The differentiation of hematopoietic stem cells was reprogrammed in advanced tumor-bearing mice

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Running title: The differentiation of HSCs was disordered in advanced tumor-bearing mice.

Key words: Hematopoietic stem cells (HSCs), multilineage differentiation, tumor-bearing mice.

Abstract: Background: Hematopoiesis and the differentiation of HSCs have been proved to not only play important roles in cancer progression but also be changed or reprogrammed by the tumor microenvironment itself. In this study, we investigated the changes of HSCs differentiation in advanced tumor-bearing mice.

Methods: The tumor-bearing mice model was established by subcutaneously inoculating with xenografts of B16-F10 mouse melanoma cells into the right back of male wild-type C57BL/6 mice. Hematopoietic stem cells and multilineage differentiation were evaluated using blood routine, HE-staining, flow cytometry assay and HSCs culture techniques. Results: The multilineage differentiation of hematopoietic stem cells was reprogrammed in vivo. Especially, the differentiations of megakaryocyte and erythrocyte were blocked, while myeloid cell and lymphoid cell differentiation was encouraged in advanced tumor-bearing mice. Conclusion: In this
study we showed the potential mechanism of hematopoietic disorder in tumor condition from a respective of hematopoietic stem cell and multilineage differentiation, which provided new knowledge regarding cachexia.

**Introduction:**

Advanced tumor patients always have bad conditions, which called wasting syndrome or cachexia\(^{[1,2]}\). As a fatal, polyfactorial, and irreversible syndrome, cachexia affects about 50–80\% of patients with various types of tumors\(^{[3]}\). Cancer cachexia has been defined by an international consensus as a multifactorial syndrome including loss of weight, muscle atrophy, weakness, anorexia, fatigue, low immunity and severe hematopoietic abnormalities\(^{[4-7]}\). It is well accepted to concentrates on cachexia as a metabolic disorder. And previous studies have attributed severe hematopoietic abnormalities to the consequence of debilitating chronic disease\(^{[8,9]}\). But the potential mechanism of severe hematopoietic abnormalities is still unknow.

Hematopoiesis and the differentiation of HSCs have been proved to not only play important roles in cancer progression but also be changed or reprogrammed by the tumor microenvironment itself\(^{[10-12]}\). For example, some of these tumor-induced cell populations, such as tumor-associated macrophages (TAMs), myeloid- derived suppressor cells (MDSCs), and regulatory T (Treg) cells are being reported as potential therapy targets\(^{[13-15]}\). Furthermore, primary tumor-induced recruitment of neutrophils to distant organs can facilitate metastasis formation\(^{[16-18]}\). And platelets can protect tumor cells from immune elimination in the circulatory system and support their establishment in secondary lesions\(^{[19,20]}\). So, in this work we try to find new evidences to explain the mechanism of severe hematopoietic abnormalities in perspective of hematopoiesis and the differentiation of HSCs.

In this work, we try to find new evidences to explain the reason of Cancer cachexia induced hematopoietic abnormalities. We focused on the changes of hematopoiesis in advanced tumor-bearing mice model. By using flow cytometry and HSCs culture techniques, we found that hematopoietic stem cell and multilineage differentiation were disordered *in vivo* and *in vitro*. Especially, Megakaryocyte and erythrocyte differentiation were blocked, while myeloid cell and lymphoid cell
differentiation was encouraged in advanced tumor-bearing mice. These results provided new knowledges regarding the changes of hematopoiesis in cachexia condition.

**Materials and Methods**

**Chemicals and reagents**

Streptavidin FITC, Anti–Mouse CD117 APC eFluor 780, Anti–Mouse Ly-6A/E (Sca-1) PerCP-Cyanine5.5, Anti–Mouse Ter119 FITC, Anti–Mouse CD71 PE, Anti–Mouse CD11b-PE, Anti–Mouse Ly-6G(Gr-1) FITC, Anti–Hu/Mo CD45R (B220), Anti–Mouse CD8a PerCP-eFluor 710, and Anti–Mouse CD4 FITC were purchased from BD-Pharmingen (San Diego, CA). Lineage Cell Detection Cocktail-Biotin and Anti–Mouse IgM FITC were purchased from Miltenyi Biotech.

**Cell culture and treatment**

B16-F10 mouse melanoma cells was obtained from American Type Culture Collection and cultured in DMEM/HIGH GLUCOSE (HyClone) with 10% FBS (Gibco) at 37 °C in a 5% CO2 humidified chamber.

