**Featured Article**

**Depleted Dopamine in Gastric Cancer Tissues: Dopamine Treatment Retards Growth of Gastric Cancer by Inhibiting Angiogenesis**

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**ABSTRACT**

**Purpose:** It has been recently shown that the catecholamine neurotransmitter dopamine (DA) strongly and selectively inhibits vascular permeability factor/vascular endothelial growth factor (VPF/VEGF)-induced angiogenesis. Gastric cancer is highly angiogenic and is dependent on VEGF for its growth and progression. Because substantial amounts of DA present in normal stomach tissues has been implicated in several gastric functions, we therefore investigated the role, if any, of this neurotransmitter in the growth and progression of gastric cancer.

**Experimental Design:** Initially, the status of DA and tyrosine hydroxylase, the rate-limiting enzyme required for DA synthesis, were determined in human gastric cancer tissues and in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer tissues of rats. On the basis of our observation of inverse correlation between stomach DA and gastric cancer growth, we determined the effect of pharmacological dose of DA on the angiogenesis and growth of MNNG-induced gastric cancer in rats and Hs746T human gastric cancer in nude mice.

**Results:** DA and tyrosine hydroxylase were absent in both human and rat gastric cancer tissues. On the contrary, a low nontoxic pharmacological dose of DA significantly retarded tumor angiogenesis by inhibiting VEGFR-2 phosphorylation in tumor endothelial cells, which expressed DA D2 receptors. This action of DA was associated with the growth inhibition of both MNNG-induced rat malignant gastric tumors and xenotransplanted human gastric cancer in nude mice.

**Conclusions:** Our study demonstrates that there is an inverse correlation between endogenous stomach DA and gastric cancer and indicates that DA already in clinical use for other purposes might have a role as an antiangiogenic agent in the treatment of gastric cancer.

**INTRODUCTION**

Besides being a predominant neurotransmitter in the brain, the physiological significance of dopamine (DA) in the periphery has now been emphasized (1). In addition to its role in renal and cardiovascular systems (1), DA also has a wide spectrum of physiological actions in the stomach (2–5). These actions of DA are mediated through DA receptors present in the stomach (1, 3, 4).

Tumor angiogenesis is essential for the growth and progression of solid tumors, including gastric cancer and vascular endothelial growth factor (VEGF) is thought to be the single most important angiogenic cytokine in malignancy (6–10). As substantial amounts of dopamine is produced by the stomach (3–5, 11, 12) and we (S. Basu and P. Dasgupta) have recently shown that VEGF-mediated angiogenesis is inhibited by DA (13), we therefore reasoned that endogenous dopamine present in stomach tissue might have a role on the growth and progression of gastric cancer in vivo. Accordingly, we investigated the status of DA in human gastric cancer tissues. As N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats is an established in vivo model for human gastric adenocarcinoma (14), we also investigated the status of DA concentration in the gastric cancer tissues of these animals. We report here for the first time that unlike normal stomach tissues, there is depletion of DA in cancer tissues of the stomach in both humans and rats. Furthermore, treatment with a low nontoxic dose of DA inhibits the growth of MNNG-induced gastric cancer in rats and human gastric cancer xenografted in nude mice.

**MATERIALS AND METHODS**

**Materials**

MNNG, domperidone, ethylene glycol tetra acetic acid, and reduced glutathione were obtained from Sigma (St. Louis, MO). DA was from TTK Healthcare (Chennai, India). OCT compound was from Miles Diagnostics (Elkhart, IN). Collagenase and DNase were from Roche Diagnostics Corporation (Indianapolis, IN). VEGFR-2, CD16/CD32, CD31 and CD34 monoclonal antibodies for flow cytometry analysis were from BD Biosciences PharMingen (San Diego, CA). CD31 monoclonal antibody for immunohistochemistry was from BD Biosciences PharMingen. DA D2 receptor, VEGFR-2, and tyrosine hydroxylase antibodies for immunoblot were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Phosphotyrosine antibody was from Upstate Biotechnology (Lake Placid, NY), and...
the ABC kit was from Vector Lab (Burlingame, CA). Mouse 
VEGF quantikine ELISA kit and tumor TACS in situ apoptosis 
detection kit (TA 5411) were from R&D Systems (Minneapolis, 
MN). 5-Bromo-2’-deoxyuridine (BrdUrd) labeling and detection 
kit was from Roche Diagnostics Corporation. Hybond-enhanced 
chemiluminescence nitrocellulose membrane and enhanced 
chemiluminescence were from Amersham (Piscataway, NJ). 
DMEM and fetal bovine serum were purchased from the American 
Type Culture Collection (Manassas, VA).

