

Contribution of very late antigen-4 (VLA-4) integrin to cancer progression and metastasis

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Abstract The integrin “very late antigen-4” (VLA-4) is expressed by numerous cells of hematopoietic origin and possesses a key function in the cellular immune response, e.g., by mediating leukocyte tethering, rolling, binding, and finally transmigration of the vascular wall at inflammatory sites. Thus, VLA-4 is a valuable target in medical sciences to interfere with pathological inflammations. In addition, leukemic cells and different solid tumors, which express VLA-4, make use of these adhesive functions and confer VLA-4 a progressive role in the metastatic spread. With a growing insight into the molecular mechanisms for creating a tumor-friendly microenvironment at metastatic sites and various tumor host interactions, the multiple functions of VLA-4 became evident recently, e.g., in leukocyte recruitment to micrometastases, the protection of tumors from immune surveillance, or contribution to a chemoresistance. Nevertheless, despite accumulating evidence for several functions of VLA-4 in tumorigenicity, a therapeutic interference with VLA-4 in cancer sciences has not been developed yet to the clinical level, undoubtedly by a marked impact on the physiological immune response. This review gives an up to date insight into the multiple functional role of VLA-4 in cancer and introduces this integrin as a promising target worthwhile to attract attention in biomedical cancer research.

Keywords VCAM-1 · VLA-4 · Integrin · Metastasis · Cancer · Angiogenesis

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1 Molecular background and physiological function of VLA-4

Integrins are cell surface receptors which are expressed by almost all cell types capable to mediate cellular contacts to other cells and to the extracellular matrix (ECM) or constituents of the blood plasma [1–4]. All integrins consist of non-covalently linked α and β subunits. In vertebrates, 18 α and 8 β subunits have been identified, forming 24 α/β pairs [5, 6]. Each integrin subunit consists of a large extracellular domain, a single-spanning transmembrane domain, and a short cytoplasmic tail [7]. Integrins are implicated in cell adhesion and migration, embryogenesis, and several other different biological processes [8–11]. The term “integrin” refers to the ability to integrate contacts with the cell microenvironment to intracellular signals, also known as outside-in signaling. Vice versa, the binding of adaptor molecules or signaling components to the integrin cytoplasmic tail can alter ligand affinity by conformational changes in the integrin structure [7, 12]. Hence, integrins provide cells a general capability to adapt to multiple physiological situations.

The functional classification of integrins into different groups is generally conducted according to the β subunits. Whereas β_1 integrins recognize components of the ECM, β_2 integrins are expressed by almost all leukocytes, displaying their crucial role in leukocyte trafficking to the sites of inflammation. As an exception, the integrin very late antigen-4 (VLA-4) ($\alpha_4\beta_1$, CD49d/CD29) has a dual role, since this leukocyte integrin mediates not only the recruitment of leukocytes to site of inflammation but also the binding to fibronectin or osteopontin as components of the ECM [13, 14].

VLA-4 was identified by Hemler and colleagues in 1987 together with VLA-3 and VLA-5 [15]. Due to its very late appearance on T cells after activation, the whole family of proteins was termed VLA-very late antigen. Subsequently, VLA-4 was found to be widely distributed on hematopoietic

cells like thymocytes, peripheral blood lymphocytes, monocytes, activated T and B lymphoblastoid, and myeloid cell lines [16]. Sequence studies revealed that the α_4 subunit consists of 999 amino acids containing three potential cationic binding sites but lacks an inserted I- or A-domain [17, 18]. VLA-4 recognizes LDV-related protein sequences in its ligands, which is comprised in vascular cell adhesion molecule-1 (VCAM-1) or fibronectin, the two best characterized interaction partners [14, 19, 20]. Further ligands for VLA-4 are osteopontin [13], ADAM7 and ADAM28 (a disintegrin and metalloprotease) [21], the LPS receptor CD14 [22], or the Lutheran blood group antigen/basal cell adhesion molecule (BCAM) [23, 24]. VLA-4 binding to mucosal addressin cell adhesion molecule-1 (MadCAM) is described controversial [25–27].