**Animals and treatment**

Male wild-type C57BL/6 mice aged 6–8 weeks old were obtained from Chinese Academy of Sciences (Shanghai, China). All mice were housed in a laboratory animal room under standard conditions. The experiments were approved by the Laboratory Animal Center of the Naval Medical University, China in conformance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The tumor-bearing mice model was established by subcutaneously inoculating with xenografts of B16-F10 mouse melanoma cells (1.0*10^6) into the right back of male wild-type C57BL/6 mice.

**Blood routine**

Peripheral blood samples obtained by cardiac puncture from mice and then were subjected to blood cell analyzer (Mindray).

**Histological examination.**

Mice femurs and spleen were removed and then fixed in 4% paraformaldehyde. The mouse femurs and spleen were then stained with hematoxylin and eosin (HE).
**Antibody staining and flow cytometry**

Bone marrow cells (BMCs), spleen cell (SPCs), thymic cells (THCs) and peripheral blood (PBCs) were isolated freshly. Then cells were strained through a 40-um strainer in the presence of phosphate-buffered saline and red blood cells were removed. Cells were stained with antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis.

**HSCs culture techniques**

MethoCult™ GF M3434, purchased from STEMCELL, is optimized for the growth and enumeration of hematopoietic progenitor cells in colony-forming unit (CFU) assays of mouse bone marrow cells.

**Statistical analysis**

Data were expressed as means ± the standard errors of means. Two-tailed Student’s t-test was used to analyze the difference between 2 groups. The data were analyzed using SPSS ver. 19 (IBM Corp., Armonk, NY, USA). P<0.05 was considered statistically significant.

**Results**

**Advanced tumor-bearing mice had loss of weight, heavier spleen.**

The tumor-bearing mice model was established by subcutaneously inoculating with xenografts of B16-F10 mouse melanoma cells. After 3 weeks, the conditions of mice were evaluated. As shown in Figure 1A, Tumor-bearing mice had loss of weight. The HE results of femur between control and test group had no significant difference (Fig 1B). But the structure of tumor-bearing mice’ spleen was totally damaged. In addition, the spleen of tumor-bearing mice was heavier than normal mice spleen (Fig 1C).
Figure 1. Advanced tumor-bearing mice had loss of weight, heavier spleen. (A). Mice weight was measured. (B). The HE staining of femur. (C) The general image and HE staining of spleen. Data are presented as mean ± s.d. *, P < 0.05; **, P<0.01.

**Advanced tumor-bearing mice had severe hematopoietic abnormalities.**

To found out the changes of hematopoietic system in the advanced tumor condition, we did blood routine and found there were severe anomalies of the hematopoietic system in the tumor-bearing mice compared to normal mice. Consistent with the clinic symptoms of cachexia, advanced tumor-bearing mice has severe anemia, thrombocytopenia, granulocytosis and mononucleosis (Fig 2 and Table 1).
Figure 2. Advanced tumor-bearing mice had severe hematopoietic abnormalities. The number of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB) and platelet (PLT) were counted in peripheral blood samples obtained by cardiac puncture from normal mice (n=3) and tumor-bearing mice (n=3). Gran, granulocytes; Lymph, lymphocytes; Mon, monocytes. Data are presented as mean ± s.d. *, P < 0.05; **, P<0.01.

Table 1. The hematologic parameters of normal mice and advanced tumor-bearing mice. Blood samples were obtained by cardiac puncture and then were
subjected to blood routine. WBC, white blood cell; Lymph, lymphocytes; Mon, monocytes; Gran, granulocytes; RBC, red blood cell; HGB, hemoglobin; PLT, platelet.

**Advanced tumor-bearing mice had a disrupted HSC repopulation capacity.**

Previous studies ascribed severe anemia to the consequence of debilitating chronic disease. But the extensive hematopoietic abnormalities might indicate multilineage disorders in HSCs and/or early progenitors (HPCs). We further assayed the proportion of HSCs/HPCs using flow cytometry (Fig. 2A). And we found that the proportion of HSCs with Lin<sup>-</sup>c-Kit<sup>+</sup> Sca-1<sup>+</sup> phenotype was decreased, while the proportion of HPCs with Lin<sup>-</sup>c-Kit<sup>+</sup> Sca-1<sup>-</sup> phenotype was increased in advanced tumor-bearing mice compared to normal mice (Fig. 2B).

