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REVIEW

Roles of neutrophils in cancer growth and progression

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Abstract

Chronic inflammation is a well-known tumor-enabling capacity, which allows nascent tumors to acquire all the hallmark capabilities, including the escape from immunosurveillance. Soluble and cellular inflammatory mediators constitute the complex network of the tumor microenvironment, in which tumors grow and with which constantly interact. Myeloid cells (e.g., tumor associated macrophages) are pivotal players of the tumor microenvironment and are characterized by plasticity, which consists of the ability to acquire distinct phenotypes in response to the microenvironment in which they reside. Neutrophils are emerging as important players of tumor microenvironment, given their heterogeneity and plasticity. Increasing evidence suggests a dual role for neutrophils in modulating tumor behavior and highlights the need for a reassessment of neutrophil functions in cancer initiation and progression.

KEYWORDS

cancer-related inflammation, cancer immunotherapy, predictive biomarker, prognostic factor, tumor-associated neutrophils

1 | INTRODUCTION

Cancer-related inflammation (CRI) was already known for a long time, so that cancer was considered as "a wound that does not heal."[1] The presence of an immune infiltrate around tumors was largely attributed to an attempt of the immune system to eradicate cancer. During the last several decades, a growing number of observations have proved that CRI promotes tumor initiation and progression and enables cancer to acquire all hallmark capabilities, including the ability to evade immunosurveillance.[2]

This new point of view emphasized the novel model that tumors should no longer be viewed merely as genetic diseases. Indeed, cancers initiate, progress, and respond to therapy within a complex microenvironment with which they continuously interact.

Tumor microenvironment (TME) is a complex network in which myeloid cells play pivotal roles in initiating and promoting cancer development. Among tumor-infiltrating immune cells, macrophages are well-known players in CRI and are typically characterized by plasticity, that is, the ability to acquire a wide spectrum of activation states in response to the signals derived from the microenvironment.[3,4]

Beyond macrophages, neutrophils have recently been shown to play many roles in the various phases of cancer initiation and progression.[5] Indeed, they represent a surprisingly heterogeneous population, endowed with unsuspected plasticity.[6]

In this review, we will recapitulate the main biological aspects of neutrophils and their roles in cancer development. We will evaluate their role(s) as prognostic and predictive biomarkers in human cancers and we will explore the functions of these tumor-infiltrating immune cells as means or targets of anticancer therapeutic approaches.

1.1 | Roles of neutrophils in tumor growth and progression

1.1.1 | Pro-tumor versus anti-tumor functions of neutrophils

Neutrophils have been shown to play key roles in CRI to exert anti-tumoral or pro-tumoral functions and to be endowed with unsuspected plasticity.[6–8] Fridlender and colleagues made the seminal observation that in tumor-bearing mice, neutrophils acquired a pro-tumoral

Abbreviations: 5-FU, 5-fluorouracil; BCG, bacillus Calmette-Guerin; CRC, colorectal cancer; CRI, cancer-related inflammation; G-MDSC, granulocytic myeloid-derived suppressor cell; HDN, high-density neutrophils; HNSCC, head and neck squamous cell carcinoma; LDN, low density neutrophils; MDSC, myeloid-derived suppressor cell; MMP, metalloprotease; NE, neutrophil elastase; NLR, neutrophil-to-lymphocyte ratio; NSCLC, non small cell lung cancer; PD-L1, programmed death-ligand 1; ROS, reactive oxygen species; $sPLA_2$, secreted phospholipase; TANs, tumor-associated neutrophils; TME, tumor microenvironment

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phenotype under the influence of TGF-β.[7] Indeed, TGF-β blockade led to the tumor infiltration of neutrophils with increased cytotoxicity, high expression of TNF-α, CCL3, and ICAM-1, and low levels of arginase-1. TGF-β inhibition also promoted a T-cell anti-tumor response, which involved neutrophils as effector cells.[7] Mirroring the M1/M2 paradigm, neutrophils were then proposed to polarize in two distinct activation states: an anti-tumor N1 or a pro-tumor N2 phenotype in response to signals derived from TME. In murine models of melanoma and fibrosarcoma, *IFN-β* deletion favored the infiltration of neutrophils characterized by a high expression of CXCR4, VEGF-A, and metalloprotease 9 (MMP-9).[9] These results suggested a pivotal role for type I IFNs in polarizing neutrophils toward a N1 anti-tumoral phenotype.[6] However, to date, these findings were obtained only in murine models and the existence of polarized neutrophil populations still need to be demonstrated in humans.

