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High-Resolution Imaging and Antitumor Effects of GFP⁺ Bone Marrow-Derived Cells Homing to Syngeneic Mouse Colon Tumors

Niklas K. Finnberg,* Lori S. Hart,[†]
Nathan G. Dolloff,* Zachary B. Rodgers,[†]
David T. Dicker,* and Wafik S. El-Deiry*

From the Penn State Hershey Cancer Institute,* Hershey, Pennsylvania; and the Department of Radiation Oncology,[†] University of Pennsylvania, Philadelphia, Pennsylvania

Bone marrow-derived cells (BMDCs) participate in the growth and spread of tumors of the breast, brain, lung, and stomach. To date, there are limited reports of bone marrow involvement in colon cancer pathogenesis, but such findings would have the potential to generate novel treatments for colon cancer patients. We have established a mouse model for imaging BMDCs from whole tumor to single-cell resolution, whereby the bone marrow of lethally irradiated host animals is reconstituted with EGFP-expressing bone marrow cells from matched TgActb(EGFP) donors. The BM transplants yield mice with fluorescently labeled bone marrow, and so BMDCs can subsequently be monitored within a tumor through optical imaging. Successful BM reconstitution was confirmed at 8 weeks after transplantation, when surviving BALB/c mice were injected with CT26 mouse colon cancer cells. We find that up to 45% of cells dissociated from the tumors are GFP⁺ and approximately 50% of Lin⁺, CD11b⁺, and CD3⁺ cells express high levels of GFP. Notably, tumor growth is reduced in BM transplanted animals, compared with untransplanted host mice or EGFP-expressing BM donor mice. A needed next step is to separate the molecular and cellular (eg, T cells, NK cells, macrophages) bases of the antitumor effect of the BMDCs from any protumorigenic effect that could be subverted for therapeutic gain. (*Am J Pathol* 2011, 179:2169–2176; DOI: 10.1016/j.ajpath.2011.07.028)

Cancer has been regarded as a disorder in which transformed cells grow in an autonomous tissue-invasive manner, and much of the effort to develop cancer therapy has been focused on targeting the transformed cell itself. It is

becoming increasingly clear, however, that to manage cancer the perspective of the disease as a single-cell disorder has to change to that of a complex disease in a heterogeneous microenvironment in which a multiplicity of cellular interactions occur within the malignant tissue.¹

Many distinct cell types populate the tumor microenvironment and play a role in tumor proliferation and progression.² These cell types include bone-marrow derived cells (BMDCs) such as monocytes (CCR2⁺, CX3CR1^{low}, and GR1⁺), macrophages (CCL15⁺, CCL20⁺, CXCL10⁺, and CXCL11⁺), hematopoietic stem cells (CD34⁺, CD59⁺, CD90/Thy1⁺, CD38^{low/-}, c-Kit^{-/low}, and Lin⁻), pericyte precursor cells (CD45⁻ and CD31/VEGFR/VE-cadherin⁺), and mesenchymal stem cells (CD73/CD90/CD105⁺ and CD14/CD34/CD45⁻).^{2–4} Laboratory data support a role for some of the cells in tumor progression. Csf1^{-/-} mice are deficient in the recruitment of monocytes to tumor sites, and subsequently display attenuation of late stage progression and metastasis.⁵ Some BMDCs may aid in the triggering of immune-mediated tumor rejection; however, cancer cells often escape immune-mediated surveillance and control BMDCs to promote invasion, angiogenesis, and metastasis by excreting soluble factors that promote the growth and expansion of new blood vessels from the pre-existing vasculature or through the remodeling of the extracellular matrix.^{1,2,6} Such soluble factors include VEGF, bFGF, TNF, HGF, EGF, PDGF, CXCL12, IL-6, IL-8, Cox-2, IGF, and Wnt.^{7,8}

Nonetheless, the presence of BMDCs in the tumor microenvironment may be associated with a favorable prognosis by inhibiting tumor growth and invasiveness.^{2,9} Melanoma patients with moderate-to-marked lymphocytic infiltrates have a significantly better prognosis and a threefold higher 5-year survival, compared with

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N.K.F. and L.S.H. contributed equally to the present work.

