Project Report

A review on

A review of tumor-associated macrophages and neutrophils and their possible interactions in the tumor microenvironment.

(This report is presented in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy)

Submitted To:

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APPROVAL

This project titled **‘‘A review of tumor-associated macrophages and neutrophils and their possible interactions in the tumor microenvironment.’’** submitted by Jahidul Hassan Simanta, ID: 191-29-195, Department of Pharmacy, Daffodil International University has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and approved as to its style and contents.

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DECLARATION

I hereby declare that this project report is done by me under the supervision of **Professor Dr. Muniruddin Ahmed**, Head of the department, Department of Pharmacy, Daffodil International University, impartial fulfillment of the requirements for the degree of Master of Pharmacy. I declare that this Thesis is my original work. I also declare that neither this project nor any part thereof has been submitted elsewhere for the award of Masters or any degree.

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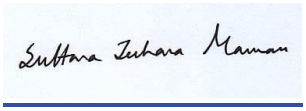

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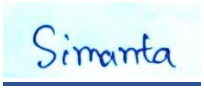

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To my beloved parents, my teachers, my supervisor, and my friends.

ABSTRACT

Cancer is influenced by the tumor microenvironment (TME), which is characterized by inflammation and a variety of stromal components. Cancer cells encounter different types of stromal and immune cells throughout the disease process. The TME is a significant predictor of tumor behavior, contrary to the traditional oncological theory that describes cancer development as a multi-stage process affecting only cancer cells. The TME includes local stromal cells such as fibroblasts and macrophages, and far-flung recruited cells such as endothelial cells, immune cells, bone marrow-derived precursor cells, and circulating platelets. Among the myeloid populations in TAMs, MDSCs, TANs, and tumor-associated dendritic cells. We will focus on TAMs and TANs, both originating from TAMCs.

Keywords: Tumor microenvironment, Inflammation, Stromal cells, Immune cells, Myeloid cells

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CHAPTER-1

INTRODUCTION

1.1 Introduction:

Cancer is one of the leading causes of death encyclopedically, and the estimated number of cancer cases is anticipated to grow to 16 million by 2020. Rare cancers make up a significant portion of adult judgments, with 13 of all cancers being rare in 2017. The majority of these cases are seen in people aged 50 to 74, but 20 rare cancer cases have passed in people aged 20 to 49. With this statistic in mind, it's clear that age isn't inescapably a factor in rare cancer judgments. Rare cancers, defined in this report as cancers with smaller than 6 cases per,100,000 people per time, frequently present unique challenges to opinion and treatment. The tumor microenvironment (TME) describes the biological setting in which tumors and cancer stem cells thrive. Tumors contain cancer stem cells, which can divide indefinitely and fuel the growth of the tumor.[1] Despite contributing greatly to our knowledge of the tumor microenvironment (TME), cancer's own special stem cells provide substantial obstacles to both cancer diagnosis and treatment. The TME consists of the cells of the immune system, blood vessels, extracellular matrix (ECM), fibroblasts, lymphocytes, inflammatory cells generated from bone marrow, and signaling chemicals in the immediate vicinity. [2,3,4] Cancers are complicated "rogue" organs where many cells are recruited and corrupted by altered cells. The tumor microenvironment (TME) is shaped by interactions between malignant and nonmalignant cells and influences tumorigenesis and metastasis.[5] Nonmalignant cells When it comes to carcinogenesis, the TME's benign cells frequently play a protumorigenic role by promoting unchecked cell division at any stage.[6] Various cellular components make up the TME.

Endothelial cells are the first, and they're crucial to tumor growth and immune system suppression. Angiogenic arteries in tumors are typically offshoots of existing vessels or are generated from endothelial progenitor cells [6]. These cells provide essential nutrients for the expansion and progression of tumors. Other crucial elements include immune cells including granulocytes, lymphocytes, and macrophages. These cells participate in a wide range of immunological reactions and activities, including inflammatory reactions that the tumor itself orchestrates to increase its

chances of survival. Macrophages are the dominant type of immune cell seen in the TME. Macrophages have multiple roles in cancer, including facilitating tumor cell dissemination and inhibiting immune system responses that target tumors. [6,7] Tumor-initiating processes that include neutrophils are complex.