1.1.2 | Neutrophil recruitment in tumor microenvironment

Within the TME, CXC chemokines produced by tumor and stromal cells and associated with cancer growth and progression also retain neutrophil-recruiting functions.[10–12] For instance, human head and neck squamous cell carcinoma (HNSCC) cell lines produced CXCL8 and macrophage inhibiting factor, which induced neutrophil chemotaxis through the engagement of CXCR2.[13,14] Hepatocellular carcinoma cells also recruited neutrophils through the production of CXCL8.[15] We recently found that thyroid cancer (TC) cells produce CXC chemokines, which were able to recruit neutrophils in a CXCR1/CXCR2-dependent manner (unpublished results). Moreover, TC-derived conditioned media were able to modify neutrophil biology, in terms of activation, modulation of survival, gene expression, and release of oxygen derivatives, suggesting an interesting role of tumor-derived chemokines in influencing neutrophil plasticity.

Several experimental tumor models showed a central role for CXCR2 in promoting lung and pancreatic cancers.[16,17] Indeed, *CXCR2* deletion or neutrophil depletion suppressed inflammation-induced and spontaneous carcinogenesis in mice.[18] In addition, in a murine model of graft tumor, CXCL17 promoted cancer growth together with the increased infiltration of a myeloid subset of $CD11b^{+}Gr1^{+}F4/80^{-}$.[19] In a conditional genetic murine model of lung cancer driven by K-ras activation and p53 inactivation, macrophages and neutrophils precursors accumulated in the spleen and relocated from the spleen to the tumor, suggesting a role for the spleen as reservoir for tumor-promoting myeloid cells.[20]

1.1.3 | Neutrophil-derived mediators in tumor growth

Neutrophils play important roles in tumor initiation. Indeed, neutrophils release oxygen and nitrogen free radicals, which promoted DNA point mutations and genetic instability.[21] Neutrophil granule proteins also play a dual role in tumor progression. Indeed, neutrophil elastase (NE) was taken up by lung cancer cells and degraded insulin receptor substrate-1, which usually inhibits PI3K. This event unleashed PI3K activation and PDGFR signaling, thus favoring tumor cell proliferation.[22] NE was also involved in neutrophil-related epithelial-to-mesenchymal transition.[23] By contrast, NE could also restrain antitumor immune response. In fact, NE taken up by breast cancer cells upregulated the expression of HLA class I in tumor cells, thus increasing the responsiveness of breast cancer cells to adaptive immunity.[24] Moreover, once taken up by breast cancer cells, NE cleaved cyclin E, which was then presented in a truncated form in HLA-I context and efficiently activated a CTL-mediated antitumor response.[25] Neutrophil-derived oncostatin M up-regulated VEGF production in breast cancer cells, promoting tumor cell detachment and invasiveness.[26] In bronchoalveolar carcinoma patients, hepatocyte growth factor in broncholavage fluid was correlated with neutrophil infiltration and was associated with poor prognosis.[27,28]

Neutrophils also release TRAIL, which retains important anti-tumoral activities.[29,30] Indeed, *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) induced the release of TRAIL from neutrophils, and this mechanism was proposed to play a role in the anticancer effects of BCG in human bladder cancer.[31] In addition, in chronic myeloid leukemia patients, the release of TRAIL from neutrophils induced by IFN-α therapy promoted apoptosis of leukemic cells.[32,33]

In patients with early-stage lung cancer, a pro-inflammatory neutrophil phenotype, with high production of CCL2, CCL3, CXCL8, and IL-6, as well as high expression of costimulatory molecules (e.g., CD86 and OX40L) was found. These tumor-associated neutrophils (TANs) stimulated T-cell proliferation and IFN-γ release, mainly in a contact-dependent manner[34] and amplified a positive feedback loop that suggested an anti-tumoral role for TANs in early stages of human lung tumor.[34]

In a mouse model of PTEN-deficient uterine cancer, an inhibitory role for neutrophils was also described. Indeed, TANs inhibited early-stage tumor growth and retarded malignant progression by inducing tumor cell detachment from the basement membrane. TANs were recruited at tumor site independently of lymphocytes and cellular senescence but in the context of the tumor's intrinsic inflammatory response to hypoxia. In humans, a neutrophil gene signature correlated with improved survival in PTEN-deficient uterine cancer.[35]

