Abstract

Although natural killer (NK) cells are recognized as direct antitumor effectors, the ability of NK cells to control cancer-associated inflammation, which facilitates tumor progression, remains unknown. In this study, we demonstrate that NK cells control tumor-promoting inflammation through functional modification of neutrophils. NK cells control the tumor-promoting function of neutrophils through an IFNγ-dependent mechanism. Tumor progression in an NK cell-depleted host is diminished when the IL17A–neutrophil axis is absent. In NK cell-depleted mice, neutrophils acquire a tumor-promoting phenotype, characterized by upregulation of VEGF-A expression, which promotes tumor growth and angiogenesis. A VEGFR inhibitor which preferentially suppressed tumor growth in NK cell–depleted mice was dependent on neutrophils. Furthermore, the systemic neutropenia caused by an antimetabolite treatment showed an anticancer effect only in mice lacking NK cells. Thus, NK cells likely control the tumor-promoting and angiogenic function of neutrophils. Cancer Immunol Res; 6(3); 348–57. ©2018 AACR.

Introduction

The tumor microenvironment consists of not only transformed cancer cells, but also various normal cells that control tumor progression (1, 2). Of such tumor stromal cells, innate and adaptive immune cells and their effector molecules regulate tumor development with an immune editing process (3–5). Natural killer (NK) cells contribute to immunological surveillance of development and subsequent growth of cancer cells through a variety of effector mechanisms, including perforin/granzymes or death receptor dependent cytotoxicity and antitumor cytokine production (6–8). Besides their direct antitumor effector function, NK cells also control subsequent adaptive immune responses (9–11).

In contrast to antitumor immune responses, tumor-promoting immune responses, often regarded as an inflammation, contribute to malignant cancer progression (2, 12, 13). The inflammatory immune cells involved in such tumor-promoting immunity include innate cells as well as adaptive lymphocytes. Such cells produce a variety of inflammatory cytokines, chemokines, and growth factors (2, 13). Neutrophils, abundant circulating innate inflammatory cells, respond to infection or tissue damage (14–16). Neutrophils mediate host defense through multiple functions including phagocytosis, reactive oxygen species (ROS) production or release of granules containing antimicrobial peptides and proteases (14–17). Neutrophils also aid tumor-promoting immune responses (18–20). Indeed, clinical studies support the use of circulating neutrophil counts or neutrophil-to-lymphocyte ratios (NLRs) as a predictive biomarker for poor outcome or distant metastasis in cancer patients (21–25). The function of neutrophils may be converted from tumor-suppressing to tumor-promoting in the tumor-bearing state. Indeed, tumor-associated neutrophils (TANs) can be classified into antitumor (N1) or tumor-promoting (N2) subpopulations (26). Although N2 TANs were driven by the presence of TGFβ (26), IFNγ polarized TANs toward N1 phenotype (27). These studies suggest that an improved understanding of how TANs switch from the antitumor to tumor-promoting state will aid efforts to control cancer progression.

Although both NK cells and neutrophils are components of tumor stroma, there is no indication of whether the interaction between NK cells and neutrophils is involved in tumor control. In this study, we demonstrate that NK cells control tumor-promoting inflammation through the functional modification of neutrophils. NK cells control the tumor-promoting function of neutrophils via an IFNγ-mediated mechanism. Therefore, tumor progression in an NK cell–depleted host is diminished when the IL17A–neutrophil axis is absent. In NK cell–depleted mice, neutrophils acquired a tumor-promoting phenotype as seen in their VEGF-A upregulation to promote tumor growth and angiogenesis. The VEGFR inhibitor suppressed tumor growth in NK cell–depleted mice through mechanisms dependent upon neutrophils. Furthermore, the systemic neutropenia induced by an antimetabolite treatment showed an anticancer effect only in mice lacking NK cells. Thus, NK cells likely control the tumor-promoting and angiogenic function of neutrophils.
Materials and Methods

