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The Stroma and Tumor Microenvironment Are Not the Most Instrumental Players in Tumor Cells Immune Evasion, Growth, and Resistance to Therapy

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ABSTRACT: The central reason behind emergence of clinically-detectable tumors is evasion from immune surveillance due to lack of cancer cells surface membrane expression of tumor-specific peptides in association with MHC class I molecules, concealment of natural killer cells-activating molecules**,** and absence of inflammation resulting from inefficient stimulation of innate immunity receptors and co-stimulatory molecules. The tumor microenvironment (TME) also contributes to tumor initiation, progression and resistance to therapeutic interventions because of its dense, fibrogenic, barrier-like composition, aberrant vasculature, and production of cytokines and chemokines responsible for recruitment of immune suppressive cells, notably myeloid-derived suppressor cells, M2 macrophages, regulatory T cells, extracellular trap-forming neutrophils, and cancer-associated fibroblasts. We herein show that the relentless efforts and strategies to overcome the TME elusive tumor-promoting impact produced contrasting, opposed, controversial effects, characterized by limited efficacy and proven adversity, and most importantly

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deterred from attempts to discover and counteract the fundamental inherent mechanisms initiating, and not consequent to, carcinogenesis.

KEYWORDS: Cancer surgery; Cancer radiotherapy; Cancer immunotherapy; Cancer stroma; Cancer-associated fibroblasts; Carcinogenesis; Cancer resistance to immunotherapy; Exosomes; Hypoxia; Neutrophil extracellular traps; Sphingomyelin; Neutral sphingomyelinase; Tumor microenvironment; Tumor-associated macrophages

Introduction

The term "tumor microenvironment" implies that carcinogenesis has been initiated, tumor clinically detected**,** and surgical, radio- or chemotherapeutic interventions have already been applied, unless at the onset of presentation, the tumor advanced stage defied removal and any treatment mode or strategy. The stroma and microenvironment composition and influence differ in these distinct settings.[1]

Methods

An extensive and comprehensive literature review was performed at PubMed. Web of Science, Science Direct, Scopus, Google Scholar, ProQuest and Embase search engines, using the keywords: Cancer surgery; Cancer radiotherapy; Cancer immunotherapy; Cancer metastasis; Cancer stroma; Cancer drugs; Cancer-associated fibroblasts; Carcinogenesis; Cancer resistance to immunotherapy; Cancer vasculature; Checkpoint inhibitors; Contact inhibition cancer; Exosomes; Hypoxia; Immune surveillance; Immune checkpoint inhibitors adverse events; Myeloid-derived suppressor cells cancer; Neutrophil extracellular traps; Sphingomyelin; Neutral Sphingomyelinase; Tumor extracellular matrix Tumor microenvironment; Tumor-associated macrophages.

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Abbreviations: CARs, chimeric antigen receptors; CTLA-4, cytotoxic T lymphocyte antigen 4; DAMP, danger-associate molecular patterns; IFN-γ, interferon-gamma; IL, interleukin; MDSC, Myeloid-derived suppressor cells; NK, natural killer; NET, neutrophil extracellular trap; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; ROS, reactive oxygen species; RNS, reactive nitrogen species; SM, sphingomyelin; TAM, tumor-associated macrophages; TGF, transforming growth factor; TME, tumor microenvironment; TNF, tumor necrosis factor; Treg, regulatory T cell; TSA, tumor-specific antigen.

Stroma and Microenvironment Impact on Unresectable Tumors

Tumors that are clinically detected at Stage III or IV and are unresectable because of wide spreading, critical location, or large size are usually discovered due to their impact on the integrity and function of neighboring vasculature, muscles, nerves, and endocrine or exocrine cells.[2,3] Such cancer cells have already evaded contact inhibition dictates and immune surveillance, likely because of surface membrane antigen concealment, resulting from critical changes in surface membrane composition, integrity, and permeability.[4] Additionally, the molecules released by dividing, dying, and necrotic cells that are able to interact with the innate immunity receptors on the surface, endosome, or cytosol of surrounding fibroblasts, macrophages, and mast cells are released only following considerable tumor growth and progression. Failure to activate the innate and acquired immune defenses results in lack or delay in generation of inflammatory mediators, and most importantly resultant edema and pain.