Patients

Gastric cancer patients of both sexes with a median age of 
45 years were selected. These patients did not receive any prior 
treatment, including medicines, and they were also free from 
disorders that could alter the status of DA in the stomach. 
Malignant gastric tumor tissue specimens were taken during 
surgery from different cancer patients. Similarly, benign control 
tissues were collected from the biopsy specimens of patients 
suffering from benign adenomatous polyps of the stomach, and 
normal tissues were collected from the histologically normal 
portions of the biopsy specimens not invaded by the tumors. 
Tissues were frozen at −80°C until assayed for DA concentra-
tion. The protocol to obtain tissue samples for this study was 
approved by the Institutional Review Board, and all samples 
were confirmed histologically.

Animals and Cell Line

Animals. Eight-week-old male Sprague Dawley rats were 
supplied from the institutional animal facility, and 4–6-
week-old male nude mice were purchased from National Centre 
for Laboratory Animal Science (Hyderabad, India). All proced-
ure in animals were approved by the Institutional Animal Care 
and Use Committee.

Cell Line. Human gastric carcinoma cell line Hs746T 
was purchased from the American Type Culture Collection and 
was maintained in culture as recommended by American Type 
Culture Collection. Briefly, the cells were cultured in DMEM 
with 4 mM L-glutamine adjusted to contain 1.5 g/liter sodium 
bicarbonate, 4.5 g/liter glucose, and 10% fetal bovine serum. 
The cultures were maintained at 37°C in an atmosphere of 5% 
CO₂/95% air. Cells were grown to ~80% confluence before 
removal for xenografting. These cells were recently reported to 
secrete VEGF (15).

Rats and Tumor Induction

Eight-week-old male Sprague Dawley rats were divided 
into two groups: control and MNNG treatment groups. The 
MNNG treatment group was given MNNG dissolved in drink-
ing water at a concentration of 80 mg/liter for the first 28 weeks 
(16). After 28 weeks, MNNG feeding was stopped, and these 
animals were given drinking water without MNNG for another 
24 weeks (total of 52 weeks). The control group was given 
drinking water without MNNG for 52 weeks. At the 53rd 
week of the experiment, all rats were sacrificed. As these tumors are 
flat, we therefore measured the tumor diameters by microcali-
pers to determine their growth, and this is the standard pro-
dure for determining the growth of these tumors (16, 17). The 
stomach was then removed, and then the stomach specimens 
were examined grossly and their histology was evaluated.

Mice and Tumor Transplantation

To obtain tumor donor animals, three male nude mice received s.c. injections of a 0.2-ml cell suspension containing 10⁵ 
cells. The well developed tumors were dissected and minced, and 2-mm³ pieces of tumor tissue were transplanted s.c. 
to both flank areas of the experimental animals (18). When tumors became measurable, experimental groups were formed 
with about equal average tumor sizes, and the control and treatment groups were selected at random. Tumor growth was 
monitored weekly, and perpendicular tumor diameters were 
measured using Vernier scale calipers, and the tumor volume 
was calculated using the formula [(x² × y)/2] for an ellipsoid 
(19).

DA Treatment

Rats. At the beginning of the 50th week, rats with 
MNNG-induced gastric cancer verified to have tumor diameters 
of 1–2 mm in laparotomy were selected for the study. Then 
these tumor-bearing rats were randomly divided into three 
groups. The first group was injected i.p. with DA at a nonno-
toxic dose of 50 mg/kg, corresponding to ~5% of the 
median lethal dose (LD₅₀) in rodents (20), beginning 50th week 
after MNNG feeding, and was continued for 10 days. This i.p. 
dose of DA raises the level of DA in rats and mice to 2.1 ± 0.04 
and 1.2 ± 0.01 nmol/ml, respectively, in plasma 1 min after DA 
administration (the normal plasma level of DA in rats and mice were 
2.4 ± 0.01 and 1.5 ± 0.01 pmol/ml, respectively) (17). 
A specific peripheral D₂ DA receptor antagonist, domperidone, was in-
jected i.p. (10 mg/kg) to the second group of rats before DA 
treatment. The third group received the vehicle only. After 
completion of DA treatment, rats from all of the three groups 
were sacrificed.