Mice

Wild-type C57BL/6 (WT) mice were purchased from CLEA Japan, Inc. IFN-γ−/− (IFN-γ KO) and IL17−/− (IL17 KO) mice on B6 background were kindly provided by Dr. Y. Iwakura (Tokyo University of Science, Chiba, Japan) and maintained at Laboratory Animal Research Center, Institute of Medical Science, University of Tokyo. In some experiments, groups of mice were treated with anti-CD4 (clone GK 1.5), anti-CD8 (clone 53.6.2), anti-NK1.1 (clone PK136), or anti-asialo-GM1 (asGM1; Wako Chemicals) on days −3 and −1, anti-CXCR3 (clone CXCR3-173, Bio X Cell) on days −1, 0, 2, 4, and 6 or anti-Ly6G (clone 1A8, Bio X Cell) on day −1 and 3 (where day 0 is the day of primary tumor inoculation). Antibodies against CD4, CD8, and NK1.1 were purified from hybridoma cells. In some experiments, mice were treated with SU5416 [AdooQ BioScience, 30 mg/kg intraperitoneally (i.p.)] for 7 days from the day of tumor inoculation. To induce systemic neutropenia, mice were injected intravenously (i.v.) with 150 mg/kg 5-fluorouracil (5-FU) at day 0 relative to the tumor implantation. All experiments were approved and performed according to the guidelines of the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences of the University of Tokyo, the Care and Use of Laboratory Animals Committee of the Graduate School of Pharmaceutical Sciences of the University of Science, Chiba, Japan) and maintained at Labora-

Cells and reagents

MCA205-Luc2 cells stably expressing luciferase with a CMV promoter were established as previously described (29). The luciferase-expressing 3LL cell line (3LL-Luc2) was prepared as previously described (29). pGL4.50 [Luc2/CMV/Hygro] vector, and n-luciferin were obtained from Promega. Lipofectamine 2000 was purchased from Invitrogen. Hygromycin B was obtained from Nacalai Tesque. Those cell lines were kept in culture no more than a month and have not been reauthenticd.

Bioluminescent imaging of in vivo cancer cell growth

MCA205-Luc2 cells or 3LL-Luc2 cells were inoculated subcutaneously (s.c.). To obtain bioluminescent images, mice were injected with D-luciferin (Promega, 150 mg/kg i.p.) and luminescence was measured with an in vivo imaging system (IVIS Lumina II) 10 minutes after the D-luciferin injection. Analyses of regions of interest were performed using Living Image 4.2 Software (Caliper Life Science) to determine the light emitted from the tumor. For each mouse, all values were determined as photons per second ( photon/sec).

Tumor-infiltrating lymphocyte (TIL) isolation and flow cytometry

MCA205-Luc2 cells (10^5) were inoculated s.c. Seven days after inoculation, tumor tissues were dissected, minced, and digested with 2 mg/mL collagenase (Roche Diagnostics GmbH) and 0.1 mg/mL DNase I (Roche Diagnostics GmbH) in serum-free RPMI 1640 for 1 hour at 37°C. Samples were further homogenized through wire mesh. For collecting tumor culture supernatant, the tumor homogenates were cultured in complete RPMI 1640 media for 24 hours and the cell-free supernatant were harvested. For flow cytometry analysis, cells were first preincubated with anti-CD16/32 (2.4G2) to avoid non-specific binding of antibodies to FcR. The cells were then
incubated with a saturating amount of fluorescein-conjugated monoclonal antibody (mAb). Antibodies against CD3ε (2C11), NK1.1 (PK136), CD11b (M1/70), Gr-1 (RB6-8C5), and Ly6G (1A8) were purchased from Biolegend, eBiosciences, or Tombo Bioscience. Flow cytometry analysis was performed with FlowJo software (Tree Star).