Tumor growth depends on adhesion of tumor cells to stromal fibroblasts or to components of the extracellular matrix that furthermore can favor usage of metabolic requirements and blunt therapeutic access.[5] To invade other cellular spaces, tumor cells must degrade

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basement membranes, extracellular matrix, collagen, epithelial compartments, and tissue parenchyma, via secretion of proteolytic enzymes, notably cysteine peptidases.[6-9] Consequently, the tumor microenvironment (TME) is essentially in a state of structural and functional chaos.

Tumor microenvironment of unresectable tumors often suffers nutrient deprivation, and lack of adequate oxygen supply (hypoxia), due to neoplastic cells high metabolic and oxygen consumption, impairment of microvasculature structure and blood flow, and tumor-associated anemia.[10-12] Hypoxia hinders cancer cell destruction by reactive oxygen species (ROS), i.e., sodotherapy,[13] and renders tumor cells resistant to conventional chemo- and radio-therapy.[14,15] In response to the hypoxia, cancer cells opt to glucose oxidation leading to generation of lactic acid, which export together with H+ prevents intracellular acidification, while acidosis, besides hypoxia, becomes the hallmark of the TME,[16] with lactates reaching concentrations of 12.5 mM versus 1.5-3 mM in healthy tissues.[17] Lack of sufficient oxygen supply to tumor or TME cells may, however, prove beneficial leading to restrained cell proliferation in addition to necrosis or apoptosis, and subsequent release of danger-associate molecular patterns (DAMP) and generation of inflammatory and pain signals. Among clinically discovered colorectal cancers, sixteen hypoxia-related genes were found to be expressed in tumor and TME cells, with six genes likely involved in drug resistance, rendering hypoxia the primary factor in prediction and prognosis of outcome.[18] Additionally, low oxygen consumption rates, and not the lack of specific antigen recognition, were recently reported to alter the ability of tumor-infiltrating CD8+ cytotoxic T cells to mediate tumor cell killing.[19]

Cell RNA sequencing and immunohistochemistry of paraffin sections of 76 cases of salivary gland carcinomas revealed the presence of M2 macrophages and myeloid-derived suppressor cells (MDSC) in the microenvironment of adenoid cystic, myoepithelial, and

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salivary duct carcinomas.[20] Absence of cytotoxic macrophages results from lack of inflammatory type 1 cytokines, notably interferon (IFN)-γ, while release of allergenic molecules favors the accumulation of alternatively-activated (M2) macrophages. T cells appeared to infiltrate salivary duct carcinomas, but were more or less excluded from the microenvironment of the other histological types. [20] The association of high levels of immune infiltrating T cells with expression of mutation- or fusion-derived neoantigens and with aggressive clinical behavior is likely due to bacterial or virus infection or activation of endogenous virions,[21-27] rather than to the exhaustion of dysfunction of the T cells.[20]

Bone TME is rich in immune cells, which are however unable to control the proliferation of primary or metastatic tumor cells, predominantly because of the paucity of T and natural killer (NK) cells.[28,29] The large numbers of regulatory T cells (Tregs) and MDSC are poised to impair the elusive antitumor activity of CD8+, CD4+, and NK cells, which are not expected in the first place to act against cancer cells that failed to express tumor specific antigens (TSA), HLA class I molecules, and NK cells-activating ligands.[4] Bone resident macrophages are dedicated to coordinating normal bone remodeling and injury repair rather than attacking tumor cells. Moreover, metastatic cancer cells often lead to osteolysis, and induce the production of growth and survival factors by bone-forming osteoblasts and bone-resorbing osteoclasts.[30] Accordingly, cancer-bone microenvironment provides an immune-privileged niche for tumor cells and contribute to the disease progression and to therapy resistance.[28-31]

Cytotoxic T cells are unable to kill tumor cells that fail to express TSA-derived peptides in association with HLA class I molecules. Down modulation of surface membrane display of the latter license NK cells to action, providing activating molecules are not concealed due to biochemical changes in the cell membrane.[4] Antibodies are still able to induce NK cells

