



Role of microenvironmental acidity and tumor exosomes in cancer immunomodulation

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Abstract: Tumor microenvironment (TME) is a complex milieu in which tumor grows, develops and progresses through a complex bi-directional cross-talk with immune-, stromal cells, and the extracellular matrix (ECM). In this context, tumor-derived exosomes (TE) drive the fate of tumor cells through a stimulatory or inhibitory role on immune system. In fact, TE can induce the apoptosis of cells of the immune surveillance, and enhance the proliferation and survival of stromal cells that sustain tumor development. However, depending on the molecular cargo, TE are also able to stimulate anti-tumor immune response. TME is mainly characterized by the acidic pH that contributes to tumor development, through multiple mechanisms. Among these, the impairment of tumor immune surveillance does occur within acidic TME, and is directly mediated by acidic pH or by molecular cargo carried by TE. Little is known about the role of TE in immunomodulation in acidic conditions. The present review summarizes the studies describing the role of microenvironmental acidity and TE in immune system modulation.

Keywords: Microenvironmental acidity; exosomes; immunomodulation; cancer

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Introduction

Tumor mass is characterized by poor blood perfusion, that reduces the supply of oxygen and nutrients, leading to hypoxia, inflammation, enhanced fatty acid metabolism, nucleotide synthesis and glutaminolysis. To support the high proliferation rate, tumor cells are forced to reprogram their metabolism and to increase the rate of glucose uptake, through the up-regulation and the enhanced activity of glucose transporters (1). This process leads to an elevated production of lactate and intracellular accumulation of protons, through aerobic glycolysis, the so-called “Warburg Effect” (2). Additional protons are generated and released by oxygenated cancer cells due the high levels of carbonic dioxide produced during mitochondrial respiration. To

avoid cytosolic accumulation of these acidic metabolites and prolonged intracellular acidosis, tumor cells redirect the trans-membrane ion fluxes through vacuolar ATPase (V-ATPase), Na⁺/H⁺ exchanger (NHE), monocarboxylate transporters (MCTs), and carbonic anhydrase (3), that actively export protons into extracellular microenvironment, thus lowering the extracellular pH up to 6.0–6.8. Such hostile milieu selects tumor cells among other non tumor cells that can not adapt.

A large body of evidence indicates that acidosis is a hallmark of almost all tumors (4) as well as a crucial determinant of tumor progression. In fact, it has been reported that, in the early stages of tumor progression, the extracellular acidity influences gene expression by up modulation of several hundred genes encoding receptors,

signal proteins, transcription factors, cytokines (5), involved in invasion, tissue remodeling, cell cycle control and proliferation (6,7), thus leading to a more malignant cell phenotype.

In this context, the immune cell subsets that are differentially sensitive to low pH, adopt different strategies in order to survive to acidic conditions, but the immune response is impaired by metabolic dysfunctions in cancer cells (8). In fact, under local acidity cancer cells erase the activity of antitumor immune effectors [T cells, natural killer (NK) cells and dendritic cells (DCs)], and at the same time favor the conversion of regulatory T (T_{reg}) cells and myeloid cells into immunosuppressive and pro-tumor cells. Protons and lactate, create a hostile milieu for T cells, while myeloid and T_{reg} cells can survive thanks to specific metabolic reprogramming and expression of pH regulators (8).

Tumor cells build a favorable milieu in the microenvironment through a dynamic interplay with stromal and immune cells based on a continuous and abundant release of exosomes. In fact, these nanosized (30–150 nm) vesicles endowed with proteins, lipids and nucleic acids (DNA, mRNA, circular RNA, miRNAs and long non-coding RNAs), can be shuttled between neighboring and distant cells, altering the physiological functions of recipient cells.

Tumor exosomes (TE) are involved in a plethora of mechanisms that sustain tumor, such as local invasion, angiogenesis (9–12), preparation of the pre-metastatic niche (13–16), metastasis (17–20) and immune modulation. Depending on their molecular content, TE can either inhibit the host immune system, or enhance anti-tumor immunity. In fact, TE induce T cell apoptosis, helping tumor cell immune escape (21), and stimulate immune cells against tumor through their endogenous tumor-associated antigens (TAA). These findings paved the way toward the development of anti-tumor vaccines in clinical trials, based on the cross-presentation of tumor Ag to antigen presenting cells (APCs) (22,23).

