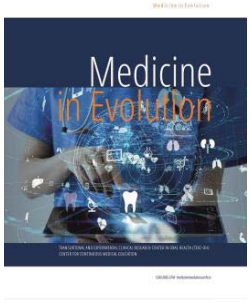
Review: The tumor microenvironment of melanoma



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Abstract

Aim and objectives: Melanoma is one of the most complex skin cancers based on its numerous somatic mutations and chromosomal instability. Besides the traditional causative factors, such as ultraviolet radiation, or genetic factors, recent studies showed that melanoma highly depends on its adjacent microenvironment. A malignant tumor, like melanoma, is composed of oncogenic cells, regular cells (melanocytes, macrophages, mast cells, keratinocytes, and others), the supporting stroma, which includes fibroblasts, endothelial cells, immune cells, soluble molecules, and the extracellular matrix (ECM). This article aims to present the tumor microenvironment's main functions in the development or progression of melanoma. **Material and methods:** we have studied some of the most recent 30 articles based on the role of the microenvironment in melanoma. **Results:** After analyzing the most recent articles regarding melanoma's tumor microenvironment (TME) and its impact, we have found that the cells that comprise the TME can affect melanoma's onset or progression. TME's cells actively interact with each other and have many functions, from tumor-suppressing roles to the initiation or progression of melanoma. **Conclusions:** Understanding the complex interplay between tumor cells, and systemic mediators of disease progression is critical for the rational development of effective melanoma treatments. Each cell involved in the TME can play an important role either in the onset or advancement of melanoma with the help of certain mutagenic factors.

Keywords: melanoma, microenvironment, mast cells, mutations

INTRODUCTION

Melanoma develops through the oncogenic transformation of melanocytes, under the influence of various mutagenic factors, these cells being derived from the neural crest [1], [2]. The malignant transformation of melanocytes begins with a proliferative stage in which the formation of common nevi occurs, then an abnormal and dysplastic growth of melanocytes takes place, with the onset of atypical nevi [2]. Subsequently, the tumor will grow radially, with the intraepidermal development of the tumor, then a vertical growth would occur, with the invasion of the dermis and subcutaneous tissue. Finally, the cancerous cells acquire the ability to metastasize to other organs [2]. When atypical melanocytes cross from the epidermis into the dermis a "trigger reaction" takes place where an inflammatory infiltrate appears, formed of macrophages, histiocytes, and lymphocytes and this could be considered the first step of the immune system in trying to block the migration of atypical cells [3]. In this process, many atypical melanocytes are destroyed, but some manage to continue their path in melanoma development and the metastasizing process [3].

The onset of melanoma is also associated, both through direct or indirect interactions, with the tumor microenvironment formed by various cells (mast cells, macrophages, keratinocytes, or other immune system cells, and cells that form the cutaneous blood vessels) that interact with each other through signaling proteins and cytokines [1]. Recent studies have shown that the cells mentioned above could be involved in all stages of melanoma: initiation, 0enhance the migration of cancerous cells and therefore could be involved in melanoma progression and the metastatic process [1].

All this considered melanoma may also arise "de novo" (de novo melanoma / DNM) under certain mutational factors, and not only on preexisting lesions (nevus-associated melanoma/NAM) [1].

Aim and objectives

This article aims to present the microenvironment's main roles in the appearance, or progression of melanoma. Some of the TME cells will be presented for their role in melanoma's initiation or progress.

MATERIALS AND METHODS

We have studied over 30 of the most recent articles regarding the role of the tumor microenvironment in melanoma, to provide a review of the possible causes of the initiation and progression of this neoplasia and the importance of the TME.