Matrigel plug angiogenesis assay

Growth factor–reduced Matrigel (BD Biosciences) containing MCA205-Luc2 cells (10^5) was s.c. inoculated and 1% Evans blue was injected intravenously into mice at 7 days after the inoculation. Ten minutes later, Matrigel plugs were removed after perfusion by saline and incubated with formamide for 48 hours. The amount of Evans blue dye was quantified by absorbance at 620 nm and normalized to the weight of Matrigel.

Neutrophil isolation and in vitro culture

To isolate tumor-infiltrating neutrophils, tumor samples were harvested 7 days after the inoculation and CD11b^+ Ly-6G^+ neutrophils were isolated by cell sorting (>90% purity, FACS Aria Special Order, BD Bioscience). To isolate peritoneal neutrophils, mice were injected i.p. with 7.5% casein solution (1 mL) in PBS the day before casein injection. Ly6G^- neutrophils were purified using a Mojosort cell separation kit (Biolegend). Briefly, cells were first incubated with CD16/32 (2.4G2) mAb to avoid non-specific binding of antibodies to FcyR. The cells were then incubated with a saturating amount of biotin-conjugated Ly6G mAb and streptavidin-conjugated magnet beads. Ly6G^- neutrophils were isolated using a Mojosort magnet. Peritoneal neutrophils (10^6 cells) were cultured with tumor supernatant (1:1 dilution with fresh media) for 6 hours. Tumor supernatant was prepared as described above and the blocking anti-IFNγ (clone XM1C12, 10 μg/mL) was added in some experiments. Gene expression of neutrophils was measured using real-time RT-PCR. RT-PCRs were performed with the following primers: ICAM-1 (f) 5’-AAATTCCACACT-GAATGCCAGCTC-3’ (r) 5’-CAAGCAGTGCCTGTCGTTCA-3’; TNFα (f) 5’-TTGTTCTTCTCAGGTGTTCTC-3’ (r) 5’-GAGGTG-ACCTTCTCTGATTG-3’; CCL-2 (f) 5’-CTCACCCTGTCACATCTTATC-3’ (r) 5’-ACTACAGCTCTTITGGGACAC-3’; VEGF-A (f) 5’-GGTGACACGACCCTTGCTTTA-3’ (r) 5’-GGTCACATACGC- GACCGAGTA-3’. To standardize the cDNA, the housekeeping gene GAPDH was tested with primer pairs: (f) 5’-GGTCTCAATCG-00000-3’; (r) 5’-TGAAGGGGTGTTGATG-3’. The protein expression of VEGF-A was determined by the specific ELISA kit (RayBiotech) according to the manufacturer’s instruction.

Immunohistochemistry and immunofluorescence staining

For frozen section preparation, tumor tissues were immediately fixed with 1% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at 4°C, and then transferred to PBS. Tissues were soaked in 30% PBS-buffered sucrose, and then the entire tissue was embedded in optimal cutting temperature (OCT) compound (Sakura Finetek) and frozen at −80°C for 20 minutes. The frozen sections were cut on a cryostat (CM 3050S-IV; Leica Microsystems). The sections were incubated at 4°C overnight with monoclonal anti-CD3 γδ, anti-CD8, anti-CD4, anti-CD25 mAb or biotin-conjugated secondary antibodies (Jackson) were used at 1:500 dilutions. Nuclei were stained with Hoechst 33258 (Nacalai Tesque). The immunofluorescence images were randomly obtained by a confocal microscope (TCS-SP5; Leica Microsystems).

Statistical analysis

All data were obtained from a group of 6 to 9 mice and are representative of at least two independent experiments. Data were analyzed for statistical significance using the Student t test. P values less than 0.05 were considered statistically significant.