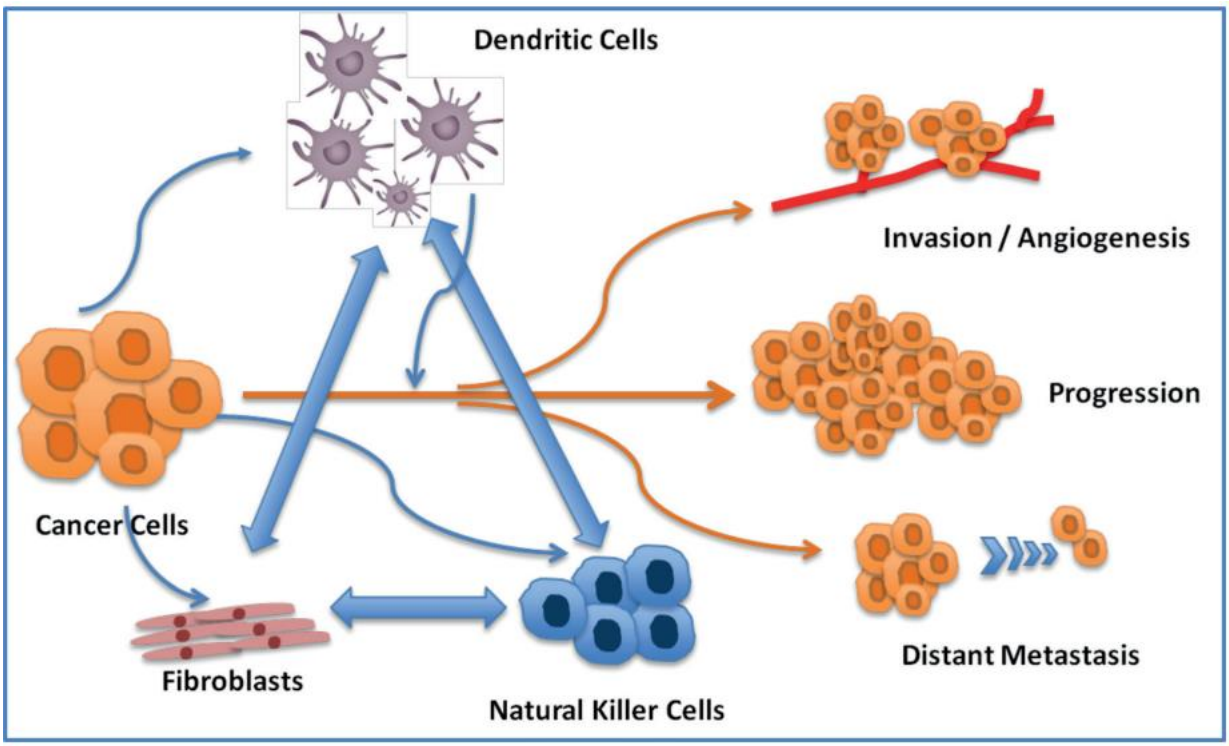

Figure 1. Tumor microenvironment shows the cell-cell interactions, which contribute to cancer cell progression, invasion, and metastasis.

Moreover, neutrophils produce oxygen and nitrogen free radicals, which enhance DNA point mutations and genetic instability. The proteins found in neutrophil granules have a dual purpose in the development of tumors. Lung cancer cells did acquire neutrophil elastase (NE) and use it in their malignant progression, damaged insulin receptor substrate-1, an inhibitor of PI3K.
Tumor cell proliferation was aided by the release of PI3K activation and PDGFR signaling. CXCL8 was produced by melanoma cells, which boosted 2-integrin expression.[116]

1.2 General characteristics of TAM:

Macrophages are highly versatile cells that play important roles in many different processes, such as tissue growth and maintenance, homeostasis, the removal of damaged cells and pathogens, and

the control of inflammation.[8] The resultant macrophage can exhibit a wide variety of phenotypes depending on the specifics of the cytokine environment and the tissue niche in which it is located.[9] Common knowledge is that TAMs can be broadly categorized into the anticancer M1 phenotype (classically activated state) and the protumor M2 phenotype (alternatively activated state), reflecting the Th1-Th2 polarization of T cells, as proposed by Mills' group. Upon arrival in the tumor microenvironment (TME), TAMs generated from peripheral blood monocytes undergo M1-like or M2-like activation in response to different stimuli. [10,11] Antigen-presenting M1-like TAMs activate Th1-type immune responses.

They produce NO, ROS, and pro-inflammatory cytokines like IL-1β, IL-6, IL-12, IL-23, C-X-C motif chemokine (CXCL) 9, CXCL10, TNF-α, and MHC molecules. [12,13,14] In response to tissue damage signals, circulating monocyte precursors in the bone marrow (BM) became TME macrophages. In mouse mammary cancers, tumor-infiltrating monocytes almost always exhibit high levels of Ly6C, a mouse monocyte marker. Ly6Chigh monocytes constantly renew all non-proliferating TAM populations, forming a heterogeneous myeloid component with M1-like MHC-IIhigh and M2-like MHC-IIlow TAM subpopulations. [15,16] The term "activation" rather than "polarization" is preferred for the classification of a functional condition of TAMs, as part of a newer trend toward a unified vocabulary for characterizing the features of the macrophages under study. As TAMs are not a polarized macrophage population, we shall use the word "activation" rather than "polarization" throughout this review.[17]

It is an oversimplification to think of TAMs as a single, skewed population of M2-like macrophages because human cancer macrophages cannot be reliably separated into these two types. The current M1 versus M2 polarization model can be expanded to a "spectrum model" with at least nine unique macrophage activation programs, according to a new study using highly standardized stimulation of human macrophages. [18,19] Activation of tumor-associated macrophages (TAMs) and overall tumor prognosis appear to be influenced by distinct signals from various places within the tumor microenvironment (TME).[20] Micro anatomical variations within cancerous tissue, such as the accumulation of cells with protumor properties in hypoxic areas and differences in inflammatory components and pathways between tumors originating from different anatomical sites, are just two examples of how TAMs can be highly complex.[21,22]

1.3 General characteristics of TANs:

Tumor-associated macrophages and stromal vascular fractional anisotropy (TAMs) are normal host cells that aid cancer cell proliferation, invasion, and metastasis in the tumor microenvironment. [28,29] It is now known that cells generated from bone marrow, such as TAMs, TANs, and myeloid-derived suppressor cells (MDSCs), contribute to tumor growth. [30] Several recent investigations used immunohistochemical techniques to show that neutrophils, another leukocyte, intermixed in cancerous tissues.