VLA-4 is generally regarded as one of the key integrins mediating tethering, rolling, firm adhesion, and the transendothelial migration of leukocytes to inflamed tissues [28–32]. Furthermore, VLA-4 is involved in immunological synapse formation, phagosome maturation in macrophages, embryogenesis, and angiogenesis in the central nervous system [8, 33–37].

The capacity of VLA-4 to conduct cell tethering or rolling before finally mediating firm adhesion under shear conditions is, in contrast to, e.g., the leukocyte integrin LFA-1 (lymphocyte function-associated antigen-1, $\alpha_L\beta_2$) [38, 39], regulated by different adhesive states in which VLA-4 is potentially existent [40]. Chigaev et al. described the existence of six different VLA-4 conformational states, varying in ligand affinity, molecular extension, and beta subunit hybrid domain exposure. Ligand binding affinity and molecular extension were apparently regulated independently either by cytoplasmatic Ca^{2+} concentrations or in a diacylglycerol-dependent manner. Whereas binding affinity primarily accounts for the duration of ligand interaction, molecular unbending contributes to improved capture affinity [18, 41, 42]. The different states of activation are regulated by a multitude of signals, varying from extracellular shear stress [43] to the activation of different G protein-coupled receptors, which can increase or downregulate VLA-4 affinity [44, 45]. Also, a direct binding of monomeric C-reactive protein (mCRP), cysteine-rich protein 61 (Cyr61), or allosteric modulation by the chemokine fractalkine can have an impact on VLA-4 affinity [46–48].

Intracellular, a lot of signaling molecules were identified, which potentially participate in VLA-4 affinity regulation. Besides the phosphatidylinositol-4,5-bisphosphate 3-kinases γ and δ , the Src kinase p56, or B-Raf, also the small GTPase Rap1, seems to interfere in VLA-4 activation [49–53]. The adaptor molecules talin-1, paxillin, and kindlin-3 connecting the cytoskeleton with the VLA-4 subunits were described to regulate adhesion strengthening [54–57].

In addition to the outstanding physiological role of VLA-4, a plenty of excellent reviews provide surveys of the involvement

of VLA-4 next to other integrins in diverse pathophysiological situations. This review shall give a broad overview of the implication of the integrin VLA-4 in tumorigenicity and cancer metastasis, a synopsis, which is to the best of our knowledge currently not available.

2 Contribution of tumor cell expressed VLA-4 to metastasis

The interaction of tumor cells (TCs) with blood components and endothelial cells (ECs) is indispensable for a successful TC extravasation into the surrounding parenchyma and finally the establishment of a metastatic niche [58]. First reports described an increase in melanoma EC interaction due to EC activation with inflammatory mediators like $\text{TNF}\alpha$ or IL-1, without shedding light on the molecular basis of this phenomenon [59, 60]. Shortly after, VLA-4 was discovered also on leukemia, lymphoma, osteosarcoma, and kidney carcinoma cells, and the process of leukocyte rolling and adhesion could be conferred to circulating TCs utilizing the same panel of adhesion receptors [61–63]. In *in vitro* studies, endothelial activation by either IL-1, IL-4, bacterial endotoxin, or $\text{TNF}\alpha$ resulted in an increased VCAM-1 expression and augmented TC adhesion to ECs [59, 61, 62, 64]. On the other hand, the treatment of melanoma cells either with $\text{TNF}\alpha$ or laser radiation augmented α_4 and β_1 integrin subunit expression which led to an elevated adhesion to collagen and invasion through fibronectin layers [65, 66]. By comparing different melanoma cell lines varying in VLA-4 affinities, Klemke and colleagues revealed that cells which display high-affinity interactions between VLA-4 and endothelial VCAM-1 possess an enhanced transendothelial migration [67].

These investigations convey the first hints that the VLA-4/VCAM-1 interaction crucially contributes to metastasis. Okahara and coworkers conducted the first *in vivo* experimental confirmation by illustrating an enhanced number of metastatic foci in the lungs of mice that received $\text{TNF}\alpha$ before B16-BL6 melanoma cell inoculation. Antibodies against VLA-4 or VCAM-1 alleviated the number of lung metastases [68]. Further studies revealed an enhancement of lung metastasis after IL-1 application or $\text{TNF}\alpha$ release due to surgical stress, which elevated VCAM-1 expression on the lung epithelium [69, 70].