Results

NK cell controls cancer growth dependent on IFNγ and CXCR3

In order to determine the role of NK cells in controlling cancer cell growth, we monitored the luminescence of MCA205-Luc2
A b s e n c e o f N K c e l l s , b u t n o t C D 4 − T c e l l s , t h e tumor (ICAM-1, TNF
regulated expression of VEGF-A mRNA as well as other anti-
involved in the promotion of cancer cell growth in NK cell–
depleted mice. CXCR3 blocking did not further
enhance MCA205-Luc2 cell proliferation in the NK cell–
depleted mice. These results suggest that NK cells control
cancer cell growth through mechanisms dependent on IFNγ
and CXCR3.

**IL17A–neutrophil axis promoted cancer cell growth in the absence of NK cells**

The cytokine IL17A supports the tumor-promoting immune response and counteracts the antitumor function of IFNγ (32–34). We found that NK-cell depletion did not affect the *in vivo* growth of MCA205-Luc2 cells in IL17A-deficient (IL17 KO) mice (Fig. 2A). This result indicates that the promotion of *in vivo* cancer cell growth in the absence of NK cells required IL17A. As IL17A attracts neutrophils to inflammatory sites (35) and neutrophils are a component of tumor-promoting inflammation (18–20), we next determined whether neutrophils are involved in the promotion of cancer cell growth in NK cell–
depleted mice. We used anti-Ly6G (clone 1A8) to deplete neutrophils as previously described (36). As shown in Fig. 2B, the promotion of MCA205-Luc2 cell growth was diminished by neutrophil depletion in the absence of NK cells. This contribution of neutrophils to cancer cell growth in NK cell–
depleted mice was confirmed in a 3LL-Luc2 model (Fig. 2C). Neither MCA205 cells nor 3LL cells in mice with intact NK cells showed differences in cancer cell growth upon neutrophil depletion (Fig. 2B and C), suggesting that neutrophils are involved in controlling cancer cell growth only in the context of NK cell absence. In order to understand whether NK cells control tumor neutrophil accumulation, we examined the infiltration of neutrophils into MCA205-Luc2 tumors by flow cytometry. Although we found that neutrophil infiltration into the tumor sites was impaired in IL17 KO mice, NK-cell depletion did not affect the proportion of tumor infiltrating neutrophils in either WT and IL17 KO mice (Fig. 3A and B). Collectively, these results indicate that neutrophils promote *in vivo* cancer cell growth in the absence of NK cells and the quality, but not quantity, of neutrophils at the tumor site can be controlled by NK cells.

**NK cells control neutrophil angiogenic switch through VEGF-A expression**

Tumors contain tumor-associated neutrophils (TAN), which are identified with either antitumor (N1) or tumor-promoting (N2) phenotypes (26). In order to examine the phenotypic difference of TANs with or without NK cells, we isolated and characterized TANs. Although we did not see any difference in the maturation status of TANs as determined by their nuclear segments (Fig. 4A), TANs in the absence of NK cells upregulated expression of VEGF-A mRNA as well as other anti-
tumor (ICAM-1, TNFα) or protumor (CCL2) markers (Fig. 4B).

Naïve peritoneal neutrophils upregulated expression of VEGF-
A mRNA upon culture with the supernatant of an NK cell–
depleted MCA205 tumor tissue culture (Fig. 5A), suggesting that NK cells control VEGF-A expression of TANs by modulating soluble factor(s) within the tumor microenvironment. To determine whether IFNγ directly inhibits VEGF production (37, 38), we tested the role of NK cells and IFNγ in causing neutrophils to express VEGF-A. Although the blocking of IFNγ

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**Figure 3.** Impaired tumor neutrophil infiltration in IL17-deficient, but not in NK cell–
depleted mice. MCA205-Luc2 cells were s.c. inoculated into WT or IL17 KO mice. To deplete NK cells, mice were treated with anti-asGM1 antibody (200 μg/mouse, i.p.) on days −3 and −1 (day 0 = tumor inoculation). A, Dot plots of flow cytometry analysis are shown. The numbers are the percentage of cells electronically gated on neutrophils (CD11b+ Ly-6G+) in tumors. B, Summary of percentage of neutrophils in tumors. Data are presented as the mean ± SEM. *, * P < 0.05, compared with WT mice. The presented data are representative of two independent experiments.