Micropeptides called chemokines to attach to G protein-coupled receptors and stimulate chemoattraction, inflammation, and/or angiogenesis. [31] They play a critical role in the dissemination of cancer. Many inflammatory chemokines, notably CXC-chemokines that recruit neutrophils, are produced by tumor cells. Neutrophils are drawn to tumors by chemokines called CXCs, which bind to CXCR1 and/or CXCR2 to initiate their migration. [32,33,34] Neutrophils were once thought to be the primary line of defense against external infections. New neutrophil function evidence has emerged. Environmental signals can polarize neutrophils toward different phenotypes, which regulate innate and adaptive immune systems [22].

Classically, they trigger phagocytosis, release lytic enzymes, and create reactive oxygen species (ROS). [35,36] Although neutrophils were originally thought to serve a defense function against tumor cells, mounting evidence has revealed their major role in penetrating tumor tissues to promote their growth, invasion, angiogenesis, and metastasis in a variety of malignancies. [37,38] TAMs are classified into two distinct populations, referred to as the anti-tumorigenic "M1" phenotype and the pro-tumorigenic "M2" phenotype, respectively. As is the case with TAMs, new research has revealed that TANs also display a significant amount of plasticity and are capable of polarization into either an anti-tumorigenic "N1" phenotype or a pro-tumorigenic "N2" phenotype. This is similar to what has been observed with TAMs. [32,39,40]

Their transcriptional regulators, cytokine patterns, and surface indicators are unknown. Neutrophils release inflammatory, immunoregulatory, and angiogenic substances like neutrophil elastase, matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), and hepatocyte growth factor, which might affect the tumor microenvironment. By producing TNF-α, ICAM-1, ROS, Fas, and lowering arginase, "N1" neutrophils boost cytotoxicity and decrease immunosuppression. Arginase, MMP-9, VEGF, and many chemokines like CCL2, CCL5, and CXCL4 are expressed by "N2" neutrophils, which promote tumor growth. [40,41,42,43]

According to research carried out by Fridlender and colleagues, the signaling of the transforming growth factor (TGF)- acts as a regulator between the N1 and N2 phenotypes. When TGF- is present in tumors, it drives differentiation in the direction of the N2 phenotype, while blocking its signaling results in the induction of an anti-tumor N1 phenotype. [39] Relatively recently, it was discovered that interferon- may also promote an N1 phenotype. When everything is considered together, the phenotype of TANs is determined by the signals that are met in the microenvironment of the tumor. [44]

Also, new research has looked into the role of neutrophil extracellular traps (NETs) in facilitating cancer cell migration and metastasis. Neutrophil extracellular traps (NETs) are protein-decorated strands of decondensed DNA. [45-47] In this way, they serve as a kind of mesh that ensnares microbes and then facilitates communication between pathogens and neutrophil-derived effector molecules.

Recent studies have revealed that NETs can act as a "snare" for circulating cancer cells, hence facilitating their migration to new locations. Cellular proliferation, migration, and invasion are triggered by NETs because they activate mitogen-activated protein kinase (MAPK) signaling, which in turn activates toll-like receptor 9 on CRC cells. [48,49,50]

Due to technological challenges in extracting TANs from human tumor tissues, the vast majority of studies on TANs have been conducted in mouse models. Unfortunately, the biology and roles of neutrophils in mice and humans are very different. Thus, the gradual growth of tumors and the complicated interactions between tumor and microenvironment cells in humans are not represented adequately in murine models of tumor progression. There is no direct evidence of pro-tumoral

activity of neutrophils in human tumor tissues, and the N1/N2 polarization of TANs in humans is still unclear [51].

However, experimental investigations on murine models have highlighted both the pro-tumor and anti-tumor activities of neutrophils in cancer. Tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) from newly formed tumors were more cytotoxic toward tumor cells, but TANs from more established tumors acquired a more protumoral character [52]. Although it is known that TAM activation is irreversible, the same cannot be said for TAN activation, and it has been hypothesized that neutrophils' N1-like and N2-like behaviors result from varying degrees of activation rather than polarization [53]. To yet, it is unclear if TANs can be regulated to enter a state of straightforward irreversible activation or whether reversible activation states are also possible.

1.4 Origin and Recruitment of TAMs in Tumor Sites:

Traditionally, it has been thought that macrophages in tumors are formed by monocytic progenitors in the local environment and attracted from the periphery by chemotaxis.

Newer research, however, demonstrates that in at least certain cancers, tissue-specific embryonic-derived resident macrophages infiltrate tumor tissues and hence represent a non-negligible input source of TAMs.[23] Although research has shown that resident macrophages derived from monocytic rather than embryonic sources can support the expanding population of TAMs in the tumor's inflammatory environment, the potential differences between monocytic- and embryonic-derived TAMs and their effects on tumor development and/or progression remain an intriguing open question.[24] Macrophages are widely understood to originate from monocytes in the bone marrow.