Several studies reported the influence of local acidity on the release of exosomes with modified protein content in some tumor models (24–26), likely responsible of disease advancement. However, a direct role of TE released under acidic condition in the modulation of the immune system was never described so far.

Despite growing literature on exosome field of study, however some clarifications are needed. Tumor cells release together with TE a plethora of extracellular vesicles (EVs) of plasma membrane origin (ectosomes) able to elicit

biological functions diverse from TE in target cells. To date, the lack of an elective technical procedure to separate these two populations is a main limitation in the study of TE. Moreover, the complexity of exosomal population has been recently demonstrated, being composed of subpopulations with different size, molecular composition, and biological role (27). All the specific issues in TE/EVs field have been treated in a consensus paper that provides guide lines for the characterization and analysis of EVs subpopulations (28).

In this review, we will provide an overview on the mechanisms of TE on immune modulation, the effects that local acidity may exert on the different players of cancer-immune cross-talk, and the possible role of TE released at low pH on immune modulation.

Tumor-derived exosomes modulation of immune cells

It is firmly established that the immune system can be reprogrammed by tumor cells to be ineffective or even acquire tumor-promoting phenotypes. In this scenario, TE play a pivotal role in modulating the immune system, thus driving the fate of tumor development (*Table 1*). TE can play dual roles—immunostimulatory or immunoinhibitory depending on their molecular content (*Figure 1*), thus stimulating immune cells against tumor, or inducing T cell apoptosis, and favoring tumor cell immune escape (57). In the following paragraphs, we will provide an overview of the studies regarding the role of TE in immunomodulation.

Stimulatory role in adaptive immune system

TE are able to activate immune cells and drive anti-tumor immunity by using the TAA present in their cargo, such as MHC molecules and chaperones (HSP70 and HSP90) (29,58) (*Table 1*). After internalization in DCs, the cargo of TE is processed by the antigen processing machinery (APM) and presented to T cells for cellular anti-tumor responses (29).

Notably, ligands carried by TE can be recognized by the cognate receptors on lymphocytes, including the co-stimulatory molecules CD40, CD80 and CD86, and the adhesion molecule CD54, (59) or, alternatively, TE antigens can bind to cellular MHC receptors. The main mechanism used by exosomes to interact with immune cells is through MHC-I complex, which enables the tumor antigen cross-presentation mediated by DCs, and the activation of T cells response, thus inducing tumor cells death (30,60).

Table 1 List of representative TE molecules involved in stimulatory and inhibitory functions in immune cells

