Dear Editor,

Please find enclosed a modified version of my Microreview manuscript “Exploring the Tumor Microenvironment Concerning Immunotherapy.” To address the concerns and comments raised by the 2 reviewers, I made the following changes to improve and clarify the manuscript. I hope that these changes make the manuscript acceptable for publication in Microreviews in Cell and Molecular Biology.

Sincerely,
Zoie Morrison

Reviewer 1:
1. The comments from reviewer 1 were adequate and I agree with what was stated about how I needed to add more context and citations.
2. The changes I made were that I elaborated more on how each component of the tumor microenvironment played its role in immunotherapy treatment. As well as I added descriptions to certain terms that were not clear. I did not leave a section unchanged and I revised many components in each paragraph.

Reviewer 2:
1. The comments from reviewer 2 were just as helpful in my opinion they had around the same ideas and suggestions. I agree with what was said and it allowed me to improve my manuscript.
2. The comments from reviewer 2 in regards to some of my paragraphs feeling unfinished was correct and with that comment I finished the paragraph and added in more information. We clarified this issue by making changes to my clarity issues towards the end of the manuscript as well as adding citations in text.

Microreviews in Cell and Molecular Biology 2022

Exploring the Tumor Microenvironment Concerning Immunotherapy

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Abstract. The Tumor Microenvironment (TME) is the blood vessels, molecules, and normal cells. The microenvironment of a tumor can alter and affect how the tumor spreads and expands. The microenvironment is a crucial part of the tumor to activate heterogeneity of a carcinoma and present studies on multi-drug resistance to a malignant cell. Heterogeneity of malignant cells is significant to cancer stem cells' capability to preserve and renew cells with different actions. In correlation with cancer stem cells of the inflammatory myeloid-derived suppressor cells (MDSCs) which mediate immune responses. Each component of the TME is very crucial to different aspects of tumor cells and will continue to expand studies to differently utilize each component. The discovery of the tumor microenvironment went on to drive an abundant amount of research on the TME and how immunotherapy implicates the components of the tumor microenvironment for cancer treatments. Durable responses presented from immunotherapy treatments have the potential to produce additional growth to successfully attack cancer cells and advance cancer patients to go into remission. This assumption is proven in the present and will undergo further research in the future.

Keywords: Tumorigenesis, inflammation, angiogenesis, hypoxia, stem cells, immunotherapy.

Introduction

The Tumor Microenvironment is an expanding field undergoing research with its correlation to malignant cells in immunotherapy treatment today. Their cell growth appears distinctly different in physical and molecular configurations that aid in the progression and growth of each one. In comparison to the tumor microenvironment, there is diversity between the benign and malignant tumor types. It has been proven that TME presents anti malignancy actions and pro malignancy actions with consideration of different conditions. Early on in tumor growth, a connection is incorporated between malignant cells and the TME to aid in metastatic distribution, survival, and invasion. This connection promotes angiogenesis which produces new red blood cells to replace the oxygen and nutrients allowing for the microenvironment to subdue hypoxia [6]. Today angiogenesis is the immune circumstance regarding tumors, along with the

interrelationships between tumor cells and fibroblasts, and pro-inflammatory intercession. The microenvironment of tumors is a major factor in cancer therapy and leads to genetic alterations of tumor cells [1]. The outcomes of genetic alterations cause hypoxia, trigger stress to the extracellular matrix (ECM), fibroblasts, macrophages, lymphocytes responses, and as stated the activation of angiogenesis. The ECM, fibroblasts, macrophages, immune cells such as T and B lymphocytes, stromal cells, and vascular systems are all components of the TME. Each one of these components contributes to cancer therapies and has led researchers to study the effects of the interactions with different malignant cells and the microenvironment of tumors. In particular, one researcher Stephen Paget contributed a significant role in the discovery of the importance of the tumor microenvironment in the progression of tumors [6]. The tumor progression and pathogenesis of cancer significantly favor the components of the Tumor Microenvironment. Paget's discovery led to the conclusion that the components of the TME can aid in the development or treatment of malignant cells [1].

Recent Progress

Many steps go into the development of treating cancer because it's continuously evolving and that is why there are constantly new ways of treatment being introduced. Immunotherapy is a form of cancer treatment that utilizes the body's natural defenses to induce substances for the immune system to attack malignant cells on its own. One way immunotherapy is implemented is through the tumor microenvironment and its components. There has been recent progress in utilizing the tumor microenvironment components to treat cancer by using cancer-associated fibroblasts (CAFs), macrophages, stromal cells, etc [6]. Utilizing the components of the TME allows activation of tumorigenesis, cancer stem cells, angiogenesis, and immunotherapy treatments such as cancer vaccines, macrophage-targeting therapy, and T-cell therapy.