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Figure 4.
Characterization of tumor-infiltrating neutrophils in NK cell-depleted mice. MCA205-Luc2 cells (10⁵ cells/mouse) were inoculated s.c. into WT mice. To deplete NK cells, mice were treated with anti-asGM1 (200 μg/mouse, i.p.) on days –3 and –1 (day 0 = tumor inoculation). Tumor samples were harvested on day 7 and CD11b⁺ Ly-6G⁺ neutrophils were isolated by cell sorting. A, Nucleus segmentation of neutrophils from control or NK cell-depleted (NK dep) mice is shown. B, Relative mRNA expressions of neutrophils are shown. The data indicate normalized mRNA expression of neutrophils in NK cell-depleted mice to control mice. Data are presented as the mean ± SEM. *, P < 0.05, compared with control tumor group. The presented data are representative of two independent experiments.

Figure 5.
NK cells control angiogenic switching of Ly6G⁺ tumor-infiltrating neutrophils. A, Purified peritoneal Ly6G⁺ neutrophils were cultured with the supernatant of MCA205-Luc2 cells (cell culture), tumors from WT mice (control), or tumors from NK cell-depleted mice (NK dep) for 6 hours. B, To block IFNγ, anti-IFNγ (α-IFNγ, 10 μg/mL) was added to the culture of peritoneal neutrophils with tumor supernatants. C, Purified peritoneal Ly6G⁺ neutrophils were cultured with the supernatant of control tumors or NK cell-depleted tumors (NK dep) from WT or IFNγ KO mice for 6 hours. Relative mRNA expressions of neutrophils are shown. The data indicate normalized mRNA expression of neutrophils cultured with MCA205-Luc2 cell supernatant. D, MCA205-Luc2 cells (10⁵) were s.c. inoculated into WT mice. Mice were treated with anti-asGM1 (200 μg/mouse, i.p.) and/or treated with anti-Ly6G (α-Ly6G, 500 μg/mouse, i.p.) on days –1 and 3. Tumor samples were harvested on day 7 and cells were cultured for 24 hours to collect the supernatant. The VEGF production was measured by ELISA. Data are presented as the mean ± SEM. *, P < 0.05, compared with control tumor supernatant group. NS, not significant. The presented data are representative of two independent experiments.
Figure 6.
Requirement of Ly6G⁺ neutrophil dependent for enhanced tumor angiogenesis in NK cell-depleted mice. Growth factor–free Matrigel containing MCA205-Luc2 cells (10⁵) was injected s.c. into WT mice. To deplete NK cells and neutrophils, mice were treated with anti-asGM1 antibody (200 µg/mouse, i.p.) on days −3 and −1 (day 0 = tumor inoculation), and/or anti-Ly6G (500 µg/mouse, i.p.) on days −1 and 3, respectively. A, Mice were i.v. injected with 1% Evans blue 7 days after the tumor inoculation and Matrigel plugs were removed and perfused 10 minutes later, then incubated in formamide for 48 hours. The amount of Evans blue dye eluted was quantified by measuring the absorbance at 620 nm and normalized to the weight of Matrigel. B, Immunofluorescence of CD31 (green) in tumor with nuclear staining (Hoechst, blue). Scale bar, 50 µm. C, Morphometric analysis of angiogenesis in tumor tissue. D, Quantitative analysis of blood vessel areas and diameters are shown. Data are presented as the mean ± SEM. *, P < 0.05; **, P < 0.01. The presented data are representative of two independent experiments.
did not affect neutrophil VEGF-A expression upon coculture with tumor tissue culture supernatants (Fig. 5B), the supernatant of IFN-γ KO tumors upregulated neutrophil VEGF-A expression regardless of NK-cell status (Fig. 5C). Expression of VEGF-A protein within tumors was elevated in the absence of NK cells, although addition of anti-Ly6G treatment abrogated the effect (Fig. 5D). These results imply that NK cells control neutrophil VEGF-A expression and that endogenous IFN-γ (Fig. 5D). These results imply that NK cells control neutrophil angiogenesis by mechanisms dependent on IFN-γ and CXCR3, in concert with our previous results (31). We further show that the IL17A–neutrophil axis is involved in such promotion of VEGF-A in tumor-infiltrating neutrophils. When NK cells were absent, neutrophil-dependent tumor-induced angiogenesis was promoted. An inhibitor of VEGFR suppressed tumor growth in