Tumor type	TE molecule	Target cell	Function	Reference
Immunostimulatory role				
Malignant ascites	TAA	DCs	T cell activation	(29)
Pancreatic cancer	MHC-I complex	DCs	T cell activation	(30)
Melanoma	HSPs, melan A, mesothelin and CEA	APCs	Anti-cancer response activation	(31)
Colon carcinoma	HSP70	NK cells	Migration and cytotoxicity activation	(32)
Multiple myeloma	HSP70	NK cells	IFN- γ production	(33)
Multiple myeloma	HSP70	DCs	Stimulation of type 1 CD4 ⁺ T-cell response	(34)
Prostate tumor	HSP70	DCs	Induction of Th17 cells and inhibition of T _{reg} cells via IL-6	(35)
Breast cancer	HSP70	Macrophage	Secretion of inflammatory cytokines and activation of NF- κ B	(36)
Immunoinhibitory role				
Ovarian cancer	TCR and FasL	T lymphocyte	Suppression of TCR and CD3- ζ expression	(37)
Mesothelioma	TGF- β	CD8 ⁺ T cells	Skewing to T _{reg} cells and impaired response to IL-2	(38)
Head and neck squamous cell carcinoma	TAA and TCR	T cells	T _{reg} cells expansion and apoptosis of CD8 ⁺ T lymphocytes	(39)
Melanoma	PD-L1	PD-1 CD8 ⁺ T cells	Inhibition of proliferation and cytotoxicity of CD8 ⁺ T cells	(40)
Lung carcinoma and breast cancer	PD-L1	DCs	Differentiation of myeloid precursor cells into CD11s ⁺ DC and induced apoptosis	(41)
Head and neck cancer	PD-L1	CD8 ⁺ T cells	Apoptosis of CD8 ⁺ T cells, suppression of CD4 ⁺ T-cell proliferation and upregulation of T _{reg} cells	(42)
Head and neck cancer	Galectin-1	CD8 ⁺ T cells	Induction of a suppressor phenotype by the loss of CD27/CD28 expression	(43)
HCC	HMGB1	CD8 ⁺ T cells	TIM-1 ⁺ B cell expansion through the TLR-MAPK pathway	(44)
Mesothelioma	NGK2D ligands	NK cells and CD8 ⁺ T cells	Impaired activation	(45)
Mammary and breast cancer	Stat-3 and IL-6	CD11b ⁺ myeloid precursors	Block of differentiation in DCs via IL-6	(46)
Melanoma	Stat-3 and IL-6	CD14 ⁺ cells	Impaired monocyte differentiation into DCs and generation in an immunosuppressive cell subset	(47)
Multiple myeloma	HSP70	MDSCs	Production of immunosuppressive cytokines	(48,49)
Multiple myeloma	HSP70	MDSCs	Enhanced survival and suppression activity	(50)
Multiple myeloma	PGE-2 and TGF- β	Myeloid Cells	Switch of the differentiation pathway of myeloid cells to the MDSC pathway	(51)
Epithelial ovarian cancer	miR-940	Macrophages	MA polarization	(52)
Epithelial ovarian cancer	miR-222-3p	Macrophages	Activation of TAM-like phenotype	(53)
Melanoma	miR-690	CD4 ⁺ T cells	Upregulation of the mitochondrial apoptotic pathway	(54)
Pancreatic cancer	miR-203	DCs	Downregulation of anti-tumoral activity via TLR-4	(55)
Pancreatic cancer	miR-212-3p	DCs	Decrease of MHCII expression via RFXAP	(56)

TE, tumor exosome; TAA, tumor-associated antigens; DCs, dendritic cells; APC, antigen presenting cell; CEA, carcinoembryonic antigen; NK, natural killer; T_{reg}, regulatory T; TCR, T-cell receptor; MDSCs, myeloid-derived suppressor cells.

