Vascular Endothelial Growth Factor Receptor-1 Signaling Promotes Mobilization of Macrophage Lineage Cells from Bone Marrow and Stimulates Solid Tumor Growth

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Abstract

Vascular endothelial growth factor and its receptors, including Flt-1 and Flk-1, are involved in angiogenesis under physiologic and pathologic conditions. Recently, Flt-1-expressing cells were reported to contribute to the intracranial growth of glioma cells. However, the role of Flt-1 signaling in solid tumor growth in s.c. tissue has not been elucidated. To investigate how Flt-1 signaling is involved in the proliferation of solid tumors, we implanted tumor cells into wild-type (Wt) and Flt-1 tyrosine kinase (TK)-deficient (Flt-1 TK⁻/⁻) mice. Growth of HSML and B16 but not Lewis lung carcinoma cell in s.c. tissue was significantly decreased in Flt-1 TK⁻/⁻ mice. Angiogenesis in HSML and B16 tumors was remarkably reduced in Flt-1 TK⁻/⁻ mice. Moreover, the infiltration of macrophage lineage cells into HSML and B16 tumors was clearly suppressed in Flt-1 TK⁻/⁻ mice. Pericyte marker⁺ cells were also reduced in Flt-1 TK⁻/⁻ mice. However, in the border area of tumor, angiogenesis and the infiltration of macrophage lineage cell were basically similar between Wt and Flt-1 TK⁻/⁻ mice. In bone marrow (BM) transplantation experiments, tumor angiogenesis, infiltration of macrophage lineage cells, and tumor growth were significantly suppressed in Wt/Flt-1 TK⁻/⁻ mice implanted with Flt-1 TK⁻/⁻ BM cells compared with those implanted with Wt BM cells. We conclude that Flt-1 signaling is involved in the function of BM-derived cell, such as the migration of macrophages into cancerous tissues, and significantly contributes to angiogenesis and tumor progression. Cancer Res; 70(20); 8211−21. ©2010 AACR.

Introduction

Vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFR), including VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), are essential for vasculogenesis and angiogenesis (1, 2). VEGF-A, the most abundant VEGF and highly active (KDR/Flk-1), are essential for vasculogenesis and angiogenesis, including VEGFR-1 (Flt-1) and VEGFR-2 (VEGFR-2). The VEGF-A gene resulted in the death of mice at embryonic days 7.5 to 8.5 (E7.5–8.5) due to a lack of vasculogenesis and hematopoiesis (8). Moreover, loss of VEGFR-2 in embryonic days 3.5 results in embryonic lethality due to cardiovascular defects (4). VEGFRs belong to the Fms/kit/platelet-derived growth factor receptor (PDGFR) super gene family and are characterized by a cytoplasmic tyrosine kinase (TK) domain with kinase insert sequence (5). The binding activity of Flt-1 for VEGF is 10-fold higher than that of Flk-1, whereas the TK activity of Flt-1 is one order of magnitude lower than that of Flk-1 (6, 7). Flt-1 binds VEGF-A, placenta growth factor (PlGF), and VEGF-B. Gene targeting studies have shown that flt-1−/− mice die by E8.5 due to a lack of vasculogenesis and hematopoiesis (8–10). On the other hand, flt-1−/− mice die at E8.5 to E9.0 because of a disorganized system of blood vessels and an overgrowth of endothelial-like cells (11, 12). Thus, flt-1 is regarded as a positive regulator for the proliferation of endothelial cell; in contrast, flt-1 is considered a negative regulator for angiogenesis in early development.

To investigate whether Flt-1 TK itself is a negative signal transducer or not, we previously generated Flt-1 TK–deficient (Flt-1 TK⁻/⁻) mice (13). Although deficient in Flt-1 signaling, these mice have the ligand-binding domain of Flt-1 and exhibited normal development. The results indicate that the Flt-1 TK⁻/⁻ mice are useful for investigating how the Flt-1 signal is important in pathologic angiogenesis (13, 14). We and others have reported that Flt-1 is expressed not only on endothelial cells but also on monocyte/macrophages and, in some conditions, on smooth muscle cells and other cell types (15–17). Flt-1 TK is involved in the survival signaling of liver sinusoidal endothelial cells and migration of monocyte/macrophages (18).

Recent studies have shown the importance of cells expressing Flt-1 in the formation of premetastatic niches in
tumor-bearing animals, reconstitution of BM tissues, expression of extracellular matrix metalloproteinase-9, and promotion of inflammatory diseases (19–22). Furthermore, Leek and colleagues and others reported that the number of macrophages infiltrated into tumors correlated with capillary density and a poor prognosis (23, 24). However, the relationship between Flt-1 signaling and tumor progression in s.c. tissue has not been clarified.