**Discussion**

Although NK cells are known for their antitumor immune responses, their role in tumor-promoting immune responses remains unknown. In this study, we demonstrated that the tumor-infiltrating NK cells control in vivo cancer cell growth through mechanisms dependent on IFN-γ and CXCR3, in concert with our previous results (31). We further show that the IL17A–neutrophil axis is involved in such promotion of VEGF-A in tumor-infiltrating neutrophils. When NK cells were absent, neutrophil-dependent tumor-induced angiogenesis was promoted. An inhibitor of VEGFR suppressed tumor growth in

**Systemic neutropenia controls cancer cell growth in the absence of NK cells**

In order to test a clinical application of our findings, we examined whether systemic neutropenia could control the growth of tumors in mice with impaired or absent NK cells. The antimetabolite 5-FU acts as an anticancer drug by targeting DNA replication (39) and inducing systemic neutropenia (40). A single treatment of 5-FU reduced the population of neutrophils in the peripheral blood, bone marrow, spleen, and tumor (Fig. 6A). At this dose of 5-FU, we did not see any antitumor effect as shown in Fig. 8B. In contrast, the same dose of 5-FU showed an antitumor effect in mice with depleted NK cells. These data suggest that chemotherapy with 5-FU may control tumor malignancy both through inducing systemic neutropenia and through direct antitumor effects when NK cells are impaired.

**Conclusion**

We have shown that the tumor-promoting immune responses remain unknown. In this study, we demonstrated that the tumor-infiltrating NK cells control in vivo cancer cell growth through mechanisms dependent on IFN-γ and CXCR3, in concert with our previous results (31). We further show that the IL17A–neutrophil axis is involved in such promotion of in vivo cancer cell growth when NK cells are absent. NK cells suppressed expression of VEGF-A in tumor-infiltrating neutrophils. When NK cells were absent, neutrophil-dependent tumor-induced angiogenesis was promoted. An inhibitor of VEGFR suppressed tumor growth in vivo.
NK cell–depleted mice in a manner dependent on neutrophils. Treatment with the antimitabolite 5-FU controlled cancer cell growth by two mechanisms: (i) inducing systemic neutropenia and (ii) direct antitumor effects when NK cells are absent. Thus, NK cells function in angiogenic switching of neutrophils to control tumor-promoting immune responses within the tumor microenvironment.

In addition to the suppressive arm of the immune response to cancer, known as cancer immune surveillance or immunoediting, evidence indicates that an inflammatory tumor microenvironment contributes to cancer promotion or malignant progression (2, 12, 13). Of the immune cells involved in tumor-promoting immune responses, neutrophils are innate immune cells that often promote tumor-associated inflammation (13, 41). Infiltration of TANs correlates with poor prognosis in both preclinical and clinical tumor settings (19, 20, 42, 43). TANs display plasticity in their function either as an antitumor (N1) or as a tumor-promoting (N2) phenotype. TGFβ can lead TANs to the tumor-promoting N2 phenotype (26, 27). Although we observed a higher production of TGFβ in NK cell–depleted tumors, we did not see any change in the maturation status of TANs in NK cell–depleted tumors as determined by their nuclear segmentation. Instead, TANs in the absence of NK cells upregulated expression of VEGF-A among the N2-associated molecules. Because the culture supernatant of NK cell–depleted tumors also upregulated VEGF-A expression in naïve peritoneal neutrophils, we hypothesized that a soluble factor produced within the tumor microenvironment would be responsible for polarization of TANs in the absence of NK cells. Corresponding with the suppressive role of IFNγ in VEGF expression (37, 38), we found that the NK-cell IFNγ regulated VEGF expression on neutrophils by an unknown mechanism. Although NK-cell IFNγ can block migration of neutrophils to inflammation sites (44, 45), NK-cell depletion did not affect the ability of neutrophils to infiltrate tumors, suggesting that NK cells control the tumor-promoting, but not tumor-infiltrating functions of neutrophils. The mechanism by which NK cells control the tumor-promoting function of neutrophils depends on IFNγ. Endogenous type I IFNs such as IFNβ also regulate tumor angiogenesis and growth in a mouse model through neutrophils (27).