RESULTS

The present study will be categorized into 3 sections, as follows: 1. The role of the tumor microenvironment in melanoma, 2. Immune system cells, 4. Vascular cells - angiogenesis and melanoma. After analyzing the latest articles on the topic of the tumor microenvironment in melanoma the following facts were found:

1. The role of the tumor microenvironment in melanoma

The tumor microenvironment (TME) composition differs from tumor to tumor, but the most frequently encountered features include immune cells, stromal cells, blood vessels, and extracellular matrix (ECM) [4]. Some studies attest that TME could be an active promoter of cancer progression, as in tumor growth there is a relationship that develops between the oncogenic cells and the components of the TME to support the survival of neoplastic cells, the local invasion and metastatic dissemination [4].

Therefore, melanoma's onset and progression are based on a complex evolution from a common nevus, at first through a radial superficial growth phase, then a vertical invasive phase that leads to an eventual metastatic process [5]. Epidermal melanocytes are attached to the basement membrane, and their migration is controlled mostly by the surrounding keratinocytes [5]. Thus, the epidermis represents the first microenvironment responsible for melanoma progression [5]. This process involves cell-to-cell interactions and the secretion of a broad range of bioactive molecules [5]. At first, there is a breakdown of the dermo-epidermal basement membrane followed by the migration of oncogenic melanocytes into the dermis [5]. Then, the microenvironment must support the multiplication and survival of melanocytes outside the normal epidermal–melanin units [5].

When atypical melanocytes cross from the epidermis into the dermis a "trigger reaction" occurs where an inflammatory infiltrate takes place, formed of macrophages, histiocytes, and lymphocytes and this may be the first step of the immune system in trying to block the migration of atypical cells [3]. In this process, many atypical melanocytes end up being destroyed, but some manage to continue their path in melanoma development [3].

The tumor microenvironment is formed of various immune cells, fibroblasts, endothelial cells, and the extracellular matrix (ECM), among others [6]. The normal tissue microenvironment can block cancer outgrowth through the suppressive functions of immune cells, fibroblasts, and the ECM [6]. However, for cancer to progress, it must evade these functions and influence cells in the TME to become tumor-promoting, resulting in increased multiplication, and local invasion [6]. TME also plays an important role in the metastatic process, stimulating cancer cell survival in the circulation, and promoting extravasation [6]. During the metastatic stages, the TME helps to control metastatic cell dormancy, and subsequent metastatic outgrowth [6].

1.1. Keratinocytes

Primary melanomas, originating from melanocytes, appear in close interaction with keratinocytes, which are known for their role in melanoma initiation, progression, and immune escape [7].

The first signs of oncogenic transformation include increased melanocyte density, changes in cellular features, and migration from the dermal-epidermal junction [7].

At first, melanoma in situ can be formed through a pagetoid growth with superficial spreading for BRAFV600E cells or through a lentiginous growth with confluent individual melanocytes along the dermo-epidermal junction for BRAFnonV600E cells, leading to the radial growth phase [7]. Later, the vertical growth phase can occur and is associated with an epithelial-to-mesenchymal-like transition or phenotypic switching of oncogenic cells triggered by certain genetic events and microenvironmental factors [7]. Therefore, some studies attest that keratinocytes are involved in melanoma initiation (through the BRAFV600E mutation), invasion/progression (eventually through activating melanoma cell Notch signaling) and may be involved in melanoma-associated inflammation (melanoma initiation, progression, and metastasis have long been associated with chronic inflammation) [7].

2. Immune system cells

Immune cells are a very important component of the TME, as immune cells can either inhibit tumor growth or initiate it [4]. The immune system is mostly known for having two main functions: non-specific and acquired immunity [8].

Adaptive immunity is activated by exposure to certain antigens and is involved in "evaluating" threats through immunological memory and in enhancing immune responses [4]. T-cells, B-cells, and NK (natural killer) cells belong to the adaptive immune response [4]. In general, macrophages and natural killer (NK) cells recognize and eliminate oncogenic cells. Innate immunity can be considered a non-specific defense mechanism that appears hours

after a foreign antigen enters the body [4]. Cells that carry out an innate immune response include macrophages, neutrophils, and dendritic cells [4]. Also, along with the dendritic cells, some studies confirm that macrophages are involved both in innate and acquired immunity [8].