Here, we show that, in solid tumor models, a deficiency of Flt-1 signal suppresses tumor angiogenesis, infiltration of macrophage lineage cells, and tumor growth. Furthermore, based on the results of bone marrow (BM) transplantation (BMT) experiments, we suggest that the Flt-1 signal on macrophage lineage cells derived from BM plays a major role in Flt-1–related tumor growth.

Materials and Methods

Cell lines and mice

A murine uterine cancer cell line HSML was kindly provided by Dr. Kudoh (Hirosaki University, Hirosaki, Japan). Lewis lung carcinoma cells (LLC) and a murine melanoma cell line B16 were purchased from the American Type Culture Collection. HSML cells were cultured in RPMI 1640 (Nacalai Tesque), LLC and B16 cells were maintained in DMEM (Nacalai Tesque). Each medium was supplemented with fetal bovine serum to 10%, and the cells were cultured at 37°C with 5% CO2.

All experiments using animal models were carried out according to guidelines, following a protocol approved by the ethics committees of the Animal Center of Institute of Medical Science in University of Tokyo and the Disease Model Research Center in Tokyo Medical and Dental University. Genotyping of all mice was performed as described previously (13). Wild-type (Wt) C57BL/6 mice (6–8 weeks old; CLEA Japan, Inc.) were used as a control in this study and as BM recipients. Flt-1 TK−/− mice were used at the same age as Wt mice. Green fluorescent protein (GFP) mouse strain (RBRC00267) was provided by RIKEN BRC through the National Bio-Resource Project of the Japanese Government Ministry of Education, Culture, Sports, Science and Technology.

In vivo tumor growth experiments

Eight-week-old mice were anesthetized and injected s.c. with 1 × 107 tumor cells. Tumors were measured every 2 days from day 7 for 3 to 4 weeks. After mice were euthanized, tumors were removed and either postfixed in 4% paraformaldehyde or directly embedded in optimal cutting temperature medium. Tumor tissues were removed and either postfixed in 4% paraformaldehyde or directly embedded in optimal cutting temperature medium. After mice were euthanized, tumors were removed and either postfixed in 4% paraformaldehyde or directly embedded in optimal cutting temperature medium. Tumor tissues were removed and either postfixed in 4% paraformaldehyde or directly embedded in optimal cutting temperature medium.

BMT

BM cells were harvested by flushing femurs and tibias of healthy Wt mice and Flt-1 TK−/− mice, and then the hematopoietic stem cell lineage-negative population was isolated by magnetic cell sorting (Miltenyi Biotec) according to the manufacturer’s protocol. BM cells were i.v. injected at 1 × 106 cells per mouse into recipient mice, which were irradiated with a single lethal dose of 10 Gy of X-ray. Wt/Wt and Wt/Flt-1 TK−/− mice (indicating donor genotype/recipient genotype) at 4 weeks posttransplantation were inoculated with 1 × 107 tumor cells and sacrificed ~4 weeks later.

Reverse transcription-PCR and real-time PCR

Reverse transcription-PCR (RT-PCR) and real-time PCR were performed following a previous report (25). Alternative splicing subtypes of mouse vegf-a were detected by RT-PCR with forward primer as 5′-ATGAACCTTCTGCTCTTG-GTGTC-3′ and reverse primer as 5′-TGGCTACCGCCCT-TGGCTTGTCAC-3′ (25).

Immunohistochemistry

Fixed mouse tissues were cut 20-μm thick by Cryostat (Leica) and stained with the following antibodies: rat anti-mouse F4/80 (Serotec), rat anti-mouse platelet/endothelial cell adhesion molecule 1 (CD31; BD Bioscience), and rabbit anti-GFP (Invitrogen). Cryosections of fresh frozen mouse tissues were stained with rat anti-CD11b (BD Bioscience), hamster anti-CD31 (Millipore, MA), mouse anti-smooth muscle actin (SMA; Sigma), rabbit anti-PDGFβ (eBioscience), and rat anti-NG2 (Millipore). Sections were incubated with appropriate secondary antibodies, with the nuclear staining dye To-Pro-3 (Invitrogen) and the in situ Cell Death Detection kit TMR red [terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL); Roche], and then analyzed with a confocal microscope (Radiance 2000, Bio-Rad).