Cardones et al. demonstrated an upregulation of VLA-4 affinity on melanoma cells by CXCR4 activation, which increased melanoma cell adhesion and the number of lesions in the lungs of mice [71]. Recent data from Rebhun imply that VLA-4 expressing B16F1 melanoma cells metastasize to lymphatic tissues due to constitutively expressed VCAM-1 on the lymphatic endothelial cells [72]. All these studies embrace the given fact of a constitutive VCAM-1 expression at the site of

metastasis offering a hospitable microenvironment to the TCs, which is triggered by inflammatory mediators (Fig. 1).

Nonetheless, under pathophysiological conditions, an endothelial VCAM-1 expression can also be induced by TC host cell contacts [73–75] or reduced shear stress [76], whereas high shear rates have anti-inflammatory effects [77–79]. After VLA-4 mediated TC adhesion to the endothelium, TCs are able to extravasate and proliferate in the adjacent tissue.

In a model of spontaneous melanoma metastasis, which is quite closer to the pathological situation than the common experimental metastasis assays, Quian et al. detected an inverse correlation between VLA-4 expression and the number of metastatic foci in the lungs. A high VLA-4 expression on B16F10 cells strongly reduced lung metastases formation and Matrigel invasion [80]. Further studies provided molecular explanations for these findings illustrating homophilic interactions between VLA-4 molecules, which generated VLA-4-mediated cell aggregation and reduced metalloproteinase secretion [81–87]. Hence, single cells in a tumor with high VLA-4 density are hindered to leave the tumor network and emigrate to different organs. These results are supported by Hart and Schadendorf, investigating the VLA-4 expression of melanocyte strains, naevi, primary malignant melanoma, and melanoma metastases. They found a significant increase in VLA-4 expression on primary melanomas which correlated with the development of metastases. Also, a negative association of VLA-4 expression and disease-free periods were feasible. In comparison to the VLA-4 increase on melanoma metastases, a decrease in VLA-1, VLA-2, and VLA-6 was detectable [81, 88–91].

Concluding, on primary/early melanoma lesions, VLA-4 seems to keep the cells together, whereas in a later phase, VLA-4 is able to support steps of the metastatic cascade by interaction with endothelial VCAM-1. Nonetheless, the fact that also VLA-4 negative cells can be highly metastatic shows the existence of different, abundant pathways mediating the critical steps of tumor cell adhesion to and transmigration through the endothelium.

3 How VLA-4 expressed by leukocytes contributes to cancer metastasis

The VLA-4 expression by TCs confers them the ability of vascular adhesion and transmigration out of the blood flow, as outlined above. Besides VLA-4 on TCs, VLA-4 expressed by myeloid cells contributes to tumorigenicity and metastasis in different manners which shall be reconsidered in this chapter.

The VLA-4 counterreceptor VCAM-1, which is usually expressed on inflamed endothelial cells, is also aberrantly expressed by several cancer cells, e.g., breast, renal, malignant mesothelioma cells, or gastric carcinomas [92–96]. Thus, VCAM-1 belongs, e.g., to the site-specific metastasis gene signature for breast cancer [97–99], since the aberrant VCAM-1 expression confers several advantages to the breast cancer cells either metastasizing to the lungs or the bones. In the lung tissue, the TCs interact with VLA-4 positive tumor-associated macrophages, which leads to a VCAM-1 clustering and subsequently a phosphorylation and binding of the adaptor molecule Ezrin to the VCAM-1 cytoplasmic tail. Ezrin in