Mice. The treatment of the mice bearing Hs746T tumors 
was started 30 days after transplantation, when the mean tumor 
volume was 100 mm³. These tumor-bearing mice were ran-
domly divided into three groups. First group was injected with 
DA i.p at a dose of 50 mg/kg/day for 10 continuous days. A 
specific peripheral D₂ DA receptor antagonist, domperidone 
was injected i.p. (10 mg/kg) to the second group of tumor-bearing 
mice before DA treatment. The third group received 0.9% NaCl, 
i.p., for 10 consecutive days. After the completion of the treat-
mant, all of the mice were sacrificed, tumors were excised and 
processed for further experiment.

Assay of DA by High-Performance Liquid 
Chromatography

Samples were processed and assayed for DA by reverse-
phase high-performance liquid chromatography with electro-
chemical detection. In short, tissue was weighed and then ho-
logenized in 10.2 ml of ice-chilled 0.6 mM perchloric acid 
containing 1.7 mg/ml ethylene glycol tetra acetic acid and 1.1 
mg/ml reduced glutathione. After centrifugation for 15 min at 
2500 × g at 0°C, 1.0 ml of the supernatant was adjusted to pH 
8.6 with 6 mM potassium hydroxide and then processed for de-

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5 S. Basu and P. S. Dasgupta, unpublished data.
termination of DA concentration after alumina-batch extraction. Twenty-five μl of 3 mM potassium chloride were added to 200 μl of the 0.2 mM perchloric acid eluate. After centrifugation, 50 μl of the supernatant were injected into the high-performance liquid chromatography system. The limit of sensitivity in the supernatant, defined as two times baseline noise, was 0.01 ng/ml for DA. If a concentration was lower than the limit of sensitivity, the concentration of the DA was assigned the value 0 (21).

Flow Cytometry Analysis
A suspension of Hs746T-malignant human gastric tumors was made by passage of viable tissue through sieve and treatment with collagenase and DNase. The cells were washed, and the RBCs were lysed with PharM Lyse (BD Pharmingen). The cell pellets were then resuspended in fluorescence-activated cell sorting buffer (1× PBS plus 1% BSA), preblocked with a Fc block (CD16/CD32), and then incubated with primary antibody on ice: phycocerythrin-conjugated anti-VEGF receptor-2 (anti-VEGFR-2), 1:100; CD31, 1:100; and CD34, 1:100. One million positive tumor endothelial cells were collected by fluorescence-activated cell sorting (22, 23).

Immunoprecipitation and Western Blot Analysis
Human and rat tissues were homogenized in 0.2% Triton in PBS (pH 7.5), assayed for protein content, and stored at −80°C. Either 20 μg of protein from human or rat stomach tissues or 2 μg of protein from rat adrenals was size fractionated by SDS-PAGE and then electroblotted onto Hybond-enhanced chemiluminescence nitrocellulose membrane at 30 V for 18 h in transfer buffer (20% methanol, 25 mM Tris, and 192 mM glycine). Blots were preblocked in 5% nonfat dry milk in Tris-buffered PBS and incubated with anti-tyrosine hydroxylase antibodies (1:500) for 1 h at 22°C. Blots were then incubated with horseradish peroxidase-conjugated mouse IgG (1:1000) for 1 h at 22°C and developed using enhanced chemiluminescence (5). Immunoprecipitation and Western blot analysis using antibodies against DA D2 receptors (1:100) were also performed on Hs746T human gastric cancer cells and tumor endothelial cells isolated from Hs746T tumors. A similar experiment was performed on the same tumor endothelial cells using antibodies against VEGFR-2 (1:100) and phosphotyrosine (1:100).

Plasma and Tumor Tissue VEGF Assays
The blood was collected from the rats in EDTA containing tubes on ice by cardiac puncture. The blood samples were centrifuged at 800 rpm for 30 min at 4°C, followed by 10 min at 3000 rpm. The plasma was collected and stored at −80°C until analysis. Plasma and tissue VEGF were then determined by ELISA following the protocol provided by the manufacturer (R&D Systems) that has been shown to recognize both rodent and murine VEGF (24, 25).

Immunohistochemistry
Tissue samples were fixed in 4% paraformaldehyde, rinsed in PBS, transferred to 30% sucrose in PBS at 4°C, and frozen in OCT compound. Immunohistochemistry was then performed on frozen tissue sections using antibody against CD31 (1:50). Microvessel density was quantitated by analyzing 10 random fields/section (26).