Recent research suggests that the recruitment of circulating monocytes is critical for TAMs accumulation, even though TAMs originate mostly from bone marrow monocytes. Multiple cytokines (CSF-1) and chemokines (CCL2 and CCL5) in the VEGF family may be responsible for

attracting circulating inflammatory monocytes to tumors. CCR2+ monocytes can be induced to become TAMs as a result of tumor development.[25,26]

In the presence of cytokines and growth factors such CCL2, CCL18, CCL20, colony-stimulating factor -1 (CSF-1), and vascular endothelial growth factors, circulating monocytes are attracted and develop into macrophages (VEGFs). In addition to existing macrophages in the lungs and peritoneum, epidermal Langerhans cells, Kupffer cells, and microglia in the brain also participate in the polarization of newly acquired macrophages. M2-polarized macrophages, also known as TAMs, are generated from M1 macrophages when stimulated with lipopolysaccharide (LPS), tumor necrosis factor-alpha (TNF-alpha), and interferon beta. HIF, CSF-1, and interleukins CXCR4, CXCL12, and IL10 are all secreted by tumors and macrophages in the TME to promote macrophage polarization. The M1 phenotype of macrophages exhibits anticancer properties, while the M2 phenotype of macrophages exhibits protumor properties. H igh IL-12 and low IL-10 phenotypes stimulate immune responses and aid in the destruction of cancer cells. Instead, during the superior.[27]

1.5 Recruitment of TANs:

Substances released by tumors can direct the recruitment of neutrophils. At this point, it is generally agreed that nearly all solid tumors release chemokines that control the number of PMNs in the tumor microenvironment. In the same way as IL-8, MIP-1/CCL3, and human granulocyte chemotactic protein-2 (huGCP2/CXCL6) "behave as powerful chemoattractants and neutrophil activators" [54,55] MIP-1, GCP-2, and KC (15–17) in mice do the same thing.
We found that neutrophils were present in the peritumoral stroma of 238 HCC patients. Neutrophil recruitment in HCC peritumoral tissues is facilitated by the proinflammatory cytokine IL-17, either directly through the synthesis of chemokines or indirectly through the activation of IL-17-producing T cells [56]. It has been determined that the spleen, in addition to the blood, is a significant TANs reservoir. This is because neutrophil precursors migrate from the spleen to the tumor stroma as the tumor grows.

In contrast, in a mouse model of lung adenocarcinoma showing activation of K-RAS and inactivation of p53, removal of the spleen surgically inhibits tumor growth by lowering the number of invading neutrophils. [57]

UV-irradiated keratinocytes secrete "High Mobility Group Box 1 protein (HMGB1)," which greatly enhances melanoma spread. The release of HMGB1 from UV-damaged epidermal keratinocytes triggers the activation of neutrophils, which is in turn mediated by Toll-like receptor 4. (TLR4). Using a genetically modified mouse model, researchers found that exposure to UV light triggers an inflammatory response that in turn promotes angiogenesis by increasing neutrophil activity and the migration of melanoma cells in the direction of endothelial cells. Histo-pathologists first identified this process, now known as "angio-tropism," as a result of the inflammatory response to UV-irradiation, which catalyzes interactions between melanoma and endothelial cells that ultimately result in invasion into the perivascular space. [58]

Mast cells in silica-exposed lungs secrete LTB4, a leukotriene early mediator of inflammation, which interacts with BLT1, a leukotriene B4 receptor, on neutrophils to recruit these cells and accelerates rapid tumor growth. In contrast, tumor growth in an implanted lung tumor model is slowed when BLT1 is deleted. [59]

Adjusting the neutrophils' lifespan is another strategy for controlling their infiltration into the tumor microenvironment. Neutrophils prevent apoptosis in Head and Neck Squamous Cell Carcinoma (HNSCC) due to macrophage migration inhibitory factor (MIF) secreted by tumor cells, as shown in. [60] In addition, neutrophils' increased autophagy may be one way in which activated immune cells contribute to tumor development. Cell survival, cell death, and metabolism are only some of the many physiological and pathological processes linked to autophagy [61]. Cancer cells engage in autophagy due to prolonged hypoxia and inflammation [62], which allows them to survive in a hostile environment [63,64].

CXCL1, CXCL2, and CXCL5 chemokines and their receptors are also involved in IFN-'s effects on neutrophil migration. Mice with tumors exhibit CXCL1 and CXCL2 gradients, with low levels of chemokine in the bone marrow (BM) and high levels in the tumor. CXCR2 expression was conversely higher on BM neutrophils and lower on TANs. When it comes to CXCR2, IFN- is

powerless, but it can control the ligands that bind to it. In this way, IFNs can play a role in cancer prevention through natural processes [65].

