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Role of LFA-1 and ICAM-1 in cancer

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Abstract
The lymphocyte function–associated antigen-1 (LFA-1) (also CD11a/CD18 and αLβ2), is just one of many integrins in the human body, but its significance derives from its exclusive presence in leukocytes. In this review, we summarize the studies relating LFA-1 and its major ligand ICAM-1 (CD54) with cancer, through the function of lymphocytes and myeloid cells on tumor cells. We consider how LFA-1 mediates the interaction of leukocytes with tumors and the role of ICAM-1 in tumor dynamics, which can be independent of its interaction with LFA-1. A more detailed examination of LFA's role within B-cell chronic lymphocytic leukemia is made. Finally, we discuss the role of LFA-1-harboring exosomes in tumor growth and metastasis.

Keywords: cancer metastasis; chronic lymphocytic leukemia; exosomes; tumor microenvironment

LFA-1 is widely expressed in hematopoietic cells, mediating intercellular interactions within the immune system and between leukocytes and non-blood cells. Several intercellular adhesion molecules (ICAM), including ICAM-1 (CD54) are common ligands for LFA-1 [1] [2]. The strength of LFA-1 adhesion varies by adjusting the affinity (conformation), the level of LFA-1 clustering and the force applied by the ligand [3] [4] [5]. LFA-1 can adopt different conformations, from a folded low-affinity inactive one to an open active conformation. The stimulation of cells by receptors like the T-cell receptor or chemokine receptors changes the adhesive properties of LFA-1 in a process called "inside-out" signaling that may open the conformation of LFA-1 and expose its ligand-binding site [6]. In addition, it should be noted that the characteristics of the external ligand binding to LFA-1 initiates a signaling cascade in LFA-1 (named "outside-in" signaling), giving rise to various cellular changes [7]. As with other integrins, LFA-1 is not a mere adhesive contact between cells, but it also mediates signals that modulate the growth, differentiation and survival of the cell.

LFA-1 participates in the cytotoxic immune response against tumors

The T cell cytotoxic response against cancer cells starts when the T cell receptor recognizes a specific tumor antigen on the surface of the cancer cell; this is normally followed by delivery of toxic granules that kill the target cell. In the cytolytic response against virus-infected cells, the adhesion between the cytotoxic lymphocyte and the target cell is not strictly dependent on LFA-1, this however is necessary in the cytolytic response to immunogenic tumors [8] [9]. LFA-1 is an essential initiator of the immunological synapse that forms between the cytotoxic T or NK cell and the cancer cell, and mediates the firm adhesion to the target cell and the polarization of cytotoxic granules towards the target [7] [10] [11]. There is a positive correlation between the maturation state of NK cells, their cytotoxic potential and the activation level of LFA-1 [12].

Tumor infiltrating lymphocytes need LFA-1 to adhere to target cells

Cancer cells can escape from the immune response by changing the tumor microenvironment into an immune-suppressive one [13]. Indeed, the presence of an immune-suppressive

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regulatory T cell infiltrate within a tumor correlates with reduced survival [14], but not if the nature of the T cell infiltrate is inflammatory [15].

The tumor infiltrating lymphocytes (TIL) can be purified from solid tumors and expanded in vitro before being used as an effective therapeutic tool. However, TIL are not fully functional when first obtained from tumors. The presence of a galectin lattice covering the surface of TIL interferes with the adhesion of T cell LFA-1 to ICAM-1 on the target cell [16]. These galectins impair the recruitment and activation of LFA-1 into the immunological synapse formed between TIL and tumor cells [16]. The extent of LFA-1-ICAM-1 interaction affects the quantity of secreted cytokines by TIL [16].

Leukocytes in the tumor stroma can play a positive or negative role in tumor growth

Cancer cells develop in a dynamically changing microenvironment that constitutes a safe zone for their survival and proliferation. Through the interaction with stromal cells, cancer cells receive survival signals and produce proteins and metabolites that suppress and modify the activity of the immune system. This dependence of the tumor on its microenvironment could also be the tumor's Achilles heel, since tumor-supporting stromal cells could be targeted therapeutically.

Myeloid cells can either, contribute to tumor development or restraint tumor growth, depending on the tumor context. Below is a summary of studies where LFA-1 has been related to myeloid cell function in the tumor microenvironment.