**Figure 2.** Advanced tumor-bearing mice had a disrupted HSCs repopulation capacity. BMCs were isolated from mice and stained with antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis. (A). The gating strategy of
HSCs (Lin-c-Kit+ Sca+) and HPC (Lin-c-Kit+ Sca-). (B). Left, the proportion of HSCs in advanced tumor-bearing and normal mice. Right, the proportion of HPCs in advanced tumor-bearing and normal mice. Results (means ± s.d.) are for three mice in each group. *, P < 0.05; **, P<0.01. Data are representative of three experiments.

**Erythroid and megakaryocytic differentiation were blocked in tumor-bearing mice.**

To find out the reason why there were a severe anemia in advanced tumor-bearing mice, we examined the process of erythropoiesis using flow cytometry. Interestingly, the proportions of Pro.E and Ery.A were significantly increased while the proportions of Ery.B and EryC were decreased, which means that erythrocyte differentiation were blocked in Ery.A (Fig. 3A). Next, we detected the number of megakaryocytes with CD41 phenotype. The gating strategy was shown as Fig 3B, and the results of flow cytometry revealed that the proportion of CD41+ cells was lower in advanced tumor-bearing mice than in normal mice. Taken together, these data explained why there were severe anemia and thrombocytopenia in advanced tumor condition.
Figure 3. Erythroid and megakaryocytic differentiation were blocked in advanced tumor-bearing mice. BMCs were isolated from mice and stained with Ter119/CD71 or CD41 antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis. (A). The gating strategy and proportion of Pro.E, Ery.A, Ery.B, Ery.C in BMCs of mice. (b). The gating strategy and proportion of megakaryocyte in BMCs of mice. Results (means ± s.d.) are for three mice in each group. *, P < 0.05; **, P<0.01. Data are representative of three experiments.

Myeloid cell differentiation was encouraged in advanced tumor-bearing mice.
The result of blood routine suggested that advanced tumor-bearing mice also had hard granulocytosis. We further examined the changes of myeloid differentiation. The results of flow cytometry showed that the proportion of Gr-1⁺CD11b⁺ cells was down-regulated in bone marrow, while B220⁺CD11b⁺ cells was increased in spleen. Moreover, consistent with the changes of blood routine, cells with CD11b⁺ phenotype were also increased in PBC. These data suggested that myeloid cell differentiation might be encouraged in advanced tumor-bearing mice.

**Figure 4.** Myeloid cell differentiation was encouraged in advanced tumor-bearing mice. BMCs, SPCs and PBCs were isolated from mice and stained with Gr-1 and/or CD11b antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis. Results (means ± s.d.) are for three mice in each group. *, P < 0.05; **, P<0.01. Data are representative of three experiments.

**B-Lymphoid phenotype was induced in advanced tumor-bearing mice.**
As showed in blood routine, lymphocytes were also increased in advanced tumor-bearing mice. Then we analyzed the expression of B220 and IgM, which were both important membrane marker in B-lymphoid differentiation process. As showed in Fig. 5A, the proportion of proper-B and mature-B was decreased, while the percentage of immature-B did not much change in BMCs of advanced tumor-bearing mice. Consistent with the bone marrow phenotype, the proportion of mature B-lymphocytes with B220+IgM+ phenotype was also decreased in spleen of advanced tumor-bearing mice compared to normal mice. And the proportion of B220+ cells were increased in PBCs of advanced tumor-bearing mice. Taken together, these data revealed that B-Lymphocytes was induced in tumor-bearing mice.

Figure 5. B-Lymphoid phenotype was induced in advanced tumor-bearing mice. BMCs, SPCs and PBCs were isolated from mice and stained with B220 and/or IgM antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis. Results (means ± s.d.) are for three mice in each group. *, P < 0.05; **, P<0.01. Data are representative of three experiments.
T-Lymphoid phenotype was induced in PBC of advanced tumor-bearing mice.

T-lymphoid differentiation were also analyzed in this work. Although lymphocyte numbers increased sharply in peripheral blood of advanced tumor-bearing mice, there was no much change in the distribution of thymic CD4, CD8 single-positive T cells and CD4/CD8 double positive T cells subpopulations. However, the CD4 and CD8 single-positive compartment were relatively increased in peripheral blood in advanced tumor-bearing mice.

Figure 6. T-Lymphoid phenotype was induced in PBC of advanced tumor-bearing mice. THCs and PBCs were isolated from mice and stained with CD4 and CD8 antibody for 20 minutes at 4°C. Then cells were subjected to flow cytometry analysis. Results (means ± s.d.) are for three mice in each group. *, P < 0.05; **, P<0.01. Data are representative of three experiments.