Tumorigenesis. Epidemiology allows us to gain insight into the multistep process within the tumor microenvironment that can alter tumors. Malignant cells are genetically and epigenetically known as Tumorigenesis [1]. Tumor progression is a process where normal cells develop with an abnormal and excessive amount of tissue. Random mutations and epigenetic changes of DNA drive this process that affects the genes which manage the survival, proliferation, and physical characteristics of malignant cells. During tumorigenesis, there has to be stability between pro-angiogenic and anti-angiogenic components that are brought forward from malignant cells and stromal cells of the TME [7]. The stability between pro-angiogenic and anti-angiogenic is important because it controls the vascular homeostasis which does not allow for endothelial cells to proliferate [7].

Cancer Stem Cells and Inflammation. Cancer stem cells' connection to the tumor microenvironment is portrayed through tumorigenesis, tumor progression, and metastasis [8]. They were first identified within a solid tumor which is a common feature of hypoxia. However, CSCs can enhance resistance to hypoxia with the initiation of angiogenesis by limiting tumor antigens and increasing anti-inflammatory growth factors and cytokines, and activating drug resistance. This ability has proved the significance of the tumor microenvironment to CSCs [3]. In correlation with the inflammatory factors of the microenvironment which activate the growth of tumors and the progression of tumors. The components of the inflammatory system of CSCs need to create new and more efficient ways to implement the body's immune system

for treatment. Malignant behavior of cancer is controlled through the tumor microenvironment and has been proven due to cancer-associated fibroblasts heterogeneity [4]. Understanding this allows for our bodies to utilize immune cells in correlation to inflammation. That is because there is constant inflammation when a chronic infection is present in the body as well as aiding in tumor formation. Several cancers have proven this such as colorectal, cervical, and hepatocellular [1]. One example of this was in pancreatic carcinoma cells where inflammatory interferons (IFNs) regulate the actions of CSCs to advance metastasis activities [8]. Alongside that, studies have shown that IFNs maintain an immunosuppressive TME, and downregulate tumor antigens. Which has allowed researchers to discover that these roles can identify tumor kinds, TME, and the stage of the tumor allowing for a positive immunotherapy treatment [8].

Angiogenesis and Hypoxia. However, platelets and certain types of T-cells are not the only things that can activate or suppress angiogenesis and metastasis. Tumor hypoxia does as well. Hypoxia is often a characteristic of solid tumors that develop because of the fast expansion of tumors which cuts off the oxygen supply and stops blood flow because of the formation of abnormal blood cells [3]. However, since hypoxia cuts off the oxygen supply and stops blood flow it does not mean that the tumor cells can not adapt. This allows for the process of angiogenesis to develop new blood vessels [3]. Hypoxia regulates angiogenesis by targeting the HIF transcription factors; which allows the new vessels to be created under hypoxic conditions. This is key for tumor survival, invasion, and malignant progression.

Platelets and T-cells. Platelets and T-cells of the tumor microenvironment have been proven to play active roles in the processes of tumorigenesis, angiogenesis, and metastasis as well[2]. They also play a critical role in immune cells in the TME. Immune cells are utilized in two ways such as suppressing tumor growth or advancing it. There are two different types of immune cells which are innate and adaptive. the adaptive immune cells hold T-cells, B-cells, and natural killer cells (NKC), and are activated by exposure to specific antigens to enhance the response of the immune system [1]. Each T-cell has its T-cell receptor which allows it to recognize each certain antigen entering the body [1]. T-cells also have several distinct cells that encourage tumorigenesis. One of those cells is known as the cytotoxic T-cell which detects abnormal tumor antigens on cancer cells and allows for an attack as well as suppresses angiogenesis. However, in most cases, if cytotoxic T-cells are present it is a positive case of cancer [1]. This has to lead to discovering how each one of these components affects the microenvironment and how they can suppress these processes explained above. However, platelets and certain types of T-cells are not the only things that can activate or suppress angiogenesis. Platelets also play significant roles in the functions stated above. These functions are carried out by the activation of platelets to form MPs and release lipids and growth factors into the bloodstream which allows for stimulation of cancer metastasis and angiogenesis [2]. This is important because, during the circulation phase of metastasis, cancer cells are presented dangerous forces caused by blood flow and attacks on the immune system [2]. When this happens platelets are the backbone of metastasis for protection from these forces.

