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Vascular Endothelial Growth Factor-D Is a Key Molecule That Enhances Lymphatic Metastasis of Soft Tissue Sarcomas

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Abstract

Studies on lymph node metastasis of soft tissue sarcomas are insufficient because of its rarity. In this study, we examined the expressions of vascular endothelial growth factor (VEGF)-C and VEGF-D in soft tissue sarcomas metastasized to lymph nodes. In addition, the effects of the two molecules on the barrier function of a lymphatic endothelial cell monolayer against sarcoma cells were analyzed.

We examined 7 patients who had soft tissue sarcomas with lymph node metastases and who had undergone neither chemotherapy nor radiotherapy before lymphadenectomy. Immunohistochemistry revealed that 2 of 7 sarcomas that metastasized to lymph nodes expressed VEGF-C both in primary and metastatic lesions. On the other hand, VEGF-D expression was detected in 4 of 7 primary and 7 of 7 metastatic lesions, respectively. Interestingly, 3 cases that showed no VEGF-D expression at primary sites expressed VEGF-D in metastatic lesions.

Recombinant VEGF-C at 10^{-8} and VEGF-D at 10^{-7} and 10^{-8} g/ml significantly increased the random motility of lymphatic endothelial cells compared with controls. VEGF-D significantly increased the migration of sarcoma cells through lymphatic endothelial monolayers.

The fact that VEGF-D induced the migration of fibrosarcomas through the lymphatic endothelial monolayer is the probable reason for the strong relationship between VEGF-D expression and lymph node metastasis in soft tissue sarcomas. The important propensities of this molecule for the increase of lymph node metastases are not only lymphangiogenesis but also down-regulation of the barrier function of lymphatic endothelial monolayers, which facilitates sarcoma cells entering the lymphatic circulation.

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Conflicts of interest
The authors have no conflicts of interest and no financial conflicts.

Keywords

lymphatic endothelial cells; lymph node; metastasis; motility; VEGF-C; VEGF-D

Introduction

Vascular and lymphatic systems are crucial for the metastasis of cancer cells. In particular, angiogenesis is well known to be important for tumor formation, progression and metastasis [1]. Soft-tissue sarcomas mainly metastasize via the blood stream to the lung [2]. Clinical trials using bevacizumb, which is a humanized anti-vascular endothelial growth factor (VEGF) antibody that suppresses angiogenesis, have already started for the treatment of soft tissue sarcomas [3, 4].

On the other hand, lymphatic metastasis is a relatively rare event in soft-tissue sarcomas [5], although several sarcomas exhibiting histologic patterns that resemble epithelial or endothelial cancers often metastasize via lymphatic vessels [6–8]. Due to its rarity, studies on lymph node metastasis of soft tissue sarcomas are insufficient, although the prognosis of soft tissue sarcomas with lymph node metastases is quite severe, with 5-year survival rates reported to be less than 30% [7, 9].

During the last decade, there has been an increase of reports on the relationship between lymphangiogenesis promoted by tumor cells and lymphatic metastasis [10–12]. VEGF-C and VEGF-D are known to regulate lymphangiogenesis [13, 14] via their receptors, VEGF receptor (VEGFR)-2 and VEGFR-3 [15–17]. As expected from their propensity to induce lymphangiogenesis, the expressions of VEGF-C and VEGF-D correlated with lymph node metastasis in various kinds of tumors [18–20]. In some tumors, however, VEGF-D expression inversely correlated with lymph node metastasis [10, 18], meaning that the roles of VEGF-D in lymph node metastasis currently remain controversial.

As for lymphangiogenesis-related molecules in soft tissue sarcomas metastasizing to lymph nodes, Lahat et al. reported that there was no correlation between the expression of VEGF-C and peritumoral lymphatic vessel density, which has been considered to correlate with lymphatic spread of the tumors in various human malignant neoplasms [1–3, 15–17, 21]. They did not, however, analyze VEGF-D expression and the molecules expressed in metastatic lymph node lesions.

In this study, we examined the expressions of VEGF-C and VEGF-D in soft tissue sarcomas metastasized to lymph nodes. In addition, the effects of the two molecules on the monolayer of lymphatic endothelial cells *in vitro* were analyzed.

Materials and Methods

Patients

Patients with a musculoskeletal malignant tumor treated in our hospital between 2000 and 2010 were analyzed. Among them, seven patients with soft tissue sarcomas had undergone surgery for both primary lesions and lymph node metastases. Histopathological findings confirmed that partial lymph node structures, such as marginal sinus, remained in 5 of 7 cases although most of the lymph nodes were invaded by tumors. In the rest of the cases, lymph node structures were completely replaced by tumor cells. None of the seven patients had undergone chemotherapy or radiotherapy before lymphadenectomy. Specimens of primary tumors and lymph node metastases were taken from the 3 male and 4 female patients (mean age 65.3 years). The sites of the primary tumors were 2 thighs, 1 lower leg, 1

knee, 1 buttock, 1 elbow, and 1 back. As a control, specimens from 6 patients (1 male and 5 females) with tumors metastasizing to non-lymph node sites, which were diagnosed radiologically, were examined (mean age 66.3) and the primary tumors originated from 5 thighs and 1 buttock.

Cell line and reagents

Human lymphatic endothelial cells (HLEC) were obtained from ScienCell Research Laboratories (Carlsbad, CA). Cells were cultured in endothelial cell medium (ScienCell Research Laboratories) with 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Human fibrosarcoma cell line HT-1080 was obtained from the American Type Culture Collection (ATCC) and human osteosarcoma cell line HuO9 was kindly provided by Dr. Hotta (Niigata University, Niigata, Japan). These two tumor cell lines were cultured in DMEM with 10% fetal bovine serum.

Goat polyclonal antibody to VEGF-C (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit polyclonal antibody to VEGF-D (Santa Cruz Biotechnology) were used for immunohistochemistry and immunoblot to stain VEGF-C and VEGF-D, respectively. Mouse monoclonal antibody to VEGFR-2 (R&D Systems, Minneapolis, MN) and VEGFR-3 (R&D Systems) were used for immunofluorescence.

Recombinant proteins of humanVEGF-A_{121}, B, C and D, platelet-derived growth factor (PDGF)-AA, fibroblast growth factor (FGF)-basic, and angiopoietin (ANG)-1 were obtained from PeproTech Inc. (Rocky Hill, NJ) and rabbit phosphoglucose isomerase (PGI) from Sigma (St Louis, MO).

Immunohistochemistry

The avidin-biotin-peroxidase complex (ABC) method was performed using the Vectastatin ABC Kit (Vector Laboratories, Burlingame, CA). Briefly, from a formalin-fixed, paraffin embedded-tumor tissue block, serial section slides of 4 μm were prepared, dried at 60°C for at least 60 min and incubated in a 0.3% hydrogen peroxide-methanol solution for 30 min to remove endogenous peroxidase. Next, the stained sections were incubated with pepsin for 10 min at room temperature and with the primary antibodies at a dilution of 1:50 overnight at 4°C. Subsequently, specimens were incubated with the corresponding biotinylated secondary antibodies. Freshly prepared ABC reagent was applied and incubated for 60 min. Finally, specimens were incubated with 0.02% 3,3-diaminobenzidine tetrahydrochloride solution containing 0.03% hydrogen peroxidase. Between each step, the sections were washed in PBS for 5 min, three times and then counterstained in Mayer's hematoxylin. Sections were scored as positive if ≥10% of the neoplastic cells stained positively.

Immunoblotting

HT-1080 and HuO9 cells expanded on 10-cm dishes were lysed in radioimmunoprecipitation assay buffer (20 mmol/L Tris, 150 mmol/L NaCl, 1% Triton X-100, 1% NP40, 10 mmol/L EDTA, and 25 mmol/L sodium deoxycholate) and centrifuged at 15,000 × *g* for 5 min. Supernatants were subjected to SDS-PAGE and transferred to a PVDF membrane, which was probed with anti-VEGF-C or anti-VEGF-D polyclonal antibody, and immunoreactive protein was detected.

Immunofluorescence

Cells were seeded on coverslips coated with bovine albumin, fixed with 4% formaldehyde and permeabilized with ice-cold methanol for 5 min at −20°C. The cells were blocked with 3.0% bovine serum albumin (BSA)/PBS for 30 min, and then labeled with primary antibodies, followed by incubation with Alexa Fluor 488 secondary antibody in the dark. To

detect nuclei, the cells were co-stained with 4′,6′-diamidino-2-phenylindole. Fluorescent images were analyzed using a KEYENCE BZ-8100 fluorescence microscope (KEYENCE, Osaka, Japan).

Phagokinetic Track and Transwell Motility Assay

For the random motility assay, uniform carpets of gold colloids were prepared on glass coverslips coated with bovine serum albumin as previously described. The coverslips were placed in 35-mm tissue culture dishes, and 2000 HLEC were plated onto coverslips coated with gold colloids. After 24-h incubation with 10^{-11} to 10^{-7} g/ml cytokines, the area of migrated cells was calculated. The phagokinetic tracks of at least 30 individual cells were measured.

The migration assay for HLEC was performed using Transwell supports with 8.0-μm pore polycarbonate membrane inserts (Corning Life Sciences, Acton, MA). A 100 μl sample of medium with 1% fetal bovine serum (FBS) including cell suspension (1×10^5 cells/ml) was added to the upper compartment, and 600 μl medium with FBS and 10^{-10} to 10^{-7} g/ml recombinant proteins was added to the lower compartment. After 24-h incubation at 37 °C, the membranes were fixed with 70% ethanol for 1 h, stained with 0.4% trypan blue, and washed with distilled water. Cells that migrated through the membrane were counted after the cells on the upper surface of the membrane had been swiped with cotton swabs.

Transendothelial Migration Assay

The transendothelial migration assay for sarcoma cells was performed using Transwell seeded HLEC. First, 5×10^4 HLEC in culture medium were added to each insert in a 24-well plate and cultured for 48h until cells formed a monolayer. Next, 100 μl medium with 1% fetal bovine serum (FBS), including sarcoma cells (1×10^5 cells/ml) treated with CellTracker Green Fluorescent Probe (Lonza, Walkersville, MD) according to the instructions, was added to the upper compartment, and 600 μl medium with FBS and 10^{-8} and 10^{-7} g/ml recombinant proteins was added to the lower compartment. After 24-h incubation, the membranes were fixed with 70% ethanol for 1 h. Cells that migrated through the endothelial monolayer and the membrane were counted under a KEYENCE BZ-8100 fluorescence microscope.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL). Phagokinetic track motility, Transwell motility and transendothelial migration assays were analyzed using analysis of variance (ANOVA) and the significance of individual differences was evaluated using the Dunnett C or Tukey test if ANOVA was significant.

Results

Pathological diagnoses

Pathological diagnoses of the tumors metastasized to lymph nodes were 3 undifferentiated pleomorphic sarcomas, 1 clear cell sarcoma, 1 myxoid liposarcoma, 1 myxoid-type extraskeletal myxoid chondrosarcoma and 1 epithelial sarcoma (Table 1) and those metastasized to non-lymph node sites (4 to lungs, 1 to spine and 1 to subcutaneous) were 2 undifferentiated pleomorphic sarcomas, 1 myxofibrosarcoma 1 myxoid liposarcoma and 1 extraskeletal myxoid chondrosarcoma (Table 2).

Expression of VEGF-C and VEGF-D

Immunohistochemistry revealed that 2 of 7 soft tissue sarcomas that metastasized to lymph nodes expressed VEGF-C both in primary and metastatic lesions (Fig. 1a, c). On the other hand, VEGF-D expression was detected in 4 of 7 primary and 7 of 7 metastatic lesions of soft tissue sarcomas that metastasized to lymph nodes, respectively (Table 2). VEGF-D was located in the cytoplasm of tumor cells and hardly at all in lymphocytes (Fig. 1b, d, f). Interestingly, three cases that did not express VEGF-D at primary sites showed VEGF-D expression in metastatic lesions (Fig. 1e, f). In addition, four cases in which primary lesions were VEGF-D-positive more strongly expressed this protein at metastatic than primary sites (Fig. 1b, d). In control cases, which were soft tissue sarcomas metastasized to non-lymph node sites, only 1 of 6 primary tumors expressed both VEGF-C and -D.

In immunoblotting, anti-VEGF-C antibody recognized two bands in the lysate of HT1080 cells, but not of HuO9, which is compatible with a report by Joukov et al. that the main form of both endogenous VEGF-C produced by HT1080 cells was a doublet of 29/31 kDa [22], Meanwhile, neither cell line expressed VEGF-D (Fig. 2a). Immunofluorescence results showed that both HT1080 cells (Fig. 2b, c) and HuO9 cells (Fig. 2d, e) expressed VEGFR-3 (Fig. 2b, d) and slight VEGFR-2 (Fig. 2c, e).