- Neutrophils

In mouse breast cancer models, neutrophils are the main drivers of metastasis [17]. By producing leukotrienes, neutrophils contribute to the expansion of cancer cells with high tumorigenic potential and to metastasis [17]. Hence, targeting the production of leukotrienes in neutrophils had a therapeutic effect on metastasis. In a similar mouse model of estrogen receptor-positive breast cancer, neutrophils were recruited into the tumor via increased expression of LFA-1, provoked by estradiol and TGFβ1 [18]. Neutrophil adhesion to endothelial cells by means of LFA-1, is associated with prolonged survival of the cell [19]. Importantly, the neutrophil-cancer cell interactions mediated by LFA-1 facilitated breast cancer dissemination [18]. In ovarian cancer patients, the adhesive properties of blood neutrophils are increased, due to a higher expression of CD11b/CD18 integrin, suggesting that the neutrophil-cancer cell contacts modify the properties of neutrophils to facilitate tumor dissemination [20].

By contrast, in other types of cancer, neutrophils play a surveillance role against tumor development [21]. When this is the case, it would be desirable to potentiate neutrophil infiltration into the tumor to increase its elimination. Due to the contrasting roles of neutrophils in cancer, it will be necessary to categorize tumors, to determine the convenience of improving the neutrophil response against the tumor or by the contrary, blocking the neutrophil help to the tumor.

- Macrophages

Macrophages are an abundant component of many solid tumors and can play varied functions, depending on the acquired phenotype of the macrophage and the tumor context. These tumor-associated macrophages contribute to the epithelial-mesenchymal transition (EMT) and tumor dissemination [22] [23] [24] [25]. The metastasis-promoting effect of macrophages is exerted through cytokine secretion such as TGFß [23]. In an in vitro model of EMT, it was shown that M2-type macrophages in direct contact with carcinoma cells facilitated the dispersion of the latter via ICAM-1 and integrin CD18 interaction [26]. Similarly, in a mouse ovarian cancer model,

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the initial steps of spheroid formation and transcoelomic metastasis were facilitated by the attachment of cancer cells to macrophages via CD11b/CD18 – ICAM-1 adhesion [25]. If binding to ICAM-1 was neutralized with antibodies, spheroid formation and ovarian cancer progression were impaired [25].

- Eosinophils

Eosinophils are potential players in the anti-tumor arsenal. In a mouse melanoma model which depends on the anti-tumor activity of cytotoxic CD8 T cells for survival, eosinophils played an essential role in the recruitment of cytotoxic T cells into the tumor [27]. In addition, in this melanoma model, eosinophils normalized the tumor vasculature and reprogrammed tumor-associated macrophages into inflammatory M1 type [27]. Similarly to eosinophils, when basophilia was induced in these mice by treatment with interleukin-13, enhanced T-cell infiltration and tumor rejection was obtained, indicating a similar anti-tumor role for basophils [28]. Other studies attribute an antitumor role to eosinophils in colon cancer [29]. The cytotoxic activity against tumor cells that eosinophils can display in vitro depends on the interaction LFA-1 - ICAM-1, which is upregulated by interleukin-18 [30].

LFA-1 in chronic lymphocytic leukemia

Because there are studies describing alterations of LFA-1 signaling pathway in chronic lymphocytic leukemia (CLL), these studies are being considered here. CLL, the most common leukemia in the Western world, is characterized by the accumulation of clonal mature B cells in blood and lymphoid tissues [31]. Circulating normal B cells continuously home to secondary lymphoid organs in search of antigen and to acquire survival signals. Similarly, B-CLL cells take advantage of this survival path by increasing the expression of homing chemokine receptors CXCR4 and CCR7, and decreased expression of the egress receptor S1P1, to home to lymphoid organs, an environment that favors clonal expansion [32]. The gathering of CLL cells with other stromal cells, macrophages and T cells defines a pseudo-follicle, which supports CLL cell proliferation [33]. The importance of migration and adhesion in determining pathogenesis in this leukemia type is exemplified by treatment with a Bruton tyrosine kinase inhibitor, that mobilizes leukemic cells out of the supportive lymphoid organs resulting in CLL regression [32] [34]. The inhibition of Bruton tyrosine kinase impedes chemokine- and B-cell receptor-derived signaling governing adhesion and migration of CLL cells.