By contrast, in colorectal cancer (CRC) patients, an increased number of TANs (defined as "PMN-myeloid derived suppressor cells") were found, which were recruited and activated by tumor-infiltrating $\gamma\delta$T cells, through the release of CXCL8/IL-8 and GM-CSF. These TANs produced high levels of arginase I and reactive oxygen species (ROS) and potently inhibited T-cell proliferation and IFN-γ production.[36]

1.1.4 | Pro-angiogenic versus anti-angiogenic functions of neutrophils

Neutrophils also play a dual role in modulating tumor angiogenesis. Neutrophils are main producers of VEGF-A and release high levels of MMP-9, which is responsible for the release of the active form of VEGF-A from the extracellular matrix.[15,37,38] Interestingly, neutrophils release MMP-9 in the absence of the inhibitor tissue inhibitor of proteases, thus further enhancing the pro-angiogenic and pro-invasive activity of MMP-9.[39] By contrast, in a murine model of breast cancer, intratumoral delivery of MMP-9 reduced tumor growth and angiogenesis, suggesting that MMP-9 also retains anti-angiogenic functions.[40] In a xenograft murine model, under the influence of G-CSF,

neutrophils released the pro-angiogenic molecule Bv8.[41] Interestingly, tumors resistant to anti-VEGF therapy showed high infiltration of neutrophils and drug resistance was associated with G-CSF-induced Bv8 neutrophil expression.[42,43]

Neutrophils also express a number of anti-angiogenic molecules. For example, NE cleaved VEGF and FGF-2, giving rise to the angiostatin-like fragments from plasminogen, which suppressed VEGF- and FGF-2-induced angiogenesis.[44,45] We found that exogenous Group V (hGV) and Group X (hGX) secreted phospholypases ($sPLA_2$s) induce the release of VEGF-A, angiopoietin 1 , and CXCL8/IL-8 from human neutrophils. Moreover, hGV $sPLA_2$ induced the secretion of the anti-angiogenic isoform of VEGF-A, namely, $VEGF\text{-}A_{165b}$.[46]

1.1.5 | Role of neutrophils in modulating metastastatic tumor behavior

Melanoma cells produced CXCL8, which up-regulated β_2-integrin expression on neutrophils. These, in turn, interacted with ICAM-1 expressed by melanoma cells, thus favoring metastatic seeding of tumor cells.[47] Neutrophil extracellular traps also captured circulating tumor cells and favored their engraftment to distant organ sites.[48] In contrast, in a murine model of transplanted breast cancer, under the influence of G-CSF and tumor-derived CCL2, neutrophils inhibited breast metastasis in the premetastatic lung in a H_2O_2-dependent manner.[49]

Interestingly, in a murine model of bitransgenic mice expressing conditioned *IL-17A* along with *Kras*, lung tumors grew more rapidly and mice displayed a reduced survival compared to wild types. Levels of IL-6, CXCL1, and G-CSF were increased in lungs of transgenic mice and TANs were increased, whereas tumor-infiltrating lymphocytes were reduced, compared to controls. Neutrophil depletion by anti-Ly6G antibodies as well as IL-6 blockage efficiently reduced tumor growth, but anti-PD-1 therapy was not effective, suggesting a role for IL-17A in promoting anti-PD-1 resistance and lung tumor growth in a neutrophil-dependent manner.[50]