Effect of angiogenic and lymphangiogenic molecules on the motility of HLEC

To examine whether VEGF-C and -D can stimulate the motility of HLEC, we utilized two independent motility assays: 1) phagokinetic track assay for measuring overall random motility (Fig. 3a) and 2) migration assay utilizing Transwell for measuring directed motility capabilities (Fig. 3b). Recombinant VEGF-C at 10^{-8} and VEGF-D at 10^{-7} and 10^{-8} g/ml significantly increased the random motility of HLEC compared with controls in the phagokinetic track motility assay (analysis of variance; $F_{15, 784} = 8.415$, $p < 0.05$). In the migration assay using Transwell, neither VEGF-C nor -D in the lower chamber significantly stimulated the directed motility of HLEC (analysis of variance; $F_{9, 90} = 1.446$, $p = 0.30$). These motility assays indicated that VEGF-C and -D stimulated random cell motility (chemokinesis) of HLEC rather than directed motility (chemotaxis). In these experiments, we used PGI because this molecule is known to be identical to autocrine motility factor (AMF), which stimulates the motility of tumor cells and human umbilical vein endothelial cells [23]. Against our expectations, PGI/AMF did not stimulate the random or directed motility of HLEC. In addition, VEGF-A and B, FGF-basic, PDGF-AA, and ANG-1 did not increase random motility (analysis of variance; $F_{15, 144} = 0.469$, $p = 0.953$) (Fig. 3c).

Effect of VEGF-C and VEGF-D on the monolayer of HLEC

Based on the results of the motility assays of HLEC, we hypothesized that VEGF-C and -D affect the stability of the endothelium of lymph vessels by up-regulating the random motility of endothelial cells, and facilitate tumor cell migration into lymphatic systems. To verify the effect of VEGF-C and -D on the endothelium of lymph vessels for sarcoma cells, the numbers of sarcoma cells that migrated through an endothelial monolayer in medium including VEGFs were analyzed. VEGF-C at 10^{-8} and VEGF-D at 10^{-7} and 10^{-8} g/ml significantly increased the transendothelial migration of HT1080 cells (analysis of variance; $F_{4, 20} = 18.438$, $p < 0.05$) (Fig. 4a–d). Meanwhile, VEGF-D at 10^{-7} g/ml, but not VEGF-C, significantly increased the transendothelial migration of HuO9 cells (analysis of variance; $F_{4, 20} = 9.041$, $p < 0.01$) (Fig. 4e). A migration assay for sarcoma cells using Transwell without the endothelial monolayer was also performed, because there was a possibility that VEGF-C and -D stimulated not only the random motility of HLEC but also the directed motility of HT1080 and HuO9 cells, in which the expression of VEGFR3 was confirmed. Neither VEGF-C nor -D, however, increased the number of HT1080 cells (Fig. 4f) and HuO9 cells (Fig. 4g) migrated through the pores of the Transwell® membrane compared

with the control (analysis of variance in HT1080 cells: $F_{4, 20} = 0.771$, $p = 0.557$, analysis of variance in HuO9 cells: $F_{4, 20} = 0.500$, $p = 0.736$).

Discussion

Immunohistochemistry in the current study revealed that all seven metastatic lesions of soft tissue sarcomas that metastasized to lymph nodes showed VEGF-D expression. It is noteworthy that three VEGF-D-negative cases at primary sites showed VEGF-D expression in metastatic lesions and the rest of the cases presented stronger VEGF-D expression in the metastatic lymph node lesion than in the primary lesion. This suggests that the metastatic lymph node lesions were probably composed of populations that acquired the propensity to strongly express VEGF-D in primary lesions and moved to lymph nodes. In contrast to sarcomas metastasizing to lymph nodes, soft tissue sarcomas that metastasized to non-lymph node sites showed VEGF-D expression in only one of seven cases. VEGF-C, the expression of which was positive in only two of seven cases, did not seem to be an essential factor for lymph node metastasis of soft tissue sarcomas. Lahat et al. have also described that no difference in VEGF-C expression was observed between metastatic lymph node-negative and -positive soft tissue sarcomas [21].

It is well known that VEGF-C and -D up-regulate the growth of lymphatic endothelial cells via VEGFR-2 and -3 and enhance lymphatic metastasis [13, 14]; however, tumor cells need to accomplish several steps for lymphatic metastasis, not only lymphangiogenesis but also other factors, such as invasion to the lymphatic system, survival in the circulation, and extravasation [24]. Vascular permeability is one of the important factors that make the vascular endothelium susceptible to tumor invasion into vascular systems [25, 26]. Several molecules involved in cell motility, such as PGI/AMF and RhoA, were reported to increase the permeability of vascular endothelium [23, 27], and therefore we investigated the effect of angiogenic and lymphangiogenic factors and PGI/AMF on the motility of lymphatic endothelial cells. PGI/AMF is a molecule secreted from tumor cells, including fibrosarcoma and osteosarcoma cell lines, and stimulates the motility of tumor cells in an autocrine or paracrine fashion [28, 29]. We expected that this molecule also affects lymphatic endothelial cell motility and affects the stability of endothelium; however, the current study revealed that only VEGF-C and -D, but not PGI/AMF, up-regulated the random motility of lymphatic endothelial cells. In addition, there was a report that aortic endothelial cells transfected with VEGFR-2 showed enhanced migration compared to control cells [30], which prompted us to perform a Transwell directed motility assay of lymphatic endothelial cells using VEGFs. None of VEGF-C, -D and PGI/AMF, however, affected directed motility, suggesting that these molecules were not chemoattractants for lymphatic endothelial cells.

After confirming that VEGF-C and -D, not PGI/AMF, are key factors affecting the random motility of lymphatic endothelial cells, we performed a transendothelial migration assay using VEGFs. The numbers of HT-1080 and HuO9 cells that migrated through the monolayer of lymphatic endothelial cells were increased by both VEGF-C and -D and by only VEGF-D, respectively. In particular, VEGF-D increased the number of sarcoma cells migrating through lymphatic endothelial monolayers with a lower concentration than VEGF-C. This result means that VEGF-D facilitates sarcoma cell entry into lymphatic systems by affecting the endothelium. He et al. also described that blocking the signals of VEGFR-3, a receptor of VEGF-C and -D, can inhibit the entry of lung cancer cells into lymphatic vessels [31]. Meanwhile, other authors have reported that VEGF-C but not VEGF-D induced vascular permeability [16, 32, 33]. This discrepancy can probably be explained by the difference in characteristics between vascular and lymphatic endothelial cells. Interestingly, VEGF-C and -D did not stimulate the migration of HT1080 and HuO9

cells through the membranes without HLEC, although the two cell lines expressed VEGFR-3. The signal pathways of VEGF-D-VEGFR3 are probably dependent on cell types.

We speculated that the fact VEGF-D induced the migration of HT1080 fibrosarcomas through the lymphatic endothelial monolayer at a lower concentration than VEGF-C can explain the strong relationship between VEGF-D expression and lymph node metastasis in soft tissue sarcomas. Soft tissue sarcomas rarely metastasize to lymph nodes compared with carcinomas [5]. Resolving why soft tissue sarcomas hardly metastasize to lymph nodes and to identify molecules similar to VEGF-D that induce soft tissue sarcomas to metastasize to lymph nodes will contribute to the regulation of lymph node metastasis of carcinomas.

There are some limitations of this study. First, the number of cases is relatively small, which prevented us from performing exact histological matching between the lymph node and non-lymph node metastasis groups, due to the rarity of lymph node metastasis in soft tissue sarcomas [5]. In addition, to avoid any modification of the specimens we omitted patients who had received radiation or chemotherapy in this study. However, patients with lymph node metastasis usually undergo radiation therapy or systemic chemotherapy before lymphadenectomy [34], which makes it difficult to collect unmodified specimens of lymph nodes. Greater accumulation of cases or a multi-institutional study may be needed, although our institute treats 40–50 patients with sarcoma every year. Second, no human sarcoma cell lines steadily metastasize to lymph nodes. It would be interesting to establish human sarcoma cell lines that metastasize to lymph nodes, for example, by transfection with genes including *vegfs*.

Conclusions

We showed that VEGF-D expression was strongly related to lymph node metastasis of soft tissue sarcomas. The important propensity of this molecule to increase lymph node metastases is not only lymphangiogenesis but also down-regulation of the barrier function of lymphatic endothelial monolayers, which facilitates sarcoma cell entry into the lymphatic circulation. Investigating VEGF-D will help to elucidate the mechanisms of lymph node metastasis.

Abbreviations

VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
HLEC	human lymphatic endothelial cells
PDGF	platelet-derived growth factor
FGF	fibroblast growth factor-basic
ANG-1	angiopoietin-1
PGI	phosphoglucose isomerase
ABC	avidin-biotin-peroxidase complex
FBS	fetal bovine serum
ANOVA	analysis of variance
MFH	malignant fibrous histiocytoma
AMF	autocrine motility factor

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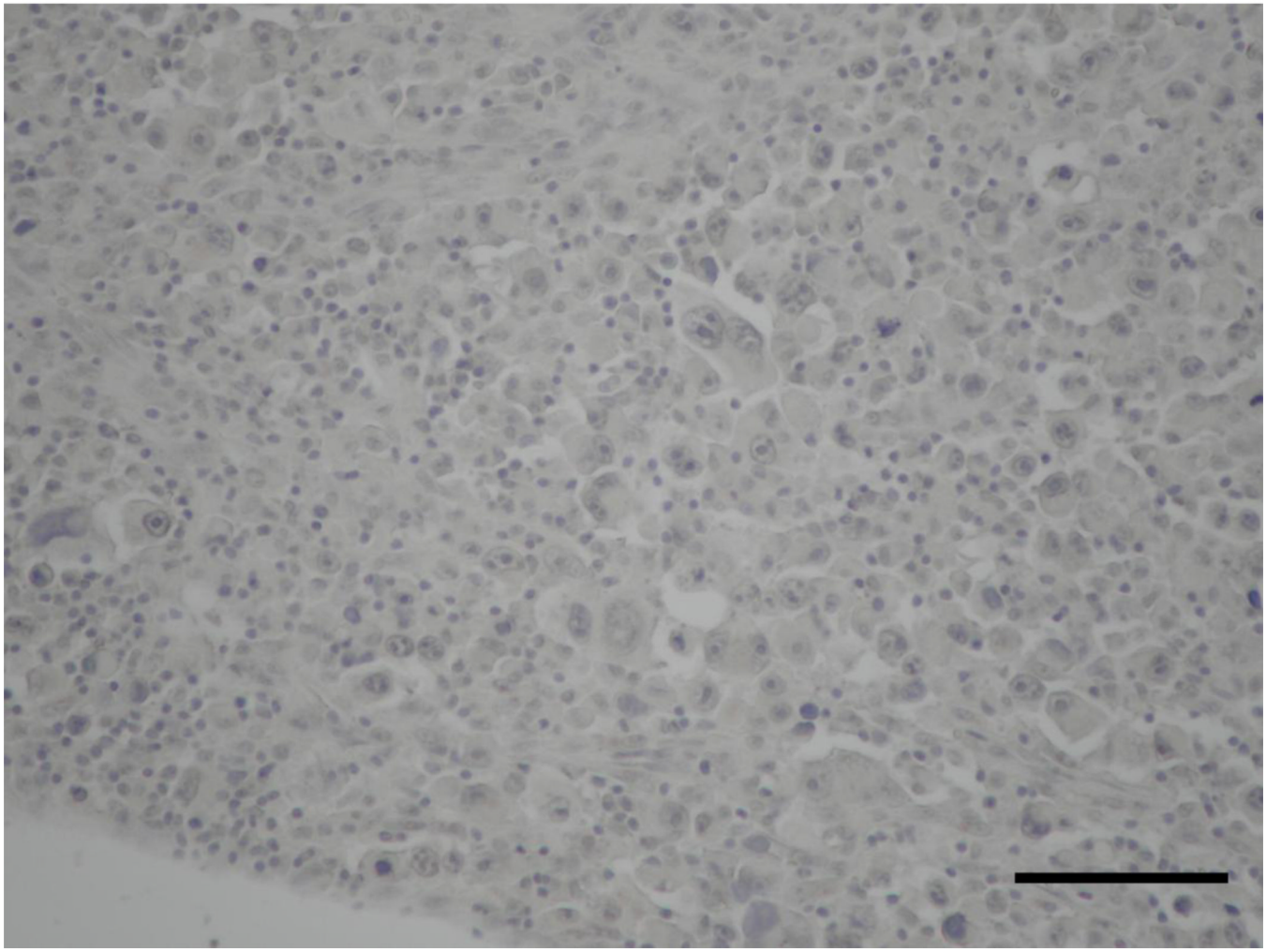