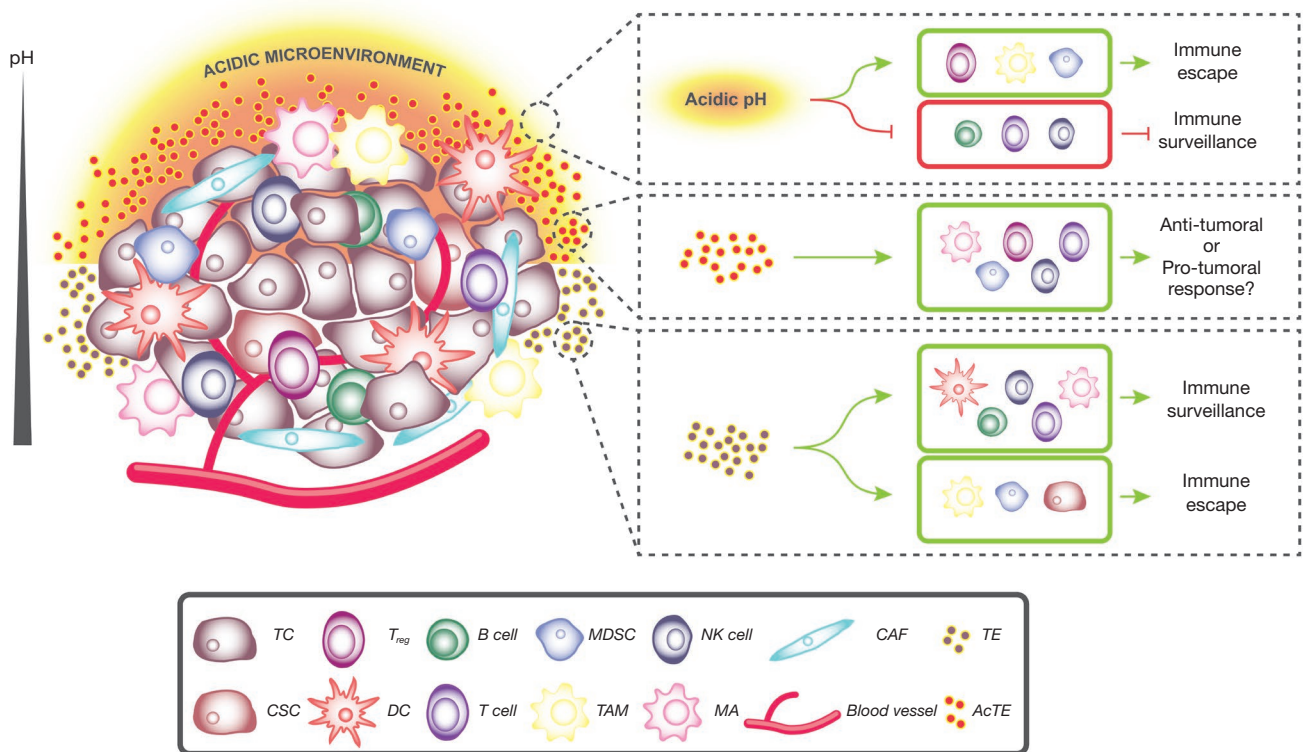


Figure 1 Schematic representation of immune modulation within TME. Cancer cells interact with immune cells through exosomes that can mediate either anti- or pro-tumor responses. Moreover, low pH *per se* is able to modulate different immune cells that promote the cancer immune escape, helping cancer progression and growth. Acidic tumor exosomes can play a role in immune modulation, activating multiple cell subsets. B, B cells; CAF, cancer-associated fibroblasts; CSC, cancer stem cells; DCs, dendritic cells; MA, macrophages; MDSC, myeloid-derived suppressor cells; NK, natural killer cells; T_{reg} , regulatory T cells; TAM, tumor-associated macrophages; TC, tumor cells; TE, tumor exosomes; AcTE, tumor exosomes released in acidic TME; TME, tumor microenvironment.

Stimulatory role in innate immune cells

TE proteomic analysis revealed that several cancer-specific antigens such as melan A, mesothelin and carcinoembryonic antigen (CEA), are responsible of the exosome-mediated immune surveillance, through the activation of APCs that support the anti-cancer immune response (31,60).

Besides indirect presentation, exosomes can also directly activate macrophages, neutrophils, NK cells and APCs. Notably, TE carrying HSP70 are able to induce NK cells migration and cytotoxicity activity (32) and the production of interferon- γ (IFN- γ) (33). Moreover, the binding of HSP70 to TLR2 stimulates DCs (34,35) and macrophages (36,61), that in turn activate T cell response and NK cell-mediated immunity.

In addition, TE create a local inflammatory environment that enhances an immune response, thus representing a powerful adjuvant in anti-cancer therapies (62).

Unfortunately, the capability of TE to stimulate the immune system is not always sufficient to prevent tumor progression, perhaps due to the dual opposite role exerted by these vesicles in the tumor microenvironment (TME).

Inhibitory role in adaptive immune system

TE are endowed with molecules (Table 1), which are able to interact with immune cells and suppress their anti-tumor activity. This process leads to a pro-tumorigenic microenvironment, resulting in an enhancement of tumor growth and shorter survival (44).

It has been reported that TE can induce the apoptosis of T lymphocytes and induce the loss of the expression of TCR-associated signal transducing ζ -chain, causing an impaired T cell-mediated immune responsiveness in cancer patients (37). TE can also inhibit the IL-2 proliferative response in $CD8^+$ T cells and favor T_{reg} cell responses (38,39).

Another systemic immune-inhibitory mechanisms used by TE is represented by activation of apoptosis in CD8⁺ and CD4⁺ T cells, mediated by the exosomal FasL or PD-L1 (40,63-66). In addition, Czystowska and colleagues demonstrated that T cell apoptosis, mediated by TE containing FasL, is significantly inhibited by T cells pre-treatment with IRX-2, a cytokine-based biological agent, thus suggesting its possible usage in cancer biotherapies (67).