Hematopoietic stem cell and multilineage differentiation were disordered by using HSC culture techniques.

To find the changes of processes of HSC differentiation, we used MethoCult™ GF M3434 to culture HSC. MethoCult™ GF M3434 has been formulated to support optimal growth of primitive erythroid progenitor cells (BFU-E), granulocyte-
macrophage progenitor cells (CFU-GM, CFU-G and CFU-M), and multi-potential granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM).

After three weeks the mice model established, the BMCs were isolated freshly and then seed into 6 wells plank. After 10 days, the photos were taken and shown as Figure 7. BFU-E in tumor-bearing mice was smaller than it in normal mice, while CFU-GM and CFU-GEMM were much bigger than that in in normal mice. Those results were consistent with the blood routine and flow cytometry results.

Figure 7. Hematopoietic stem cell and multilineage differentiation were disordered by using HSCs culture techniques. BMC (5000 cells/well) were isolated freshly from normal mice and advanced tumor-bearing mice, and then cells were seeded into 6 wells plank. The photos were taken after 10 days.

Figure 8. The differentiation of HSCs educated in the advanced tumor-bearing mice. The differentiation of HSCs educated in the advanced tumor-bearing mice tend to differentiate to lymphocyte and leukocyte subsets originated from CLP
(Common Lymphoid Progenitor) and CMP (Common Myeloid Progenitors) cells, and the proportion of MEP (Megakaryocyte-Erythroid Progenitor cell) which differentiate to erythrocytes and platelets was decreased.

**Discussion**

At present, when the number of cachectic patients and therefore cancer rates are increasing, it seems particularly appropriate to analyze the different pathophysiologic adaptations that are involved in cancer, with the aim to facilitate the development of a more focused therapeutic approach[21-23]. In this research, tumor-bearing mice models were used to extrapolate advanced tumor patients.

After three weeks the tumor-bearing mice model established, we measured the changes of mice’s body weight and found that the body weight of tumor bearing mice were much lower than the body weight of normal mice, which proved that the cancer cachexia model was succeed established. Then the hemopoietic system, including bone marrow and spleen, were analyzed by using HE staining. To our surprised, the structure of tumor-bearing mice’s spleen was totally damaged. In addition, the spleen of tumor-bearing mice was heavier than normal mice spleen. But the structure of femur between control and test group had no significant difference. The blood routine shown that peripheral blood of tumor-bearing mice had severe hematopoietic abnormalities, severe anemia, thrombocytopenia, granulocytosis.

Previous studies have attributed severe anemia to the consequence of debilitating chronic disease[24, 25]. But in this work, we focused on the changes of hematopoiesis in advanced tumor-bearing mice model. By using flow cytometry assay and HSCs culture techniques, we found that tumor-bearing mice had a disrupted HSCs repopulation capacity. The erythrocyte differentiation was blocked in Ery.A, which was responsible for the severe anemia. The decreased CD41+ megakaryocyte contributes to thrombocytopenia observed in the mice bearing the B16-F10 melanoma. As for Myeloid cells, those cells’ differentiation was induced in tumor-bearing mice, which caused granulocytosis. Another mechanism that is involved in granulocytosis is inflammation. Advanced tumor-bearing mice always have inflammation[26, 27], but in this study, we cannot exclude the role of inflammation in
the myeloid cell differentiation. The differentiation of lymphocytes was also analyzed, the flow cytometry results showed that B-Lymphoid phenotype was induced in tumor-bearing mice, while CD4, CD8 single-positive T-Lymphoid phenotype was only induced in peripheral blood samples of tumor-bearing mice. Finally, to find the changes of processes of HSCs differentiation, we used MethoCult™ GF M3434 to culture HSCs. The results showed that BFU-E (Burst Forming Unit Erythroid) in tumor-bearing mice was smaller than it in normal mice, while CFU-GM (Colony Forming Unit-Granulocyte and Macrophage) and CFU-GEMM (Colony Forming Unit-Granulocyte, Erythrocyte, Monocyte and Megakaryocyte) were much bigger than that in in normal mice. Those results were consistent with the blood routine and flow cytometry results.