Two components of the innate immune system, neutrophils and NK cells, were found in this study to counter-regulate tumor progression. In their anti-inflammatory role, NK cells regulate neutrophil function through the NKG2A receptor pathway in a colitis model (46). Human NK cells trigger neutrophil apoptosis through activating NK-cell receptor NKP46- and Fas-dependent

Figure 8. 5-FU-induced systemic neutropenia controls cancer cell proliferation in NK cell–depleted mice. MCA205-Luc2 cells (10⁶ cells) were s.c. inoculated in WT mice (control) or NK cell–depleted mice (NK dep). Mice were treated with anti-asGM1 (200 µg/mouse, i.p.) on days –3 and –1 (day 0 = tumor inoculation) and/or 5-FU (150 mg/kg, i.v.) on day 0. A, Peripheral blood mononuclear cells (PBMC) were collected on day 7 and the dot plots (left) and summary (right) of flow cytometry analysis are shown. The numbers are the percentage of cells electronically gated on neutrophils (CD11b+ Ly-6G+) in PBMC. B, The bioluminescence of MCA205-Luc2 tumors was monitored. Luminescence was normalized by that of the individual mouse on day 0. Data are presented as the mean ± SEM. *P < 0.05; **P < 0.01, compared with untreated mice. The presented data are representative of two independent experiments.
pathways (47, 48). The NK cell–produced cytokines, IFNγ and TNFα, convert neutrophils from tumor promoting to tumor suppressing (49). TANs also influence tumor control by coordinating with other components of immune cells through their ability to suppress antitumor immunity (43, 50, 51). Although a suppressive function of TANs in the activity of antitumor CD8+ T cells was reported (43), we did not see any difference in the status of tumor-infiltrating CD8+ T cells as determined by their effector or memory marker CD62L/CD44 expression or activation marker PD-1 expression (Supplementary Fig. S2). Moreover, NK cells, but not CD8+ T cells, temporally and dominantly control in vivo cancer cell proliferation by modifying neutrophil function through an IFNγ-dependent mechanism. Further study is required to ascertain the relevance of our findings to later stage tumors. Nevertheless, we conclude that TANs in the absence of NK cells promote tumors rather than suppress antitumor CD8+ T-cell responses.

Regarding the clinical implication of our findings, neutrophil-to-lymphocyte ratios correlate with prognosis for cancer patients (21–25). We demonstrated that systemic neutropenia induced by 5-FU treatment controlled cancer cell proliferation separately from its direct antitumor effect in NK cell–depleted mice. In alignment with our preclinical findings, a clinical study indicates that peripheral NK-mediated cytotoxicity is negatively associated with survival of patients treated with 5-FU (52). We propose that a therapeutic approach involving functional impairment of neutrophils in cancer patients may improve their prognosis, particularly for tumors that lack NK-cell activity or accumulation. Our findings offer an opportunity to improve cancer therapy and predict the therapeutic outcome in the context of NK cell–neutrophil counterregulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

NK Cells Control Tumor-Promoting Function of Neutrophils

NK Cells Control Tumor-Promoting Function of Neutrophils in Mice

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