Using highly purified neutrophils from healthy donors, thyroid cancer cell medium stimulated neutrophil chemotaxis by IL-8 and survival by GM-CSF. Neutrophils underwent many morphological and activation alterations, including CD11b, CD66b, and CD62L upregulation. IL-8, VEGF-A, TNF-α, and MMP-9 were also upregulated. [66]

1.6 Possible Interaction of TANs with TAMs:

Is it TANs' job to activate macrophages in the TME in an M2-like fashion, or do they also play a role in recruiting TAM precursors to the tumor site? The release of IL-8 and TNF- from active neutrophils is known to activate and recruit macrophages to the site of inflammation.[67] MPO is secreted by neutrophils, and when it binds to the MMR, it triggers the release of IL-8, TNF-, and GM-CSF, all of which contribute to the persistent inflammation that characterizes conditions like rheumatoid joints. [68]

M2-like macrophages are characterized by their low levels of HLA-DR and IL-1beta expression in comparison to their high levels of macrophage mannose receptor (MMR) and IL-10. [69] Massive MPO-positive neutrophil infiltration was discovered in established cases of colorectal cancer [70] and lung cancer [71], despite the fact that we still lack direct data that supports the connection between TAN and TAM via MPO and the MMR. Additionally, the fact that TGF- has a similar effect on neutrophil and macrophage activation (M2-like and N2-like, respectively) suggest that TAMs and TANs are closely related in the same TME and raises the possibility that macrophage recruitment by neutrophils may occur before their N2-like polarization. The relationship between TANs and TAMs in the TME needs to be compared to the well-known

interactions between neutrophils and macrophages in a nontumoral chronic inflammatory milieu. Tumor-bearing mice were physically transferred from the spleen to the tumor stroma. Furthermore, they discovered that the recruitment of tumor-promoting spleen-derived TAMs needed CCR2 signaling, establishing the spleen as a key location of TAM and TAN amplification (Cortez-Retamozo et al., 2012).

Proliferation may contribute to macrophage buildup during type II inflammation (Jenkins et al., 2011) and inflamed peritoneum of mice (Davies et al., 2011) or in cancer, according to recent findings. Despite the fact that gliomas release a wide range of chemokines, mouse microglia cells are self-sustaining and do not require continual replenishment from circulation (Ajami et al., 2007). M-CSF/CSF-1 and IL-4 may promote the proliferation of macrophages, including TAMs. Our lab discovered a paracrine loop in the regulation of TAM proliferation in murine sarcoma in the early 1990s, including M-CSF/CSF-1 released by sarcoma cells and acting on c-fms expressing TAMs (Bottazzi et al., 1990). More recently, a paracrine loop involving tumor cell-expressed M-CSF/CSF-1 and macrophage-derived epidermal growth factor (EGF) has been shown to promote macrophage survival and recruitment during solid tumor formation (Condeelis and Pollard, 2006).[72]

1.7 Nuclear Extracellular Trap (NET) Formation in the TME:

Activated neutrophils release DNA-histone complexes and proteins to form NETs. NETs are involved in autoimmune diseases and neutrophil innate immune responses.

diseases include systemic lupus erythematosus, rheumatoid arthritis, psoriasis, and non-infectious pathologies such as coagulation disorders, thrombosis, diabetes, atherosclerosis, vasculitis, and cancer. NETs have been linked to cancer progression and metastasis in animal models and humans. In primary and metastatic cancers, NET development has been linked to cancer cell recruitment of

tumor microenvironment neutrophils (TANs). NETs can potentially collect and spread malignant cells.

Wakes dormant cancer cells, promoting relapse and metastases. NETs' pro-tumorigenic activity in malignancies makes them a possible cancer treatment target.[73] Animal models and human patients have both been found to have NETs in their blood and tumors, where they may play pro- or antitumoral roles depending on the state of the immune system and the tumor microenvironment. [74.75] There have been studies looking at the link between NET development and the onset, progression, and dissemination of tumors, including reports of NETs' direct activity on tumor cell proliferation via their proteases or activating signals.[76] It has also been shown that Tumor-Associated Neutrophils (TANs) are present in the tumor microenvironment and are linked to the creation of The NET and that cancer cells can induce NETosis in vivo and in vitro. [77]

1.8 The Role of Platelets in Macrophage and Neutrophil Recruitment:

One of the most well-known functions of platelets in circulation is their role in vascular hemostasis, thrombosis, atherosclerosis, and inflammation. It is less well understood, however, how platelets interact with cancer cells. When you consider that blood arteries are among the most important anatomical channels for cancer cell dispersion, this finding comes as a bit of a surprise. Circulating tumor cells communicate with endothelial cells, white blood cells, and platelets within the blood vessel. [78]

In order to survive, tumor cells that make it into the bloodstream must withstand intense shear forces and immunological surveillance, such as the attack of natural killer cells. This mechanism is extremely inefficient since only a tiny fraction of circulating tumor cells eventually reach metastatic foci. By encasing them in a thrombus, platelets prevent cytolysis by natural killer cells, which would otherwise destroy circulating tumor cells (CTCs). Hypercoagulation and a higher risk of thrombosis are caused by the tumor cells' activation of platelets through separate processes, allowing for persistent attachment between platelets and tumor cells. [79,80,81] ADP, thromboxane A2, and high-mobility group box 1 (HMGB1) are soluble mediators released by tumor cells that bind toll-like receptor 4 (TLR4) and cause local platelet activation. [82]

Consequently, tumor cells can produce localized coagulation and platelet activation. Tumor cells and platelets agglomerate after platelet activation employing several adhesion receptors. After tumor cell injection into Sprague-Dawley rats' tail veins, Chew and Wallace found fibrin accumulation on the platelet and tumor cell covering. After tumor cell injection, fibrin deposition peaked at 1 h and vanished at 9 h. At the early stages of tumor cell dispersion, solitary neutrophils, and lymphocytes were seen in the developing aggregates. [83] Platelet-dense granules are responsible for the secretion of ATP upon the platelet-mediated arrest of the tumor cell embolus at the arterial wall.