Address reprint requests to Wafik S. El-Deiry, M.D., Ph.D., Hematology/Oncology Division, Penn State Hershey Cancer Institute, 500 University Drive, Room T4423, Hershey, PA 17033. E-mail: weldeiry@hmc.psu.edu.

patients with minimal lymphocytic infiltration.^{10,11} CD8⁺ T cells possess the ability to directly kill cancer cells, and there is an association between patient survival and CD8⁺ T cell infiltrates.¹² Tumor-infiltrating B cells (TIL-B cells) are present in 25% of breast cancers and comprise up to 40% of the TIL population.¹³ TIL-B cells are often found together with CD4⁺ and CD8⁺ T cells and dendritic cells. Lymph node-negative breast cancer with a gene signature indicative of TIL-B cells has a better prognosis than lymph-node negative BC without the gene signature.¹⁴ Likewise, together with CD8⁺ and CD4⁺ T cells, TIL-B cells have been implicated in favorable survival rates in medullary breast cancer.^{15–17}

Thus, there is a high level of complexity with regard to assessing the influence of BMDCs on tumorigenesis. The temporospatial pattern of the inflammatory response may determine whether BMDC infiltration supports or inhibits tumor growth,¹ and elucidating the dynamics of BMDC migration in relation to changes in tumor volume may be essential to resolving this question. To this end, we developed an *in vivo* model to investigate the dynamics of BMDC tumor infiltration. We transplanted BM from TgActb(EGFP) mice to nontransgenic recipient mice injected subcutaneously with syngeneic colorectal tumor cell lines and then monitored infiltrating BMDCs through noninvasive, high-resolution optical imaging. TgActb(EGFP) is a transgenic gene that expresses green fluorescent protein under the control of a chicken β -actin promoter. This model may help in elucidating the temporal and spatial dependency of different hematopoietic lineages, and may also help to elucidate the cell types influencing the tumorigenic processes. As a consequence, novel cellular targets for cancer therapy may be identified.

Materials and Methods

Mice

Four- to five-week-old male BALB/cByJ and CByJ.B6-Tg(CAG-EGFP)1Osb/J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All mice were housed in a controlled environment with regard to light, temperature, and humidity. Mice were euthanized through carbon dioxide-induced asphyxiation. All animal care and treatment procedures used were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Bone Marrow Transplantation

Recipient mice were given water containing autoclaved, acidified antibiotics (10 mg/mL polymyxin B sulfate and 100 mg/mL neomycin; both from Sigma-Aldrich, St. Louis, MO) 1 week before irradiation and 2 weeks after irradiation. Mice were exposed to whole-body irradiation with a single lethal dose of 7.8 Gy from a ¹³⁷Cs source (at 1.4 Gy/min). Four hours after the irradiation, mice were injected intravenously with 5×10^6 syngeneic BM cells isolated from the femur and tibia of wild-type donor mice (C57BL/6J or BALB/cByJ) or TgActb(EGFP) mice [C57BL/6-Tg(CAG-EGFP)1Osb/J and CByJ.B6-Tg(CAG-

EGFP)1Osb/J]. Recipient mice were used for syngeneic tumor cell injection 8 weeks after bone marrow transplantation (BMT).

Cell Lines and Syngeneic Tumor Engraftment

CT26 mouse colon carcinoma cells (ATCC, Manassas, VA) were maintained and cultured in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin streptomycin. Mice subjected to BMT were injected subcutaneously under ketamine/xylazine anesthesia on the left and right flank with 1×10^6 CT26 (BALB/cByJ) cells mixed with 50% (v/v) Matrigel (BD Biosciences, San Jose, CA). Either the injected cells were allowed to engraft in the recipient for 3 weeks and were subsequently subjected to noninvasive imaging or the mice were euthanized and BMDCs isolated from the tumor tissues.