Taken together, the differentiation of HSCs educated in the advanced tumor-bearing mice tend to differentiate to lymphocyte and leukocyte subsets originated from CLP (Common Lymphoid Progenitor) and CMP (Common Myeloid Progenitors) cells, and the proportion of MEP (Megakaryocyte-Erythroid Progenitor cell) which differentiate to erythrocytes and platelets was decreased (Fig. 8). Finally, these changes lead to anemia, thrombocytopenia, granulocytosis and mononucleosis in advanced tumor-bearing mice in vivo.

By focusing on the changes of hematopoiesis in advanced tumor-bearing mice model, we found a series of significative evidences. Of course, this study had some shortcomings. Such as that the tumor models grow faster than tumors found in humans; the tumor models are carried out using subcutaneous inoculation and therefore may not reflect the behavior of a tumor in the correct anatomical location.

**Conclusion**

In conclusion, in this work we try to find new evidences to explain he potential mechanism of hematopoietic disorder in tumor condition from a respective of hematopoietic stem cell and multilineage differentiation. And understanding the disorders of hematopoiesis in tumor-bearing mice model may possibly accelerate the development of novel therapeutic interventions against cancer-induced bad conditions.
**Abbreviations**

HSCs: Hematopoietic Stem Cells, HPCs: Hematopoietic Progenitor Cells, BMCs: Bone Marrow Cells, SPCs: Spleen Cells, PBCs: Peripheral Blood Cells, THCs: Thymic cells, CFU: Colony Forming Unit, BFU-E: Burst Forming Unit Erythroid, CFU-GM: Colony Forming Unit Granulocyte and Macrophage, CFU-GEMM: Colony Forming Unit Granulocyte, Erythrocyte, Monocyte and Megakaryocyte, CLP: Common Lymphoid Progenitor, CMP: Common Myeloid Progenitors, MEP: Megakaryocyte-Erythroid Progenitor cell, HE: Hematoxylin and Eosin, WBC: White Blood Cells, RBC: Red Blood Cells, HGB: Hemoglobin, PLT: Platelet, Gran: Granulocytes, Lymph: Lymphocytes, Mon: Monocytes.

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**Authors’ contributions**

Cong Liu was responsible for the conception and design, collection and assembly of data, data analyses, and manuscript writing. Jicong Du, Yuan Gao, and Penglin Xia were responsible for the collection and/or assembly of data and data analyses and interpretation in the animal experiments. Ruling Liu, Ying Cheng and Jianguo Cui were responsible for the collection and assembly of data and data analyses in the cell biology experiments. Fu Gao and Hu Liu were responsible for the conception and design, and manuscript writing. All authors read and approved the final manuscript.

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**Availability of data and materials**

Not applicable.

**Ethics approval and consent to participate**
The animal experiments were approved by the Laboratory Animal Center of the Naval Medical University, China in conformance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Reference

1. Barton MK. Cancer cachexia awareness, diagnosis, and treatment are lacking among oncology providers[J]. CA: a cancer journal for clinicians, 2017;67(2):91-92. doi:10.3322/caac.21364.
2. Lok C. Cachexia: The last illness[J]. Nature, 2015;528(7581):182-183. doi:10.1038/528182a.
3. Sadeghi M, Keshavarz-Fathi M, Baracos V, et al. Cancer cachexia: Diagnosis, assessment, and treatment[J]. Critical reviews in oncology/hematology, 2018;127(91-104. doi:10.1016/j.critrevonc.2018.05.006.
4. Bhullar AS, Anoveros-Barrera A, Dunichand-Hoedl A, et al. Lipid is heterogeneously distributed in muscle and associates with low radiodensity in cancer patients[J]. Journal of cachexia, sarcopenia and muscle, 2020. doi:10.1002/jcsm.1252410.1002/jcsm.12533.
5. Zhang Y, Wang J, Wang X, et al. The autophagic-lysosomal and ubiquitin proteasome systems are simultaneously activated in the skeletal muscle of gastric cancer patients with cachexia[J]. Journal of cachexia, sarcopenia and muscle, 2020. doi:10.1002/jcsm.1253310.1093/ajcn/nqz347.
6. Liva SG, Tseng YC, Dauki AM, et al. Overcoming resistance to anabolic SARM therapy in experimental cancer cachexia with an HDAC inhibitor[J]. 2020:e9910. doi:10.1093/ajcn/nqz34710.1093/emmm.201809910.
7. Ahmadabadi F, Saghebjoo M, Huang CJ, et al. The effects of high-intensity interval training and saffron aqueous extract supplementation on alterations of body weight and apoptotic indices in skeletal muscle of 4T1 breast cancer-bearing mice with cachexia[J]. EMBO molecular medicine, 2020. doi:10.15252/emmm.20180991010.1139/apnm-2019-0352.
8. Scherbakov N, Doehner W. Cachexia as a common characteristic in multiple chronic disease[J]. Journal of cachexia, sarcopenia and muscle, 2018;9(7):1189-1191. doi:10.1002/jcsm.12388.
9. Belloum Y, Rannou-Bekono F, Favier FB. Cancer-induced cardiac cachexia: Pathogenesis and impact of physical activity (Review)[J]. Oncology reports, 2017;37(5):2543-2552. doi:10.3892/or.2017.5542.
10. Shalapour S, Font-Burgada J, Di Caro G, et al. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy[J]. Nature, 2015;521(7550):94-98. doi:10.1038/nature14395.
11. Peng D, Kryczek I, Nagarsheth N, et al. Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy[J]. Nature, 2015;527(7577):249-253. doi:10.1038/nature15520.

