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Non-tumor cells role in the tumor microenvironment (TME) of head and neck cancer (HNC)

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ABSTRACT

OBJECTIVE

Although it is known that the tumor microenvironment (TME) is composed of a heterogeneous group of cells, which includes tumor cells, as well as non-tumor cells, such as stromal cells associated with the tumor and some leukocytes types, the objective of this systematic review was to provide updated information on the participation of non-tumor cells present in the TME on tumor progression of head and neck cancers.

METHODS

A bibliographic review was carried out, through an online search between May and October 2020, of scientific articles published in Portuguese and English between 2002 and 2020 in the public health databases: LILACS, SCIELO, PubMed, and Google Scholar, following the question guiding: what is the role of non-tumor cells present in the TME in the progression of head and neck cancer?, and using the descriptors: neoplasia; tumor microenvironment; head and neck cancer; infiltrating tumor cells; stromal cells; fibroblast; leukocytes; T lymphocytes; macrophages, myeloid-derived suppressor cells.

RESULTS

Initially 158 articles were selected, of which 60 were excluded because they were duplicated, 58 because they did not address the theme of the study, and 18 because they did not answer the guiding question. Thus, 22 studies were used in this review.

CONCLUSIONS

TME is a specific tumor site where there is an intense interaction between molecules and cells and, in general, the non-tumor cells present in the TME, both fibroblasts, a stromal cell, and leukocytes act favoring the progression of head and neck cancer

DESCRIPTORS

Cytokines. Chemokines. Fibroblast. Macrophages. T cells. Myeloid-derived suppressor cells.

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INTRODUCTION

Cancer is a disease characterized by a significant and progressive alteration of the cell cycle, which leads to this cell to present an evident loss of its proliferation control [1, 2]. Alterations such as mutations or translocations in proto-oncogenes genes, as well as in tumor suppressor genes are responsible for inducing the transformation of normal cells to the malignant profile observed in neoplastic cells, a process known as tumorigenesis[1-4].

Based on the literature, the tumorigenesis process it is initiated from the accumulation of deletions, amplifications, mutations, translocations, inversions, or even by the insertion of viral DNA in the genetic regions containing proto-oncogenes or tumor suppressor genes, which drastically affects their gene expressions[3-5]. According to the World Health Organization (WHO), cancer ranks 4th place among the main causes of death in the world [6]. In relation to Brazil, it is estimated that 625.000 new cases will be diagnosed by 2022, and regarding the data presented in 2019 by the National Cancer Institute of Brazil (INCA), cancers from head and neck are the 5 most frequent types in Brazil, in both sexes[6].

Head and neck cancers (HNC) are composed of a broad and heterogenic group of neoplasm, which can develop in the oral and nasal cavity, paranasal sinuses, pharynx, larynx, thyroid, and salivary glands[7, 8]. Among these different types, head and neck squamous cell carcinoma (HNSCC) has a higher incidence worldwide, being the oral cavity the most prevalent site, whereas thyroid cancer is the second most common neoplasms in the head and neck[7, 9, 10].

It is well-known that smoking and alcoholism are the main carcinogenic agents that promote tumorigenesis for HNC, followed by human papilloma virus (HPV)[7, 11]. Furthermore, it is noteworthy to highlight that, even with a significant advanced in the diagnosis and therapeutic methods, the HNC is characterized as a disease of poor prognosis presenting a life expectancy of 5 years due to its aggressive behavior[7, 8]. Studies have reported that the tumor malignancy found in HNC is closely associated with the presence of inflammatory and immunosuppressive tumor microenvironment (TME), which can putatively favor not only tumor progression but also the prominent resistance to conventional therapies, such as chemotherapy and radiotherapy, observed in these cancers[7, 8, 11].

Since Virchow's reported, in the mid-eighties of the 19th century, evidencing that inside the tumor there is a significant infiltration of non-tumor cells, such as leukocytes and stromal cells, several types of research until now are developed with the objective to clarify the real role of these cells in the tumor microenvironment (TME), beyond of understanding how tumor cells can suppress the immune response in this specific site[12]. Taken into account the current data, this immunosuppressive area found in TME is generated not only by the expression of some types of cytokines and chemokines, as well as by the interaction between different types of cells present in the TME, which, in a general way, promote an environment favorable to tumor progression[7, 10, 11].

Based on these pieces of information, the aim of this systematic revision was to provide updated information about the participation of non-tumor cells present in the TME and also discuss their importance in tumor progression of the mains HNC.

METHODS

This study was carried out in a systematic literature review format, which allows critical analysis of current scientific knowledge on the main theme purposed, besides contributing to evidence-based health practice. In this sense, five steps were carried out: 1) definition of the theme and main study´s objective, 2) establishment of criteria for inclusion of studies, 3) definition of selected studies, 4) methodological evaluation of the included studies, and 5) interpretation of main findings.