Detection of Apoptotic Tumor Cells
Tumor cell apoptosis was assessed by measuring DNA fragmentation in a standard terminal deoxynucleotidyl transferase-mediated nick end labeling assay according to the instruction of the manufacturer (R&D Systems). The number of apoptotic cells was expressed as an apoptotic index (8).

Cell Proliferation Index
The cellular kinetics of the tumor specimens was determined by immunohistochemical staining of BrdUrd. One h before sacrificing the animals, 20 mg/kg body weight of BrdUrd were injected i.p. into each animal. Subsequently, the animals were sacrificed, and tumors were excised and fixed in buffered formalin and embedded in paraffin. Cross-sections of the tissues were cut 4-μm thick. Tissue sections were then immunostained by using BrdUrd labeling and detection kit. The percentage of stained cells was counted and expressed as labeling index (16).

Statistics
Mean and SE were calculated. Differences among groups were evaluated by ANOVA and the unpaired Student’s test or Dunn’s multiple comparison tests.

RESULTS
MNNG-Induced Intestinal Type of Gastric Adenocarcinoma
MNNG-treated rats developed a single (Fig. 1A) intestinal type of adenocarcinoma of the stomach similar to human gastric cancers (Fig. 1B), and our results are in conformity with other studies (14, 27).

Absence of DA and Tyrosine Hydroxylase in Gastric Carcinoma
In normal stomach tissues of humans and rats substantial amounts of DA (Table 1) and tyrosine hydroxylase (Fig. 2, A and B), the rate-limiting enzyme required for DA synthesis (5) was observed. On the contrary, there was total absence of endogenous DA (Table 1) and tyrosine hydroxylase (Fig. 2, A and B) in malignant tumor tissues of the stomach collected from humans and rats.

Significant Secretion of VEGF and Striking Angiogenesis in Malignant Gastric Tissues
MNNG induced gastric cancer tissues showed striking angiogenic response as evident from CD31 expression (Fig. 3A). In contrast, we found CD31 expression in normal rat gastric tissues to be very low or insignificant (20–30/10 randomly chosen high power fields), and our results are in conformity with a recent study that indicated similar results (28). Furthermore, these malignant tumor tissues also showed a significantly higher concentration of VEGF in comparison to normal controls (Table 2; 6.7 ± 0.8 versus 0.7 ± 0.02 ng/g tissue, mean ± SE, P < 0.05). The plasma VEGF levels were also significantly higher in the malignant tumor-bearing rats when compared with normal controls (Table 2; 25.7 ± 1.6 versus 8.8 ± 1.0 pg/ml, mean ±
These results corroborate the striking angiogenesis observed in these tumors and, to our knowledge, is the first article that indicates that MNNG-induced malignant gastric tumors in rats also secrete VEGF.

DA Treatment Decreased Tumor Diameter and Angiogenesis and Increased Tumor Cell Apoptosis

**Rats.** DA when administered to gastric adenocarcinoma-bearing rats showed 3-fold decrease ($P < 0.5$) in tumor diameter than control animals (Table 3). This was also associated with significant inhibition of tumor cell proliferation as evident from reduction of BrdUrd incorporation in malignant stomach tissues after DA treatment in both rats and mice (Table 4). These DA-treated animals with decreased tumor diameter also showed significant inhibition of tumor angiogenic response than untreated controls as evidenced from decreased frequency of CD31$^+$ cells in DA-treated tumor tissues (Fig. 3, A–D). Decreased angiogenic response in tumor tissues after DA treatment was also associated with increased apoptosis of tumor cells when compared with controls (Fig. 4, A–D). The increase in apoptosis of tumor cells after DA treatment are in conformity with other studies that indicate that inhibition of angiogenesis results in apoptosis of tumor cells (8, 26). In contrast, DA did not inhibit tumor growth (Table 3) in rats that received prior treatment of domperidone (10 mg/kg i.p.), a specific peripheral D$_2$ DA receptor antagonist, thereby confirming that the action of DA is through D$_2$ DA receptor. The other two catecholamines, epinephrine and norepinephrine, did not inhibit either angiogenesis or tumor growth (data not shown).