Western blotting

Cell lysate was prepared with a lysis buffer containing 1% Triton-X 100 to solvate cytosomal. After centrifugation at 4°C to remove the supernatant, a one-fourth volume of 5× sample buffer containing 10% SDS for solving nucleus fraction was added to the precipitate. Aliquots were applied to the gel and transferred to a membrane, and then the membrane was incubated with rabbit anti-fibroblast growth factor-2 (FGF-2) antibody (Santa Cruz) or mouse anti-actin antibody (Millipore) as a primary antibody. Anti-rabbit IgG or anti-mouse IgG antibody was used as a secondary antibody, which was conjugated with alkaline phosphatase (Promega).

RNA interference experiment

Stealth RNA interference (RNAi; Invitrogen) for impairment of FGF-2 mRNA was transfected into LLC by Lipofectamine RNAi MAX (Invitrogen). Transfected cells were collected and implanted into s.c. tissue of Wt and Flt-1 TK−/− mice. An aliquot of cells was used to detect the levels of FGF-2 inhibition by real-time PCR. Tumor volume, degree of angiogenesis, and infiltration of macrophage lineage cells were examined.

Transfection of FGF-2 cDNA into B16

FGF-2 cDNA was transfected into B16 tumor cells by using pMX-puro containing mouse retroviral vector and Fugene 6 transfection reagent (Roche). After overnight infection, the culture medium was replaced to fresh medium containing 1.5 μg/mL puromycin. FGF-2–expressed clones were obtained after 1 to 2 weeks.
mouse macrophages as well as monocytes were identified by immunohistochemical staining using F4/80 antigen. As a control, we confirmed that the basal level of resident macrophage lineage cells in s.c. tissues in healthy Wt mice did not differ from that in healthy Flt-1 TK−/− mice (Supplementary Fig. S1). The infiltration of macrophage lineage cells into HSML and B16 tumor tissues was strikingly suppressed in Flt-1 TK−/− mice. In contrast, numbers of macrophages in the border area of tumor tissues in Flt-1 TK−/− mice were similar to those in Wt mice (Fig. 2A). The infiltration of macrophages into tumors was 15% (HSML), 39% (B16), and 78% (LLC) in Wt mice (Fig. 2B). These results suggest that the infiltration of macrophage lineage cells into some tumor tissues is at least partly dependent on Flt-1 signaling, although the border area of tumors is not affected.

**Myeloid cells and perivascular cells were also decreased in tumors inoculated in Flt-1 TK−/− mice**

Recently, Shojaei and colleagues have reported that CD11b+ Gr-1+ cells derived from BM mediate tumor refractoriness to antiangiogenesis therapy involving BV8 and granulocyte colony-stimulating factor (27–32). Immunohistochemical staining by anti-CD11b antibody was carried out to evaluate the infiltration of myeloid cells in these tumors. Numbers of myeloid cells in tumor tissue were significantly lower in Flt-1 TK−/− mice than Wt mice (Fig. 2C and D). We also examined perivascular cells, including pericytes and smooth muscle cells, that may contribute to the vasculature in these tumors. Immunohistochemical staining revealed that the numbers of SMA+ and PDGFRβ+ perivascular cells in tumor tissue were decreased in Flt-1 TK−/− mice (Fig. 3). These results indicate that SMA+ and PDGFRβ+ cells are also less numerous in tumors under Flt-1 TK−/− conditions, although it is not clear whether the decrease is a direct effect of Flt-1 TK−/− or not.