In order to perform the present study is configured as a systematic review and therefore a bibliographic review is carried out through an online search of scientific articles in public health databases: LILACS (Latin American and Caribbean Literature on Health Science), SCIELO (Scientific Electronic Libary Online), PubMed (US National Library of Medicine National Institutes of Health), and Google Scholar, published between 2002 and 2020.

After defining the theme and the study´s main objective, the following guiding question was elaborated: What is the role of non-tumor cells present in the tumor microenvironment to the head and neck cancer progression? The inclusion descriptors used in the study were: neoplasia; tumor microenvironment; head and neck cancer; head and neck squamous cell carcinoma, thyroid cancer; tumor-infiltrating cells; stromal cells; fibroblast; leukocytes; T lymphocytes; macrophage, myeloid-derived suppressor cells. The systematic reviews, metanalyses, and original articles published both in Portuguese and English languages in the databases previously cited during the period between 2002 and 2020 were included. All the data analyses were carried out in the months of May to October 2020.

RESULTS AND DISCUSSION

Following the selection of the articles, initially, among the 158 articles that met the inclusion criteria, 60 were excluded because they were duplicated. In addition, 58 articles were excluded because they did not address the theme of the study, and 18 studies were excluded because they did not answer the guiding question. Thus, 22 studies that met completely the inclusion criteria recommended for this systematic review article were used, according to the flow diagram shown in Figure 1.

The main findings concerning the role of non-tumor cells, such as stromal cells (fibroblasts), as well as leukocytes (particularly the T lymphocytes, macrophages, and myeloid-derived suppressor cells) that infiltrate the TME of HNC are showed below separately.

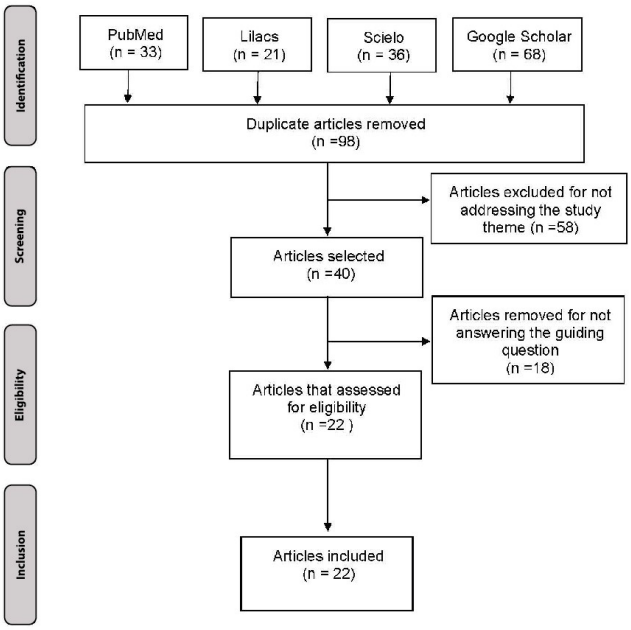

Figure 1. Flow diagram of the article's selection process.

Tumor microenvironment (TME) infiltrating cells

As formerly mentioned and also showed in Figure 2, TME is composed of a heterogeneous group of molecules and cells, highlighting the fact that, beyond tumor cells, there are two other main groups of cells present in this site: the tumor-associated stromal cells, in special the fibroblasts, and also the immune cell infiltrates, such as T lymphocytes, macrophages, and myeloid-derived suppressor cells[9, 11, 13, 14].

Although the observation of leukocytes infiltrates the TME can putatively represent that an immune response against tumor cells was elicited, according to the literature, the presence of different types of signaling molecules, particularly cytokines, chemokines, and a set of immune checkpoint proteins, such as the programmed cell death 1 receptor (PD-1) and its ligand programmed cell death ligand 1 (PD-L1) expressed by many cells in the TME, impacts the immune cells leading them to alter its anti-tumor profile to a protumor profile, which, consequently, contributes to tumor progression[9, 13, 14]. Moreover, it is of utmost to highlight that the presence of inflammatory sites inside TME is recognized as the corollary factor to promote the attraction of stromal and immune cells to TME and, thus, generate the favorable TME to tumor progression[9, 11, 13, 14].

Based on these pieces of information, it is evident that the infiltration of both stromal and immune cells in the TME plays a remarkable role in the tumor progression[9, 13, 14]. In agreement with the literature, immune system cells moving to TME are submitted to a remodeling process that occurs in three stages: firstly, there is the recruitment of these cells through chemokines, such as MCP-1 (monocytes chemotactic protein-1); secondly, there is an association between these cells through cytokines, such as tumor necrosis factor-alpha (TNF-α), macrophage migration inhibitor factor (MIF) and IL-6; lastly, the differentiated immune cell release cytokines and growth factors that are used by tumor cells to promote their proliferation and also to maintain a tumor immune tolerance[14, 15].