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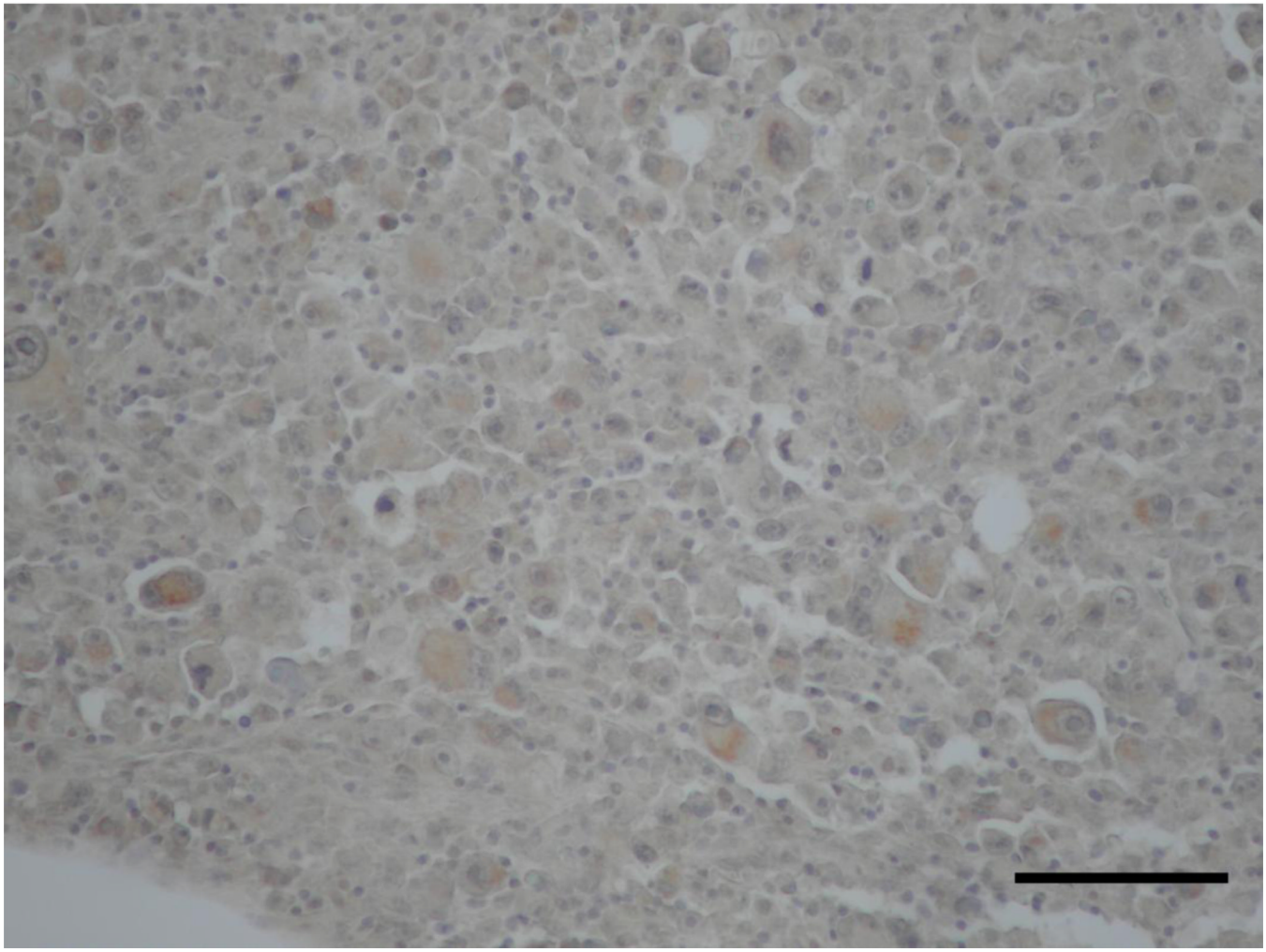

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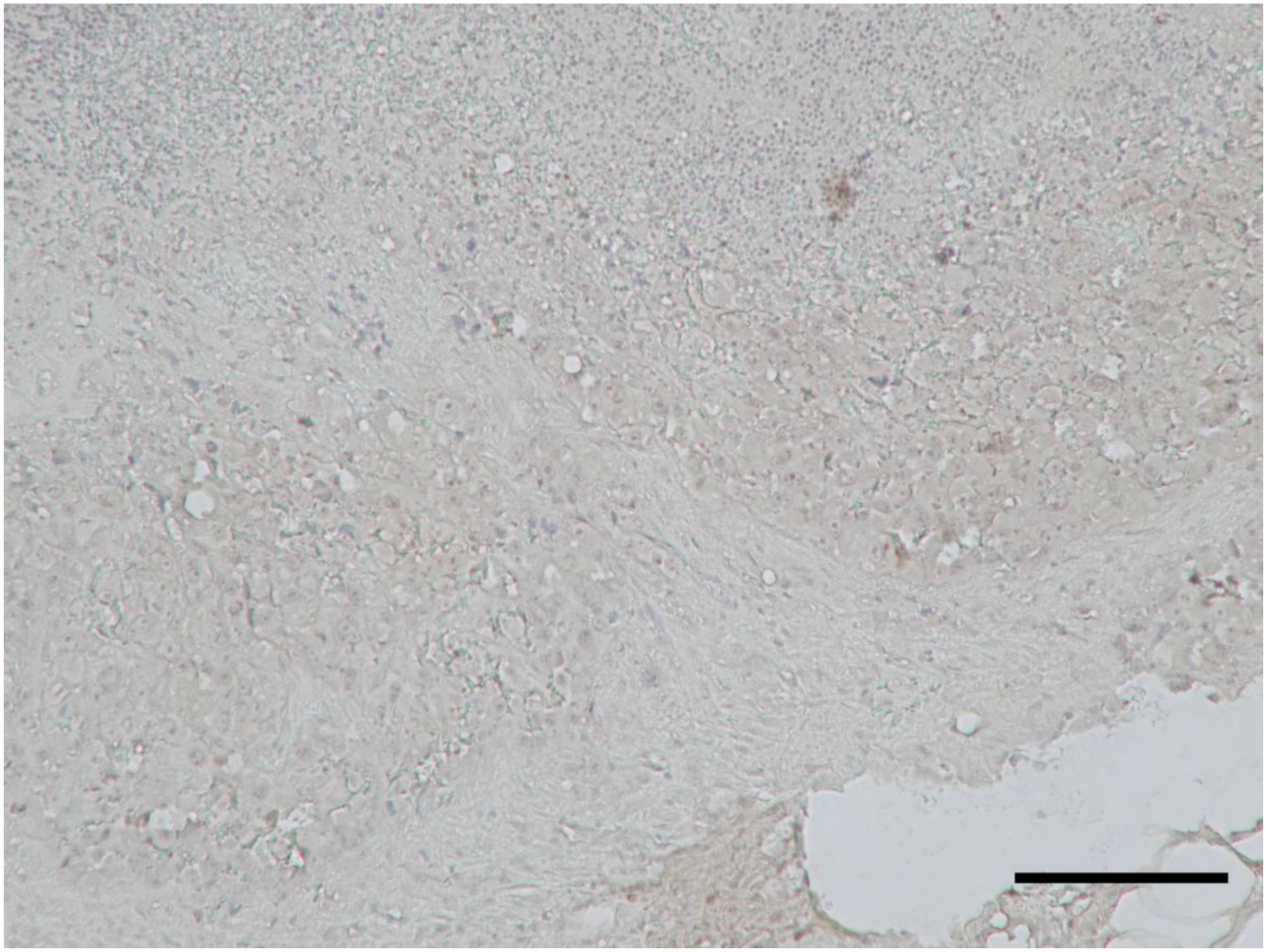

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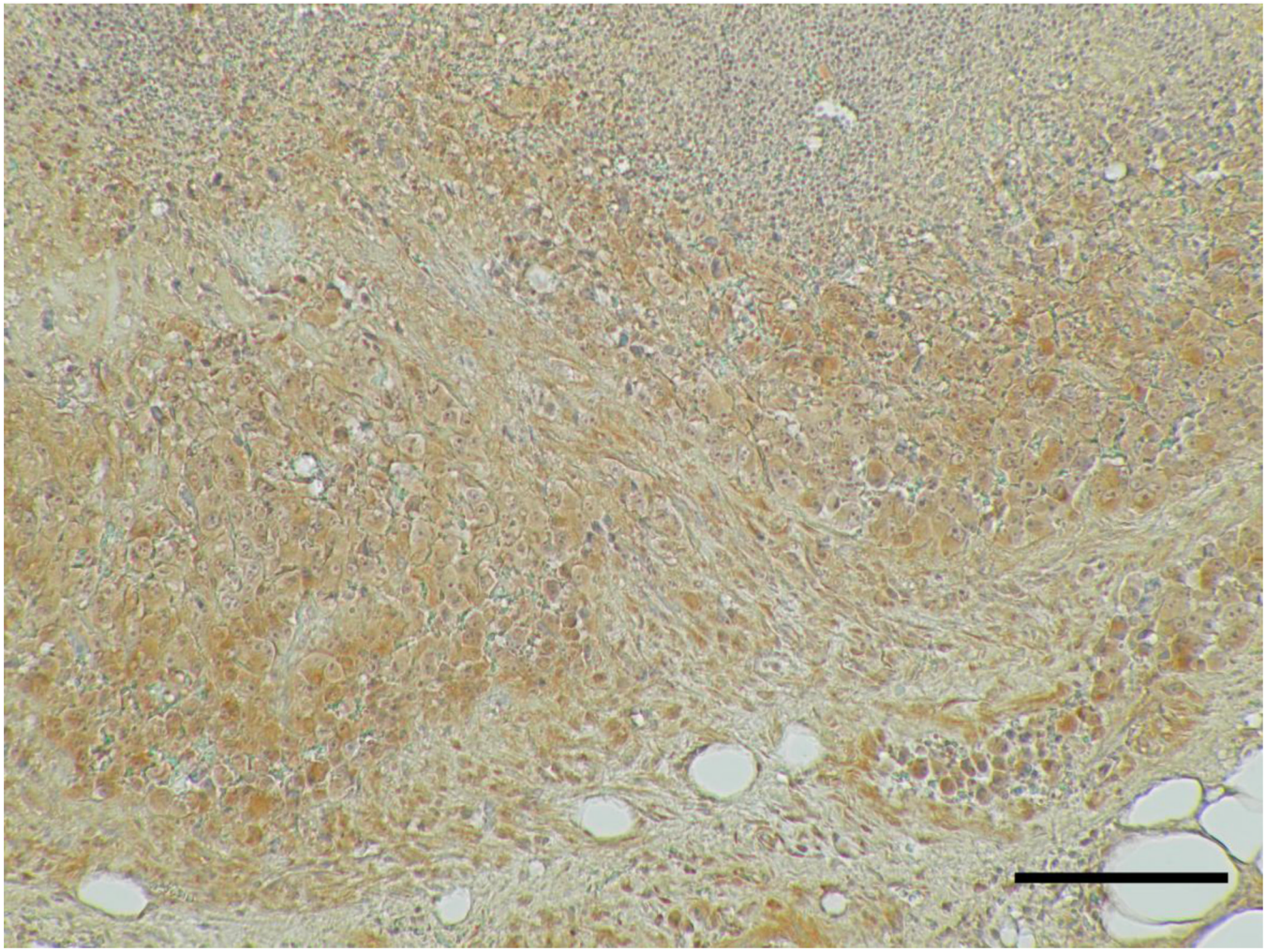

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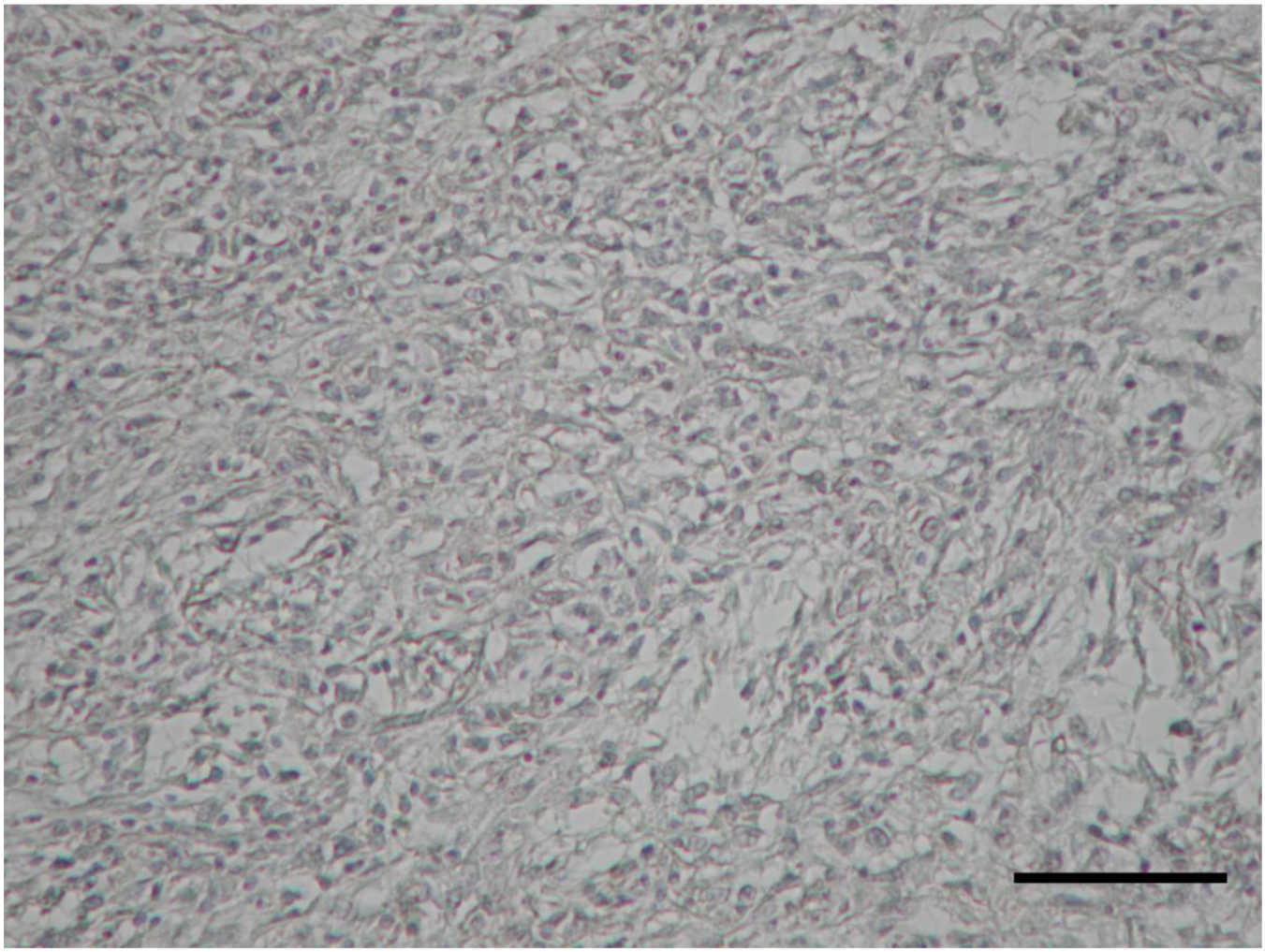