This, in turn, binds to endothelial P2Y2 receptors and activates these receptors. After this, the endothelium barrier is breached, which makes it easier for tumor cells to transmigrate and extravasate, ultimately leading to the formation of metastatic foci [84]. Platelet activation results in the release of more than 300 bioactive chemicals, in addition to ATP, which are then sequestered into the immediate surrounding environment [85]. Several of these chemicals have been gifted with the power to stimulate the proliferation of tumor cells and to accelerate the development of tumors. In a mouse model of orthotopic pancreatic cancer, daily injection of a P2Y12 inhibitor called clopidogrel resulted in a considerable reduction in both the growth rate of the tumor and the number of metastatic foci [86]. Blocking integrin IIb/III led to a reduction in the migration, invasion, and proliferation of endothelial cells. Moreover, the mean tumor volume of melanoma cells injected subcutaneously into nude mice and rats was significantly decreased [87].

Female nude mice injected with ovarian cancer cells into the peritoneal cavity showed an increase in tumor weight after receiving a platelet transfusion. Decreases were also seen in levels of cleaved caspase-3, an enzyme involved in apoptosis. Docetaxel's anti-tumor effects in tumor-bearing mice may be restored by platelet transfusion [88]. Ovarian cancer cells are protected against the apoptosis generated by docetaxel in vitro when cocultured with platelets across a membrane [89]. Hence, the physiologically active chemicals found in platelets can serve as indicators of tumor development and apoptosis.

Platelets are vital to the process of angiogenesis, as shown by in vitro and in vivo studies employing angiogenic assays [90-94]. Platelets contain the proangiogenic chemicals VEGF, PDGF, bFGF, and EGF. There are several antiangiogenic chemicals present in platelets, including endostatin,

angiostatin, PF4, and thrombospondin [95-98]. Elevated levels of VEGF in the blood are linked to advanced disease and a bad prognosis in a number of cancers [99,100]. Possible link between VEGF and tumor angiogenesis. Tumor endothelial cells that express TF can also engulf platelets. As a result, TF creates thrombin and activates platelets via PAR-1, leading to local platelet settling and growth factor release [101]

Platelets release SDF-1, which is essential for the recruitment and placement of macrophages in tumors, and they play a significant role in directing homing and retention signals for bone marrow-derived cells (BMDCs) and cancer cells. There are a number of ways in which platelets might entice neutrophils to an injury site, including the creation of platelet/leukocyte complexes, the release of serotonin, and the activation of P-selectin on platelets and ICAM-1 and v3 on endothelial cells.[102] Platelets stimulate angiogenesis in a variety of different ways; however, the specific methods by which platelet granules secrete the many proangiogenic and antiangiogenic substances are still partially understood. Platelets promote angiogenesis in a variety of distinct ways.

New therapeutic possibilities for the modification of platelet-mediated support of angiogenesis will become available once we have a clearer understanding of the signaling pathways, receptors, and granule organization that are involved in platelets. T. cruzi, as shown in studies of human and mouse macrophages, elicits Ca2+ flux for invasion of immune and non-immune cells and signals low levels of NADPH oxidase mediated oxidative burst, inducible nitric oxide synthase mediated nitric oxide production, and a delayed inflammatory cytokines/chemokines response in innate immune cells. [103,104] This means that M may not be able to eliminate the parasites and may even help them spread to other tissues. [105] In individuals with CD whose status is shifting from indeterminate to chronic, parasitemia is under tight control due to the activation of adaptive B and T cell response. However, during the transition from the indeterminate to the chronic phase of CD, patients show a systemic increase in inflammatory markers (N-terminal pro-brain natriuretic peptide, C-reactive protein), inflammatory cytokines (eg, IL1, IL6, IFN, TNF), and chemokines (eg, CXCL9, CXCL10), as well as free radical-induced oxidative lesions of lipid In addition to elevated numbers of TNF+ monocytes and a proinflammatory transcriptome and proteomic profile in peripheral blood, CD patients also exhibit these characteristics. There is an overall increase in

the tissue distribution of the inflammatory cytokine Mo/M in patients with chronic CD. Hence, Mo/M are understood to be critical regulators of inflammation in CD.

Chapter 2:
literature review

2.1 Objectives of the Study

The purpose of this study is to gather the most promising data about Tumor-associated Macrophages and Neutrophils, and their possible interaction with the tumor microenvironment. Our goal in reviewing this article is to :

- To know about the tumor and its microenvironment.
- To know about tumor-associated macrophages.
- To know about tumor-associated neutrophils.
- To learn about the interaction of macrophages and neutrophils with the tumor microenvironment.
- To learn about the origin and requirements of tumor-associated macrophages for tumor sites.
- To learn about the clinical implication

Chapter 3:

Methodology

3.1 Method of searching

The following terms were used to analyze traditional books and databases like SciFinder, Elsevier, Springer, Sci-hub, PubMed, and Google Scholar between 2000-2022 on the Tumor Microenvironment and the interaction occur in it.