Cancer vaccines. Vaccines for cancer have been an ongoing process and continue to develop today. Many aspects of the TME are utilized in the success of vaccines. However, it has been shown that vaccines will be combined with other immunotherapy treatments such as targeting immune-evasive actions of tumors, regulating immune cells, and the responsibilities

of T-cells [10]. T-cell checkpoint inhibitors have become ample in immunotherapy treatments. However, for cancer vaccines to progress in the future, an act of trying to target tumor antigens is what will aid in this process. Tumor mutated specific antigens (TMSAs) also known as neoantigens the main objective is to aim checkpoint blockade immunotherapy and utilized in vaccines that influence this rejection response. T cells contribute significantly to many tumor-specific mutant antigens [10]. Therefore this rapid identification by the usage of bioinformatics and genomic approaches can improve cancer immunotherapy treatments when paired together.

Macrophage-Targeting Therapy. Macrophage therapy has shown significant therapeutic efficiency in immunotherapy regarding malignant tumors when paired with angiogenesis inhibitors; which allow a blockade of the blood vessels of expanding tumors [10]. This has allowed researchers to conclude that macrophages within the TME associated with malignant tumors are significant connectors to aid in recognizing how the immune system causes tumor progression in the early stages of cancer.

Immunotherapy of Tumor Microenvironment. The tumor microenvironment (TME) is important in immunotherapy by concealing the heterogeneity of the antitumor immunity. Currently, cancer immunotherapy beneficially activates the production of antitumor defects in the immune system [5]. The microenvironment of the tumor consists of tumor cells that create immune outflow by creating an immunosuppressive microenvironment. However, this creation involves activation of cancer-associated fibroblasts (CAFs), stroma modeling, development of vascular tumors, concealed receptors on tumor cells, and others stated above. Therefore, the tumor microenvironment is surrounding the surface of tumor cells which allows them to be in a defective state. This is why each component of the TME plays an additional significant role in immunotherapy.

Conclusion

From examining many studies I have concluded that immunotherapy involving the tumor microenvironment is emerging immensely. Each component of the TME plays a significant role in creating a successful chance in immunotherapy treatments as concluded above. Therefore, a therapy that activates white blood cells to enhance their attack on tumor cells has fewer side effects and can overall be more successful. This is why more cancer treatments have been exploring the safer route of immunotherapy. The present findings confirm a therapy that uses your immune system functions is less harmful than conventional therapy such as chemotherapy or radiation. This allows for more studies to be concluded for future treatments and the potential to treat cancer. Despite many limitations in clinical trials to immunotherapy treatments, there is constant growth within the Tumor Microenvironment.

1. Anderson, N. M. & Simon, C. M. *The Tumor Microenvironment* (2020).
2. Huong, P. T., Nguyen, L. T., Nguyen, X. B., Lee, S. K. & Bach, D. H. The role of platelets in the tumor-microenvironment and the drug resistance of cancer cells. *Cancers* (2019).
3. Emami Nejad, A. *et al.* The role of hypoxia in the tumor microenvironment and development of Cancer Stem Cell: A novel approach to developing treatment - cancer cell international. *BioMed Central* (2021).
4. Bu L, Baba H, Yoshida N, Miyake K, Yasuda T, Uchihara T, Tan P, Ishimoto T, Biological heterogeneity and versatility of cancer-associated fibroblasts in the tumor microenvironment. *Oncogene* (2019).
5. AR; R.-R. C. M. R. B. P. V. F. (REVISE) Targeting tumor microenvironment for cancer therapy. *International journal of molecular sciences* (2019).
6. Karyn Hede, Environmental Protection: Studies Highlight Importance of Tumor Microenvironment, *JNCI: Journal of the National Cancer Institute*, Volume 96, Issue 15, 4 August (2004) Pages 1120–1121
7. Fengjuan Fan, Alexander Schimming, Dirk Jaeger, Klaus Podar, "Targeting the Tumor Microenvironment: Focus on Angiogenesis", *Journal of Oncology*, vol. 2012, Article ID 281261, 16 pages, (2012)
8. Zhang, S., Yang, X., Wang, L. & Zhang, C. Interplay between inflammatory tumor microenvironment and cancer stem cells. *Oncology letters* (2018).
9. Whiteside, T. L. The tumor microenvironment and its role in promoting tumor growth. *Oncogene* (2008).
10. Grenier, J. M., Yeung, S. T., & Khanna, K. M. (2018). Combination Immunotherapy: Taking Cancer Vaccines to the Next Level. *Frontiers in immunology*, *9*, 610.