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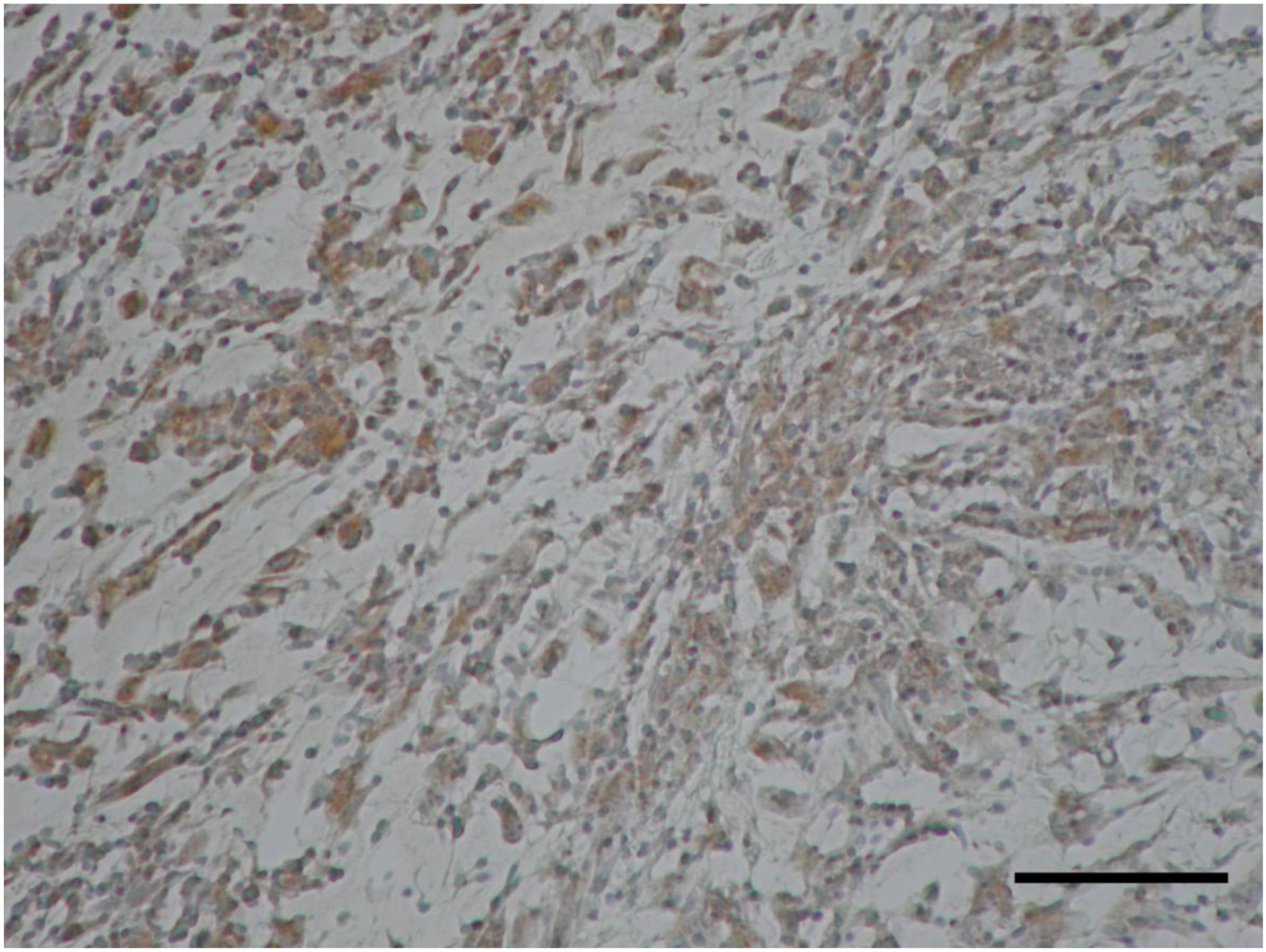
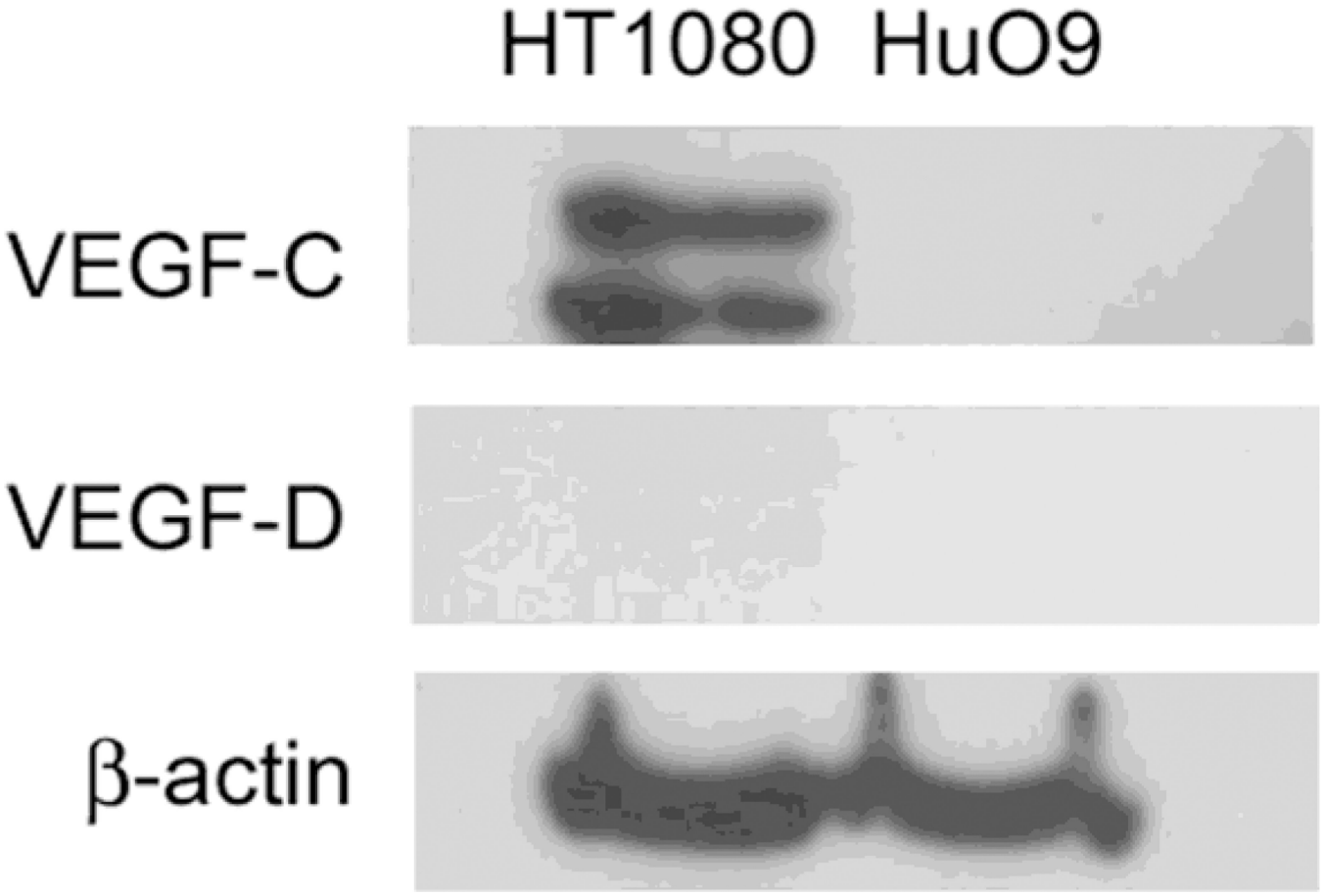


Figure 1. Expression of vascular endothelial growth factors in soft tissue sarcomas

Primary (a, b) and metastatic (c, d) lesions from a 60-year-old man with MFH. Neither the primary nor metastatic tumor expressed VEGF-C (a, c). The metastatic lesion (d) expressed VEGF-D more strongly than the primary lesion (b).

Primary (e) and metastatic (f) lesions from an 85-year-old woman with myxoid-type extraskeletal chondrosarcoma. The metastatic lesion (f) expressed VEGF-D, although the primary lesion (e) did not.

2a



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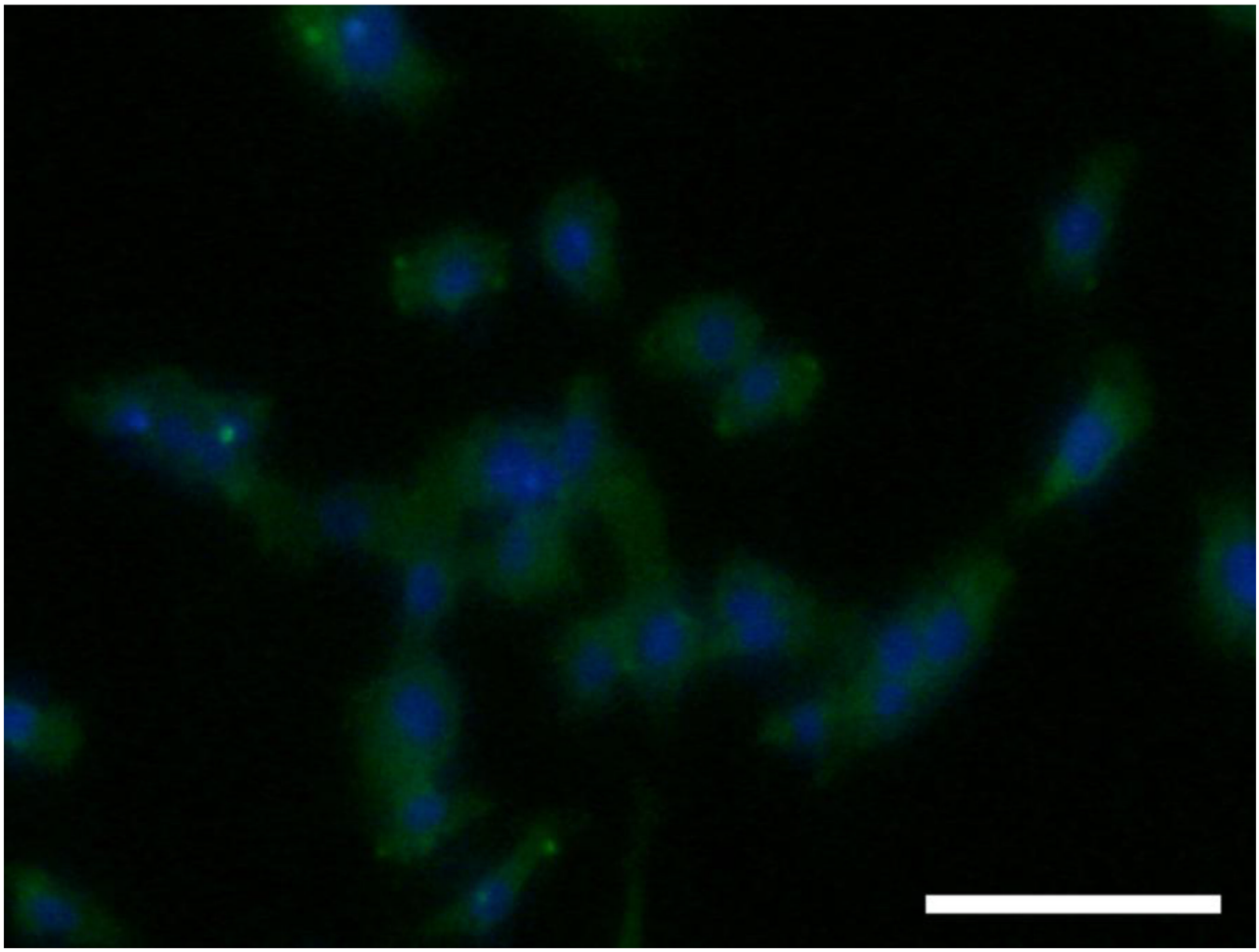