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and macrophages to kill tumor cells if there is a surface membrane antigen to access. Instead of focusing on the exact reason of immune evasion, studies have accused innate immune cells, especially macrophages, invading the tumor stroma, of tumor progression and therapy resistance.[30] The reason is the wide distribution of tumor-associated macrophages (TAM) that may constitute to up to 50% of some unresectable tumors,[31,32] in response to the surface membrane changes in necrotic cells and to DAMP. A large percentage of this macrophage population is not destined to kill, but to clean cell debris, and repair and heal lesions, thus providing proliferating cells with trophic and nutritional support. Interaction of DAMP with TAM innate receptors leads to release of inflammatory mediators, notably the endogenous pyrogen, interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-α. Interleukin-6 was recently found to act on macrophages in a paracrine manner to release cathepsin B and metalloproteinases-2 and 9, factors instrumental in degradation of the extracellular matrix and other components hindering tumor cells migration and metastasi.[34] Of note, TAM-derived IL-8 was recently reported to be responsible for breast cancer cells resistance to the chemotherapeutic drug, lapatinib.[35] Additionally, inflammatory products may induce macrophages and neutrophils to release surface membrane arachidonic acid (ARA), a documented tumoricide [4,36,37] and an activator of tumor- and TME- infiltrating neutrophils, macrophages, and T and NK cells.[38] Free ARA activates the NADPH oxidase system, resulting in generation of tumor cell-toxic ROS and reactive nitrogen species (RNS). However, lysosomal enzymes, singlet oxygen, and peroxides are more apt at attacking normal TME and endothelial than the sphingomyelin (SM)-cloaked tumor cells.[4] Impairment of lymph and blood vasculature endothelium and structure promotes metastasis. Lesions in stromal cell and nerve endings integrity and function finally alert to the devastation and danger.

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Fibroblasts, prominent components of the TME have also been implicated in tumor progression, via providing support and nutrients to the tumor. Tumor-associated DAMP may access fibroblasts innate immunity receptors, inducing the release of inflammatory cytokines, which promote extracellular matrix remodeling, and angiogenesis, tumor cells metastasis, but also edema- and pain-inducing alerting signals.[39-42] However, surrounding fibroblasts may certainly not directly abrogate the function of cytotoxic lymphocytes, or be responsible for inhibiting killing of tumor cells.[39]

Stroma and Microenvironment Impact on Surgically-Removed Tumors

Surgical intervention aims at removing the whole tumor, and often the entire organ if possible as in breast, prostate, and kidney cancers. However, resecting the entire tumor cells is almost impossible, and residual cell(s) may always grow and metastasize.[1] Removal of the large and necrotic mass and surrounding TME cells may provide space, nutrients, and oxygen required for vigorous proliferation. Severed blood vessels promote migration and spread. Accordingly, recurrence and metastasis to multiple organs, and poor prognosis are recorded in more than 40% of patients within 5 years following surgical removal of the primary tumor.[43,44] Generation of an immense plethora of DAMP in the microenvironment, together with likely bacterial or virus infection, lead to accumulation of inflammatory cytokines and cells, including neutrophils, macrophages, NK and helper and cytotoxic T cells. The inflammatory mediators are responsible for much of the pain and swelling recorded sometimes long after surgical tumor removal. The edematous parenchyma may shield nutrients and oxygen from the dividing tumor cells in the absence of direct cytotoxic T cell or antibody-dependent cell-mediated (ADCC) hit, due to the sturdiness, impermeability, and inaccessibility of the tumor cell surface. Thus, surgical intervention is not responsible for immunosuppression or rapid tumor recurrences or metastases.[45] Additionally, it is not

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clear how M2 macrophages, MDSC, and CD4+Foxp3+ Tregs allow the expansion of residual cells, and how exactly is the immunosuppressive network in the TME suppressing?[45-48] Cancer vaccines have shown inefficacy for multiple reasons among which the unavailability of TSA,[4] while the TME bears limited respomsibility.[45-48] More intuitively clear, surgical stress has recently been shown to promote recurrence and metastasis via activation and aggregation of platelets, generation of thrombin, fibrinogen, and fibrin, and increased neutrophil extracellular trap (NET) formation.[49-54]