1.1.6 | High-density versus low-density neutrophils and MDSCs

An increasing number of observations have highlighted the surprising plasticity and heterogeneity of neutrophils, in humans and in experimental cancer models. Indeed, peripheral blood human neutrophils are usually purified on a discontinuous density gradient (Ficoll). Following this separation, neutrophils segregate in the high-density granulocytic fraction, whereas peripheral blood mononuclear cells (PBMC) are found in the low-density (LD) mononuclear fraction.[51] However, in chronic inflammatory conditions such as HIV infection, autoimmunity and cancer, neutrophils can be also found in the LD fraction.[52–55] This proportion of low density neutrophils (LDNs) increases with cancer progression, displays T-cell suppressive functions, and is represented by both mature and immature granulocytes.[56] Immature LDNs have been considered as granulocytic myeloid-derived suppressor cells (G-MDSCs). MDSCs are a heterogeneous subset of myeloid cells that are expanded in peripheral blood and spleen of tumor-bearing mice and cancer patients. These cells are characterized by the capacity of suppressing T cell activation and proliferation.[57,58] In previous papers, some authors described G-MDSCs as immature myeloid cells, which inhibited $CD8^+$ T-cell mediated anti-tumor immune response through the release of arginase I.[59] By contrast, several authors described these cells as a subpopulation of activated degranulated neutrophils, inhibiting T-cell response through the release of arginase I[54,60] or ROS.[55] Transcriptomic analysis performed on naive neutrophils, TANs, and G-MDSC in tumor-bearing mice found that TANs and G-MDSC are distinct populations[61] and that naïve neutrophils and G-MDSC are more closely related to each other than to TANs.[62] G-MDSCs and LDNs display a number of similarities, such as the myeloid origin, morphological aspects, and surface markers, as well as the tumor-promoting properties. For these reasons, there is no clear consensus on the differences between these two cell populations. In tumor-bearing mice as well as in cancer patients, among LDNs two subsets were distinguished: a mature segmented one and a banded immature one, namely G-MDSC.[63] While high-density neutrophils (HDN) displayed anti-tumoral functions, LDN showed reduced chemotactic activity, phagocytosis, oxidative burst, no significant cytotoxic activity against tumor cells, and T-cell suppressive properties. Both mature and immature (G-MDSCs) LDNs displayed these cancer-promoting activities. Moreover, the authors showed that HDN can go through LDN transition under the influence of TGF-β and acquire T-cell suppressive properties, thus suggesting that part of the LDN fraction is a subset of highly activated mature neutrophils, with reduced inflammatory properties. They also proposed that LDN can switch to HDN, but to a lesser extent than the opposite transition.[63] These observations suggest that neutrophils are a heterogeneous population, composed not only of terminally differentiated cells as always thought. Indeed, these findings highlight the heterogeneity and plasticity of circulating neutrophils in cancer and recall for a rigorous reassessment of neutrophil characterization in cancer patients.

A schematic view of the roles of neutrophils in CRI is summarized in Figure 1.

1.2 | Neutrophils as prognostic/predictive biomarkers in cancer patients

The relationship between TANs and prognosis in human cancers has been already extensively discussed.[64] Neutrophil infiltration within human tumors was correlated with poor patient outcome in metastatic and localized clear cell carcinomas, bronchioloalveolar, liver, uterine cervical cancer, colorectal (CRC), and HNSCC.[15,27,65–68] In addition, neutrophil infiltration was associated with high tumor grade in human gliomas and with aggressive pancreatic tumors.[69,70] Recently, among the tumor-infiltrating cells, neutrophils were found the most prevalent immune cell type in non small cell lung cancer (NSCLC) tissues, negatively correlated with tumor-infiltrating $CD8^+$, $CD4^+$, Th1, and Th17 cells and efficiently predicted patient mortality.[71,72] By contrast, TANs were associated with good patient prognosis in gastric[73] and colorectal cancer.[74,75] These apparently controversial results could depend on the type/subtype of tumors and on the techniques used to identify