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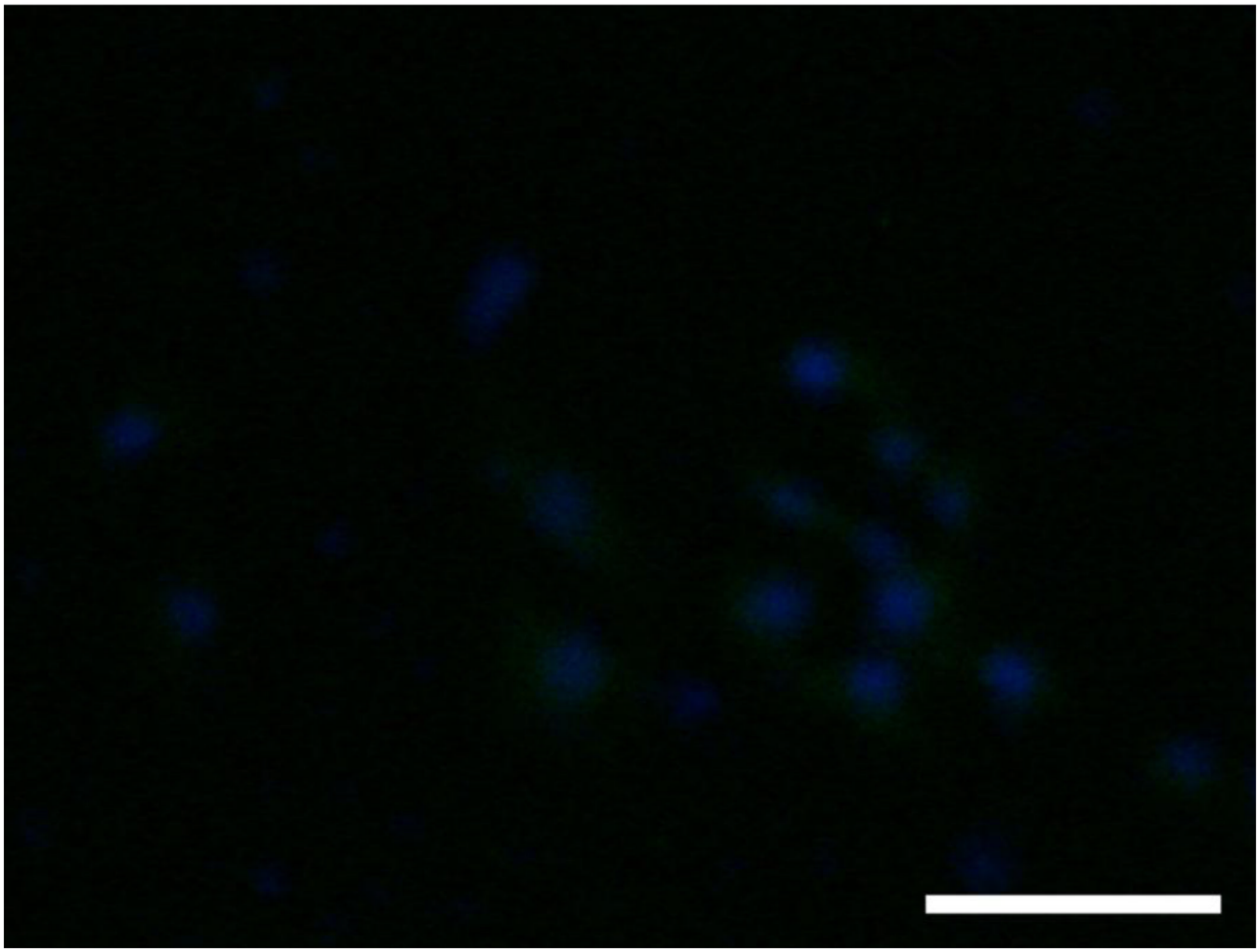

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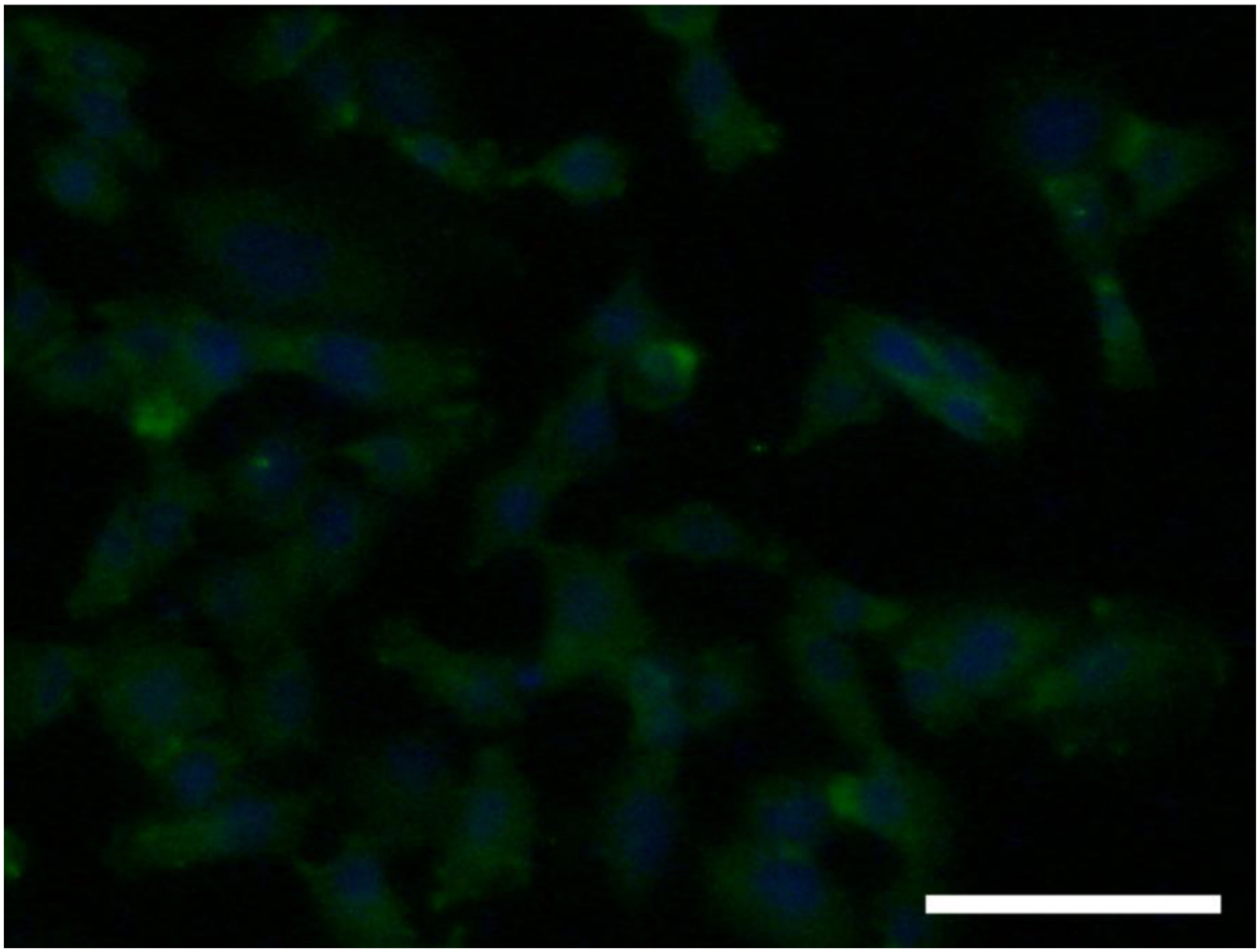

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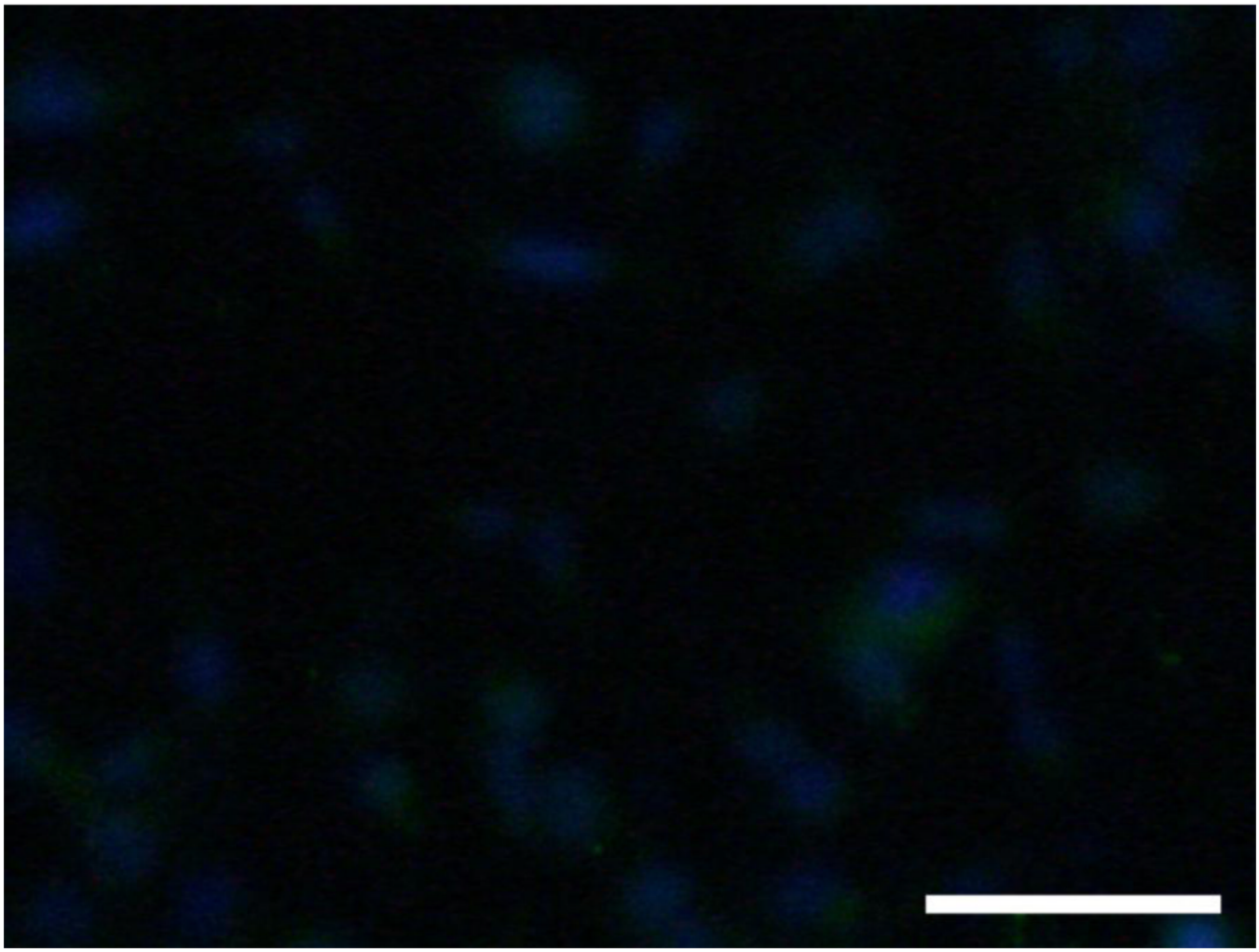

Figure 2. Expression of vascular endothelial growth factor receptors in soft tissue sarcoma cell lines

Immunoblotting revealed that HT1080 cells, but not HuO9, expressed VEGF-C (29/31kDa), while neither cell line expressed VEGF-D.

Immunofluorescence revealed that both HT1080 cells (b, c) and HuO9 cells (d, e) expressed VEGFR-3 (b, d) and slight VEGFR-2 (c, e). Bars on photographs indicate 100μm.