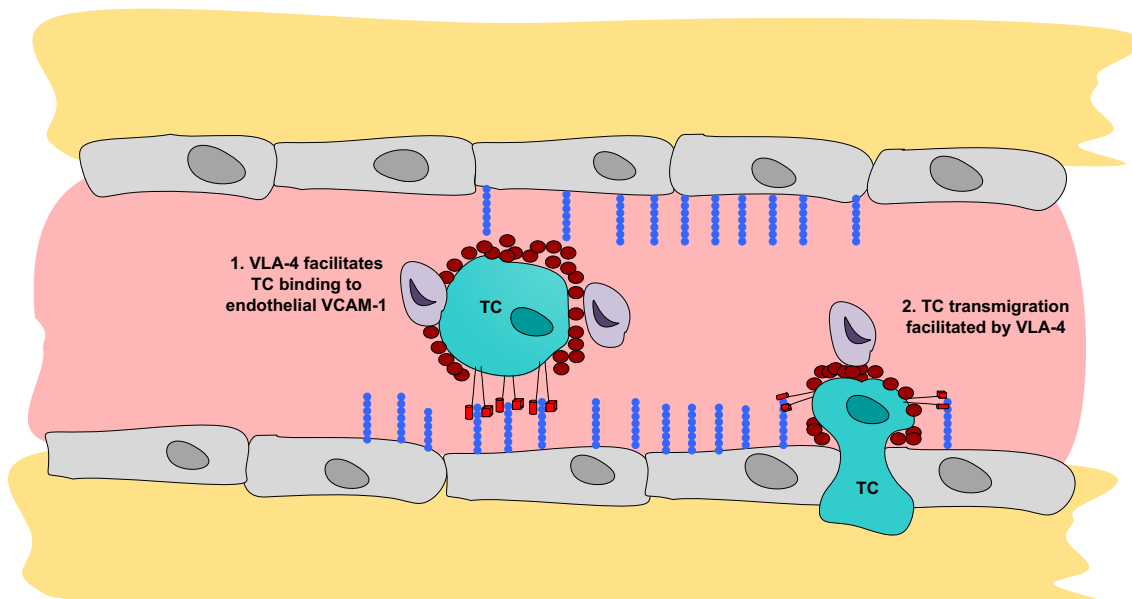


Fig. 1 Interaction of VLA-4 expressing TCs with endothelial VCAM-1. TCs entering the blood are immediately surrounded by platelets and different leukocytes. This clot can become stuck in tight vessels and finally enable the TCs to transmigrate in the surrounding tissue to

establish metastatic foci. TCs expressing VLA-4 have the additional opportunity to adhere to endothelial expressed VCAM-1 and subsequently leave the blood flow in a VLA-4/VCAM-1 facilitated manner

turn activates phosphoinositide-3-kinase (PI3K) finally leading to an Akt (protein kinase B) activation, which conveys strong pro-survival signals to the TCs [92, 100]. In the bone marrow, indolent breast cancer micrometastases awake from dormancy and attract VLA-4 positive osteoclast progenitor cells by the novel secretion of VCAM-1 in a NF- κ B-driven manner [100–102]. The osteoclast progenitor cells adhere to the breast cancer cells in a VLA-4 dependent fashion, mature to osteoclasts, and resorb the bone matrix, which leads to a discharge of growth factors. These growth factors in turn activate the TCs to support osteoclast progenitor maturation. Hence, the VLA-4/VCAM-1 interaction incites the vicious cycle of breast cancer bone metastasis and osteolysis.

Wu and coworkers proposed to study a TC immune escape and created a human papillomavirus-16 E7-expressing cancer cell line and a mouse vaccine containing E7 [93, 103–105]. Mice vaccinated with E7 showed a reduced tumor growth and an augmented number of CD4⁺ and CD8⁺ T cells. Finally, TCs were isolated, which exhibited a complete resistance against vaccine-induced immune response. Gene expression analysis revealed a strong VCAM-1 upregulation in resistant cells compared to susceptible TCs. Due to the fact that CD8⁺ cells express high levels of VLA-4, the authors proposed that CD8⁺ T cells migrate away from the TCs in a VLA-4 dependent mode, and the contact between TC MHC receptors and T cell receptors is alleviated [93, 105]. It is a well-known fact that VLA-4 can foster cell migration either directly or via the transregulation of the integrin $\alpha_1\beta_2$ [32, 106–109].