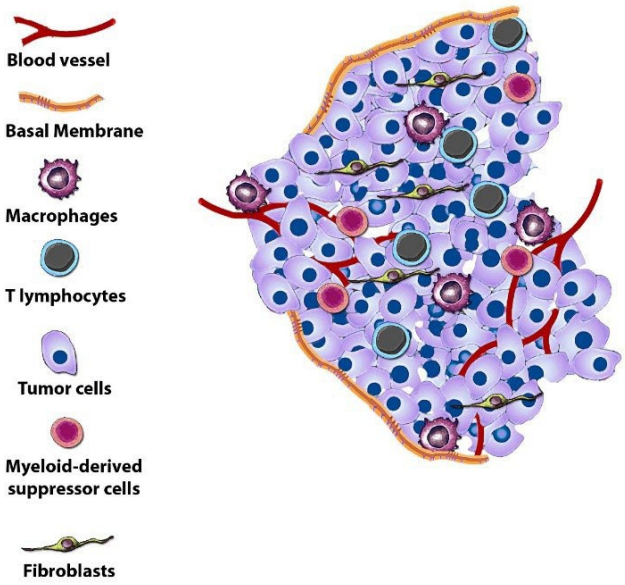

Figure 2. Representative figure of the main cellular components presents in the tumor microenvironment of head and neck cancers.

Fibroblasts

Among stromal cells found in the TME, fibroblasts stand out. Fibroblasts are derived from primitive mesenchyme that presents an essential function in tissue remodeling mainly by its secretion of structural proteins, such as collagen, elastin, laminin, and proteoglycans, as well as some cytokines, which, in a general way, act preferentially in a paracrine way in the tissue.[7, 16].

In relation to TME, cancer-associated fibroblasts (CAFs) alter their phenotype leading them to resemble myofibroblasts since these cells present the acquisition of higher motility and proliferative capacity, besides, when activated, these cells express some specific markers, such as alpha-smooth muscle actin (α-SMA) and vimentin[7, 11, 17]. CAFs are chemoattracted and activated by interleukins and growth factors, such as the transforming growth factor-beta (TGF-β), present in the TME[7, 17]. Furthermore, Scutti et al. (2016), showed that the mutation of the p53 gene also can favor the recruitment and migration of CAFs to the TME in HNSCC[7]

It has been reported that, in TME, CAFs not only show an intimate communication but also present a prominent metabolic association with tumor cells, demonstrating that CAFs play an initial and central role in the tumor progression[16, 17]. In addition, by its capacity to degrade and restructure the proteins of the extracellular matrix (ECM), including fibronectin, laminin, and type I and IV collagen, associated with the expression of integrin α6, CAFs creates favorable conditions for tumor invasion and metastasis[7, 11, 17]. The matrix remodeling function presented by CAFs is associated with its production and secretion of matrix metalloproteinases (MMPs), which facilitates tumor cell migration in TME[11, 16, 17]. Interestingly, it was also postulated that CAFs can be one of the factors responsible for resistance to therapeutic drugs such as chemotherapy[7].

Myeloid-derived Suppressor Cells

Regarding the literature, the myeloid-derived suppressor cells (MDSC) represent a heterogeneous compound of immature myeloid cells with immunosuppressive potential[18]. It has been documented that these cells are attracted to TME by some molecules, in special by the granulocyte-macrophage colony stimulating factor (GM-CSF)[11]. Since these cells show a remarkable immunosuppressive property, its presence in the TME potentially can suppress the T-cell activity against the tumor. In this sense, it was reported that this immunosuppressive potential of MDSCs is related to the induction of the arginase pathway, in which the enzyme arginase-1 can generate reactive oxygen and nitrogen species, such as nitric oxide (NO)[7, 10, 11, 18]. In agreement with Wang et al. 20019, the induction of this pathway leads to the depletion of several amino acids and also increases the expression of immune checkpoint protein PD-L1, which prevents an effective immune response against the tumor[11].

Furthermore, the presence of MDSCs into the TME is also associated with tumor progression and metastasis, mainly due to its property to induce neoangiogenesis through the secretion of vascular endothelial growth factor (VEGF), hypoxia inducible factor 1 (HIF-1), and basic fibroblast growth factor (bFGF)[7, 10, 11].

Macrophages

It is well-known that macrophages are immune system cells derived from the myeloid lineage, which not only shows an evident phagocytic ability, it also plays an important role in activating the adaptive response. It is paramount to highlight that macrophages are present in all tissues and in the bloodstream are evidenced as monocytes[7, 11].