2.1. Macrophages

Macrophages are noted in some studies as an important part of the tumor microenvironment in melanoma, as they are considered to have multiple effects on this neoplasm [9]. Macrophages may also have an important role in the immune system and their presence in a tumor could be associated with a poor prognosis [9].

Phenotypically, macrophages can be classified into two categories: classically activated (M1) and alternatively activated (M2) macrophages [9]. M1 macrophages can activate the adaptive immune system and may have antitumor abilities due to their pro-inflammatory response and the production of pro-inflammatory factors such as IL-6, IL-12, tumor necrosis factor (TNF), etc. As opposed to the M1 macrophages, alternatively activated (M2) macrophages have pro-tumor abilities and are associated with poor survival [9], see Table 1. Moreover, some studies show that macrophages may participate in tumor progression and immunosuppression, and can also promote tumor proliferation, lymphangiogenesis, therapy resistance, immune evasion/invasion, and metastasis [6], [9].

Table 1. M1 and M2 macrophages effects on melanoma [9]

Classically activated (M1) macrophages	**Alternatively activated (M2) macrophages**
Inhibits the invasion and migration of tumoral cells in melanoma	Promotes the invasion and migration of tumoral cells in melanoma
Decreasing the metastatic ability of tumoral cells	Increasing the metastatic ability of tumoral cells
Both types of macrophages may inhibit the growth of melanoma	
Triggers immune responses and normalizes irregular tumor vascular network	Promotes angiogenesis
Improves the efficacy of PD-1 immunotherapy and of the doxorubicin chemotherapy	Induces melanoma resistance to PD-1 inhibitors - resulting in anti-PD-1/PD-L1 therapy resistance

2.2. Mast cells

Mast cells originate from the bone marrow and possess many properties that enable them to participate in a diverse range of biological activities [10], [11]. They phagocytose, process antigens, produce cytokines, and release preformed (histamine, proteoglycans, proteases) and newly formed (leukotrienes, prostaglandins) mediators [11].

Mast cells (MC) are granulocytes that are involved in mediating the host defense and in the maintenance of homeostasis by degranulating histamines, cytokines, and chemokines [6]. They are known for their role in allergies and autoimmunity, but they can also infiltrate tumors [6], [10]. MC can exert both pro- and anti-tumorigenic activities depending on the microenvironmental stimuli [6], [10]. They can directly target tumor cells, but they mainly regulate the recruitment and activity of other immune populations and the endothelium [6]. From our research, regarding melanoma, mast cells can be present in lymph node metastases, in the vicinity of vessels in the intratumoral area (known for their role in promoting angiogenesis), and in the areas bordering the melanoma.

It seems that the tumor microenvironment can be either an ally or an enemy in cancer development, as MC infiltration could be critical in remodeling the TME by regulating immune and inflammatory reactions [10].

MC degranulation might be determined by tumor hypoxia and thus MCs can produce reactive oxygen species that are functionally correlated with their activation [10]. MCs can release not only pro-angiogenic factors, such as bFGF, VEGF, transforming growth factor β, TNF-α, and IL8, but also heparin and proteases that liberate pro-angiogenic factors [10].

Therefore, the importance of MCs lies in the genesis, growth, and metastasis of skin cancer [10]. Their wide biological characteristics and distribution (strategic locations near blood vessels, nerves, inflamed tissues, and neoplastic foci) enable them to play a crucial role in a multitude of pathologic processes [11].

3. Vascular cells - angiogenesis and melanoma

Angiogenesis, the process of developing new blood vessels, is essential for the formation of tumors and once a tumor grows beyond 1–2 mm, it must establish its vascular supply of oxygen and nutrients [6]

Endothelial cells (ECs) form a single cell layer that lines all blood vessels and display a remarkable heterogeneity and plasticity, as they control the passage of proteins, cells, oxygen, and fluid into the surrounding tissue [6]. Still, ECs that line tumor blood vessels differ from normal ECs [6]. Tumor ECs express low levels of adhesion molecules, which causes an impaired barrier function, and they express increased levels of inhibitory immune checkpoint molecules, which contributes to immunosuppression [6].