Flow Cytometry

Flow cytometry was performed using an Elite ESP flow cytometer (Beckman-Coulter, Miami, FL). To analyze BM from donor mice and to confirm donor BM reconstitution in transplanted mice, mice were euthanized (at least 8 weeks after BMT) and BM was harvested by flushing the femur and tibia with PBS containing 1% FBS. Single BM cells were analyzed based on forward scatter, side scatter, and EGFP fluorescence. Additional immunofluorescence and flow cytometry was performed to detect Bmi1⁺ (ab14389; Abcam, Cambridge, MA), CD11b⁺ (ab6332; Abcam), and lineage-negative (Lin⁻; lineage antibody cocktail; BD Pharmingen, San Diego, CA) cells among the EGFP⁺ BM subpopulations. To analyze immune cell infiltration of tumors, the tumors were excised, minced, and incubated with collagenase (5 mg/mL; Sigma-Aldrich) for 1.5 hours at 37°C. Dissociated single cells were washed two times with PBS, and immunofluorescence analysis was performed with the lineage antibody cocktail to detect the lineage status of EGFP⁺ tumor-infiltrating BM cells.

Immunofluorescence and Immunohistochemistry

After necropsy, tumor grafts were collected and fixed in 4% paraformaldehyde overnight at 4°C, embedded in paraffin, and cut into 4- μ m sections. Cut sections were stained for CD3 (Leica Microsystems-Novocastra Laboratories, Newcastle upon Tyne, UK), B220 (Abcam), NK1.1 (Invitrogen-Caltag, Carlsbad, CA), and EGFP (Santa Cruz Biotechnology, Santa Cruz, CA). Briefly, slides were rehydrated and subjected to antigen retrieval through boiling in 1 mmol/L citric acid buffer (pH 6.0). For immunohistochemistry, endogenous peroxidases were blocked by submerging slides in 3% H₂O₂. Primary antibodies were left on sections overnight at 4°C. Sections were washed and primary antibodies were conjugated using either the ImmPRESS peroxidase polymer detection reagent (Vector Laboratories, Burlingame, CA) for immunohistochemistry or DyLight 488- and DyLight 549-conjugated donkey antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) for immunofluores-

cence. In the case of DyLight-conjugated antibodies, counterstaining was performed using DAPI. Representative depiction of immunohistochemistry and immunofluorescence was made using iVision 4.0.16 software (BioVision Technologies, Exton, PA).

Noninvasive High-Resolution Optical Imaging

Mice were anesthetized (intraperitoneal ketamine/xylazine) and shaved. Detection with live imaging was performed using a Maestro version 2.10.0 *in vivo* imaging system and Nuance version 2.4.0 high-resolution stereomicroscope imaging system with background autofluorescence correction (both from CRI-Caliper Life Sciences, Hopkinton, MA). Regions of interest were identified over the tumor areas and image cubes captured using the blue filter setting (Maestro) or were custom built (acquisition wavelength 500 to 550 nm; Nuance).

Results

To detect the migratory patterns of BMDCs, we decided on an approach in which bone marrow from syngeneic TgActb(EGFP) transgenic animals expressing enhanced GFP under the chicken β -actin promoter was transplanted into wild-type (nontransgenic) recipient mice (Figure 1). Syngeneic mouse colon carcinoma cells

(CT26) were then injected into syngeneic BALB/C and mice. After 3 weeks of subcutaneous engraftment of the cell lines, we performed noninvasive, high-resolution imaging of the established syngeneic tumors to track BMDC migration.