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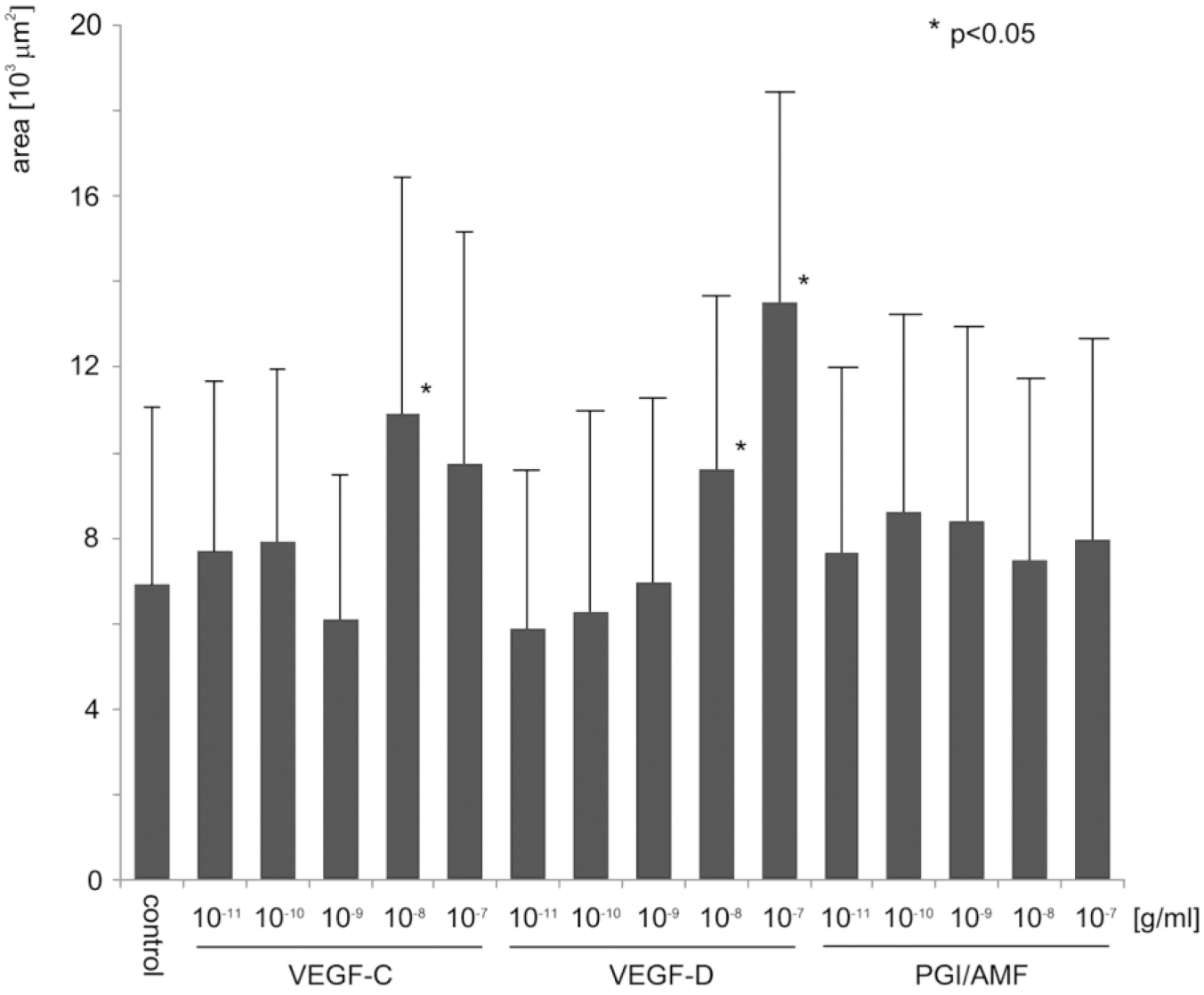

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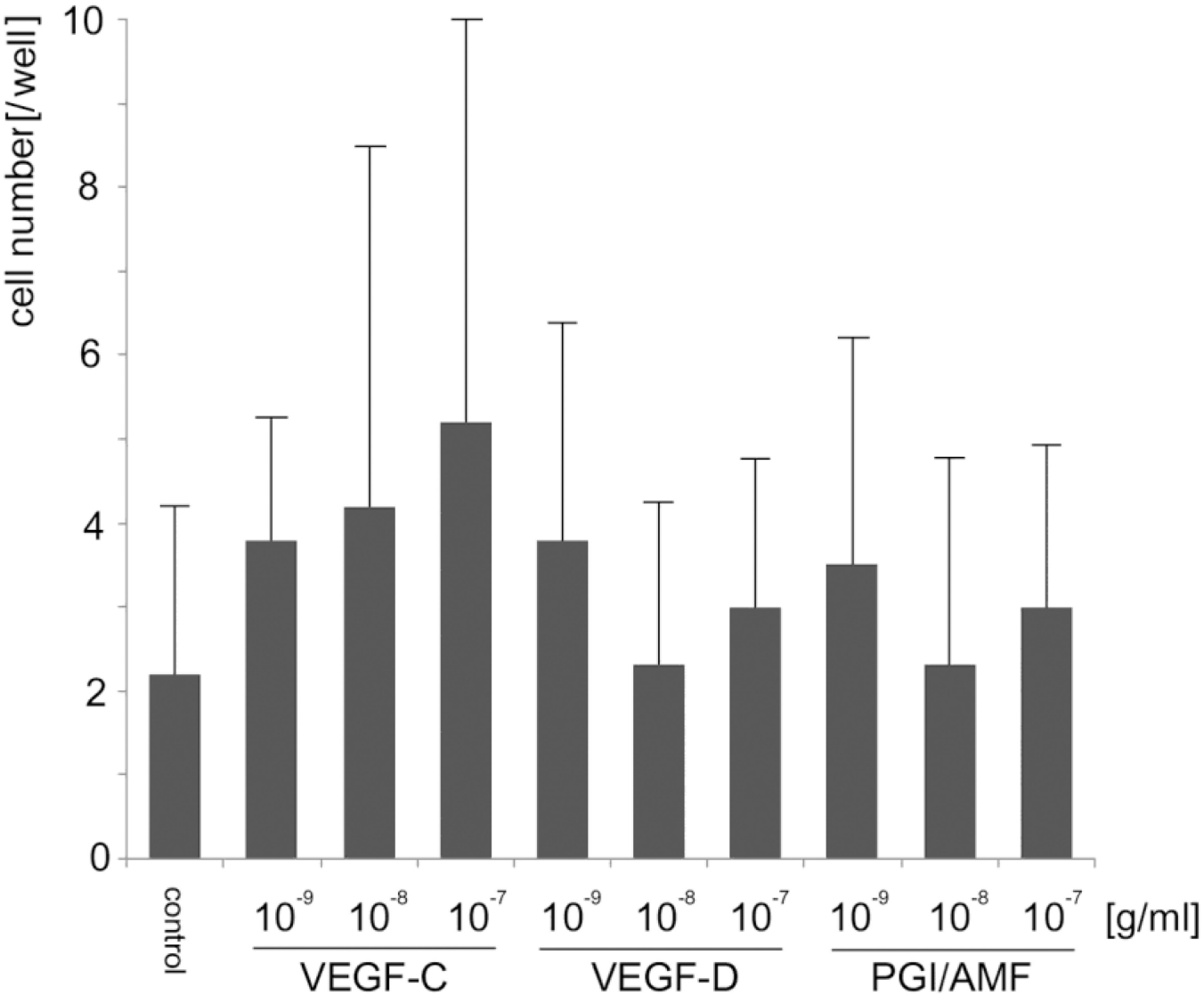

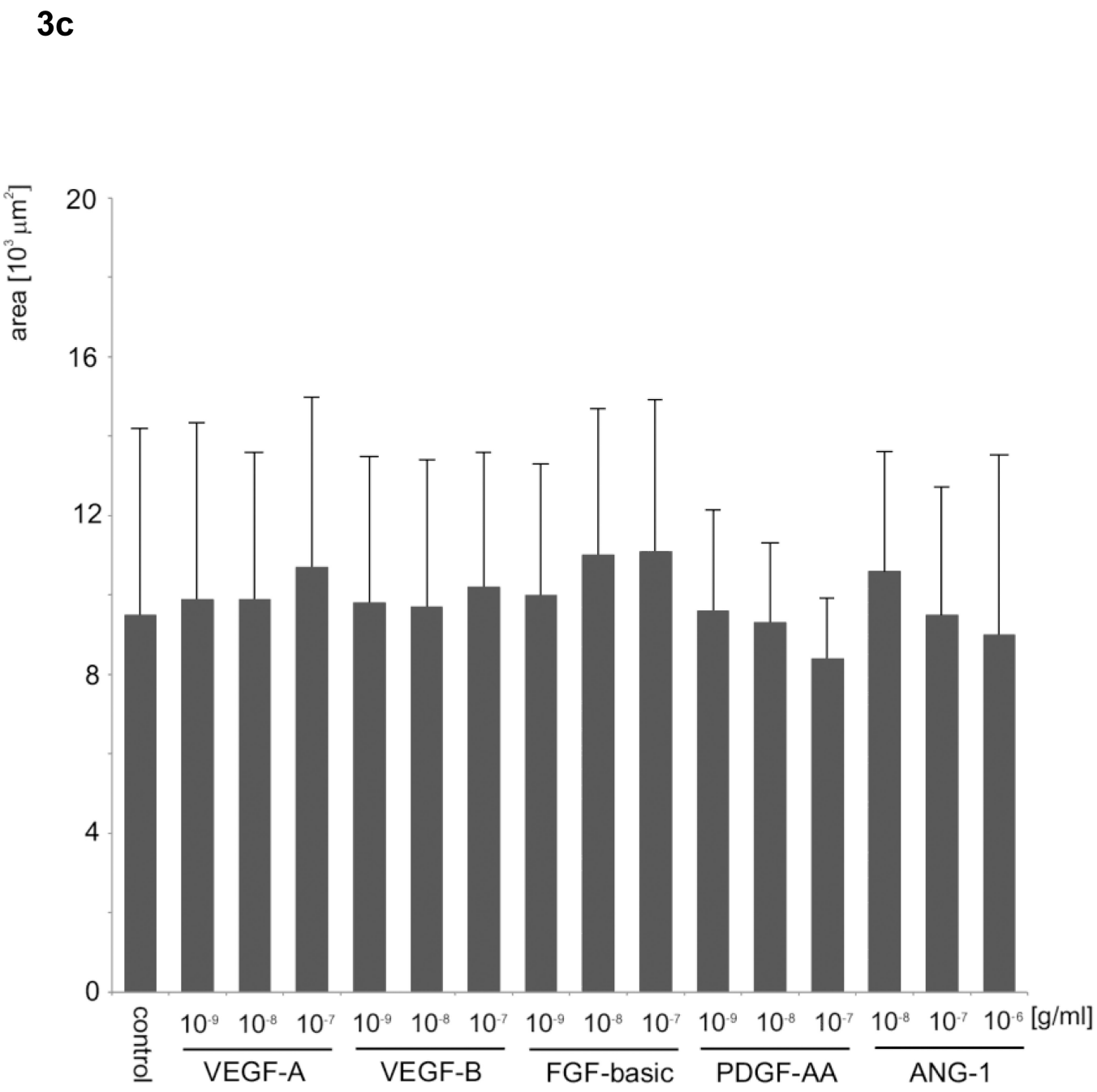

Figure 3. Effects of vascular endothelial growth factors on lymphatic endothelial cells

a. Phagokinetic track assay for measuring random motility revealed VEGF-C at 10^{-8} and VEGF-D at 10^{-8} and 10^{-7} g/ml up-regulated the motility of HLEC.

b. Transwell assay revealed that VEGF-C and VEGF-D did not affect the directed motility of HLEC. Error bars indicate standard deviation.

c. VEGF-A and B, FGF-basic, PDGF-AA and ANG-1 did not affect random motility of HLEC compared with controls in the phagokinetic track motility assay.

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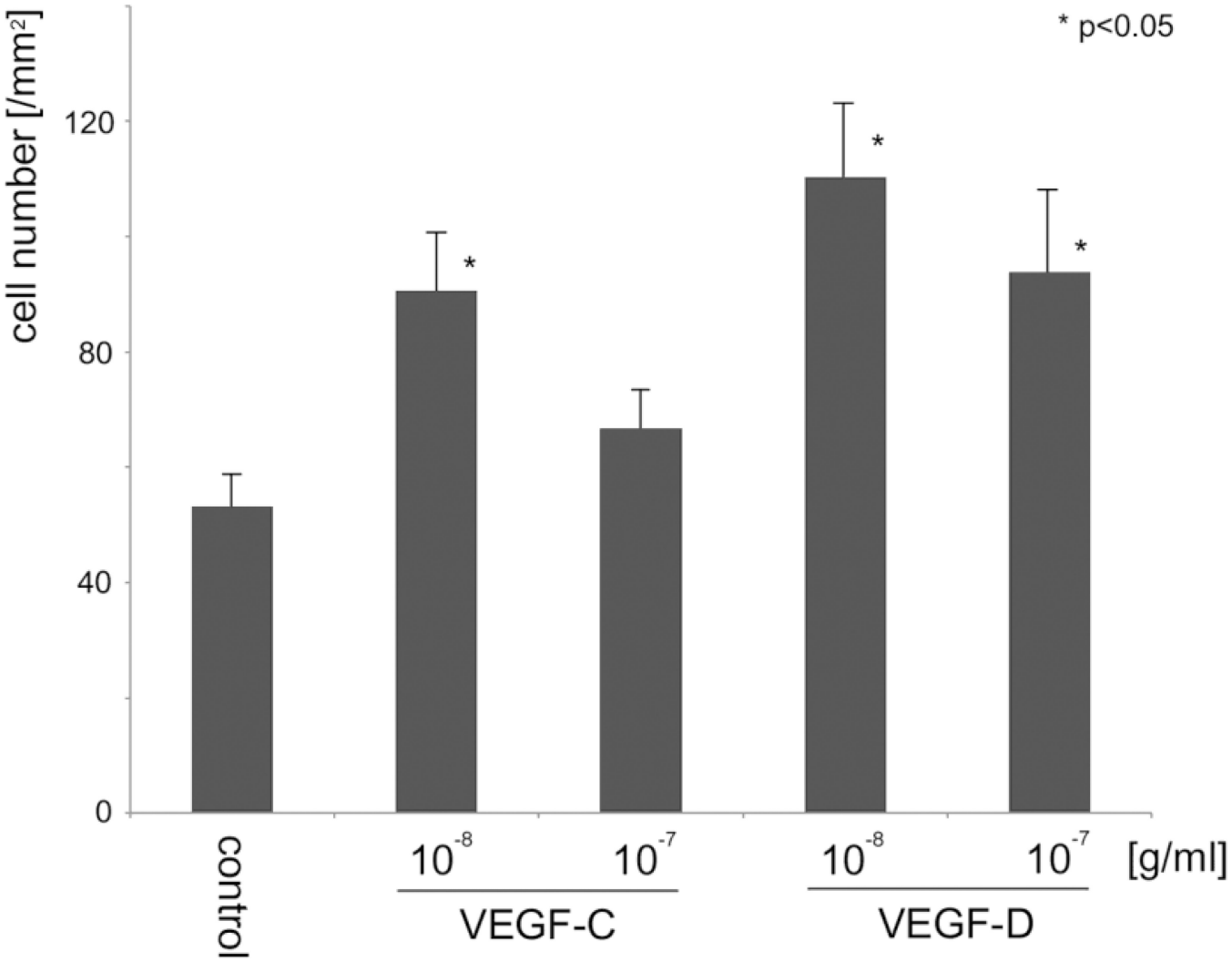

