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The unusual paradox of cancer-associated inflammation: an update

O intrigante paradoxo da inflamação associada ao câncer: uma atualização

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ABSTRACT

The inflammatory response represents a fundamental component of the tumor microenvironment and is responsible for mediating the biological communication network and the molecular signal flow that characterize the neoplastic tissue. Thus, influenced by the inflammatory process, neoplastic and non-neoplastic cells (recruited stromal and circulating cells) interact in a autocrine and paracrine mechanism to control, delineate and model the tumor growth, which is driven by a dynamic mechanism of production of cytokine, growth factors and remodeling enzymes of the extracellular matrix, creating a system of multidirectional influence that, in an accurate analysis, creates a new scientific concept of cancer, now understood as a complex tissue society, in which most of the members cooperate facilitating for neoplasia growth, for the subversion of the immune resistance and favoring metastatic dissemination.

Key words: neoplasms; inflammation; tumor microenvironment.

RESUMO

A resposta inflamatória representa um componente fundamental do microambiente tumoral, sendo responsável por mediar a rede de comunicação biológica e o fluxo de sinalização molecular, que caracterizam o tecido neoplásico. Desse modo, influenciadas pelo processo inflamatório, células neoplásicas e não neoplásicas (estromais e circulantes já recrutadas) interagem de forma autócrina e parácrina para controlar, delinear e remodelar o crescimento tumor, que é impulsionado por um mecanismo dinâmico de produção de citocinas, fatores de crescimento e enzimas remodeladoras da matriz extracelular, criando um sistema de influência multidirecional que, em última análise, faz emergir, cientificamente, uma nova definição do câncer, agora entendido como uma sociedade tecidual complexa, em que a maioria dos integrantes coopera para a facilitação do crescimento da neoplasia, para a subversão da resistência imune e para o favorecimento da disseminação metastática.

Unitermos: *neoplasias; inflamação; microambiente tumoral.*

RESUMEN

La respuesta inflamatoria representa un componente fundamental del microambiente tumoral, y es responsable por mediar la red de comunicación biológica y de señalización molecular que caracteriza el tejido neoplásico. Así, influenciadas por el proceso inflamatorio, células neoplásicas y no neoplásicas (estromales y circulantes ya reclutadas) interactúan de forma autocrina y paracrina para controlar, delinear y remodelar el crecimiento del tumor, que es impulsado por un mecanismo dinámico de producción de citocinas, factores de crecimiento y enzimas remodeladoras de la matriz extracelular, creando un sistema

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de influencia multidireccional. Esto, en última instancia, crea, científicamente, una nueva definición de cáncer, ahora comprendido como una sociedad compleja de tejidos, en la que la mayor parte de los miembros colabora para facilitar el crecimiento de la neoplasia, derrocar la inmunidad y favorecer la difusión metastásica.

Palabras clave: *neoplasias; inflamación; microambiente tumoral.*

INTRODUCTION - THE CONCEPT OF TUMOR MICROENVIRONMENT (TME)

The knowledge on the pathogenic relationship between inflammation and cancer is not new. In 1863, the German pathologist Rudolf Carl Virchow (1821-1902) raised the hypothesis that malignant neoplasms may arise in sites of chronic inflammation, assuming that inflammation would increase cell proliferation, maximizing the risk of tumor development[1, 2]. In fact, groups of inflammatory cells are histological findings common in tumor biopsies. However, the understanding of cancer as a proliferating cell set has proven incomplete and reductionist, arising, in the light of new genetic, biochemical and molecular studies, the concept of TME[3].

TME can be defined as a biologically complex tissue that exhibits important distortions of the original tissue homeostasis, in which non-neoplastic cells (which often present lack deregulated proliferation rates or increased genetic instability) are reprogrammed to act in accordance with this new tissue dynamics, mainly dictated by neoplastic cells[3]. Neoplastic cells and non-neoplastic elements of the tumor represent it. These include fibroblasts, immuno-inflammatory cells and cells that make up the blood vessels (endothelium and pericytes). It also concerns all the signaling molecules (positive and negative) produced by the cellular elements of the tumor which reflect a powerful network acting on the tumor sites. Therefore, TME contains, in addition to the neoplastic cells and the surrounding stroma (fibroblasts, endothelial cells, pericytes and extracellular matrix proteins), innate immune cells, including macrophages, neutrophils, mast cells, myeloid-derived suppressor cells lineage, dendritic cells, natural killer (NK) cells and adaptive immune cells (T and B lymphocytes).