TgActb(EGFP) mice express EGFP ubiquitously in all tissues that can be detected using excitation light, except for red blood cells and hair.¹⁸ To verify EGFP expression in different cell lineages of the bone marrow in TgActb(EGFP) mice, we performed flow cytometry analysis. We used the side-scatter and forward-scatter parameters to distinguish cellular constituents from debris and noncellular elements. Through this analysis, we readily identified three distinct cell populations present in both nontransgenic and transgenic bone marrow (Figure 2A), consistent with the presence of lymphocytes and primitive blast cells (population 1), monocytes (population 2), and granulocytes (population 3), as previously reported.¹⁹ We labeled the bone marrow cells with antibodies for Bmi1^{20,21} (an adult hematopoietic stem cell marker) and CD11b²² (a macrophage, granulocyte, NK cell, and monocyte marker). Cells from population 1 were Bmi1⁺, whereas cells from populations 2 and 3 were found (weakly) positive for CD11b, suggesting that the forward scatter and side scatter analysis did identify distinct BM cell populations of varying differentiation (Figure 2B).

Analysis of EGFP expression in cell populations 1, 2, and 3 showed that high EGFP expression could be de-

Figure 1. Schematic of the experimental BMDC transplantation approach. EGFP-expressing bone marrow was isolated from BALB/c TgActb(EGFP) mice and transplanted into lethally irradiated wild-type (nontransgenic) BALB/c recipient mice. Transplanted bone marrow was allowed to engraft for 8 weeks, and then syngeneic colorectal tumor cells (CT26) were injected subcutaneously into recipient mice. Three weeks later, the CT26 syngeneic grafts were monitored for EGFP-expressing cells by noninvasive, high-resolution imaging.

Figure 2. Characterization of bone marrow GFP expression in TgActb(EGFP) mice. Undifferentiated BM cells expressing GFP were analyzed by flow cytometry using side scatter (SS) and forward scatter (FS) analysis. **A:** Three distinct populations were identified in the bone marrow of TgActb(EGFP) mice. **B:** The blast and lymphocyte BM population 1 expresses high levels of Bmi1, relative to the other bone marrow populations, and population 3 expresses higher levels of CD11b. **C:** High EGFP expression predominates in BM populations 1 and 2 in TgActb(EGFP) mice, but a substantial number of population 3 BM cells also express high levels of EGFP. **D:** Flow cytometric analysis of EGFP expression (high EGFP expression, GFP hi; low to absent EGFP expression, GFP lo) in different BM lineages from TgActb(EGFP) mice.

ected in a large portion of all three populations (Figure 2C); however, a larger proportion of population 1 and 2 cells expressed high levels of EGFP, compared with population 3 cells. Overall, the number of bone marrow nucleated cells (BMNC) expressing high levels of EGFP (GFP^{hi}) was 31.4%, compared with 68.6% for cells with absent to low expression levels of EGFP (GFP^{neg/lo}) (Figure 2D). Approximately 80% of all BMNCs were Lin⁺ (ie, the cells expressed one or more BM lineage markers). A total of 40.4% of the Lin⁺ BMNCs were also GFP^{hi}, whereas 38.7% were GFP^{lo}, suggesting that approximately half of the lineage-committed cells in the bone marrow expressed high levels of EGFP (Figure 2D). Similar distribution of GFP^{hi} versus GFP^{neg/lo} was also found in CD11b⁺ and CD3⁺ (T cell) cells (Figure 2D). This suggests that imaging of tumor-infiltrating TgActb(EGFP) BMDCs, using GFP fluorescence as a marker, captures a substantial proportion of the population of total BMDCs, and that important BMDC modulators of tumor growth such as macrophages and T cells can be readily identified.