In head and neck cancer, CD8⁺ T lymphocytes were sensitive to apoptosis via PD-L1 pathway, likely responsible of a reduced immune competence in cancer patients (42,68). Moreover, in lung carcinoma and breast cancer it has been shown, that exosomal PD-L1 block the differentiation of CD4⁺IFN- γ ⁺ Th1 cells, inhibit the maturation and migration of DCs and induce their apoptosis (41). Hence, PD1/PD-L1 is a key regulator of immune response in T cells and its inhibition represents a treatment for some tumors (69). Accordingly, Chen and colleagues, indicated a correlation between a higher level of circulating exosomal PD-L1 and a poorer clinical outcomes (40), thus providing a rationale for the use of exosomal PD-L1 in cancer therapy.

TE can impair the immune surveillance mediated by CD8⁺ T cells through several pathways in different tumor models. In head and neck cancer, TE affect CD8⁺ T cells by inducing a loss of CD27/CD28, together with decreased levels of the antitumor cytokine IFN- γ (43). In hepatocellular carcinoma (HCC), CD8⁺ T cells activity may be down-regulated via the TLR2/4-MAPK pathway, triggered by exosomes containing high mobility group box 1 (HMGB1), in turn leading to immune surveillance failure (44).

Finally, in a melanoma model, exosomal miRNA-690, targeting Rab27a, transferred to CD4⁺ T cells accelerated mitochondrial apoptosis by down-regulating the expression of anti-apoptotic proteins, such as B-cell lymphoma-2 (BCL-2), induced myeloid leukemia cell differentiation protein (Mcl-1) and B-cell lymphoma-extra large (BCL-xL) (54).

Inhibitory role in innate immune system

Recent studies indicated that TE can also affect NK cells. Ligands, such as MIC-A/B, ULBP-1/2/3, carried by TE are able to inhibit the expression of receptors NKG2D, NKP30 and NKP46, that activate NK, thus impairing NK cytotoxic activity (45,70). NK cell function can also be regulated by exosomes through TGF- β signaling (71).

TE interfere also with the activity and numbers of

DCs. The decrease of DCs activity is mediated by the phosphorylation of Stat-3 and the expression of IL-6, that inhibits also the differentiation of CD14⁺ monocytes into DCs (46,47). In this context, CD14⁺ cells skew their differentiation toward immunosuppressive CD14⁺ HLA-DR-/low cells, which release TGF- β to inhibit T cells (47). Also exosomal miRNAs are able to modulate multiple pathway of DC, such as TLR4 (55,72) and the regulatory factor X-associated protein (RFXAP) (56), in order to down-regulate immune activity, thereby promoting proliferation and migration of cancer cells.

Other key players in inflammation during disease progression are macrophages (MA). TE through miR-940 present in their cargo, can modify the polarization of macrophages (M2), promoting cancer cell proliferation and migration (52). It has also been described, in epithelial ovarian cancer, that exosomal miR-222-3p is able to target MA, inducing a switch to a tumor-associated macrophage (TAM)-like phenotype (53).

Inhibitory role in myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs), a heterogeneous population of immune cells (73), are key players in immune escape of cancer. The increased numbers of MDSCs in neoplastic lesions are correlated with the neoplastic progression and decreased patient survival, through the inhibition of NK, CD4⁺ and CD8⁺ lymphocytes (42,74,75). Within TME, MDSCs produce numerous immunosuppressive inhibitory factors, including nitric oxide (NO) and reactive oxygen species (ROS), which cause T-cell apoptosis, T cell receptor (TCR) nitration (76), and depletion of arginine and cysteine, preventing T-cell activities (77).

TE are able to promote the survival and activity of MDSCs (50,51), which in turn secrete exosomes that can polarize monocytes to M2 phenotype, inducing the formation of a tumor-promoting microenvironment (78). TE modulate MDSCs through PGE-2, TGF-B, IL-2 and IL-10, and miRNAs such as miR-21, miR-10a, miR-155, miR-126-3p, miR-27b, miR-320, and miR-342-3p and miR-29a [for a detailed review see (79)].

Other studies reported that MDSCs after the activation by exosomal HSP70 and HSP72 can induce the production of immunosuppressive cytokines, through TLR2 engagement (48,49). Therefore, this immunoinhibitory role of HSPs seems in contradiction with the immunostimulatory activity reported on NK (80-82). This

can be related to the interaction of HSPs with immune cell receptors with divergent properties.