**FGF-2 was highly expressed in LLC cells compared with other tumor cells and contributed to tumor progression**

Because LLC cell growth in Flt-1 TK−/− mice was similar to that in Wt mice, the question arises whether this refractory feature of Flt-1 signaling is due to high levels of other angiogenic factors different from the VEGF-VEGFR system. Real-time PCR was performed to assess the expression patterns of angiogenic factors and chemoattractants at the mRNA level in vitro (Fig. 4A). LLC cells expressed FGF-2, a major angiogenic factor, at levels of two orders of magnitude higher than that in HSML and B16 cells. By Western blotting, FGF-2 protein was detected in LLC cell lysate but was undetectable in the conditioned medium (Fig. 4B). The FGF-2 expressed in LLC cells had a molecular weight of 24 kDa, the largest of the four subtypes (18, 21, 22.5, and 24 kDa). FGF-2 was highly expressed in LLC cells compared with HSML and B16 cells. By Western blotting, FGF-2 protein was detected in LLC cell lysate but was undetectable in the conditioned medium (Fig. 4B). The FGF-2 expressed in LLC cells had a molecular weight of 24 kDa, the largest of the four subtypes (18, 21, 22.5, and 24 kDa).
Figure 1. Deficiency of Flt-1 TK suppressed tumor growth in vivo. A, tumor growth curves of HSML (a), B16 (b), and LLC (c) cells. The red line indicates tumor volume in Wt mice, and the blue line indicates tumor volume in Flt-1 TK−/− mice. B, representative micrographs (10×) of immunohistochemical staining indicate HSML (a, b), B16 (c, d), and LLC (e, f) cells in Wt mice or Flt-1 TK−/− mice with CD31 (red) used for endothelial cells and To-Pro-3 (blue) for the nucleus. B and T indicate the border area of tumors and the tumor tissue itself, respectively. Scale bars, 150 μm. The dotted line indicates the margin between tumor tissue and the border area of tumors such as the dermis. C, the degree of angiogenesis quantified using an Angiogenesis Image Analyzer (KURABO). The CD31+ area was calculated in each section (n = 10). *, P < 0.03; **, P < 0.0005. D, the survival rates of Wt mice and Flt-1 TK−/− mice implanted with HSML (a), B16 (b), and LLC (c) tumors.
When we used two independent siRNAs efficiently suppressing FGF-2 mRNA, interestingly, the LLC with FGF-2 silencing but not the control LLC with scrambled siRNA showed slower tumor growth in Wt mice (Fig. 5B). Furthermore, the growth rate of the LLC with FGF-2 silencing in Flt-1 TK−/− mice was significantly slower than that in Wt mice. These results indicate that FGF-2 contributes to LLC tumor growth in vivo, and FGF-2 expression in LLC is a factor to mask the different tumor growth rates between Wt and Flt-1 TK−/− mice.

To further examine the role of FGF-2, we introduced FGF-2 cDNA into B16 cells, which express very low levels of FGF-2. Expression of FGF-2 in B16 was confirmed by Western blotting (Fig. 5C). The B16 transfected with control vector showed tumor growth slower in Flt-1 TK−/− mice than in Wt mice. However, the tumor growth rate of FGF-2-expressing B16 in

Figure 2. Suppression of the infiltration of macrophage lineage cells into tumors in Flt-1 TK−/− mice. A, macrophage lineage cells identified by anti-F/80 antibody (green), which recognizes the mouse macrophage marker. To-Pro-3 (blue) for nuclear staining. B, quantification of number of macrophage lineage cells in tumor tissue and in the border area of tumors (n = 10). *, P < 0.03; **, P < 0.0005; ***, P < 0.5 × 10−10. C, CD11b+ cells identified as by anti-CD11b antibody (green), which recognizes the mouse monocyte/macrophage marker. To-Pro-3 (blue) for nuclear staining. A and C, all confocal images (10×) represent HSML (a, b), B16 (c, d), and LLC (e, f) cells in Wt mice or Flt-1 TK−/− mice. B and T indicate the border area of tumors and tumor tissue, respectively. Scale bars, 150 μm. The dotted line indicates the margin between the tumor tissue and the border area of tumors such as the dermis. D, the number of CD11b+ cells in tumor tissue (n = 3). The score was obtained using 10 micrographic sites per mouse. *, P < 0.05; **, P < 0.005.
Flt-1 TK−/− mice was similar to that in Wt mice (Fig. 5D). These results further support the above suggestion that FGF-2 expression modifies tumor growth rate in Wt and Flt-1 TK−/− mice and masks a difference. In both RNAi and transfection of FGF-2 experiment, FGF-2 had a minor effect on angiogenesis and infiltration of macrophage lineage cells (Supplementary Fig. S2).

Macrophage lineage cells derived from BM play an important role in the promotion of solid tumors via Flt-1 signaling

The Flt-1 is mainly expressed in two cell types, vascular endothelial cells and macrophage lineage cells. To clarify which of these cell types is the major contributor to tumor growth using Flt-1 signal, we carried out BMT to make transplanted Wt/Wt and Wt/Flt-1 TK−/− mice. Genotyping of DNA from the ear as a control for Wt, Flt-1 TK+/−, and Flt-1 TK−/− and DNA from the tail (T−) as well as peripheral blood (PB−) of BM-transplanted mice were performed to check the efficiency of transplantation (Supplementary Fig. S3). The DNA from peripheral blood but not that from the tail in the Wt/Flt-1 TK−/− mice 4 weeks after transplantation showed the DNA pattern of the Flt-1 TK−/− type, indicating that BM was mostly replaced with Flt-1 TK−/− cells.