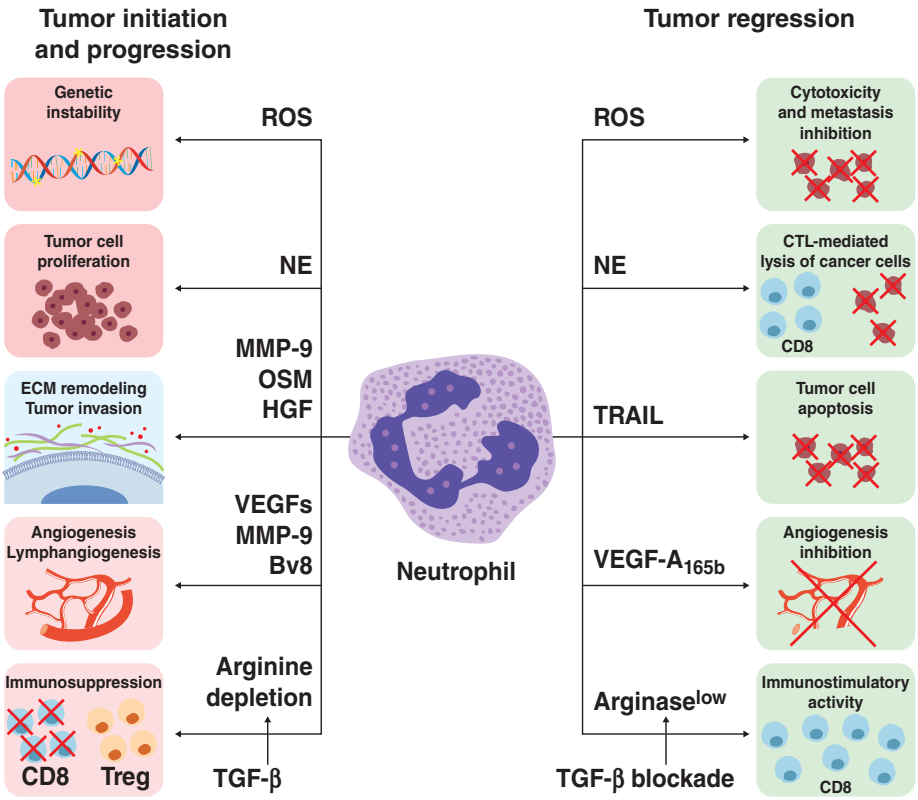

FIGURE 1 **Dual role of neutrophils in cancer-related inflammation.** Neutrophils can exert both pro-tumoral or anti-tumoral functions. TANs can mediate cancer cell killing (through the release of ROS and neutrophil elastase) and apoptosis (through the release of TRAIL) and inhibit angiogenesis (through the release of the anti-angiogenic VEGF-A_{165b}) and potentiate T-cell adaptive immune response upon TGF-β inhibition. On the other hand, TANs can induce genetic instability (through the release of ROS), favor tumor growth (through the production of growth factors and NE), promote the remodeling of the extracellular matrix (ECM) and tumor cell invasive capabilities (through the release of proteases, hepatocyte growth factor [HGF] and oncostatin M [OSM]), support angiogenesis and lymphangiogenesis (through the release of VEGFs, MMP-9, and Bv8), and suppress anti-tumoral adaptive immunity (through arginine depletion and expression of suppressive soluble and membrane molecules, such as PD-L1). See the text for details

neutrophils within the tumors (e.g., hematoxylin–eosin stain vs. immunohistochemistry).

An important task will be to investigate the predictive role of TANs in cancer patients. In the only published study, performed on Stage III CRC patients, TAN infiltration was associated with better response to 5-fluorouracil (5-FU) based chemotherapy but with poor prognosis in patients treated with surgery alone.[74] These results suggest a dual clinical significance of TANs, depending on the administration of chemotherapy and highlight the need for a re-evaluation of the role of TANs as predictive factors for response to therapy in human cancers.[74]

Several studies have evaluated the prognostic and predictive value of neutrophil-to-lymphocyte ratio (NLR) in peripheral blood of cancer patients. NLR is considered an indicator of systemic inflammation and predicted patient clinical outcome in several human cancers, such as rectal,[76] esophageal,[77] prostate,[78] pancreatic,[79] and breast cancer.[80] Moreover, a high NLR score was associated with worst survival and displayed a more consistent prognostic value among patients with advanced disease.[81] The advantage of this score is that it can be easily measured. However, its prognostic power is controversial. Indeed, NLR is not a specific biomarker since it can be confounded by other variables that influence the count of peripheral blood neutrophils and/or lymphocytes.[82] Moreover, since circulating neutrophils are not a homogeneous population, the mere count could not be a reliable biomarker of their biological functions in cancer patients. In addition, circulating neutrophils may not faithfully mirror the tumor-related ones. Thus, further studies aimed at investigating circulating neutrophil-related markers that more likely reflect the TME are needed to identify more specific biomarkers in cancer patients.

1.3 | Neutrophils in anticancer therapeutic responses

Myeloid cells can influence the effectiveness of chemotherapeutic regimens. Indeed, chemotherapeutic drugs exert their effects not only acting on tumor cells, but also on tumor-related immune cells. Indeed, some chemotherapeutic drugs, such as doxorubicin, determine an "immunogenic cell death": tumor cell death induces the expression of "danger signals" (i.e., calreticulin, ATP, and HMGB-1), which recruit and activate myeloid DC-like cells. These cells are particularly efficient in capturing and presenting tumor cell antigens and give rise to an effective anti-tumor immune response.[83,84]