Next to the direct interaction between VLA-4 positive host cells with VCAM-1 expressing TCs, myeloid cells are crucially involved in the creation of permissive metastatic niches. Lyden et al. impressively demonstrated that Vascular Endothelial Growth Factor Receptor-1 (VEGFR1)- and VLA-4 positive hematopoietic bone marrow-derived cells (BMDCs) instigate a pre-metastatic niche [110]. Mice were implanted with either Lewis lung carcinoma (LLC) or B16 melanoma cells, and by day 14, before a metastasis of the tumors occurred, BMDC clusters appeared in the lungs to prepare a pre-metastatic surrounding (Fig. 2). Also, the administration of melanoma-conditioned media mobilized immature BMDCs from the bone marrow, which created a conducive microenvironment in the lungs for incoming tumor cells. The sites of BMDC clusters were tumor-type specific, LLC cells dictated cluster formation mainly to the lungs and liver, whereas B16 melanoma cells induced clusters in the liver, testis, spleen, and kidney. Following TC implantation, but before BMDC arrival, an augmented fibronectin expression by fibroblast-like stromal cells in adjacency to the future metastatic lesion was detectable (from day 3 on), which supported VLA-4⁺-BMDCs adhesion and cluster formation. For successful TC engraftment in the pre-metastatic niche, a matrix metalloproteinase 9 and SDF-1 (CXCL12) expression was observed in a completely formed cluster, which may attract CXCR4⁺ TCs to

the pre-metastatic niche. Hence, an aggregation of VLA-4 and VEGFR1-positive hematopoietic bone marrow progenitor cells can allude to a pre-metastatic nodule.

Besides CXCR4, also CCR7 is a chemokine receptor often expressed on TCs, navigating the way to the organ of final destination [111–114]. Recently, also CCL2 (Monocyte Chemoattractant Protein-1, MCP-1) was associated with cancer metastasis, recruiting myeloid cells to the nascent metastatic nodules [115, 116]. Colon cancer cells, which metastasize to the liver, recruited a distinct myeloid subset of cells (CD11b/Gr1^{mid}) 14 days after TC inoculation to hepatic metastatic foci by the secretion of CCL2, which subsequently supported TC survival and proliferation [117, 118]. Similar observations were made for breast cancer cells engrafting in lung tissue, which attracted inflammatory monocytes and afterwards macrophages in a CCL2-dependent manner [119, 120]. Macrophage depletion inhibited TC pulmonary seeding and growth. Muschel et al. revealed a tissue factor-dependent clot formation of melanoma and breast cancer cells in the lungs of mice. This clot immediately activated the TC adjacent ECs to an elevated VCAM-1 and vascular adhesion protein-1 (VAP-1) expression [121, 122]. The subsequent myeloid cell recruitment was crucially related to VCAM-1 and VAP-1 expression in TC vicinity, referring to a strict VLA-4 dependency of the recruiting mechanism.

Thus, also shortly after TC engraftment in the lungs, the distinct populations of myeloid cells/macrophages participate in the creation of a supportive niche indispensable for early steps of metastasis.

In the later stage of cancer progression, an abundance of different cells of the myeloid lineage are detectable within tumors and metastatic lesions, supporting angiogenesis and tumor growth and mediate immune suppression in the tumor microenvironment [123–126]. The mechanisms, how myeloid cells traffic into the tumor microenvironment, have not been elucidated for a long time. Varner confirmed that VLA-4 is the key integrin for recruiting monocytes to tumors and thereby acts as an essential factor for tumor angiogenesis. The blocking of VLA-4 reduced the number of macrophages in Lewis lung carcinoma tumors and colon carcinomas [127]. Later on, the γ -subtype of PI3K was shown to activate VLA-4 on myeloid cells in a Pap1a-, RIAM-, talin-, and paxillin-dependent fashion, which is a prerequisite for myeloid cell trafficking to tumor microenvironment [50].