3.2 Inclusion criteria

- ✓ Tumor microenvironment
- ✓ Tumor-associated Macrophages
- ✓ Tumor-associated Neutrophils
- ✓ Clinical implication

3.3 Data analysis

To study and create the objects, an exploratory reading of the numerous articles was done while evaluating the work's title and abstract. After finishing the exploratory analysis, read just the papers that discussed the interaction between macrophages and neutrophils on tumor microenvironment, and the research and clinical implication. Making a primary file, working on paraphrasing, and using Grammarly before producing a final review paper

Chapter-4:

Result and Discussion

4.1 Clinical Implication:

Cancer treatments include surgery, radiation, chemotherapy (CT), and immunotherapy (IT). Since they attack the tumor from different perspectives, several anti-tumor therapies (e.g., numerous cycles of chemotherapy) function better than one. Several CT regimens can reduce T and NK cells, which can hamper immune-based therapy. We hypothesized that macrophages (M/) may be more resistant to CT than other immune cells and that IT targeting M/ activation may synergize with CT. Malignancy is promoted by the high number of macrophages seen in tumor microenvironments. Differentiated macrophages called metastasis-associated macrophages (MAMs) are critical in facilitating tumor cell metastasis, dissemination, and establishment of a metastatic niche at distant sites. Reduced host-protective macrophage properties in the TME can be restored by therapeutic intervention.

Repolarization of M2-like protumoral macrophages to antitumoral M1-like cells, as well as the ablation of macrophages and the reduction of myeloid cell migration, have all been studied as potential treatments for cancer in recent years. N2 TANs have the potential for therapeutic use. Neutrophil-secreted enzymes or cytokines, crucial in various stages of tumor progression, might be another effective technique. Neutrophil-macrophage interaction suggests that reducing TANs may have a knock-on effect on TAM levels. Using coumarins or warfarin for UFH in the early clinical trials was successful in treating coagulation issues brought on by malignancy. Clotting issues in patients with cancer have led researchers to test out new forms of heparin and hirudin. Research has shown that heparin derivatives (namely LMWH) are the most effective anticoagulants for preventing recurrent DVT.

An excellent but out-of-date research examined the effects of warfarin and dipyridamole, two non-heparin anticoagulants, on tumor growth. Warfarin did not slow the development of colorectal, head, or neck cancers, according to these studies. It was shown that the use of warfarin increased survival rates for patients with small cell lung cancer, non-small cell lung cancer, prostate cancer, and melanoma. When it came to cancer therapy, both dipyridamole and coumarin were met with skepticism. Targeted reduction of TF signaling or platelet activity could be advantageous in therapeutic conditions because of the risk of bleeding problems linked with harsh anticoagulant therapy for cancer patients. There is presently no evidence to support the assertion that direct

platelet receptor antagonists enhance the prognosis of cancer. Nevertheless, this does not rule out the possibility that they do so. In a similar vein, there is insufficient data to justify the practice of combining antiplatelet medications with conventional chemotherapeutic drugs.

Platelet mimicry is the process by which tumor cells alter their gene expression profiles in order to acquire a genophenotype that is similar to that of platelets. These tumor cells also express several megakaryocytic genes (adhesion receptors IIb3, thrombin receptor, and PECAM/CD31 and/or platelet-type 12-LOX) in order to activate platelets or the coagulation cascade. Platelet mimicry can be used to treat a variety of conditions, This well-described epiphenomenon encourages the hematogenous dispersion of tumor cells during the process of metastasis. Given this, it is reasonable to hypothesize that the identification of genetic pathways to modify platelet mimicry may possibly give innovative therapeutic methods. Neutrophils are attracted to early metastatic niches in part through the CXCR2 receptor for the granulocyte- and platelet-derived ligand CXCL5/7; CXCR2 inhibitors also impair granulocyte recruitment at initial tumor sites. [C]XCR2 inhibitors. Targeting the CXCR2-CXCL5/7 axis could be effective in clinical settings, despite the fact that the majority of patients can tolerate anti-CXCR2 medications that are currently being investigated in the clinic for inflammatory disease.

4.2 Relatively New Developments in the Study of the Tumor Microenvironment:

While traditional cancer therapies are very successful against cancer cells, they also have a negative impact on healthy tissues and cells.

As a result of advances in our understanding of cancer's molecular underpinnings, novel approaches to cancer therapy are increasingly being put into reality.

Oxygen-starved or hypoxic zones inside a tumor develop when the blood arteries supplying the tumor with oxygen and nutrients cannot supply. Supply the tumor with oxygen and nutrients is unable to keep up with the tumor's erratic or fast development. Research into the tumor microenvironment will allow for testing several therapeutic options in clinical trials, including novel medications, novel surgical and radiation treatment techniques, and novel treatment combinations.

Due to the variable degrees of the effect exerted by various cancer treatment modalities, the tumor microenvironment is regarded as significant for the management of this disease. The conventional methods of tumor treatment have a number of drawbacks, such as the tumor vascular structures being leaky and highly disoriented, which prevents oxygen from reaching the tumor core and rendering it resistant to radiation therapy, and the fact that cells located far from blood vessels require — but do not receive — the necessary nutrients or any chemotherapy substances. These limitations highlight the importance of understanding the tumor microenvironment and using an efficient therapeutic approach when assessing and treating cancer.

Although the effectiveness of anticancer therapy varies greatly between tumors and normal tissues, researchers are concentrating on the microenvironment to find effective ways to treat tumors. The tumor microenvironment is currently being studied as an independent entity linked to cancer that may be attacked. By focusing on the tumor microenvironment, new approaches have been discovered that are both successful and promising. As a result of oxygen deprivation, cancer cells are sensitive to radiation treatment, and the tumor microenvironment is heavily exploited in the tumor vasculature.