In this context, the inflammatory process stands out as a fundamental component of the TME, since it can be understood as part of the important network of communicability that characterizes it. Influenced by the immuno-inflammatory process of TME, diverse cells interact in an autocrine and paracrine mechanism to control and delineate tumor growth, which is continuously remodeled.

The inflammation associated with TME ultimately acts as mediator of the interaction between neoplastic and stromal cells, which is driven by a dynamic mechanism of production of cytokines, growth factors and remodeling enzymes of the extracellular matrix, creating a system of multidirectional influence, which interferes greatly in the development of the tumor. Nevertheless, the expression of the various immuno-inflammatory mediators, as well as the numerical density and the activation state of the different types of cells of this inflammatory component of the tumor microenvironment are events that can be controlled by the neoplastic cells.

As Grivennikov *et al*. (2010)[4] state, the tumor may direct the inflammatory behavior, in both for its favor – may incline it towards the promotion of its growth – and for host resistance – antitumor immunity. However, it has been shown in several studies that, in established tumors, this balance is deeply inclined to the pro-tumor profile, presenting the suggestion of a new understanding of inflammation, the idea of tumor-associated inflammation.

According to Suarez-Carmona *et al*. (2017)[5], while acute and transient inflammation is a factor in the control and repair of tissue damage, inflammation associated with the tumor is of a chronic, non-resolving type that promotes tumor progression.

TME IMMUNO-INFLAMMATORY CELLS MAY BE PRO-TUMOR

According to Grivennikov *et al*. (2010)[4], among the most common immuno-inflammatory cells found in TME, are the macrophages.

In analogy to the subdivision of T cells into Th (T helper) 1 and Th2, macrophages can be classified into M1 and M2 types. M1 macrophages, activated by interferon-gamma (IFN-γ) and microbial products, express high levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, IL-12 or IL-23, major histocompatibility complex (MHC) molecules, and are capable of destroying pathogens and organizing antitumor immune responses. In contrast, activated

macrophages with M2 profile negatively regulate MHC class II and IL-12 expression and show increased expression of the anti-inflammatory cytokines IL-10 and arginase-1.

In damaged tissue or during repair, macrophages play key roles, and are involved in extracellular matrix remodeling, apoptotic cell removal, epithelial migration, and angiogenesis. In tumor tissue, where macrophages are called tumor-associated macrophages (TAMs), macrophages predominantly exhibit the M2 profile and show a decrease in their immune function, with an exacerbation of their trophic function, promoting tumor mitotic events, apoptosis and angiogenic inhibitors[3].

High densities of M2 macrophages in neoplastic tissue were related to low prognostic values in breast, uterine and ovarian cancers. It was also seen that the cells of these lesions presented colony-stimulating factor type 1 (CSF-1) overexpression, a macrophage growth factor.

TAMs are therefore, an important source of trophic effects cytokines, affecting tumor growth, with significant implications in the invasion and metastasis processes. As already mentioned, in many tumors, TAMs overexpression is correlated with poor prognosis.

Other inflammatory cells, also present in TME, affect tumorigenesis, such as neutrophils, which may induce tumor promotion, depending on their differentiation state, in addition to B lymphocytes, mast cells and dendritic cells. It was found that neutrophils from the tumor microenvironment, stimulated by transforming growth factor beta (TGF-β), exert pro-tumor effects[6].

Thus, the sustained inflammatory process of the tumor microenvironment induces cell proliferation, promotes angiogenesis and increases tumor cell survival (because inhibits their apoptosis), which influences their migratory behavior and contributes to dissemination and metastasis[7].

