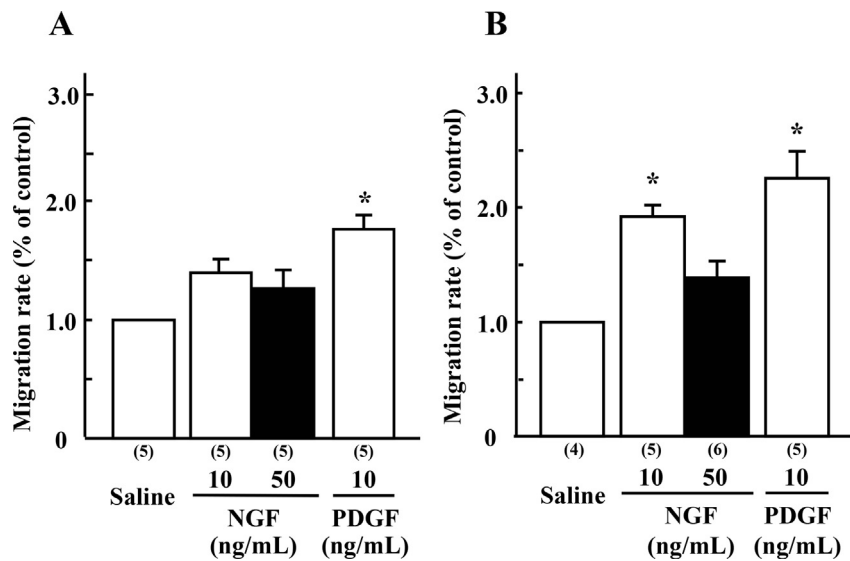
Fig. 6. Effects of nerve growth factor (NGF; 10 and 50 ng/mL) and platelet-derived growth factor (PDGF; 10 ng/mL) on the migration of primary endothelial (A) and smooth muscle cells (B) *in vitro*. Each bar indicates the mean ± S.E.M. *P < 0.05 vs. Saline (Dunnet's test). (n); number of samples.
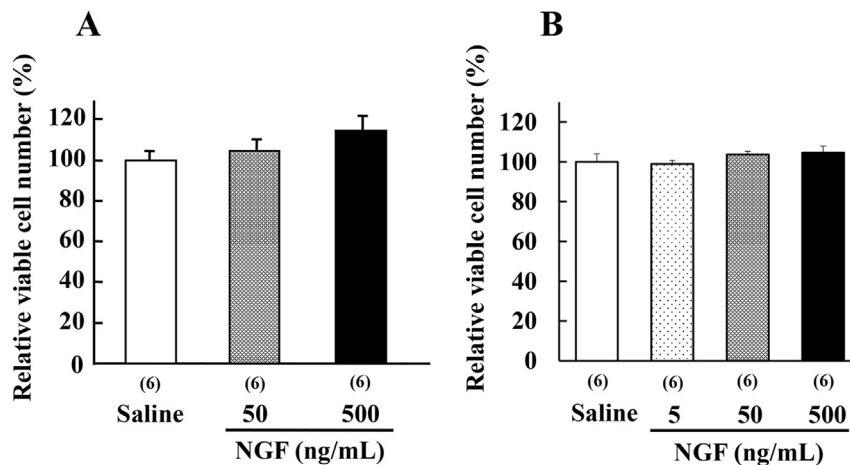
Fig. 7. Effects of nerve growth factor (NGF) on viability of HT1080 (A) and HepG2 (B) cancer cells. The viability was assayed by the WST-8 reduction method *in vitro*. Each bar indicates the mean ± S.E.M. (n); number of samples.

close with neovessels.[7] Therefore, it appears that these tumor neovessels had fewer, if any, perivascular nerves. The reason for the lack of a nervous system in tumor vessels may be that tumor tissues produce and release inhibitory substances, such as semaphorin 3A, which prevent the distribution of perivascular nerves.[20–22]

The present study also demonstrated that PGP9.5-ir nerves appeared in HT1080 tumor tissue after the treatment with NGF but not with saline, and they were in close contact with and accompanied α-SMA-ir neovessels, suggesting that NGF facilitates the innervation of perivascular nerves into neovessels in the tumor. Our previous studies have confirmed that perivascular nerves, which were reinnervated by the treatment with NGF following phenol-induced nerve injury in the rat mesenteric artery, have an active function to regulate vascular responses and blood flow.[11] Additionally, NGF pronouncedly increased a SMC recruitment, and also facilitated the innervation of perivascular nerves, resulting in decreasing blood flow toward the tumor neovessels via neural regulation, as shown our previous reports.[7] The peripheral nerves determine the blood vessel branching and arterial differentiation in the skin,[18] and enhanced blood vessel maturation inhibits tumor growth without suppressing angiogenesis. Moreover, no bleeding and/or blood leakage from DU145 or HT1080 tumor tissues in the mouse cornea appeared after treatment with NGF, contrary to with saline treatment.[7] Taken together, it is likely that NGF develops neovessels derived from HT1080 and HepG2 cells into mature and stable vessels. Therefore, it is strongly suggested that NGF facilitates the maturation of tumor neovessels by smooth muscle cell migration via the innervation of perivascular nerves, which may regulate the blood flow toward the tumor tissues, and result in the suppression of tumor growth. This notion is supported by the present findings that the increased blood flow in the HepG2 tumor was prominently cancelled to the control level by NGF treatment. Since the neovessels in tumor tissue were frangible and easy to cause bleeding, the hypoxia levels in tumor area have been reported to be relatively higher than normal area and thereby increase angiogenesis, which induces the increased blood flow in the tumor area.[23] Therefore, it is likely that the maturation of neovessels by NGF maintains and normalizes the blood flow to tumor tissue and reduces the hypoxia levels in tumor area, which inhibits angiogenesis and proliferation of tumor cells.

The present study employed human NGF, because of the treatment of human tumor cells implanted into the nude mouse, and the report that mouse NGF have no effects on the proliferation of TF1 cell with human receptor TrkA.[24] Paoletti and colleagues also reported that human NGF and mouse NGF have a high degree (ca. 90%) of sequence identity.[24] Although it is unknown whether human NGF could bind to mouse NGF receptor (TrkA) to promote perivascular innervation of neovessels, it has widely assumed that the two proteins are functionally interchangeable, that is, human NGF is likely to act on mouse TrkA. Additionally, we have reported that human NGF has function of innervation in mouse neovessels[10,25] and re-innervation of rat perivascular nerves injured by phenol.[11] These studies strongly indicate that human NGF could bind mouse and rat TrkA to promote perivascular innervation.

In conclusion, the present results suggest that NGF facilitates the innervation of perivascular nerves in order to mature neovessels and regulate blood flow toward the tumor tissues. NGF may suppress tumor growth by accelerating the maturation of neovasculatures and innervation of perivascular nerves in tumor tissues. Additionally, in the preliminary study, combination of NGF with an anti-vascular endothelial growth factor (VEGF) drug, bevacizumab, enhanced further inhibitory effect of NGF alone on HepG2 tumor growth. We expect that NGF, through perivascular innervation, stabilizes and matures the neovessels, which remained after anti-VEGF therapy. It is suggested that NGF and/or combined anti-cancer drug with NGF may have the potential to be a novel anticancer therapy.

Conflict of interest

The authors indicated no potential conflicts of interest.

Acknowledgments

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