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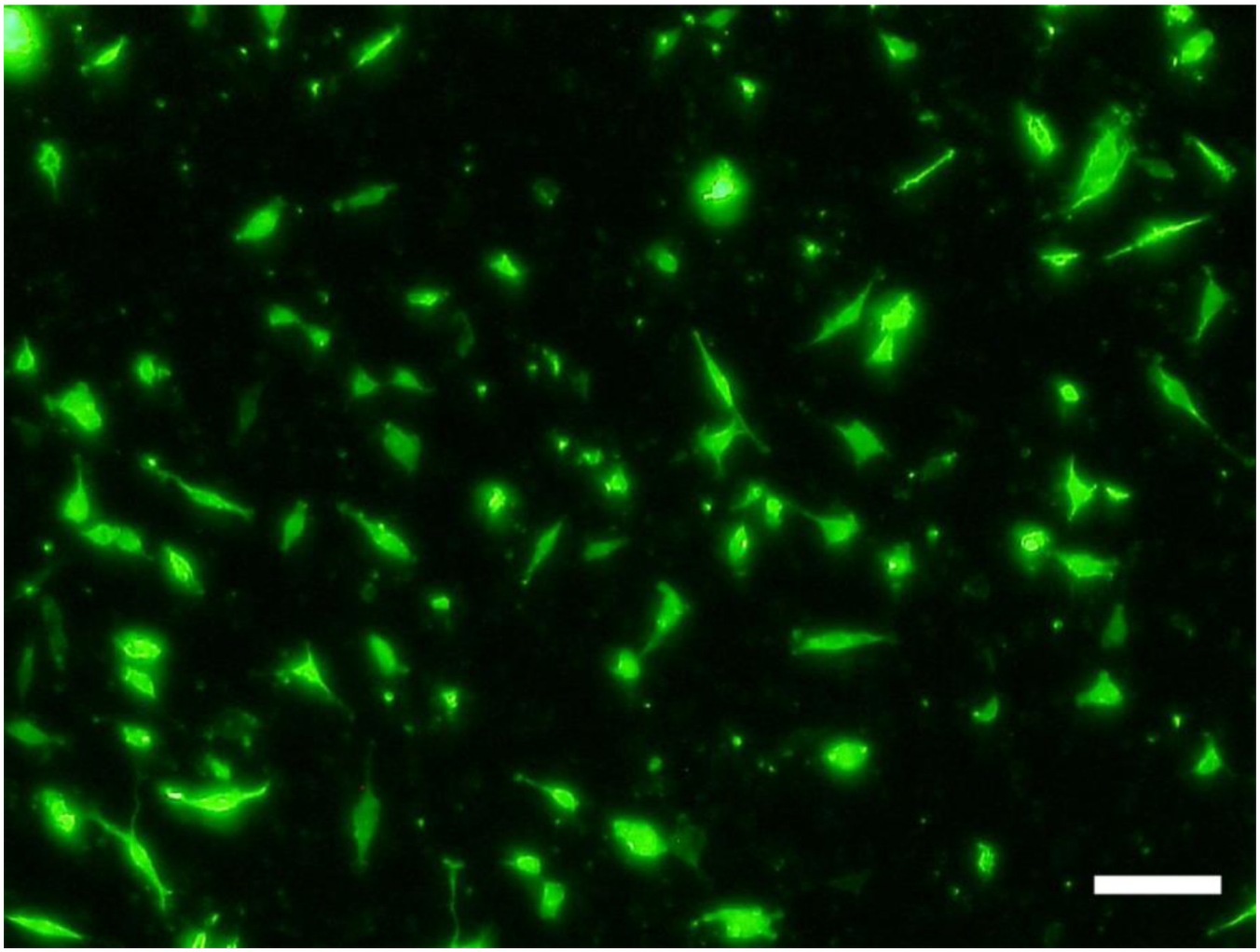

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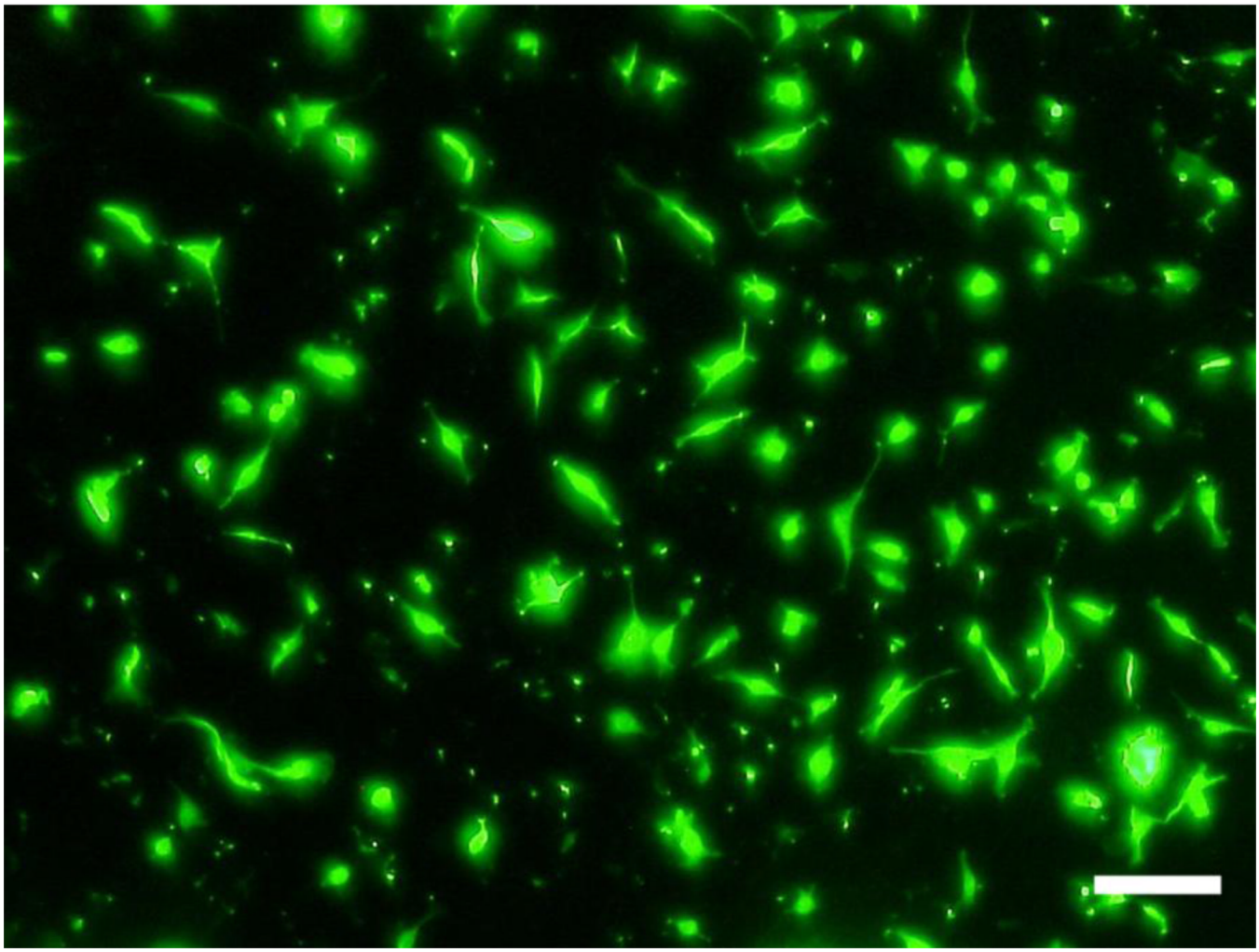

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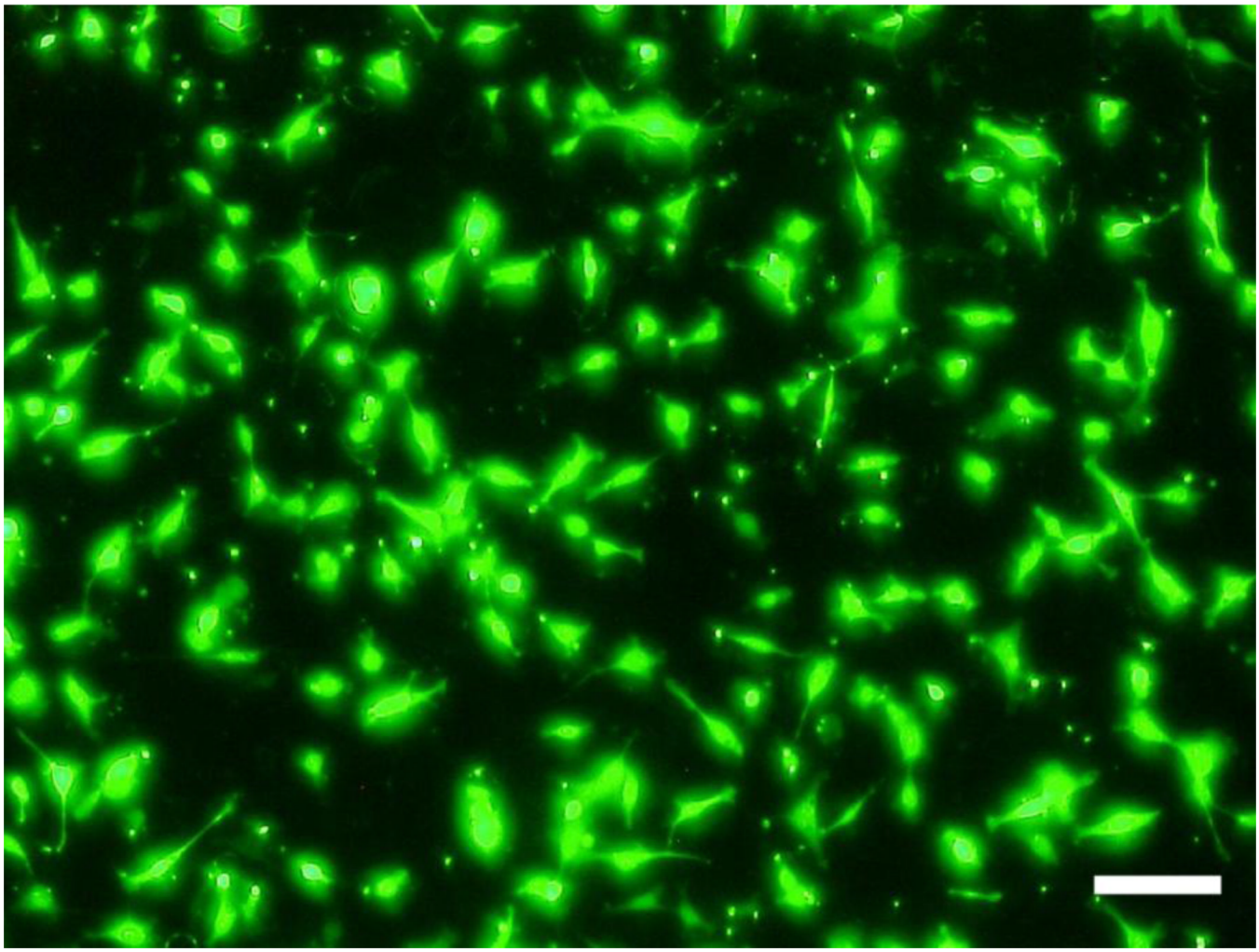