Mice were subjected to BMT with TgActb(EGFP) bone marrow (Figure 3A), and the engrafted bone marrow was analyzed 8 weeks after transplantation. As evident from flow cytometry analysis for EGFP expression, mice subjected to BMT with TgActb(EGFP) marrow retained EGFP expression, in contrast to TgActb(EGFP) mice not receiving any BMT (Figure 3B). The proportion of GFP^{hi} Lin⁺ cells were slightly increased in BMT mice, relative to that in TgActb(EGFP) mice (30.5% versus 49.5%, respectively) (Figure 3C). Of note, there was a significant increase in the number of GFP^{hi} CD3⁺ cells in BMT mice, compared with TgActb(EGFP) mice (Figure 3D). EGFP expression in the bone marrow of BMT mice was retained or even somewhat increased, suggesting that tumor-infiltrating BMDCs can be visualized using high-resolution imaging optical imaging for GFP.

Tumors were established at 3 weeks after subcutaneous injection of syngeneic CT26 colon carcinoma cells (Figure 4A). EGFP fluorescence was not detected by optical imaging in tumors of wild-type mice (BALB/c-wt) that were not subjected to BMT, but was readily detected in TgActb(EGFP) mice (not otherwise subjected to BMT) and mice subjected to BMT with TgActb(EGFP) bone marrow [BMT (EGFP)] (Figure 4B). Surprisingly, CT26 tumors grew approximately four times larger in mice that were not subjected to BMT [BALB/C and TgActb(EGFP)], compared with mice receiving BMT [BMT(EGFP)] (Figure 4, A and C).

The noninvasive, high-resolution imaging showed that EGFP expression was abundant around tumor vasculature, although the vasculature itself remained negative (Figure 5A), suggesting that BMDCs did not incorporate into the tumor vasculature. Immunohistochemical analysis for GFP showed that a larger portion of EGFP-expressing cells were present in CT26 tumors growing on TgActb(EGFP) mice, compared with BMT mice (Figure 5B). Cells staining positively for EGFP frequently included stromal cells or fibroblasts in the TgActb(EGFP) mice (Figure 5B), but such cells did not stain positively for EGFP in BMT mice (data not shown). Furthermore, in BMT

Figure 3. Characterization of transplanted TgActb(EGFP) bone marrow. **A:** Survival curve for animals receiving TgActb(EGFP) bone marrow (BMT) and mock injection (no BMT) after a single lethal dose (7.8 Gy) of γ irradiation (IR). **B:** A comparative analysis of retained EGFP expression in transplanted bone marrow cells 8 weeks after BMT (BMT7 and BMT5) to that of bone marrow cells from transgenic TgActb(EGFP) mice [TgActb(EGFP)]. Analysis of GFP expression in Lin⁺ and CD3⁺ bone marrow cells (**C** and **D**) from wild-type (BALB/c-wt) mice, TgActb(EGFP) mice, and recipients of EGFP-expressing bone marrow [BMT(EGFP)].

mice EGFP⁺ BMDCs localized around necrotic areas of the tumor, but the distribution of EGFP⁺ cells in CT26 tumors of TgActb(EGFP) did not show such heterogeneity (Figure 5C). Thus, BMDCs could be detected in syngeneic CT26 tumors growing subcutaneously on mice receiving BMT from syngeneic TgActb(EGFP) mice, and these BMDCs did not include common components of the cellular matrix such as fibroblasts.

Similar numbers of nonadherent cells, isolated from CT26 tumors growing subcutaneously in TgActb(EGFP) mice and BMT mice, expressed low to high levels of EGFP (Figure 5D). Approximately 8% of these cells were GFP^{hi}, and 3.46% were GFP^{hi}/Lin⁺ (Figure 5D). Of the GFP^{neg/lo} cells, 69% expressed no lineage marker and 23.3% were Lin⁺ (Figure 5D). Immunofluorescence of slide sections from CT26 tumors in BMT mice indicated a certain level of overlap between EGFP⁺ cells and the expression of B220 (B cell marker), CD3 (T cell marker), and NK1.1 (NK cell marker) (Figure 5E), suggesting that some of the EGFP⁺ BMDCs were B, T, and NK cells. Although readily detected by optical imaging, a relatively