Lymphatic ECs (LECs) form the walls of lymphatic vessels and in the TME, they provide a dissemination route for cancer cells in addition to blood vessels [6]. Some studies note that LECs have recently been recognized as direct regulators of anti-tumor immunity and immunotherapy response, as LECs can present tumor antigens but also immune checkpoint molecules [6].

As the malignant tumor develops, new vessels need to be formed to maintain an adequate local supply of nutrients and oxygen, a process driven by the imbalance between pro- and anti-angiogenic mediators [10]. Developing a rich vascular network seems vital for melanoma cells during the vertical growth phase, as melanoma cells require nutrients and oxygen to sustain their vertical growth [12]. Therefore, angiogenesis is essential for the occurrence and development of melanoma.

Hypoxia, the lack of oxygen in tissue, is a major trigger for angiogenesis [6]. Many molecules that respond to hypoxia can promote angiogenesis, of which vascular endothelial growth factor (VEGF) and its downstream signaling pathway are the predominant drivers [6]. In melanoma patients, high intratumoral and systemic VEGF levels correlate with poor disease outcomes across cancer types [6].

Generally, in a normal state, pro-angiogenic and anti-angiogenic factors should be in a dynamic balance [12]. However, this balance of angiogenesis is often out of control in melanoma, therefore, large amounts of pro-angiogenic factors are released which play a dominant role in angiogenesis, leading to the formation of new blood vessels [12]. Then, with an adequate supply of nutrients, tumor cells can increase rapidly without control and become more invasive, ultimately leading to metastasis [12]. Pro-angiogenic factors are released by melanoma cells and can bind receptors expressed on endothelial cells, which determines the initiation of the downstream signaling effects to stimulate melanoma proliferation, metastasis, and differentiation [12].

Similarly, tumor lymphatics also have important immunoregulatory properties [6]. Like blood ECs, lymphatic ECs can suppress T cell responses through various mechanisms, including expression of immune checkpoint molecules and antigen presentation in the absence of co-stimulatory molecules [6]. High levels of VEGF-C, the predominant driver of lymphangiogenesis, are associated with increased metastasis and reduced survival [6].

DISCUSSIONS

Melanoma can be considered a complex ecosystem comprised of tumor cells and a multitude of non-cancerous cells, embedded in an altered extracellular matrix [6]. The tumor

microenvironment (TME) is formed of a multitude of immune cell types, endothelial cells, pericytes, and other tissue-resident cell types [6]. Primary melanoma tumors can develop on pre-existing nevi ("nevus-associated melanoma") or spontaneously ("de novo").

Melanocytes are specialized cells that synthesize and distribute melanin, a pigment with a role in the pigmentation of the skin, hair, eyes, and inner ear. Approximately 128 genes are involved in skin pigmentation and ensure this process through a complex mechanism. The disruption of the functions of these genes can cause the onset of pigmentary pathologies, affecting development (specification, migration, survival, proliferation), and melanocyte differentiation. Differentiated melanocytes produce melanosomes, organelles specialized in melanin synthesis. Melanosomes are distributed by melanocytes, at the level of suprabasal keratinocytes, which multiply towards the surface of the skin, where they form a protective barrier of the skin against various environmental factors (especially ultraviolet radiation). The density and differentiation of melanocytes are influenced by the environment, respectively by ultraviolet radiation, and by the factors secreted by keratinocytes and fibroblasts. Thus, melanoma has a heterogenous pathophysiology, caused by genetic mutations that imply cell multiplication, differentiation, and survivance.

The immune system is important for protection against various pathogens, for wound healing, and for the elimination of damaged cells [6]. To execute these roles, the immune system is incredibly diverse and adaptable, but despite the ability of adaptive immune cells to recognize and eliminate pathogens, cancer cells can escape destruction and form tumors [6]. At the earliest stages of tumor initiation, cancer cells could be targeted for destruction by the immune system [6]. Fibroblasts and macrophages may help inhibit tumor growth initially, but they can be influenced by the developing cancer to gain pro-tumorigenic functions [6]. For example, macrophages can support angiogenesis and invasion by secreting growth factors, cytokines, and proteases [6].