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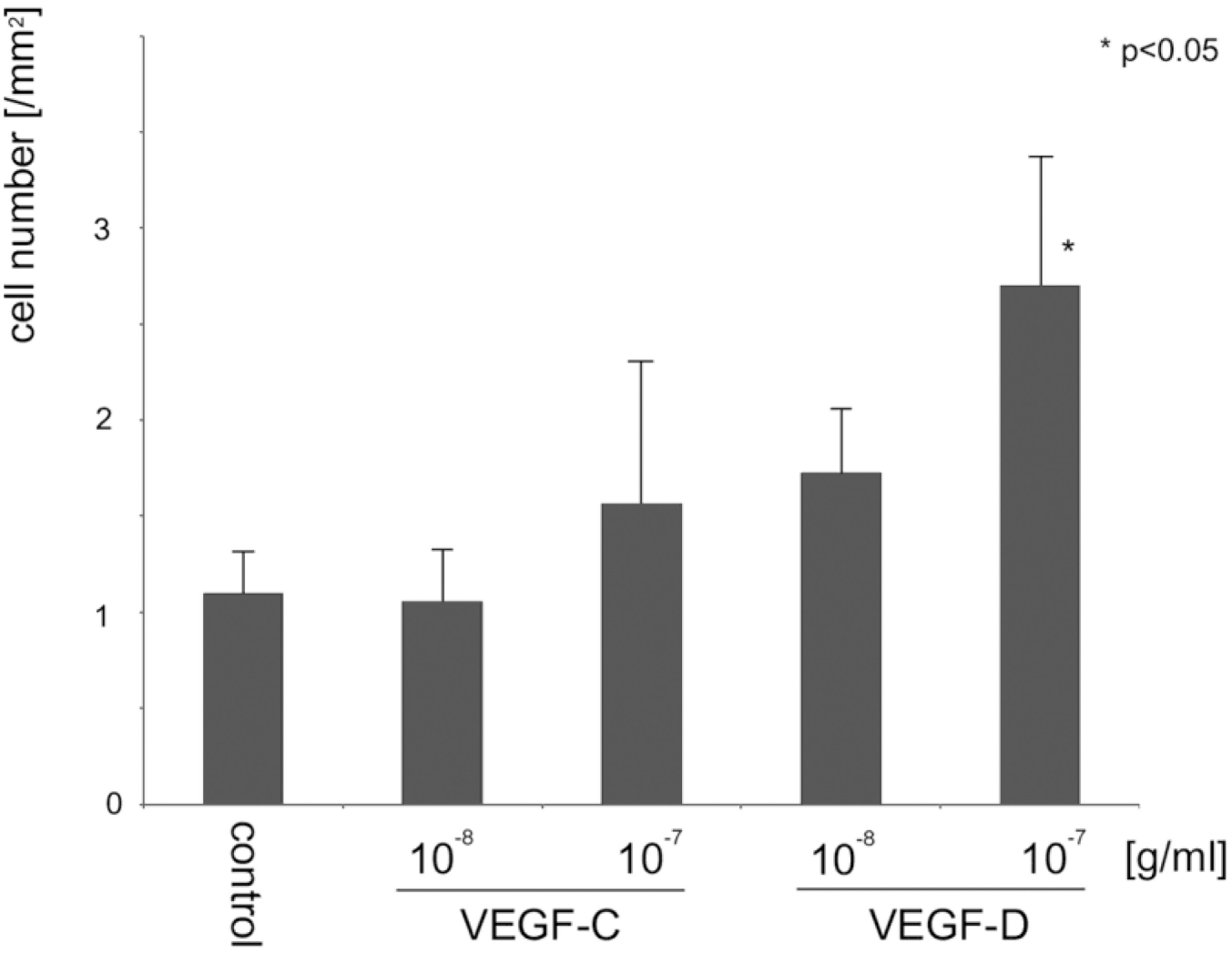

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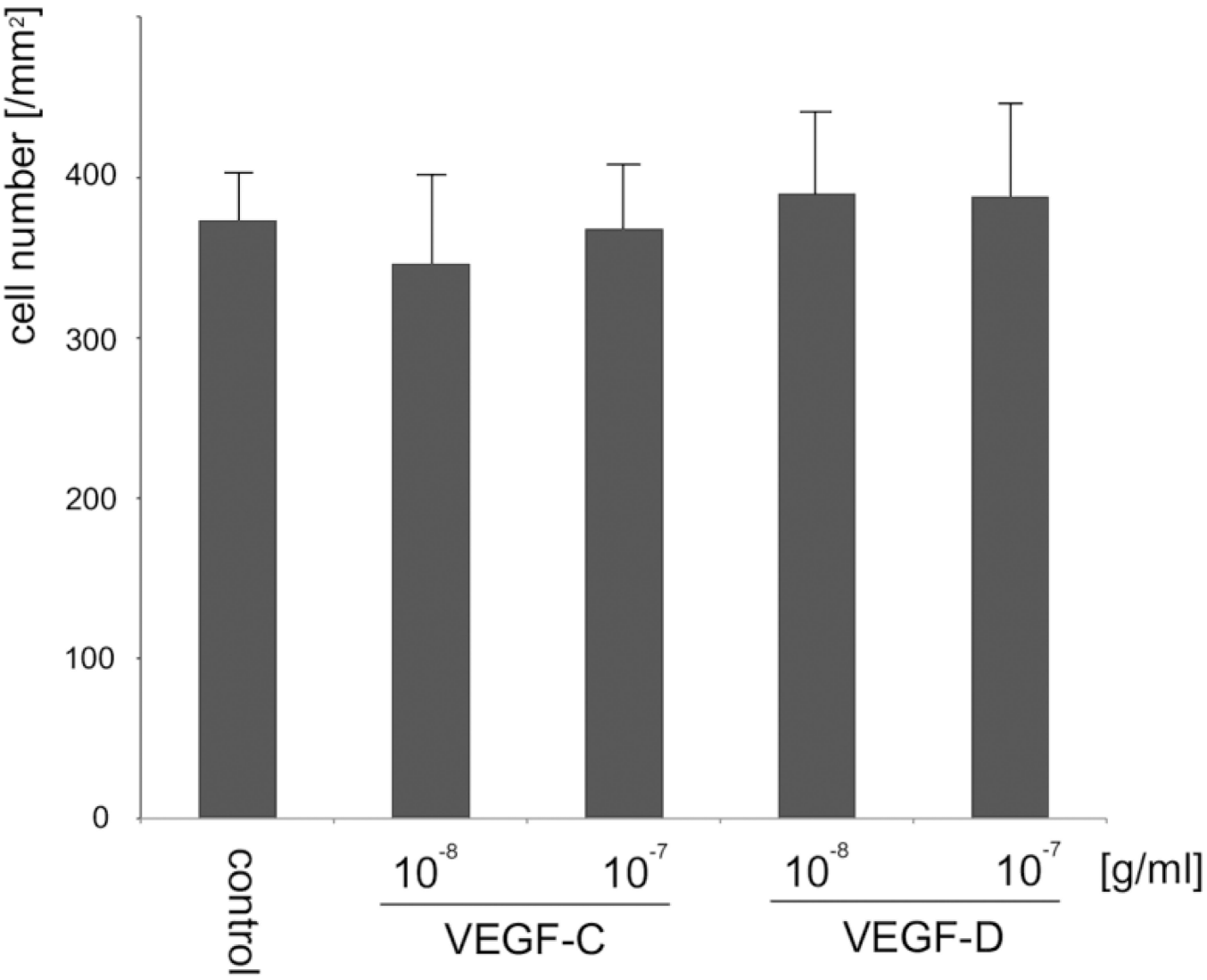

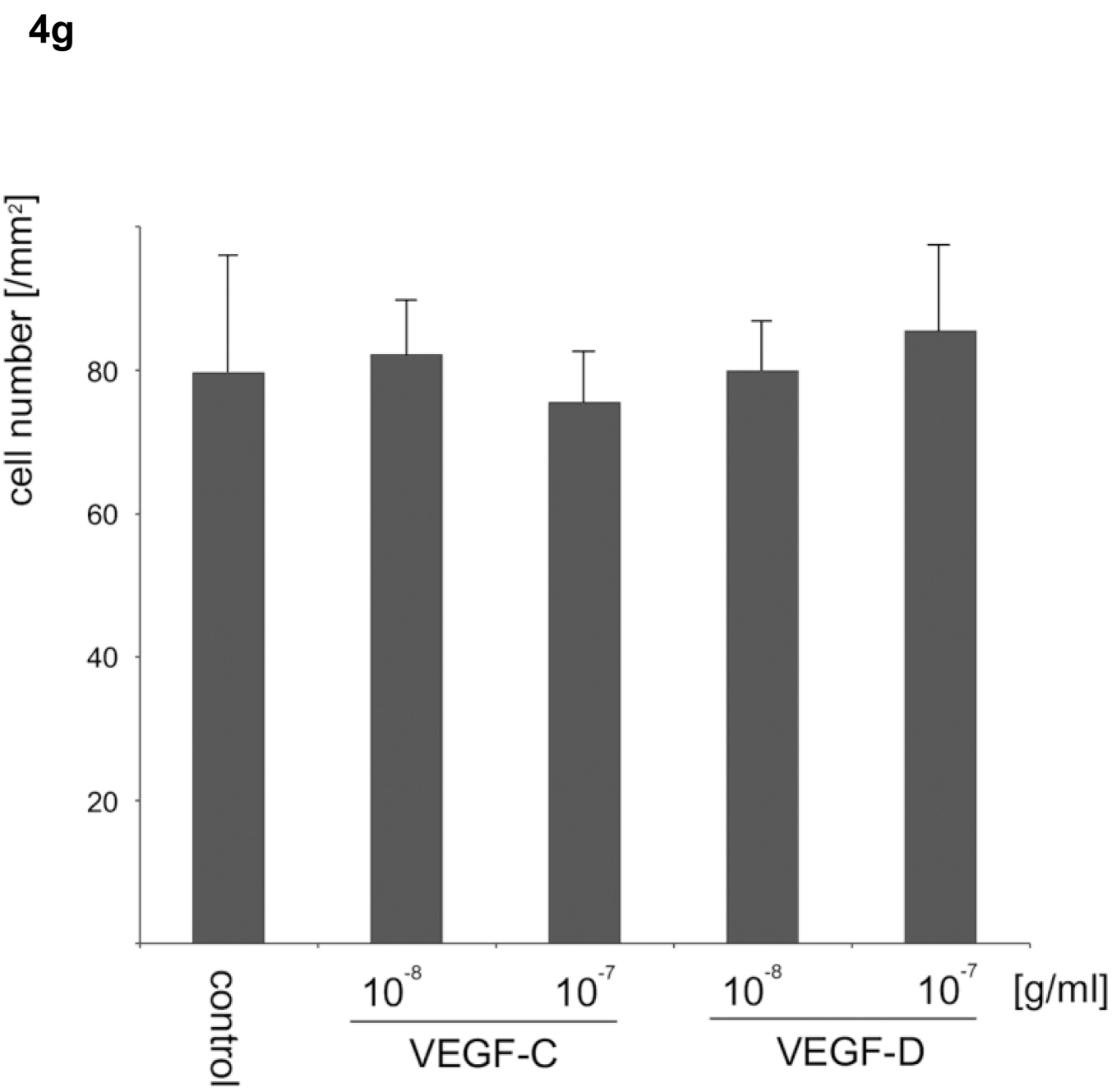

Figure 4. Effects of vascular endothelial growth factors on the monolayer of lymphatic endothelial cells and sarcoma cells

VEGF-D at 10^{-8}, 10^{-7} g/ml and VEGF-C at 10^{-7} g/ml significantly augmented the transendothelial migration of HT1080 cells through a lymphatic endothelial monolayer compared with the control (a). Representative images of control (b), VEGF-C (c) and VEGF-D (d) at 10^{-8} g/ml are shown. VEGF-D at 10^{-7} g/ml, but not VEGF-C, significantly increased the transendothelial migration of HuO9 cells (e). Neither VEGF-C nor -D increased the number of HT1080 cells (f) and HuO9 cells (g) migrating through the pores of the Transwell® membrane compared with the control. (N=5) Error bars indicate standard deviation. Bars on photographs indicate 100μm.

Table 1

Patient with lymph node metastasis

Patient No.	age	sex	pathological diagnosis	site	primary		LN metastasis	
					VEGF-C	VEGF-D	VEGFC	VEGFD
1	52	F	clear cell sarcoma	elbow	−	−	−	+
2	85	F	extraskeletal myxoid	buttock	−	−	−	+
3	68	M	myxoid liposarcoma	back	+	+	+	+
4	62	F	undifferentiated pleomorphic	knee	−	−	−	+
5	66	M	epithelial sarcoma	thigh	+	+	+	+
6	64	M	undifferentiated pleomorphic	lower leg	−	+	−	+
7	60	M	undifferentiated pleomorphic	thigh	−	+	−	+

Exp Cell Res. Author manuscript; available in PMC 2013 April 15.

Table 2

Patient with metastasis to non-lymph-node sites

Patient No.	age	sex	pathological diagnosis	site	primary		site of metastasis
					VEGF-C	VEGF-D	
8	81	F	undifferentiated pleomorphic sarcoma	thigh	−	−	lung
9	82	F	leiomyosarcoma	thigh	−	−	lung
10	63	F	myxofibrosarcoma	thigh	−	−	lung
11	72	M	extraskeletal myxoid	thigh	+	+	lung
12	51	F	myxoid liposarcom	thigh	−	−	spine
13	49	F	undifferentiated pleomorphic sarcoma	buttock	−	−	subcutaneous

Exp Cell Res. Author manuscript; available in PMC 2013 April 15.