Neutrophils were found to provide the first response to surgical trauma. Released lysosomal enzymes and toxic granule proteins severely impair the surface membrane of residual tumor cells, especially if trapped in NET.[53] Tumor cells sequestered in NET are not expected to be transported by the dying or dead neutrophils, and metastasize.[49,50,51, 53,54] Activated neutrophils lead to local accumulation of tumor-toxic ROS and RNS, and, thus, provide anti-tumor responses, an asset to surgical treatment.[49] However, release of matrix metallo-proteinase 9 (MMP-9), cathepsin C and G, and neutrophil elastase lead to digestion of the extracellular matrix and blood capillaries basement membrane, thus facilitating tumor cells movement and migration.[34,35,47,50,51] Additionally, activated neutrophils secrete cytokines implicated in stimulation of resident mast cells, resulting in increased blood capillary permeability and diameter that facilitates tumor cells metastasis.[53] Accordingly, accumulation of neutrophils and NET formation were reported to be instrumental in tumor metastasis in vitro, and post tumor surgery.[49-52] However, it is not clear how neutrophils and NET formation may be targeted for cancer therapy.[51,54] Conversely, inhibition of the lysosomal cysteine dipeptidyl aminopeptidase. cathepsin C, required for the activation of pro-inflammatory neutrophil serine proteases is now considered for cancer treatment.[55]

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Stroma and Microenvironment Impact on Radiation-Treated Tumors

In several instances, tumors regress following distant thermal or radiation intervention, a phenomenon termed the abscopal effect, whereby dead or dying cells release a plethora of DAMP and tumor-related molecules, which activate the immune system and lead to eradication of residual and metastatic tumor cells.[4,56,57] Radiation therapy induces vascular destruction, injury, and dysfunction, thus interfering with tumor cells metastasis and eliciting hypoxia. Stromal response to radiation is expressed by release of a plethora of inflammatory cytokines that promote recruitment and activation of immune cells, resulting in inflammation-mediated confinement of residual tumor cells and depletion of their metabolic requirements.[58,59] Reduction of the stromal mass is a major radiation therapy benefit, as it facilitates access of chemo- or immunotherapeutic molecules to the residual tumor cells.[60] However, it is difficult to understand how radiotherapy may transform tumors into "in situ vaccines". Previously absent co-stimulatory molecules and cytokines are now amply available; yet, the initial signal of immune sensitization with surface membrane TSA remains lacking.[57-61] Radiation therapy remodeling of the TME may not resolve the thorny issue of poor expression of tumor-specific neoantigens, rendering immune responses rather inoperative, except for some NK cell functions. The progressive inability of NK cells to access tumor cells should be explored and solutions identified instead of hanging to immune responses nothing ignite in the first place. Surgery and radiotherapy elicits the generation of a plethora of inflammatory signals and molecules that have, however, limited effect on residual or metastatic tumor masses. Conversely, Studies using several cancer cell lines indicated that radiation sensitizes tumor cells to NK cell attack, resulting in an increased tumor cell killing, and increased the intra tumor areas percentage of NK cells following gamma-ray irradiation of endometrial endometrioid adenocarcinoma.[62]

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Inability to reach correct answers led to implicating the gut microbiome in tumor initiation, growth, and radio sensitivity and resistance. In support, microbiota and germ-free mice develop larger tumors than wild type counterparts. Broad-spectrum antibiotic depletion of the microbiome resulted in radioresistance of cervical cancer to a single fraction of 6 Gy. Decrease in gut microbiome-derived short chain fatty acids appeared to sensitize tumors to radiotherapy via interfering with TME elusive immunosuppression and tolerance.[63] Even more complicated efforts to modify the TME involved combining radiotherapy with antibody to the T and B cell surface programmed cell death protein 1 (PD1) PD-1 and to the chemokine receptor, CCR2 to abolish infiltration of Tregs cells, MDSC and M2 macrophages and enhance effector and memory T cell infiltration in pancreatic ductal adenocarcinoma.[64] Management of cancer was recently found to require besides surgery and radiotherapy metabolic reprogramming of cancer cells and immune cells in the TME, which was stated to be responsible for "initiation" and progression of bladder and other cancers.[65,66]

A most potent impact of radiotherapy is on the TME fibroblasts, subsequently to vascular damage and leakage of serum proteases, leading to extracellular matrix remodeling, fibroblast activation, and fibrosis.[67] Activated fibroblasts produce and organize large amounts of collagen in dense fibrillary tissue with opposing actions on residual tumor survival: increased hypoxia and decreased access to nutrients versus increased cancer cell survival due to integrin β1 signaling and TGF-β1 release.[67] Antibody-mediated TGF-β neutralization during radiation therapy effectively generated carcinomas regression in mice and in humans, provided co-administration of anti-PD-1 and anti-CD137 (TNF receptor superfamily member 9) immunostimulatory monoclonal antibodies.[68-70] However, several clinical trials concluded that patients eventually relapse, and that all these treatments ended by being ineffective.[71]