12. Han Y, Liu Q, Hou J, et al. Tumor-Induced Generation of Splenic Erythroblast-like Ter-Cells Promotes Tumor Progression[J]. Cell, 2018;173(3):634-648.e612. doi:10.1016/j.cell.2018.02.061.

13. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment[J]. Cancer cell, 2012;21(3):309-322. doi:10.1016/j.ccr.2012.02.022.

14. Elinav E, Nowarski R, Thaiss CA, et al. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms[J]. Nature reviews Cancer, 2013;13(11):759-771. doi:10.1038/nrc3611.

15. Finn OJ. Immuno-oncology: understanding the function and dysfunction of the immune system in cancer[J]. Annals of oncology : official journal of the European Society for Medical Oncology, 2012;23 Suppl 8(viii6-9. doi:10.1093/annonc/mds256.

16. Coffelt SB, Kersten K, Doornebal CW, et al. IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis[J]. Nature, 2015;522(7556):345-348. doi:10.1038/nature14282.

17. Masucci MT, Minopoli M, Carriero MV. Tumor Associated Neutrophils. Their Role in Tumorigenesis, Metastasis, Prognosis and Therapy[J]. Frontiers in oncology, 2019;9(1146. doi:10.3389/fonc.2019.01146.

18. Wu L, Awaji M, Saxena S, et al. IL-17-CXC Chemokine Receptor 2 Axis Facilitates Breast Cancer Progression by Up-Regulating Neutrophil Recruitment[J]. The American journal of pathology, 2020;190(1):222-233. doi:10.1016/j.ajpath.2019.09.016.

19. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis[J]. Nature reviews Cancer, 2011;11(2):123-134. doi:10.1038/nrc3004.

20. Gaertner F, Massberg S. Patrolling the vascular borders: platelets in immunity to infection and cancer[J]. 2019;19(12):747-760. doi:10.1038/s41577-019-0202-z.

21. Kennedy LB, Salama AKS. A review of cancer immunotherapy toxicity[J]. CA: a cancer journal for clinicians, 2020. doi:10.3322/caac.21596.

22. Salama AKS, Irvine DJ. Enhancing cancer immunotherapy with nanomedicine[J]. CA: a cancer journal for clinicians, 2020. doi:10.3322/caac.2159610.1038/s41577-019-0269-6.

23. Topalian SL, Taube JM, Pardoll DM. Neoadjuvant checkpoint blockade for cancer immunotherapy[J]. 2020;367(6477). doi:10.1126/science.aax0182.

24. Roberts EW, Deonarine A, Jones JO, et al. Depletion of stromal cells expressing fibroblast activation protein-alpha from skeletal muscle and bone marrow results in cachexia and anemia[J]. The Journal of experimental medicine, 2013;210(6):1137-1151. doi:10.1084/jem.20122344.

25. Saitoh M, Hatanaka M, Konishi M, et al. Erythropoietin improves cardiac wasting and outcomes in a rat model of liver cancer cachexia[J]. International journal of cardiology, 2016;218(3):312-317. doi:10.1016/j.ijcard.2016.05.008.

26. Herremans KM, Riner AN. The Microbiota and Cancer Cachexia[J]. 2019;20(24). doi:10.3390/ijms20246267.
27. Stubbins R, Bernicker EH, Quigley EMM. Cancer cachexia: a multifactoral disease that needs a multimodal approach[J]. Current opinion in gastroenterology, 2020;36(2):141-146. doi:10.1097/mog.0000000000000603.