In conclusion within TME, TE play a key role in the tumor immune escape, thus allowing tumor proliferation and metastatization in secondary sites. This occurs through multiple mechanisms and interactions with immune cells, leading to apoptosis of T cells, impairment of NK, DCs, MA activities, to reduce their anti-tumor role and down-regulating inflammation. This in turn leads to the recruitment of cells that inhibit the anti-tumor responses such as TAM, T_{reg} and MDSCs.

Role of microenvironmental acidosis in immune cells

Adaptive immune system

Acidosis within TME plays an important role in tumor progression and metastasis, by promoting angiogenesis and invasion and playing a role in cancer immune escape (83) (Figure 1).

TME acidification may affect the anti-tumor response of immune cells, through the metabolic competition between T and tumor cells and extracellular accumulation of lactate and/or H⁺. Pathological concentrations of lactic acid are associated with T and NK cells inhibition, leading to poor outcome in tumor patients (84). This can be explained by the capacity of lactic acid to inhibit the upregulation of nuclear factor of activated T cells (NFAT) in T and NK cells, resulting in diminished IFN- γ and cytokines production (83). These cytokines are known to sustain antitumor immunity, and develop long-term immunological memory (8). Therefore, the decreased IFN- γ secretion under acidosis can determine a failure of the immune surveillance.

Another study reported that low pH values (6–6.5), cause anergy in both human and mouse tumor-specific CD8⁺ T lymphocytes, thus decreasing the cytolytic activity, the expression of the α -chain of the interleukin-2 receptor (IL-2R α) and T-cell receptor (TCR), as well as a diminished activation of STAT5 and ERK in response to TCR signalling (85).

Innate immune system and MDSCs

It is well established that acidity, is a major “attractor” of myeloid cells to tumor site, and contributes to alter their anti-tumor response. In fact, a study indicated in prostate

cancer, that extracellular tumor acidity (pH 6.8) contributes to carcinogenesis by altering the state of macrophage infiltration, through the modulation of MHC I (86). In another study, lactic acid was described to induce M2-like polarization of TAMs through HIF-1 α , arginase I and VEGF (87).

Other studies about the activation of DCs in acidic TME highlight conflicting evidences. It was reported that extracellular acidosis improves the capacity of DCs to Ag-presentation, together with the induction of CD8⁺ cytotoxic T lymphocytes (CTLs) responses *in vivo* (88). These effects are probably mediated by acid-sensing ion channels (ASICs) (89). On the contrary, other studies described that extracellular acidosis induces in DCs a tolerogenic phenotype and an impaired migratory response to CCL-19, a lymph node-derived chemokine (90,91), thereby triggering an immunosuppressive state within TME.

Acidity is also a direct regulator of MDSCs survival and activity in TME. MDSCs are attracted to the tumor site by selective inflammatory chemokines (e.g., CCL-2 and CCL-5) and homing factors. When MDSCs reach the tumor site, they mediate immune tolerance and promote tumor growth by favoring angiogenesis, matrix remodeling and epithelial to mesenchymal transition (EMT). MDSCs within TME can differentiate into TAMs or tumor-associated neutrophil (TANs), thus preserving their pro-tumor inflammatory properties (92).

Exosomes released in acidic condition as potential modulators of immune cells

In the acidic TME, TE play a key role in tumor development through the continuous exchanges of molecular content between cancer and stromal cells. These intercellular communications can drive metastatic properties either at intratumoral level or at distant sites by means of pre-metastatic niche formation and metastases onset (93). It was reported that acidic pressure increased the release of TE in several tumors (prostate, colorectal, lung, melanoma) (7,94,95) and the efficiency transfer of exosomes in target cells (24). As a consequence, recipient cells enhance proliferation, local invasion and metastasis.

A precise role of TE released at acidic pH was recently described in a human melanoma model (26). In this study, by means of an innovative cell labeling strategy it was possible to estimate in acidic condition the enhanced release of exosomes endowed with pro-migratory and pro-

invasive molecules: proto-oncogenes (HRAS, NRAS), metalloprotease (TIMP3), heat shock protein isoforms (HSP90AB1, HSP90B1, HSPAIL, HSPA5), enzyme (GANAB), and actin-binding proteins (GSN, CFL2). To date, these molecules are involved in signaling pathways triggered in tumor advancement.