After BMT, HSML and B16 tumors whose growth was suppressed in Flt-1 TK−/− mice were implanted in Wt/Wt or Wt/Flt-1 TK−/− mice. Interestingly, HSML and B16 tumor growth was significantly slower in Wt/Flt-1 TK−/− mice on days 20 and 21 after inoculation compared with that in Wt/Wt mice (Fig. 6A). These results indicate that Flt-1 signaling in BM cells is directly involved in tumor growth.

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**Figure 3.** Several perivascular markers on stromal cells in tumor tissue. SMA, NG2, and PDGFRβ were used as major markers of perivascular cells including pericytes, fibroblasts, and perivascular progenitor cells. A, representative micrographs (20×) indicate each perivascular cell (green) in Wt mice or Flt-1 TK−/− mice with CD31 (red) and To-Pro-3 (blue). Scale bars, 75 μm. Graphs indicate HSML (B), B16 (C), and LLC (D) cells. Numbers of positive cells were quantified in confocal macrographs (n = 3). Results were obtained using 10 micrographic sites per mouse. *, P < 0.0005; **, P < 0.05 × 10−4.
Blood vessels in HSML and B16 tumors in BMT mice were examined by immunohistochemical staining. Tumor angiogenesis was significantly suppressed in Wt/Flt-1 TK−/− mice compared with that in Wt/Wt mice (Fig. 6B, a–d). The infiltration of macrophage lineage cells detected by F4/80 staining was also dramatically decreased in the tumor tissue of Wt/Flt-1 TK−/− mice (Fig. 6B, e–h). However, angiogenesis and the infiltration of macrophage lineage cells in the border area of tumors were not significantly different between Wt/Wt and Wt/Flt-1 TK−/− mice (Fig. 6C).

To evaluate whether the reduction of macrophage lineage cell infiltration in Flt-1 TK−/− condition is due to a decrease in survival of these cells or not, we checked the apoptotic cells in tumor tissue with TUNEL staining in BM-transplanted mice. In HSML tumor tissue, GFP+ myeloid cells were non-apoptotic (Supplementary Fig. S4). B16 tumor contained some apoptotic sites, but apoptotic myeloid cells (GFP+ and TUNEL+ double-positive cell) were not detected. Thus, it suggests that Flt-1 signal is used for infiltration but not for survival of macrophage lineage cells; however, we cannot rule out the possibility that Flt-1 signal is used for retention of cells in tumor tissue.

These results indicate that Flt-1 signaling on BM-derived cells such as macrophages is important for tumor angiogenesis and tumor growth in HSML and B16 cells.

Discussion

In this study, we have shown that the s.c. growth of HSML uterine tumors and B16 melanomas, but not Lewis lung carcinomas, is significantly dependent on Flt-1 signaling. Also, we showed that in Flt-1 signaling–deficient mice, slower tumor growth was associated with less tumor angiogenesis and less infiltration of monocyte/macrophage lineage cells into tumor tissue. Furthermore, by BMT, we found that Flt-1 signaling in BM-derived cells, most likely in macrophage lineage cells, is important for rapid tumor growth and aggressiveness; thus, the Flt-1 kinase itself and its downstream signaling are attractive targets for suppression of primary tumor growth and metastasis in cancer patients.

In mammals, Flt-1 protein is highly expressed in vascular endothelial cells and monocyte/macrophages. Under hypoxic conditions, smooth muscle cells were also reported to express Flt-1 in vitro. The question is which of these Flt-1–expressing cells are important for the promotion of tumor growth. Although PlGF, a Flt-1–specific ligand, promotes the survival of endothelial cells in vitro (6, 33), transplantation experiments using Flt-1 TK−/− BM cells clearly indicated that tumor growth was slower in Wt/Flt-1 TK−/− mice than in Wt/Wt mice (Fig. 6). Meanwhile, Flt-1 signal does not affect the apoptosis of myeloid cell in this model by TUNEL assay (Supplementary Fig. S4). Therefore, the regulation of tumor growth through Flt-1 signaling is related at least partly to BM-derived cells such as macrophages but much less so to vascular endothelial cells.