Immunotherapy with checkpoint inhibitors is an established part of the therapeutic strategies for an increasing number of solid and hematologic tumors.[85] Recent evidence indicates that programmed death-ligand 1 (PD-L1) is also expressed on neutrophils and is associated with the development of numerous diseases, including HIV,[86]

sepsis,[87] *Burkholderia pseudomallei* infected disease,[88] tuberculosis,[89] and autoimmunity.[90] Recently, neutrophils accumulating in gastric cancer tissues displayed a prolonged survival, an activated phenotype, and high levels of PD-L1 expression. Increased infiltration of PD-L1$^+$ neutrophils was associated with disease progression and poor patient survival. Moreover, neutrophils purified from peripheral blood of healthy donors in vitro up-regulated PD-L1 when stimulated with gastric cancer cell line conditioned media. Finally, activated PD-L1$^+$ neutrophils inhibited the proliferation and activation of T cells, dampening anti-tumor T-cell-mediated adaptive response to promote gastric cancer.[91]

Since neutrophils can retain tumor-promoting functions, targeting these cells could be desirable. However, their depletion could lead to deleterious "side effects". Indeed, neutrophils play a central role in host defense against pathogens and their depletion could lead to immunosuppression. Neutrophil neutralization could be obtained by inhibiting their recruitment or their effector molecules. In a murine model of fibrosarcoma and prostate cancer, the inhibition of TAN recruitment through CXCL8/IL-8 blockage reduced tumor growth and angiogenesis.[92] In addition, in multiple murine models of inflammation-driven and spontaneous carcinogenesis, genetic or pharmacologic abrogation of CXCR2 inhibited tumor development.[18] Repertaxin, a small inhibitor of CXCR1 and CXCR2, selectively targeted human breast cancer stem cells and inhibited tumor growth in xenograft murine models.[93] The combination of repertaxin and 5-FU increased gastric cancer cell apoptosis and inhibited proliferation, migration, and invasion.[94] Clinical trials investigating the role of repertaxin in breast cancer patients, alone or in combination with chemotherapeutic drugs (paclitaxel), are in progress (www.clinicaltriasl.gov).

The inhibitor of NE sivelastat inhibited breast cancer cell proliferation and enhanced the anti-tumor effect of trastuzumab through restoring the expression of Her2/Neu.[95] Genetic deficiency and inhibition of NE reduced the incidence of ultraviolet B-induced tumors in mice.[96] The NE inhibitor ONO-5046 reduced both primary and metastatic growth of NSCLC in severe combined immunodeficiency mice.[97] NE inhibitors are currently undergoing clinical trials for treatment of cystic fibrosis and respiratory diseases (www.clinicaltrials.gov) and these results could also be useful for cancer research.

A neutrophil-based drug delivery system has been proponed in a murine model of glioblastoma. Interestingly, neutrophils were loaded with a cationic liposome containing paclitaxel (PTX-CL) and were injected intravenously in tumor-bearing mice after surgical removal of the primary tumor. After injection, neutrophils homed to the surgical margins where inflammatory cytokines were abundant, and released PTX-CL, giving rise to a high local delivery of PTX. By this therapeutic approach, tumor cells were killed and glioma recurrence was delayed. Despite its limitations, this study shows that neutrophils could be successfully harnessed to deliver drugs into the brain across the blood/brain barrier.[98]

2 | CONCLUSIONS

Cellular and humoral components of TME play important roles in cancer initiation and progression and in the response of most tumors to therapy. Neutrophils are main components of CRI and participate in the various phases of tumor initiation and progression. Cancer cells as well as tumor-associated immune cells release a wide spectrum of pro-tumorigenic and pro-angiogenic cytokines/chemokines. Targeting these mediators as well as blocking pro-tumor functions could be useful to inhibit tumor growth. On the other hand, fostering anticancer immune responses by blocking immunosuppressive molecules (TGF-β, IL-10, CTLA-4, PD-1, and PD-L1) expressed either by cancer cells or by tumor-infiltrating immune cells appears a promising therapeutic strategy in different tumors.

A deeper insight into the molecular mechanisms regulating the link between tumor-infiltrating immune cells and cancer cells could lead to the identification of new prognostic/predictive biomarkers, as well as a wider view of cancer immunotherapy, in an even more personalized therapeutic approach.

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AUTHOR CONTRIBUTIONS

M.R.G. conceived and designed the review. All the authors contributed intellectually and to the writing of the submitted version of the manuscript.

DISCLOSURES

The authors declare no conflict of interest.

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