Interestingly, it was reported a role for some of these proteins in tumor immune modulation (Figure 1). For instance, in thyroid cancer the activation of HRAS together with the loss of PTEN increased the secretion of SDF-1, I-TAC, CCL9/10, and MCP5. These cytokines induced the chemotaxis of MA, MDSCs, and Foxp3⁺ T_{reg} cells at the tumor stroma, and the secretion of factors that support tumor cell growth and angiogenesis, ultimately leading to tumor progression and metastasis (96).

In breast cancer, GSN was associated to late stage of the tumor, and induced both a Th1 CD4⁺ and CD8⁺ T cell immune response against the tumor (97). HSPs on the surface of exosomes are recognized by CD91⁺ tumor cells and fibroblasts, CD91⁺ SREC1⁺ TLR⁺ professional APCs and CD94⁺ cytolytic immune cells, thus driving anti-cancer responses. Therefore, HSPs can activate malignancy events in tumor cells and, in contrast, can trigger antigen cross-presentation and cross-priming by APCs and stimulate the cytolytic immune cells [for a detailed review see (98)]. High expression of HSP90 was reported in several cancer models, (lung, breast, colon, and blood), and correlates with poor prognosis (99,100). Hence, targeting HSP70/90 resulted in reduced induction of immune suppressive cells, e.g., preventing the monocyte differentiation into suppressive immune cells (101).

Altogether, these studies provide evidences that acidic TME induces an abundant release of exosomes, whose specific molecular content has the potential to modulate immune cell functions.

Conclusions

TME actively regulates the onset and growth of the tumor (102), principally through an impairment of the immune system. One of the features of TME is acidity, which takes place in tumor cells after the metabolic switch to aerobic glycolysis, and drives tumor proliferation and metastatization.

It has been described that TME acidity, and especially lactate, is a central regulator of cancer immunity, able to coordinate both local and systemic immunosuppression, thus preventing activation of anti-tumor responses. This

deregulation derives from multiple mechanisms that shorten the lifespan or inhibit the proliferation of several immune cell types, such as CD4⁺ and CD8⁺ CTLs, tumor infiltrating CTLs, DCs and NK cells (8).

The study of the impact of acidic microenvironment on immune cells represents an emerging field of research, and it can provide tools to identify promising candidates for immunomodulation in clinical setting. For instance, several pre-clinical and clinical trials examined the impairment of lactate homeostasis as a possible approach for cancer therapeutics (103). The treatment with lactate inhibitors in synergy with other adjuvant therapies might be applied also to potentiate the efficacy of tumor immunotherapy (104).

The dysfunction of the host's immune system represents one of the major mechanisms by which tumors evade immunosurveillance. This impairment can be mediated by TE that have the ability to interact with various factors and modify the phenotype of recipient cells.

The powerful role of these vesicles in the development of tumors has been clearly addressed at several phases during carcinogenesis, in microenvironment reprogramming, pre-metastatic niche formation (a step required for tumor cell dissemination in target organs), and apoptosis or inhibition of immune cells of innate and adaptive immunity. Hence, depending on cargo and origin, TE can also stimulate the immune anti-tumor response, paving the way for the use of exosomes on immune therapy. However, further studies are needed to produce exosomes that fit the requirements to mediate strong co-stimulatory signals.

The two apparent divergent functions of TE on immune system can be explained by the existence of subpopulations of TE with stimulatory or inhibitory molecules, which can alter the functional status of different immune system components within the TME.

It is worth notice that, TE immune stimulation is an important feature for his use as anti-cancer vaccines. In fact, modifying APC or mesenchymal stem cells (MSCs) function *in vivo* or *in vitro* to facilitate release of immunosuppressive EVs could be used to enhance the cytotoxic response against cancer (105,106).

Little is known about the functions exerted by TE secreted within the acidic TME. In melanoma, exosomes released under acidosis contain specific pro-invasive and pro-migratory molecules, which correlated with disease poor prognosis. This enlightened the increased release of acidic TE as a mechanism required for tumor advancement.

Several molecules specifically included within acidic exosomal cargo were described to modulate the immune

cells. However, some of these molecules have been described to mediate an anti-cancer response and others to have pro-tumoral functions. Therefore, further investigation are needed to unveil a possible role of acidic TE in immunomodulation.

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