In addition to chemokines, migration of CLL cells requires the participation of the two lymphocyte integrins VLA-4 (CD49d/CD29) and LFA-1, which show varied levels of expression in CLL cells [35]. VLA-4 (CD49d/CD29) contributes to homing of CLL cells to bone marrow [36]. However, the activation of LFA-1 is impaired in CLL cells due to defective signaling by Rap1 GTPase, a major signaling element of the inside-out signaling cascade, impeding proper clustering of LFA-1 [37]. Moreover, signaling by Rac1 and CDC42 GTPases to activate LFA-1 shows extensive degrees of alteration in CLL patients, so that some authors suggested that progression to CLL requires bypassing these GTPases [38]. The defective LFA1-dependent endothelial transmigration could play a role in survival of CLL cells [39]. Despite the necessity of CLL B cells to home to lymph nodes and bone marrow, it is not uncommon to observe a lower CD18 expression in B-CLL than that of normal B cells in healthy individuals [35]. Recently, a CD18 variant present in CLL patients has been associated with increased disease susceptibility [40]. The variant shows a glutamate to lysine E630K change, which probably impairs CD18 function. The expression of the CD18 variant in patients' B cells is even lower than that of the wild-type form [40]. By contrast, a CLL subgroup of patients harboring trisomy 12 (approximately 16% of CLL patients) show an important increase of LFA-1 and other integrins, associated with high cell proliferation and lymph node infiltration [41] [42]. These data do not clarify the role of LFA-1 in disease progression in CLL and suggest a complex interplay with other adhesion molecules and signaling pathways.

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Patients with CLL show impaired T cell function which results from the direct contact of normal T cells with B-CLL [43] (reviewed in reference [44]). Thus, the cytotoxic activity of CD8 T cells and the differentiation of T helper 1 are deficient in CLL patients. The inefficient T cell function probably contributes to CLL expansion. These T cells display low conjugation to CLL B cells *in vitro* and impaired immune synapses, characterized by impaired LFA-1 clustering at the immune synapse [45]. Importantly, pretreatment of CLL B cells with anti–ICAM-1 monoclonal antibody improved the conjugation of CLL B cells with T cells and F-actin polymerization at the immune synapse [45]. Furthermore, T cells in CLL patients exhibit defective LFA-1-mediated migration, due to dysregulated Rho GTPase signaling [46]. Treatment with lenalidomide, a clinically active drug used in hematologic cancers [47], restored Rho GTPase signaling in T cells, rescued LFA-1 function [46] and improved immune synapse formation between T cells and B-CLL [45]. It is surprising that T cells in CLL patients show a dysfunctional adhesion and signaling through the LFA-1/Rho-family GTPase pathway, resembling that of B-CLL, what highlights the importance of the LFA-1 signaling pathway in this leukemia.

Targeting LFA-1 in cancer

Tumors of hematopoietic origin normally express LFA-1, and could potentially be targeted with anti-LFA-1 antibodies [48]. In certain conditions LFA-1 is expressed by tumor cells as well, such as in brain metastasis [49] [50]. In a mouse model of a brain tumor, the presence of LFA-1 on metastatic cells contributed importantly to tumor growth [50]. LFA-1 has also been found on *in vitro* cultured melanoma cells, allowing their transmigration through endothelial cells [51]. However, whether LFA-1 is also expressed in melanoma cells *in vivo* is unknown. The presence of LFA-1 on tumors of non-hematopoietic origin will need further confirmation before it can be considered a therapeutic target.

A different approach could be targeting LFA-1 in the leukocyte infiltrates, which often play a pro-tumorigenic role. For instance, the presence of regulatory T cells, which are immunosuppressive, in the microenvironment of many solid tumors and the importance of LFA-1 in the function of these cells [52], suggests that targeting LFA-1 in these tumors would limit the function of regulatory T cells and improve the action of the immune system against the tumor [27] [28]. The acquired knowledge from targeting LFA-1 in the treatment of inflammatory diseases can be helpful here [53].

Unfortunately, attempts to functionally antagonize integrins in tumors have generally failed [54] [55]. This is the case of efalizumab, a humanized monoclonal antibody directed against CD11a, which blocks the interaction between LFA-1 and ICAM-1 [56]. Initially used in the treatment of psoriasis, it had to be withdrawn in 2009 after the unexpected appearance of progressive multifocal leukoencephalopathy in 4 patients receiving it. Unforeseen effects when targeting LFA-1 are not uncommon, for instance, the use of antibodies against CD11a/CD18 can affect indirectly the function of the integrin α4β1, inhibiting it selectively [57].

Recently, a different approach has been tested, where the host immune system is redirected against integrin-bearing tumors [58]. Also, the design and applicability of new LFA-1 antagonists is a potential field for therapy development [53] [59].