Durden and colleagues could identify a unique role of VLA-4 on tumor-associated macrophages in the transition of macrophages from M1 to a M2 metastatic phenotype. VLA-4 transmits signals from the ECM to activate PI3K, which subsequently activates the small GTPase Rac2. Rac2 in turn initiates the M1 to M2 phenotype shift (Fig. 2, right). VLA-4 with a point mutation in the α_4 cytoplasmic tail had reduced levels of Rac2-GTP levels

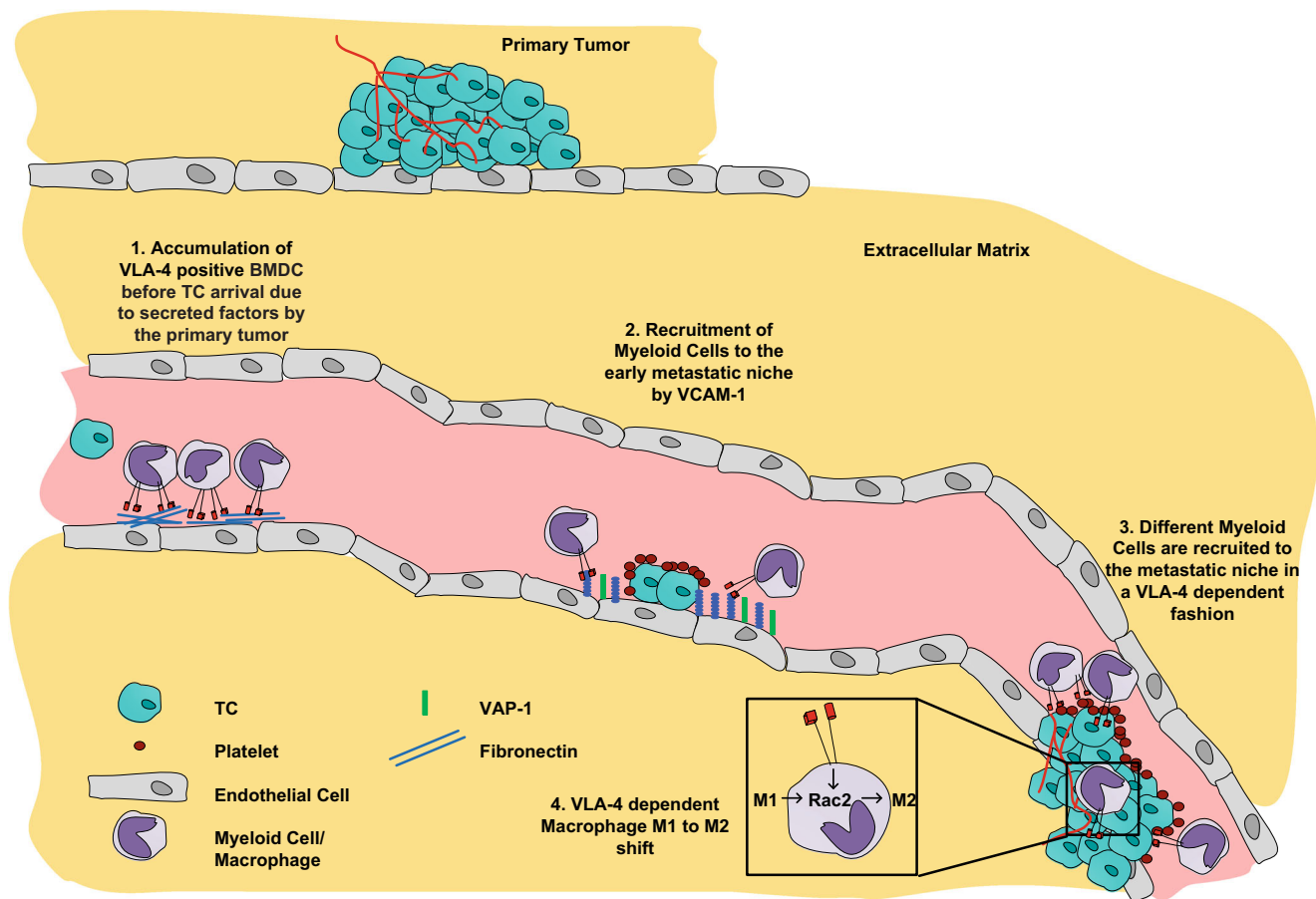


Fig. 2 Involvement of VLA-4 expressing myeloid cells in the establishment of a metastatic niche. A primary tumor secretes soluble factors, which stimulate fibroblast-like stromal cells to express fibronectin. Bone marrow-derived cells adhere to fibronectin via VLA-4, create a cluster and thereby a conducive microenvironment for TCs arriving in the pre-metastatic niche posterior (1). Cells of the myeloid lineage are also recruited to the early metastatic nodule in a VCAM-1- and VAP-1-