TME STROMAL CELLS CAN SUPPRESS ANTITUMOR RESPONSES

There are two main types of TME stromal non-lymphoid cells with pro-tumor and immunosuppressive activities: fibroblasts, also tumor associated fibroblasts (TAFs), and myelomonocytic cells. These are the most heterogeneous population and include the population of myeloid-derived suppressor cells (MDSCs) lineage and inflammatory monocytes, which can differentiate into TAMs.

According to Pinto (2015)[8], suppressor cells of myeloid origin originate in the bone marrow (myeloid progenitor) and expand into chronic infections and cancer. Inhibit the immune response by the competitive use of substrates required for regulatory T cell activation, such as arginine, cysteine and nitric oxide.

Myeloid cells, on the presence of hypoxia (a common event in tumors), then, express high concentration of programmed death 1 (PD-1) protein, inhibiting antitumor responses mediated by regulatory T cells.

TAFs secrete C-X chemokine receptor type 4 (CXCR4), also called cluster of differentiation 184 (CD184), a cytokine (chemokine) that recruits T cells into the TME, inducing their differentiation into regulatory T cells (TREGs), and recruiting myeloid suppressor cells to the tumor. They are also capable of inhibiting NK cells.

For Shalapour *et al*. (2015)[9], the most numerous cells of the tumor microenvironment are the myeloid-derived suppressor cells, neutrophils and macrophages, all with pro-tumorigenic activities, such as cell proliferation, inhibition of apoptosis, angiogenesis and induction of epithelial-mesenchymal transition.

Among macrophages, the M2 phenotype predominates. It has been proven that neoplastic cells secrete CSF-1 and TGF-β, which induce switch to M2, i.e. deviates the differentiation into M2 phenotype.

In the tumor, M2 macrophages produce the vascular endothelial growth factor (VEGF), which promotes a substantial increase in oxygenation and nutrition, an event known as angiogenic switch, which facilitates migration and metastasis.

Tumor-associated neutrophils produce genomic instability by releasing reactive oxygen species and also produce VEGF, supporting angiogenesis.

Therefore, in addition to pro-tumorigenic effects, tumor-associated inflammation also plays a key role in suppressing antitumor immunity. It is a peculiar and paradoxical type of chronic inflammation that, due to its characteristics, may be called "immunosuppressive inflammation".

TME AND ITS INFLUENCE ON METASTASIS

From the clinical point of view, metastasis is the most critical event of tumorigenesis, since 90% of cancer mortality is related to metastatic dissemination[4].

Recent studies clearly show that metastasis requires close collaboration between neoplastic cells, immune-inflammatory cells and stromal elements of TME, for example fibroblasts, endothelium and pericytes[4].

In epithelial tumors, the metastasis process may be divided into four main stages. The first stage is represented by the mesenchymal-epithelial transition – MET, in which the neoplastic cells acquire fibroblastoid characteristics – which increase the motility and allow the epithelium lining transposition – and reach blood and efferent lymph vessels. Loss of E-cadherin expression (an epithelial cell marker) is seen as a key event in this process and appears to be related to the overexpression (stimulated by the inflammatory environment) of the Snail gene, an E-cadherin transcription repressor.

TGF-β is also an important regulator of MET. It activates the subunit-mothers against decapentaplegic (SMAD) and mitogen-activated protein kinase (MAPK) transcription factors pathways, which control the expression of other MET regulator, such as SLUG, a transcription factor with anti-apoptotic activity (in drosophila, the *SLUG* gene is essential for the mesoderm formation, and its lethality leads to a circularization of the embryo axis, named "SLUG").

Other mechanisms by which the inflammatory environment may affect MET is the induction of pathways mediated by signal transducers and activators of transcription-3 (STAT3) and nuclear factor kappa B (NF-κB), expression of the TWIST embryonic gene.

The name TWIST, according to Bastid *et al*. (2009)[10], is due to the fact that, in drosophila, the *TWIST* gene was considered essential for appropriate gastrulation, as well as for generation of neural crest cells. The loss of the gene is lethal for the gastrulation and for the formation of mesoderm-derived tissues, resulting in an endoderm invagination, which leads to a twisting of the dorsal-ventral axis of the embryo, named TWIST.