We reported that VEGF-dependent and PlGF-dependent migration activities were significantly decreased in the peritoneal macrophages derived from Flt-1 TK−/− mice compared with those from Wt mice. In addition, we showed a remarkable reduction of mRNAs for IL-6 and VEGF in peritoneal macrophages obtained from Flt-1 TK−/− mice (13, 26). Furthermore, Kerber and colleagues (34) examined migration activity of monocytes isolated from the venous blood of...
Flt-1 TK−/− mice and showed that the activity toward VEGF and PlGF was significantly suppressed. These experiments strongly suggest that a lack of Flt-1 signaling attenuates infiltration of macrophages into tumor tissue via VEGF or PlGF, which is secreted from tumor cells and other cells in tumor microenvironment, and that tumor angiogenesis stimulated by tumor-infiltrated macrophages is decreased in Flt-1 TK−/− mice.

Tumor progression of LLC even in Flt-1 TK−/− mice

Although the growth of HSML and B16 tumors was highly dependent on Flt-1 signaling, the growth rate of LLC was similar between Wt and Flt-1 TK−/− mice. We hypothesized that another angiogenic factor(s) is expressed in LLC and overcomes the deficiency of Flt-1 signal in host cells. The mRNA levels of VEGF-A were not significantly different among HSML, B16, and LLC, and the expression patterns of VEGF-A subtypes were also similar to these cells (Fig. 4C). We found that FGF-2 mRNA levels were >10-fold higher in LLC cells than in HSML and B16 cells (Fig. 4A). Recently, FGF-2 has been reported to play an important role in the resistance of tumors to anti–VEGF-VEGFR therapy in mice (35).

To examine the role of FGF-2 in LLC tumor progression, we suppressed FGF-2 expression with RNA silencing method. Interestingly, the LLC with FGF-2 silencing showed slower tumor growth in the Wt mice. Furthermore, growth rate of the LLC with FGF-2 silencing in Flt-1 TK−/− mice were significantly slower than that in the Wt mice (Fig. 5B). In another experiment, B16 cells transfected with FGF-2 cDNA mimicked the phenotype of original LLC (Fig. 5D). These results indicate that (a) FGF-2 contributes to LLC-tumor growth in vivo and (b) FGF-2 expression in LLC is a factor to mask the different tumor growth rates between Wt and Flt-1 TK−/− mice.

Another question is which form(s) of FGF-2 in LLC, secreted form or intracellular form, is important for these phenotypes. In the literature, several groups already showed that 24-kDa intracellular-type FGF-2 localizes in the nuclei and promotes cell proliferation (36–39). We found that FGF-2 expressed in LLC is mostly the intracellular type (Fig. 4B), which might directly stimulate cell proliferation. A secreted type of FGF-2 seems a minor population in LLC.

Flt-1 could be an attractive target for suppression of tumor growth

Lutten and colleagues recently published that the MF1 antimouse Flt-1 neutralizing antibody from ImClone has a clear tumor-suppressing effect, although antimouse VEGFR-2 neutralizing antibody DC101 had a better effect (40). In addition, several reports described that antihuman Flt-1 neutralizing antibodies had a cancer-suppressive
Figure 6. Lack of Flt-1 signaling on BM cells inhibits tumor progression. A, growth curves for HSML (a) and B16 (b) tumors s.c. injected in the BMT mice. Tumors were measured every 2 days from day 7 postinoculation (n = 13–15). *, P < 0.05; **, P < 0.01. Recipient Wt mice transplanted with BM derived from Wt mice or Flt-1 TK−/− mice are indicated as Wt/Wt and Wt/Flt-1 TK−/− mice, respectively. B, tumor angiogenesis (a–d) and infiltration of macrophage lineage cells (e–h) detected by immunohistochemical staining for CD31 or F4/80. Confocal micrographs (20×) of the tumor sections are displayed for CD31+ cells (red), F4/80+ cells (green), and the nucleus (blue). B and T indicate the border area of tumors and tumor tissue, respectively. Scale bars, 150 μm. The dotted line indicates the margin between tumor tissue and border areas such as the dermis. C, tumor angiogenesis (a) and number of macrophage lineage cells (b) in the border area of tumors and tumor tissues (n = 13–15). *, P < 0.01.
effect when Flt-1—expressing human lymphoma or breast cancer cells were inoculated in immunodeficient mice (41–44). Because Flt-1 has a 10-fold higher affinity to VEGF compared with VEGFR-2, a higher concentration of anti-Flt-1 antibody may be required to obtain a better tumor-suppressive effect. Furthermore, some neutralizing antibodies to Flt-1 compete with the VEGF and release it from Flt-1. In this case, VEGF released from Flt-1 might bind and activate VEGFR-2. Thus, it seems to be important to develop Flt-1 blocking antibody with better quality such as noncompetitive type.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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