The effect of leukotoxin

The liganded and unliganded states of integrins can determine the life or death of the cell, respectively [60]. It is therefore not surprising that pathogens may utilize these membrane receptors to modulate the immune response. This is the case of leukotoxin, a protein produced by *Aggregatibacter actinomycetemcomitans,* that induces apoptosis in leukocytes [61]. It binds

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to activated LFA-1 and induces apoptosis by several mechanisms [62]. Leukotoxin shows a tendency to kill leukemic cells in a LFA-1-dependent manner [63] [64]. Whereas normal hematopoietic cells might be partially sensitive, leukotoxin shows preferential activity against active LFA-1 and spares most blood cells. The death of tumor lymphocytes occurred by a Fas-dependent mechanism [64]. Besides the advantage of counting with a potential therapeutic tool, working out the mechanism behind leukotoxin's action on LFA-1 leading to cell death, will provide new knowledge linking adhesion to cell fate decisions.

The role of ICAM-1 in tumors

ICAM-1 is expressed in several tumors, and as a major LFA-1 ligand, it may help in the immunosurveillance process [65] [66] [67] [68] [69] [70] [71] [72] [73]. Along this line, the presence of ICAM-1 in colorectal cancer has been associated with better prognosis [71] [72]. Moreover, the transfection of ICAM-1 in colorectal cancer cell lines inhibits tumor growth and metastasis [74]. Similar observations were obtained from colon epithelium cell lines derived from mice presenting transforming mutations in the adenomatous polyposis coli gene, the gene mutated in patients affected by familial adenomatous polyposis. When these colonic cell lines were incubated with intraepithelial T lymphocytes, they expressed ICAM-1, which mediated the interaction with T cells [75].

The production of prostaglandin E2 in the tumor microenvironment limits the expression of ICAM-1 on tumor cells, reducing the cytotoxic effectivity of T cells [76]. Mouse melanoma tumors that regress due to adoptive T cell therapy, show increased expression of ICAM-1 [77]. By contrast, upon tumor relapse, ICAM-1 and other adhesion molecules are found in lesser levels [77].

Other potential mechanisms by which ICAM-1 could retard tumor cell metastasis have been proposed. Hence, the inhibitory effect of cannabinoids on lung cancer cell invasion and metastasis has been suggested to occur via up-regulation of ICAM-1, which then increases the tissue inhibitor of matrix metalloproteinases-1 [78]. ICAM-1 has also been suggested to mediate the differentiation properties of gastrin-releasing peptide on colon cancer cells by enhancing cell-matrix attachment [79].

On the contrary, in some reports, the expression of ICAM-1 has been correlated positively with a more aggressive tumor phenotype and metastatic potential [70] [80]. Hence, the invasiveness of breast cancer cells has been positively correlated with the expression of ICAM-1 [81]. Also, an ICAM-1 – ICAM-1 homophilic interaction between breast cancer cells and mesenchymal stem cells in bone marrow has been proposed to mediate the metastatic expansion of cancer cells, displacing hematopoietic stem cells of its niche [82].

Importantly, tumor-associated fibroblasts in colorectal cancer tissue sections also show increased ICAM-1 expression in comparison to healthy mucosa [83]. When colorectal cancer tissue sections are examined for expression of ICAM-1, the number of positive fibroblasts is increased in comparison to healthy mucosa [83].

Exosomes carrying LFA-1 and ICAM-1

It is increasingly clear that exosomes released by cancer cells play a key role in metastasis [84]. Homing of exosomes released by cancer cells to specific body tissues is mediated by integrins [84]. However, the function of LFA-1 in exosome-directed mutagenesis and metastasis is poorly defined. LFA-1 is present in exosomes released by mast cells, dendritic cells and T cells [85] [86] [87], and mediates exosome uptake during T cell - dendritic cell contact [86] [87] [88]. Exosomes harboring ICAM-1 can be captured by LFA-1 present in dendritic cells [89]. ICAM-1-presence in

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exosomes released by dendritic cells is necessary for stimulation of naive T cells [90] [91]. However, ICAM-1–bearing exosomes produced by cancer cells, can block leukocyte adhesion to endothelial cells [92].

The cellular origin of exosomes is important in determining their inhibitory or activation function. Thus, dendritic cell-derived exosomes target other recipient dendritic cells via LFA-1-ICAM-1 and increase their capacity of stimulating T cell tumoricidal activity [93], whereas T cell-derived exosomes, when introduced in mice, target dendritic cells via LFA-1 and modulate their function, inhibiting CD4 and CD8 T cell anti-tumoral activity [87] [88].

Despite the great potential of exosomes in cancer therapy, their capacity to interact with different cellular partners and produce varied biological outcomes requires a deeper knowledge of their biology, before a therapeutic use is possible.

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Conflicts of Interest:
The authors declare no conflict of interest.

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