TWIST proteins in adult humans are mainly expressed in precursor cells, including myogenic, osteoblastic, chondroblastic, odontoblastic and myelomonocytic lineages, in which the gene is responsible for maintaining its undifferentiated state. On the other hand, the *TWIST* gene expression has been shown to be active in multiple carcinomas (breast, bladder, lung, kidney, colon, gastric, pancreas, ovary, prostate, oral cavity and esophageal) and also in melanomas and sarcomas.

By promoting MET, the TWIST proteins provide the neoplastic cells with motility, acquisition of self-renewal capabilities, chromosomal instability and the possibility of secreting angiogenic factors, which facilitates dissemination and metastasis[11].

Migration of neoplastic cell clones into vessels requires extensive proteolysis of the extracellular matrix on the invasive front. Therefore, inflammatory cells are important sources of matrix metalloproteinases (MMP) enzymes, proteases that degrade the extracellular matrix, changing its stiffness, making it looser, facilitating the migration of tumor cells to the capillaries.

The chemokine C-C ligand 9 (CCL9), cytokine produced by neoplastic cells, can recruit myeloid cells from the circulation, which secrete matrix metalloproteinases types MMP2 and MMP9. In addition, IL-1, TNF-α and IL-6 promote the MMPs expression[4].

In the second stage, tumor cells invade the intravascular space (blood and lymphatic vessels), and the inflammatory component of TME will produce mediators that increase the vascular permeability. It has been already shown that perivascular macrophages establish connections with endothelium and pericytes, facilitating their displacement to vascular lumen. Once in the intravascular environment, the survival of these circulating cells (third stage) is affected by inflammatory mediators released by the TME cells, such as TNF-α, IL-6 and epiregulin, which can mediate the molecular connections of neoplastic cells in their traffic through the circulation, with platelets or macrophages, transcending intravascular immune surveillance – an action usually mediated by NK cells. In the fourth and last stage, isolated cells or metastatic clones extravasate (such as via receptors expression for integrins), interact with stromal elements, and begin to proliferate. In this section, it is important to highlight that several studies have already shown that neoplastic cells, with metastatic potential, settle the future metastatic site before their arrival (establish the so-called "pre-metastatic niche").

Extracellular vesicles of the exosomes type, for example, may be involved in this process. Such soluble signaling structures travel in the circulation, allowing the interlocution of neoplastic cells with tissues distant from the primary tumor[12]. Therefore, the initiation of the metastatic process is not restricted to the late stages of tumor progression.

ANTITUMOR IMMUNITY AND ITS SUPPLANTING

Paul Ehrlich (1854-1915) argued that the immune system could eliminate tumors. Subsequently, Frank Macfarlane Burnet (1899-1985) and Lewis Thomas (1913-1993) formalized the immune surveillance thesis, a theory that basically supports the hypothesis that tumor cells express neoantigens (tumor-specific antigens and other antigens associated with it) that could activate antitumor immunity, which in some cases, could lead to the rejection of early neoplasia[9].

This theory has gained great relevance in recent times, with the development of the immune checkpoint blockade therapy, in

which it was found that reactivation of cytotoxic T lymphocytes (by blocking their negative signaling) could lead to rejection and elimination of tumors.

The specificity of T cells to their targets is mediated by the interaction of receptors on their surface [T cell receptor (TCR)] with MHC associated with antigenic peptides present on the surface of cells presenting antigen or tumor cells[8]. However, the response to the antigen presentation signaling is regulated by a series of co-regulatory receptors (co-receptors) expressed in the T cell, which recognize additional ligands present on the surface of abovementioned cells. These co-receptors can either induce positive (stimulatory) or negative (inhibitory) intracellular signaling cascades by modulating T cell activities related to cytokine proliferation, secretion, and cell lysis. These molecules of the immune system, which can stimulate and inhibit signals, are known as immunological checkpoints. Among these molecules, PD-1 stands out; this has been identified in cells of several solid tumors, namely lung cancer, breast cancer, glioblastoma, oral cancer and gastric cancer, and is related to the immune evasion of tumor cells.