Figure 4. Reduced tumor growth after BMT (**A**) and detection of GFP-expressing BMDCs (**B**) in syngeneic grafts of mouse colorectal tumor cells. CT26 syngeneic tumors engrafted in BALB/c-wt mice, TgActb(EGFP) mice and mice receiving EGFP-expressing bone marrow [BMT (EGFP)] were imaged for GFP fluorescence by noninvasive optical imaging. The tumor surface area (**C**) was significantly larger ($P < 0.05$, Student's *t*-test) in mice not subjected to BMT, compared with mice receiving BMT ($n = 4$ in each group). Error bars indicate 1 SD.

Figure 5. High-resolution imaging and fluorescence-activated cell sorting analysis of tumor-infiltrating BMDCs. **A:** Tumor-infiltrating EGFP⁺ BMDCs were readily detected with high-resolution optical imaging in CT26 colorectal tumors engrafted in the flank of BALB/c mice subjected to EGFP⁺ BMT. **B:** Infiltration of EGFP⁺ cells was confirmed by immunohistochemistry for EGFP. **C:** Immunohistochemical analysis of tissue sections from mice receiving EGFP⁺ bone marrow [BMT (TgActbEGFP)] shows BMDCs primarily localizing around necrotic areas in the tumor microenvironment, in contrast to CT26 tumors growing in TgActb(EGFP) mice. **D:** Quantification by flow cytometry of the number of EGFP⁺ cells infiltrating the CT26 colorectal tumor grafts and the number of EGFP⁺ cells expressing a lineage marker or markers (Lin⁺) using isotopic nonspecific phycoerythrin-conjugated antibodies (Ig-PE) as negative control. **E:** Immunofluorescence suggests overlap between cells expressing EGFP [α-GFP (DyLight488)] and those expressing B220 (B cell marker), CD3 (T cell marker), and NK1.1 (NK cell marker) (DyLight549) located in syngeneic subcutaneous CT26 grafts. DAPI was used to visualize cellular nuclei. Arrowheads represent GFP⁺ cells that are also positive for markers of various lineages.

small number of BMDCs expressed high levels of EGFP and slightly less than half of those cells were committed to a particular BM lineage, such as B or T cell lineage.

Discussion

Cancer is not a monocellular disease characterized simply by autonomous invasive proliferation of transformed cells, but rather is a disease that relies on signals from the tumor stroma. Tumor-infiltrating BMDCs have the potential to modulate the aggressiveness of tumor growth. Thus, elucidating the role of BMDCs in tumor development may be another step toward the goal of providing novel therapies. To address the dynamic role of BMDCs, we developed a model in which we transplanted bone marrow from TgActb(EGFP) mice to wild-type recipient mice; we then injected syngeneic CT26 colon carcinoma cells subcutaneously to establish tumors, and monitored the migratory pattern of BMDCs from the EGFP-expressing bone using noninvasive, high-resolution imaging.

Notably, TgActb(EGFP) transgenic mice expressed high levels of EGFP in several hematopoietic lineages (Figure 2), and EGFP expression was particularly high among undifferentiated cells in the bone marrow. Overall, one third of all BMNCs expressed high levels of EGFP, as did approximately 50% of Lin⁺, CD11b⁺, and CD3⁺ cells derived from the bone marrow (Figure 2D). Compared with other mouse models that express EGFP (under, for example, the ROSA26 locus), TgActb(EGFP) mice have

cells that display bright fluorescence and have particularly high expression of EGFP in the bone marrow.²³ We expected that the TgActb(EGFP) bone marrow would increase the optimal sensitivity for noninvasive optical imaging. Notably, the BMT did not negatively influence the proportion of green fluorescent cells in the bone marrow (Figure 3). In fact, a slight increase in the number of Lin⁺/GFP^{hi} and a substantial increase in CD3⁺/GFP^{hi} bone marrow cells were observed after BMT (49.5% versus 30.5%, and 1.68% versus 0.24%, respectively) (Figure 3, C and D). This was reflected in an overall increase [2.69% versus 1.32% for BMT(EGFP) and TgActb(EGFP) mice, respectively] in the total number of CD3⁺ cells in the bone marrow of animals subjected to BMT, as well as an enrichment of highly green fluorescent CD3⁺ cells (Figure 3D).