Once tumors have established the reinforcing connections between angiogenesis, inflammation, and fibrosis, they can develop local invasion [6]. Invasion is a complex, multi-step process that involves cancer cells detaching from each other, migrating from the primary tumor, and invading the surrounding stroma [6].

The next step in the metastatic process is the intravasation of cancer cells into the blood or lymphatic stream. The mechanisms by which cancer cells cross enter the circulation are complex, and influenced by cancer cell-intrinsic characteristics, the physical properties of the ECM and type of vessels, microenvironmental factors, and hypoxia [6]. The integrity of the blood vessels in tumors is often impaired, which cancer cell intravasation [6]. Lymphatic intravasation is another route that cancer cells may choose to disseminate, although the underlying mechanisms are not fully understood [6].

Regarding angiogenesis and melanoma, the main mechanisms of blood vessel formation described in this cancer are the formation of new blood vessels, the movement of cells along the surface of blood vessels, vasculogenic mimicry (melanoma cells remodel and form patterns of loops and channels that mimic vascularization) and vasculogenesis (the formation of blood vessels from endothelial progenitor cells). Similarly, it seems that tumor lymphatics may also have important immunoregulatory properties as they can suppress T cell responses through various mechanisms, including expression of immune checkpoint molecules and antigen presentation in the absence of co-stimulatory molecules [6]. For example, a high level of VEGF-C is associated with increased metastasis and reduced survival [6].

The literature is more focused on the study of tumoral cells, but recently, studies related to the importance of the tumor microenvironment have been published. The interrelationships between melanoma and the tumor microenvironment include cancer-associated fibroblasts, myeloid-derived suppressor cells, tumor-associated macrophages,

clustered differentiation of lymphocytes, dendritic, endothelial, lymphatic cells, and mast cells (MC). Mast cells play one of the most important roles in melanoma as they can be involved in the development, progression, and metastasis of this neoplasm through the secretion of proteases, and pro-angiogenic factors - both pro-inflammatory and immunoinhibitory mediators. The role of mast cells as an important player in angiogenesis is well known. This is due to the release of pro-angiogenic mediators such as IL-8, NGF, TNF-alpha, TGF-beta, urokinase-like plasminogen activator, promotion of endothelial cell proliferation, breakdown of connective tissue matrix, histamine, and release of VEGF-A, VEGF-B, VEGF-C, VEGF-D [6]. Similar to MC, other cells such as keratinocytes and macrophages can also promote angiogenesis, promote the invasion/migration of melanoma cells, and play a role in metastasis.

From our research, it seems that each cell involved in the TME can play an important part in either the onset or advancement of melanoma and later on in metastatic melanoma.

CONCLUSIONS

Melanoma is a type of skin cancer that develops from the cancerous transformation of the melanin-producing cells located in the basal layer of the epidermis. At first, the unrestrained multiplication of melanocytes can form a nevus, but over time, that nevus can turn into melanoma, under the influence of various mutagenic factors. Still, some tumors can develop in a spontaneous way (de novo melanoma) more often than on pre-existing nevi (nevus-associated melanoma).

The pathophysiology of melanoma is not known in its entirety, but several factors may develop or stimulate this malignant process. The tumor microenvironment (TME) seems to play a crucial role in melanoma, and it is formed of diverse immune cells, fibroblasts, endothelial cells, pericytes, and various other tissue-resident cell types. Each cell involved in the TME can play an important part in either the onset or advancement of melanoma with the help of certain mutagenic factors.

All this considered, this study aimed to present a review of the most recent articles on the tumor microenvironment and to observe the role of each TME cell in the onset, or progression of melanoma. In the future, the various functions of TME might be exploited to develop antitumoral therapeutic strategies.

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