**Mice.** Similarly DA when administered to Hs746T-bearing mice also showed 3-fold decrease ($P < 0.5$) in tumor volume than control animals (Table 5). This was also associated with significant inhibition of tumor cell proliferation as evident from reduction of BrdUrd incorporation in malignant stomach tissues after DA treatment in both rats and mice (Table 4). These DA-treated animals with decreased tumor diameter also showed significant inhibition of tumor angiogenic response than untreated controls as evidenced from decreased frequency of CD31$^+$ cells in DA-treated tumor tissues (Fig. 3, A–D). Decreased angiogenic response in tumor tissues after DA treatment was also associated with increased apoptosis of tumor cells when compared with controls (Fig. 4, A–D). The increase in apoptosis of tumor cells after DA treatment are in conformity with other studies that indicate that inhibition of angiogenesis results in apoptosis of tumor cells (8, 26). In contrast, DA did not inhibit tumor growth (Table 3) in rats that received prior treatment of domperidone (10 mg/kg i.p.), a specific peripheral D$_2$ DA receptor antagonist, thereby confirming that the action of DA is through D$_2$ DA receptor. The other two catecholamines, epinephrine and norepinephrine, did not inhibit either angiogenesis or tumor growth (data not shown).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Normal/Benign tissue (ng/g tissue)</th>
<th>Tumor tissue (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>22</td>
<td>40.0 ± 3.4</td>
</tr>
<tr>
<td>Adenomatous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyp</td>
<td>6</td>
<td>41.7 ± 5.9</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>22</td>
<td>Not detected</td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>24</td>
<td>21.4 ± 1.7</td>
</tr>
<tr>
<td>MNNG-induced adenocarcinoma-bearing rats</td>
<td>18</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

*MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; DA, dopamine.*

* $P < 0.05$ when compared with adenocarcinoma tissues. Tissue DA was measured by high-pressure liquid chromatography with electrochemical detection.

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**Fig. 1** N-methyl-N'-nitro-N-nitrosoguanidine induced gastric adenocarcinoma in a rat. Gross (A) and H&E staining (B) show intestinal type of adenocarcinoma of the stomach. *B, scale bar: 75 μm.*

**Fig. 2** Western blotting of tyrosine hydroxylase in normal and malignant tumor tissue of stomach in humans (A) and in rats (B). Tyrosine hydroxylase is present in normal stomach but absent from tumor tissue in both cases. The figures are representatives of six separate experiments with similar results.
tissues after DA treatment in mice (Table 4). Decreased angiogenic response in tumor tissues after DA treatment was also associated with increased apoptosis of tumor cells when compared with controls (Fig. 5). The increase in apoptosis of tumor cells after DA treatment are in conformity with other studies that indicate that inhibition of angiogenesis results in apoptosis of tumor cells (8, 19). These DA-treated animals with decreased tumor volume also showed significant inhibition of tumor angiogenesis (Table 5) in mice that received prior treatment of domperidone (10 mg/kg i.p.), a specific peripheral D2 DA receptor antagonist, and 0.02 (mm) and continued for consecutive 10 days at a dose of 50 mg/kg/day i.p. Domperidone (10 mg/kg/day) was administered before DA treatment everyday. Tumor diameter was measured 24 h after the completion of treatment.

Table 3 Effect of dopamine administration on the growth of MNNNG induced gastric adenocarcinoma in rats

<table>
<thead>
<tr>
<th></th>
<th>No. of animals</th>
<th>Tumor diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated</td>
<td>18</td>
<td>4.4 ± 0.02</td>
</tr>
<tr>
<td>DA treated</td>
<td>18</td>
<td>1.3 ± 0.01b</td>
</tr>
<tr>
<td>Domperidone + DA</td>
<td>18</td>
<td>4.1 ± 0.04</td>
</tr>
</tbody>
</table>

*a* MNNNG, N'-methyl-N'-nitro-N'-nitrosoaniline; DA, dopamine. 
b *p < 0.05* when compared with vehicle-treated control. DA treatment started when the tumor diameter was 1.2 ± 0.01 (mm) and continued for consecutive 10 days at a dose of 50 mg/kg/day i.p. Domperidone (10 mg/kg/day) was administered before DA treatment everyday. Tumor diameter was measured 24 h after the completion of treatment.