The melanoma cells become immune to the targeted therapies as well as standard chemotherapy. Not every melanoma patient responds well to standard treatments like chemotherapy and targeted therapies that use immune checkpoint inhibition. Resistance of tumor cells to therapy results from a combination of variables including genetic alterations, cellular physiology, and tumor heterogeneity. Several studies have shown the importance of the inflammatory tumor microenvironment. It has also been shown that a multi-modal strategy, which includes attacking the microenvironment in addition to malignant cells, is required for a more effective therapeutic outcome.

The process through which new blood vessels are generated is referred to as angiogenesis, and it is controlled by chemical signals that are produced by the body. The process of angiogenesis includes the movement, growth, and differentiation of endothelial cells, which are the cells that line the inside wall of blood arteries. The process of controlling angiogenesis is an essential component of both the therapy for cancer and the prevention of the disease. By gaining a better

knowledge of the role of the tumor microenvironment in the control of angiogenesis and the method used to unravel molecular linkages, it may be possible to improve both the prognosis and the therapy that is specifically targeted.

Chemoprevention is the use of pharmacologic or natural therapies that stop or reverse the development of premalignant cells in which DNA damage has already occurred. This stops or reverses the proliferation of premalignant cells, which prevents the invasive form of cancer from forming.

Studies 39 of early disruption in the tumor microenvironment and the dysfunction of the epithelial layer are essential for the process of carcinogenesis, and in particular for the process of cancer chemoprevention. It is possible that hypoxia-induced tumor cells will be resistant to therapy now that the illness has progressed to an advanced stage. Antagonize These Cells

differentiation of cancer cells and actively contribute to the continuation of the existence of cancer stem cells. They were shown to play a crucial part in the process of molding the stromal microenvironment of the tumor throughout the course of time.

Since hypoxia has the ability to support the establishment of a microenvironment that distinguishes tumor cells and stromal cells, targeting hypoxic stem cells may be critical for successful tumor treatment. This is because hypoxia causes tumor cells to behave differently from stromal cells.

4.3 How the Tumor Microenvironment Will Influence Future Drug Development:

Current developments in cancer research have centered on the microenvironment of tumors. This includes the cellular and molecular components that make up the tumor as well as the influence that this environment has on the progression of the tumor. The chemicals that are released by tumor cells have a substantial impact on the progression of tumors. They include pro-inflammatory and anti-inflammatory molecules as well as pro-angiogenic and anti-angiogenic mediators, all of which contribute to the formation of the microenvironment around the tumor.

The processes and molecules involved in this kind of back-and-forth communication within the microenvironment of the tumor have emerged as potentially alluring therapeutic targets for the treatment of cancer. It is essential to investigate the stroma that surrounds cancer cells while studying the development, progression, and behavior of a tumor. The stroma is the connective tissue that surrounds the cancer cells. According to Report 54, one of the most important aspects of knowing how cancer starts and spreads is paying attention to the interactions that take place between the stroma and neoplastic cells.

The study of how tumors develop has benefitted significantly by improvements in our knowledge of the microenvironments inside tumors. The idea of treating tumor cells depending on the microenvironment in which they are located has a lot of potential, and this innovative technique will also help in the development of new treatments to fight cancer.

The progression of human tumors is a challenging topic to research due to the fact that tumors fulfill many functions, such as growing and invading healthy tissue and spreading to other sites, respectively. Since it supplies the required nutrients, the microenvironment of a tumor is an essential component in the progression of the malignant process. The physiology, structure, and functioning of tumors are wholly distinct from one another and are entirely reliant on the local microenvironment. The growth, progression, and development of potentially lethal metastasis by tumor cells are critically dependent on a fundamentally aberrant connection between tumor and stromal cells.

This connection can only exist when tumor cells and stromal cells interact in a way that is fundamentally abnormal. Since stromal cells in the tumor microenvironment are genetically stable, using them as a therapy option is a good choice that significantly reduces the likelihood of the tumor returning. By gaining a clearer knowledge of this interaction, it is possible that novel and helpful treatment targets for cancer therapy, risk assessment, and prevention may become apparent. There are a lot of different factors that might lead to the development of cancer, some of which include normal cells, compounds that are secreted by cancer cells, and stromal cells.

Chapter-5:

Conclusions

5.1 Conclusions and Perspectives:

Myeloid cell activity in the tumor microenvironment (TME) and tumor growth has been the focus of significant effort and success made by leading researchers in the area.

Sadly, there is an overwhelming amount of data now available on the function of macrophages and neutrophils in the tumor microenvironment (TME) is poorly understood, leading to a splintered understanding of protumoral myeloid cells in cancer.[13] Moreover, the existence of certain myeloid subgroups in cancer has been hidden due to the lack of distinct markers to identify each subset.

The role of TAMs and TANs in tumor growth is well established, however, positive outcomes have been shown in animal model-based or preclinical investigations. More advanced tumor models and methods to distinguish between myeloid cell subsets in vivo are on the horizon, and we expect this to yield fundamental insights into the potential spatial and temporal modulation of myeloid cells, including their interaction with platelets, at each stage of tumor progression.

Chapter-6:

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