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Stroma and Microenvironment Impact following Tumor Chemotherapy

Pancreatic ductal adenocarcinomas are challenging tumors due to late stage of diagnosis, extensive and dense fibrous stroma, and resistance to chemotherapy.[72] The TME forms adhesions or fibrous connective tissue within the tumor (desmoplastic). Inhibition of immune checkpoint molecules has been exceptionally ineffective and the reason was attributed to the TME being immunosuppressive. Chemotherapy with gemcitabine led to increased tumor growth, and it was necessary to combine gemcitabine treatment with galunisertib, a TGF-β pathway inhibitor, anti-PD ligand and anti-CTL4 monoclonal antibodies to inhibit tumor growth in mice and induce infiltration of M1 macrophages.[73] One wonders about the cost and efficacy of applying such treatments in humans, especially that M1 macrophages and infiltrated cytotoxic T cells are not specific to tumor cells. In analyses of 450 patients with anal melanomas, chemotherapy and immunotherapy conferred no advantage on survival over surgery alone.[74] The adjuvant therapy failure may be construed to support a limited TME influence. Nevertheless, every chemotherapy failure was attributed to a TME flaw.[75,76] Gemcitabine treatment was reported to inhibit the expansion of MDSCs, concurrently induce type 2 cytokines and polarization of macrophages towards the M2 phenotype and subsequently, impact pancreatic cancer cells to secrete MDSCs-recruiting cytokines. Cisplatin or carboplatin increased the potency of tumor cell lines to secrete IL-6 that activates IL-10-producing M2 polarized TAMs, while IL-6 appears to activate macrophages-secreting factors instrumental in degradation of the extracellular matrix thus hindering tumor cells migration and metastasis.[34]

Immunotherapy is now required to complement chemotherapy of pancreatic ductal adenocarcinoma to counteract the activity of the TME cancer fibroblasts, provided the surface markers of their heterogeneous subsets, aggressive, tumor-supporting, versus mild or

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normal, tumor-restricting, are defined and proper antibodies generated. The rational is based on discovery of the impact of chemotherapy-mediated cancer cell apoptosis on the TME, namely quite an elusive education of fibroblast and immune cells towards a pro-tumorigenic phenotype.[77-83] However, efforts to target CAF have, to date, shown disappointing results in clinical trials of pancreatic and other carcinomas.[82,83] Indeed, more than forty separate clinical trials for pancreatic cancer, testing drugs that target different TME components in combination with chemotherapy delivered variable results.[84] Therefore, attention is now directed to pancreatic TME stellate cells, the resident lipid-storing cells of the pancreas, as they can transdifferentiate into highly proliferative myofibroblasts following tumor chemotherapy, leading to fibrosis that may shield residual cancer cells from chemo- or immuno-therapeutic interventions.[85] Additionally, efforts reverted to the use of nano-formulated anti-oxidants, like curcumin, irinotecan and paclitaxel, to enhance chemo therapeutic treatment of pancreatic cancer.[86]

Stroma and Microenvironment Impact in the Context of Tumor Immunotherapy

Immunotherapy was initially planned to directly attack inoperable cancer or residual tumor cells following surgical, radio- or chemotherapeutic interventions. Treatments aiming at immune checkpoint (PD-1/PD-L1; CTLA-4/B7-1/B7-2) blockade often lead to life-threatening autoimmunity episodes.[87-96] Treatment failure was ascribed to T cells limited tumor cells recognition and poor persistence, and above all to loss of tolerance driven by the TME.[94,97] The TME contribution to non-specific T cell activation was attributed to the expression of PD ligands on TME fibroblasts and vasculature, while the mechanism of tolerance breaking appeared elusive and obscure, because of the ubiquitous distribution of the antibody target(s).[94] CTLA-4, cytotoxic T lymphocyte antigen 4 (CTL-4) is a negative

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regulator of cytotoxic T cells and is constitutively expressed on Treg cells and it is a rational approach to target it, especially using a fully human monoclonal antibody, Ipilimumab. Yet, adverse reactions are expected since the molecule is expressed on a variety of other cells, including stem and pituitary gland cells.[96]