In relation to the neoplasias, the literature pointed out that, in the TME, the tumor-associated macrophages (TAMs) can present two different phenotypes: type 1, in which the TAM shows a pro-inflammatory property, and those of type M2, in

which TAM shows a suppressor property. Based on these pieces of information, it is clear that TAM demonstrates opposite functions in the TME[7, 10, 11, 19]. In this way, whereas M1-type TAM is associated with better prognosis due to its anti-tumor action promoted by releasing cytokines, such as Interferon-gamma (IFN-γ), IL-12, and IL-23, which contribute to elicit an immune response to the profile Th1 (T helper 1), the M2-type TAM, which is differentiated by the presence of IL-4, IL-10, and IL13, is associated with worse prognosis since these cells show an opposite action of M1-type TAM in the TME not only by releasing cytokines with anti-inflammatory properties, such as IL-10 and TGF-β, which inhibits the M1-type TAM profile activation, but also prevents the response of T lymphocytes through the arginase pathway, as previously mentioned[7, 11 17, 19].

Another point that renders to be highlighted is that, in accordance with some studies, the secretion of colony-stimulating factor 1 (CSF-1) by tumor cells induces the M2 macrophages accumulation near the vessels in the TME. In this site, these cells are stimulated to secrete the epidermal growth factor (EGF), which induce tumor proliferation and, by chemotaxis, also attracts tumor cells, thus inducing their migration in the TME[7, 11, 19].

T lymphocytes

Particularly in terms of TME, studies have been demonstrated that the presence of different types of T lymphocytes is closely associated with the possibility to induce both anti-tumor and pro-tumor activities[9, 10, 19]. In this sense, whereas the presence of TCD8+ lymphocytes and TCD4+ lymphocytes with Th1 profile characterize better prognosis due to its effective anti-tumor action, the presence of regulatory T lymphocytes (Treg) are associated with worse prognosis[10, 13, 19].

Since the T lymphocytes show different actions in the TME, it is of utmost better understand how these cells are induced to present a pro- or anti-tumor property.

It is broadly accepted that TCD8+ lymphocytes, also named cytotoxic cells, are the main player of acquired immunity against the tumor cells by its capacity to directly eliminate the malignant cells[13, 19, 20]. Concerning its activation and anti-tumor effect, it is well-known that TCD8+ lymphocytes activation occurs through the recognition of an epitope (an antigenic fragment) binding with human leukocyte antigen (HLA) class I by the T cell receptor (TCR), together with co-stimulators. After the activation, the cytotoxic action of this type of T lymphocyte can occur through cell death receptors expression, such as FasL or CD95, in its cell membrane, or by the secretion of proteins, such as granzyme and perforin that present cytolytic actions, or even by secretion of the cytokine's TNF-α and IFN-γ[9, 10, 20].

In relation to TCD4+ lymphocytes presenting Th1 profile, its anti-tumor property is associated with the action to secrete cytokines, such as IL-2 and IFN-γ, which helps the activation of effector TCD8+ lymphocytes, as well as increases the anti-tumor action of these cytotoxic cells[10, 20, 21].

According to the literature, activated regulatory T lymphocytes (Treg), recognized by the TCD4+CD25+FoxP3 phenotype, are responsible for controlling immune responses, inducing immune tolerance, inhibiting the exacerbated action of the adaptive immune system, and preventing autoimmunity[10, 22]. In the neoplasia context, in agreement with Wang et al. 20019, these type of T lymphocytes are attracted into the TME by chemokines, such as CCL28-CCR10 and CXCL12-CXCR4, and, due to its properties, Treg lymphocytes present a potent immunosuppressive action of anti-tumor responses[7, 11, 22]. For instance, the presence of these cells in the TME favors the induction of PD-L1 expression in tumor cells and, as formerly mentioned, the interaction between PD-L1 and PD-1, expressed in activated T lymphocytes, inhibits the T lymphocyte action, blocking its proliferation and/or even inducing apoptosis of effector T cells[7, 9, 10]. In addition, Treg lymphocytes also suppress the TCD8+ lymphocytes response against tumor cells by secretion of cytokines, such as IL-10, IL-35, and TGF-β[11, 19].

CONCLUSION

As appealing as the tumorigenesis and clinical studies could be in order to provide relevant information on tumor progression, based on the pieces of information presented in this systematic review study, it is evident that the comprehension of the role of TME in the neoplasia context is also fundamental.

The tumor microenvironment (TME) is a specific tumor site where there is an intense interaction between molecules and cells, and, depends on how this interaction occurs, can or not favor tumor development and progression, especially head and neck cancers. In terms of non-tumor cells presenting into the TME, in a general way, both fibroblasts, a stromal cell, and immune cells can help the tumor progression, in different ways and stages.

Finally, it is worth mentioning that the improvement of knowledge on the particularities of TME in head and neck cancers can lead to the development of new strategies of interventions, such as immunotherapy with antibodies, which can provide great progress in the treatment of patients with hea and neck cancers.

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