Table 4 BrdUrd incorporation in MNNNG induced rat and Hs746T human gastric tumor tissues

<table>
<thead>
<tr>
<th></th>
<th>No. of animals</th>
<th>Cell proliferation index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>24</td>
<td>9.12 ± 0.8</td>
</tr>
<tr>
<td>Vehicle-treated tumor-bearing rats</td>
<td>24</td>
<td>23.41 ± 1.1b</td>
</tr>
<tr>
<td>DA-treated tumor-bearing rats</td>
<td>24</td>
<td>12.33 ± 0.7c</td>
</tr>
<tr>
<td>Nude mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle-treated Hs746T-bearing mice</td>
<td>12</td>
<td>19 ± 3.7</td>
</tr>
<tr>
<td>Dopamine treated Hs746T-bearing mice</td>
<td>12</td>
<td>10 ± 2.1a</td>
</tr>
</tbody>
</table>

*a* BrdUrd, 5-bromo-2'-deoxyuridine; MNNNG, N'-methyl-N'-nitro-N'-nitrosoaniline; DA, dopamine. 
b *p < 0.05* when compared with normal control. 
c *p < 0.05* when compared with vehicle-treated tumor-bearing rats. Cell proliferation index was determined by counting the percentage of BrdUrd-positive cells.

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Table 2 VEGF concentrations in plasma and stomach tissue of normal and MNNNG-induced gastric adenocarcinoma-bearing rats

<table>
<thead>
<tr>
<th></th>
<th>VEGF concentration in plasma (ng/ml)</th>
<th>VEGF concentration in tissue (ng/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>22</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>Vehicle-treated tumor-bearing rats</td>
<td>18</td>
<td>25.7 ± 1.6b</td>
</tr>
<tr>
<td>Dopamine treated tumor-bearing rats</td>
<td>18</td>
<td>23.2 ± 1.2b</td>
</tr>
</tbody>
</table>

*a* VEGF, vascular endothelial growth factor; MNNNG, N'-methyl-N'-nitro-N'-nitrosoaniline. 
b *p < 0.05* when compared with normal control.

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Fig. 3 Evaluation of microvessel density by immunohistochemical analysis of CD31+ in N'-methyl-N'-nitro-N'-nitrosoaniline-induced rat malignant gastric tumor (A–D) and in Hs746T gastric cancer-bearing nude mice (E–H). Significant expression of CD31+ cells in vehicle-treated tumor tissue (A, E, D, and H). Dopamine (DA)-treated tumor tissue showed marked reduction in CD31+ cells (B, F, D, and H). Negative control consisted of sections in which primary antibody was replaced with nonimmune serum of the same IgG subclass (C and G). Microvessel density was measured by counting the number of microvessels in 10 randomly chosen high power microscopic fields within the sections showed that microvessel density was significantly reduced (*, *p < 0.05*) in DA-administered tumor-bearing animals (B and F) when compared with vehicle-treated tumor-bearing animals (A and E). A, B, C, E, F, G, scale bars: 50 μm.
D₂ DA receptors in tumor endothelial cells isolated from these tumor-bearing mice (Fig. 6A). The D₂ DA receptors were absent in Hs746T gastric cancer cells (Fig. 6A). Furthermore, VEGF is thought to induce angiogenesis by phosphorylating VEGFR-2, and our recent article had indicated that DA inhibits VEGFR-2 phosphorylation in vitro (13). In the present investigation, we found that DA treatment strongly inhibited VEGF-induced phosphorylation of VEGFR-2 in tumor endothelial cells isolated from Hs746T-malignant gastric tumors (Fig. 6B) and pretreatment with domperidone, a specific peripheral D₂ DA receptor antagonist, significantly abrogated this effect (Fig. 6B), indicating the specificity of DA action. The other two catecholamines, epinephrine and norepinephrine, did not inhibit either angiogenesis or tumor growth (data not shown).

### DISCUSSION

Endogenous DA produced in substantial amounts in normal stomach tissues regulates several physiological functions of the stomach, including reduction of gastric acid secretion, stimulation of bicarbonate, and mucus secretion (2–4). Here, we show for the first time that DA is depleted in both human gastric cancer and MNNG-induced adenocarcinoma of the stomach in rats. Furthermore, this finding is also significant because our results indicate that there is a significant retardation in the growth of gastric cancer after treatment with exogenous DA at a low nontoxic dose.