In tumor cells, overexpression of PD-1 could be associated with the emergence of more aggressive clones, more apt to promote activated T-cell anergy, especially cytotoxic T lymphocytes, leading to dysregulation of immune response mechanisms and, subsequently, to tumor progression[13].

The therapeutic blockade of this protein, which suppresses its signaling via TCR, is used in immunotherapy for melanomas, for example. Such therapy is termed the T cell checkpoint antagonist.

For Shalapour and Karin (2015)[9], in the tumor microenvironment, one of the mechanisms of immune surveillance evasion is the interaction between PD-1 expressed in neoplastic cells and their PD-L1 and PD-L2 ligands, triggering a inhibitory signal on activated T cells which, consequently, leads to decreased cytokine production (e.g., IFN-γ), to increased T cell apoptosis and to reduction of effector T cell proliferation, facilitating immune escape. According to the authors, immune tolerance to tumor may also be activated by recruitment from the immunosuppressive cells circulation into the tumor microenvironment, such as regulatory T cells (TREG), regulatory B cells (BREG), from the myeloid-derived suppressor cells lineage, in addition to immunosuppressive plasmocytes.

Fearon (2017)[14] states that immunosuppression present in the tumor microenvironment can be so strict that the tumor appears to be "immunologically silent".

For Grivennikov *et al*. (2010)[4], in most established tumors, the presence of lymphocyte infiltration is insufficient to reduce tumor growth. Such considerations gave rise to a revised version of the theory of immune surveillance called immunosuppression (immunomodulation). According to this concept, neoplastic cells constantly edit, adapt or modulate the host antitumor immune response, while the host immune response may also modulate the immunogenicity of the tumors. During this process, a balance between the antitumor response and tumor promoting immunity can be achieved, which may explain decades of cancer "dormancy".

However, when the balance is directed in favor of tumor growth (which can be achieved, for example, when malignant cells begin to readjust their repertoire of tumor antigens for less immunogenicity), there is tumor escape and reconfiguration of the tumor microenvironment to an immunosuppressive profile, which may be facilitated by immunoelection mechanisms, down-regulation of MHC and loss of antigenic variants. This could lead to pre-selection of clones of more aggressive and resistant cells, particularly prone to dissemination, even in less permissive conditions.

However, it is worth noting that in some tumors, cells continue to express a sufficient amount of tumor antigens, despite such escape mechanisms. It shows the relevant success (in some cancers) of antineoplastic therapy based on immunologic checkpoint inhibitors.

CONCLUSION

Neo-concepts of tumor microenvironment and inflammation associated with tumors has emerged by new research in the field of cancer immunopathology and they already aim to the definition of cancer as a complex tissue society – an almost ecological understanding –, in which most members cooperate to direct the balance of the tumor environment in order to facilitate the neoplasia growth, the immune resistance implantation and the metastatic dissemination.

Probably, even in early stages of tumor development, regarding the immune resistance mechanisms, the reprogramming and the genetic modification, they can create clones of more resistant cells, which survive and are more aggressive. These clones now establish communication with the non-neoplastic cells involved in the TME (immuno-inflammatory and stromal), with the proteins of the extracellular matrix and even with the circulating cells.

In the most stabilized TME, this intricate communication network is controlled by neoplastic cells, which program non-

neoplastic cells to suppress antitumor responses and, mainly, to promote growth, dissemination and metastasis.

According to Onuchic *et al*. (2010)[3], there is a true "co-optation" of immuno-inflammatory and stromal cells by the TME neoplastic cells, which use them in their favor.

Therefore, neoplasia exerts not only an internal control over the tumor environment, but also externally to the tumor, influencing the circulating cells recruitment to support the growth and dissemination of the neoplasia, and may induce, not infrequently, through suppressor signals, the almost failure of host resistance mechanisms.

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