Intriguingly, syngeneic CT26 tumors showed attenuated growth in BMT mice, compared with either wild-type mice or TgActb(EGFP) mice (Figure 4, A and C). The reason for this is not known. Some data suggest a role for graft-versus-leukemia effect in samples from patients after allogeneic or syngeneic BMT.²⁴ Other preclinical data suggest that the graft-versus-leukemia effect is primarily an allogeneic effect that is inseparable from graft-versus-host disease.²⁵ Thus, it is unlikely, given the syngeneic nature of our BMT, that the reduced tumor growth observed in mice subjected to BMT was a result of graft-versus-host disease. Furthermore, we did not find any evidence suggestive of graft-versus-host disease in the established CT26 tumors (data not shown). It is unclear

what the underlying reason might be for the tumor-suppressive role of BMT. Given the lethal nature of the radiation exposure, it is difficult to tease out whether late radiation effects on nonhematopoietic tissues or the transplanted bone marrow itself contributed to the growth inhibitory effect seen on the subcutaneous tumor grafts. Mice receiving BMT did have a larger proportion CD3⁺ cells than TgActb(EGFP) mice (Figure 3D), indicating that mice receiving BMT had more T cells in their bone marrow, which could potentially elicit more vivid antitumor immunity. On the other hand, the transplanted bone marrow may have lost some of the tumor-promoting properties of the original bone marrow in the transplantation process. Future experiments should characterize how BMT can alter tumor-promoting and tumor-suppressive functions of the bone marrow in the context of solid malignancies. We are currently undertaking experiments to elucidate more precisely the mechanism or treatment that inhibits the growth and expansion of the CT26 cells in syngeneic mice after BMT.

Using noninvasive, high-resolution optical imaging, we successfully monitored EGFP-expressing BMDCs infiltrating our CT26 tumors (Figure 5A) and verified that a population of infiltrating BMDCs expressed high levels of EGFP concomitant with the expression of BM lineage markers B220, CD3, and NK1.1 (Figure 5, D and E). Our data suggest that our optical imaging method may provide us with greater sensitivity, compared with flow cytometry analysis, and that cells that would be classified as GFP^{neg/lo} under flow cytometry are readily recognized in the tumor grafts by noninvasive optical imaging (data not shown).

Others have shown, using invasive real-time multi-color imaging cell tracking techniques with spinning disk confocal microscopy, that regulatory T lymphocytes (Treg cells) migrate in proximity to blood vessels and that dendritic-like cells, myeloid cells, and carcinoma-associated fibroblasts exhibit greater mobility in the tumor periphery than in the tumor core.²⁶ That study also addressed the effect of tumor hypoxia on the migratory pattern of the stromal cells. We are currently investigating whether similar data can be generated through noninvasive imaging.

In conclusion, we show that TgActb(EGFP) transgenic mice express GFP in several hematopoietic lineages and that transplantation with TgActb(EGFP) bone marrow may allow for the detection of tumor-infiltrating BMDCs in syngeneic tumor models. The EGFP-expressing BMDCs populating the tumor microenvironment were of different lineages; these BMDCs did not represent cellular matrix components such as tumor-associated fibroblasts, which may narrow down the cell types detected through this method. The present study provides an excellent *in vivo* experimental model in which the temporal dynamics of tumor-infiltrating BMDCs may be monitored in an immuno-competent host. With this model, novel therapies targeting BMDCs for the inhibition of tumor progression may be investigated, and it may be possible to use specific identified tumor-infiltrating BMDCs to deliver therapeutic cargo.

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