VEGF is thought to be the single most important cytokine essential for tumor angiogenesis (6–10), and we had recently demonstrated that DA specifically and significantly inhibits

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**Table 5** Effect of DA administration on the growth of Hs746T human gastric tumor-bearing nude mice

<table>
<thead>
<tr>
<th></th>
<th>No. of animals</th>
<th>Tumor volume (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated</td>
<td>12</td>
<td>311.5 ± 11.9</td>
</tr>
<tr>
<td>DA treated</td>
<td>12</td>
<td>106.0 ± 7.4</td>
</tr>
<tr>
<td>Domperidone + DA</td>
<td>12</td>
<td>302.7 ± 12.8</td>
</tr>
</tbody>
</table>

* DA, dopamine.

* P < 0.05 when compared with the vehicle-treated control group.

DA treatment started when tumor volume was 100 mm³ and continued for 10 consecutive days at a dose of 50 mg/kg/day i.p. Domperidone (10 mg/kg/day i.p.) was administered before DA treatment everyday. Tumor diameter was measured 24 h after the completion of treatment.

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**Fig. 4** Effect of dopamine (DA) on tumor cell apoptosis in N-methyl-N'-nitro-N-nitosoguanidine-induced gastric cancer in rats as determined by terminal deoxynucleotidyl transferase-mediated nick end labeling assay. A, negative control (without terminal deoxynucleotidyl transferase enzyme). Vehicle treatment (B) and DA treatment (C) show increased apoptosis of tumor cells. Apoptotic index was determined by the ratio of terminal deoxynucleotidyl transferase-mediated nick end labeling-positive cells and total number of cells (*, P < 0.05). A–C, scale bars: 50 μm.
VEGF-mediated angiogenesis (13). DA acted through $D_2$ DA receptors to induce endocytosis of VEGFR-2, which is critical for promoting angiogenesis, thereby preventing VEGF binding, receptor phosphorylation, and subsequent signaling steps (13). Malignant human gastric tumors secrete VEGF and therefore are highly angiogenic tumors (8). In the present experiment, MNNG-induced malignant gastric tumor-bearing rats showed significant elevation of plasma VEGF, and this correlated well with their angiogenic nature. There is also a recent article that indicates the human gastric cancer cell line Hs746T selected for our experiment also secretes considerable amount of VEGF (15). Therefore, one of the important mechanisms by which DA may retard gastric cancer growth is through inhibition of VEGF-mediated angiogenesis. Our present results confirm that DA retards the growth of gastric cancer by inhibiting VEGF-induced angiogenesis. The underlying mechanism of this action has been shown to be mediated by inhibition of VEGFR-2 phosphorylation in tumor endothelial cells involving DA $D_2$ receptors present on these cells as treatment with domperidone, a specific peripheral DA $D_2$ receptor antagonist, showed significant abrogation of this inhibitory effect of DA. However, there may be other mechanisms also by which DA may inhibit tumor growth (29). It would also be noteworthy to mention here that there are few contradictory studies that have shown that administration of a DA agonist stimulated MNNG-induced carcinogenesis and a DA antagonist inhibited MNNG-induced carcinogenesis (30–32). However, in those articles, the authors had only shown the effects of DA agonist and antagonist on the process of MNNG-induced carcinogenesis (incidence of malignant gastric tumors), and nothing was mentioned regarding the effects of those agonist and antagonist on the growth and progression of a developed gastric cancer. In addition, there is also an article that indicates that DA inhibits the process of MNNG-induced glandular stomach carcinogenesis in rats (33). However, that study was also limited to the incidence of gastric cancer in the rats, and no effect of DA on the growth and progression of malignant gastric tumor was mentioned (33). Therefore, in brief, the present results are of both physiological and clinical significance because it has been shown for the first time that there is an inverse correlation between endogenous stomach DA and gastric cancer. These results also indicate a direct gastric cancer growth inhibitory action of nontoxic dose of DA in vivo.

Finally, gastric cancer is one of the leading causes of cancer death worldwide (34). Radiation therapy or chemotherapy does not affect the length and quality of life of patients with advanced gastric cancer (8). Thus, novel therapies are needed to target the progression of these tumors. DA is already in clinical use with an established safety record (35). Therefore, DA can be tested without the usual delays that would be required to assess the safety of a new drug in patients with gastric cancer in which VEGF-mediated angiogenesis has an important pathogenic role (8). In addition, information concerning the potent antitumor angiogenic activity of DA in gastric cancer needs to be disseminated widely and rapidly so that clinical trials can be started as soon as possible to combat this very common cancer, which, as of yet, has no significant treatment.

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