Difficulty in accepting the lack of TSA in clinically-detected tumors led to genetic modification of T cells to express chimeric antigen receptors (CARs) using viral vectors, notably lentivirus and retrovirus. The constructs are engineered to express a light or heavy chain variable region specific to a putative TSA and the T-cell receptor intracellular signaling domain, to allow CD8+ T cells to target cell surface makers independently of MHC class I presentation.[98-100] The costs, challenges, and dangers of administering cancer patients with T cells modified with vectors carrying CAR genes do not appear as yet to balance their benefits, restricted to hematologic malignancies.[100] Failure to improve outcome in solid malignancies was attributed to the TME hindering access of the infused CAR T cells to their target cancer cells, and immunosuppressive factors, destined to inhibit the activity of normal not genetically-engineered T cells.[101] Several clinical trials showed the benefit of devising CAR T cells that resist TME immunosuppression via expressing chemokines, targeting the tumor vasculature, or injection directly in the tumor mass, especially in the brain, liver, breast, lung. [101] Clinical trials targeting colorectal, ovarian and prostate cancer, methothelioma, and lymphoma were also conducted using CAR T cells further engineered to resist TME immunosuppression via suppressing TGF-β or producing pro-inflammatory cytokines, notably IL-2 and IL-7.[101]The cycle of disorientation continues by ascribing poor response to immunotherapy and TME-related immunosuppression to low tumor expression of β2 microglobulin and MHC class I molecules.[102]

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Future Directions

Each and every cell of the TME of every cancer type has been identified, accused of initiating and promoting carcinogenesis and metastasis, leading to generation of specific drugs and inhibitors and an array of clinical trials, with resulting limited anti-tumor efficacy and a plethora of adverse reactions.[103-107] Exosomes are endosomes-derived, minute, extracellular vesicles that contain non-coding RNA, proteins and lipids, originating from cancer and TME cells. Exosomes have also been implicated in cancer initiation, progression and therapy resistance via exchanging diverse signaling interactions that vary extensively by organ, cancer type, and patient, and are thus extremely difficult to control.[108-110] Accordingly, it is recommended to presently disregard the TME, and focus on the instrumental mechanism(s) of carcinogenesis. It is likely that the tumors that succumbed to immune surveillance have been associated with bacterial or viral infections, which have rendered the cancer cells fully immunogenic, targets for cytotoxic and helper T and NK cells. Future directions should mine such associations, which have now been documented,[111,112] and exploited with advantage.[113] Of importance too, is to examine in depth the metabolic changes that render the cell, and especially the cell surface membrane deaf to contact inhibition signals, and unable to properly display MHC class I molecules, Fas ligand and other activators and targets of cytotoxic immune cells. [4,102] Very recently, abundant cholesterol sulfate-producing cancer cells were found to exhibit remarkable resistance to cancer-specific T-cell transfer and immunotherapeutic interventions.[114] Numerous articles have documented the role of excessive content of surface membrane sphingomyelin and down-regulation of the membrane-associated neutral sphingomyelinase in tumor immune evasion, initiation, growth and drug resistance of hepatic, breast, ovarian, brain, and bone cancer growth and metastasis.[4,115-122] In that context, it is timely to recall that potent activators of neutral sphingomyelinases, notably long-chain polyunsaturated fatty acids such as arachidonic acid,

are increasingly documented as safe and efficacious in prevention and therapy of clinically-detected tumors.[4,123-125]

Conclusions

Every cell of the TME was thought as pivotal in cancer initiation, progress and metastasis. Strategies, drugs, and immunotherapeutic approaches were devised to counteract the TME elusive and obscure immunosuppression. Extensive trials documented anti-tumor effects in a few patients, and adverse and fatal impact in many. In every instance, clinically-detected cancers are still met with only surgery, radio- and chemotherapy. Therefore, it is recommended to redirect the TME-related tremendous efforts and costs to discovering the reasons instrumental in cancer cell escape from contact inhibition and immune surveillance, and devising the appropriate strategies for prophylaxis and therapy.

Conflict of interest's statement

The authors declare absence of conflicts of interests.

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Author Contributions

Both authors wrote, revised, edited, and submitted the manuscript for publication.

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