dependent manner providing a permissive metastatic environment (2). In a major tumor, an abundance of myeloid cells is found within the tumor tissue comprising several pro-tumorigenic effects like immune suppression. VLA-4 is crucially involved in the attraction of different myeloid cells to the tumor vasculature (3) and is also entangled in the transition of M1 macrophages to a M2 phenotype (4)

and mice with this mutation exhibited decreased tumor growth [128].

Concluding, the direct interaction between aberrantly VCAM-1 expressing TCs and VLA-4 positive host cells can provide proliferative and pro-survival signals to the TCs and additionally mediate a hospitable environment for the growing tumor in the bone tissue. Besides this, cells of the myeloid lineage are recruited in a VLA-4 dependent mode by the primary tumor to the potential sites of metastasis, when TCs have not yet arrived, and generate a permissive niche for incoming TCs. Also, in the very early phase of the development of metastatic foci, TCs recruit myeloid cells in CCL2 and VCAM-1-related manner which facilitates TC proliferation and survival. Once the metastatic foci are grown, an abundance of myeloid cells is detectable in the tumor tissue. Here, VLA-4 is involved in the macrophage recruitment and shift from a M1 to an immunosuppressive M2 type. Thus, VLA-4 positive myeloid cells conduct pro-tumorigenic signals in nearly every stadium of cancer progression.

4 Role of VLA-4 in angiogenesis and lymphangiogenesis

Tumors exceeding a size bigger than 1–2 mm³ are obliged to obtain access to the vascular system to ensure the continuous supply of oxygen and nutrition [129]. In 1939, the first pictures of tumor implant neovascularization were recorded in the Sandison-Clark rabbit ear chamber assay by Ide and colleagues [130]. Later on, the angiogenic processes of wound healing and tumor vascularization were described in more detail and differences thereof could be delineated [131]. Folkman et al. finally inaugurated the hypothesis that “tumor angiogenesis factors” are mitogenic to capillary endothelial cells and proposed angiogenesis as a target to inhibit the growth of solid tumors [129, 132, 133]. The implication of integrins in angiogenesis and tumor angiogenesis has been subject of intensive research and is reviewed in several brilliant articles [134, 135]. VLA-4 contributes to tumor angiogenesis in three different modes. This multitude of capacities is unique within the class of integrins.

First, VLA-4 is expressed on bone marrow-derived progenitor cells/stem cells and promotes progenitor cell homing to the tumor periphery (Fig. 3). Although the existence of endothelial precursor subpopulations in bone marrow-derived cells has for a long time not been recognized, recent studies have identified a common $\text{Lin}^-/\text{Sca1}^+$ endothelial progenitor cell lineage which is capable to differentiate into all hematopoietic cell lineages as well as ECs [136–140]. After progenitor cell homing to VCAM-1 or fibronectin in tumor periphery in a VLA-4 dependent fashion, the progenitor cells can differentiate into endothelial cells, thereby participating in angiogenesis. Other integrins or Lin^+ progenitor cells did not contribute to vessel formation [141, 142]. Up to 16 % of tumor neovasculature can originate from bone marrow-derived cells, as Ruzinova revealed in 2003 [143].

Second, monocytes or myeloid cells in general are recruited in numerousness into tumor tissue and are capable to emit either angiogenic or angiostatic signals [123, 144]. The finding that various myeloid cell types like hemangiocytes [110, 139, 145], monocytes [115, 146], TIE2-expressing monocytes [147, 148], immature dendritic cells [149], myeloid-derived suppressor cells [125, 150], neutrophils [124, 151], eosinophils [152], or immature mast cells [153] are present in tumor tissue is well-known and extensively reviewed elsewhere [123]. Since myeloid cells express several different integrins as well as other adhesion receptors, it has long time been

unclear how these cells might enter the tumor microenvironment from the blood circulation. Varner and coworkers finally revealed VLA-4 as the key integrin responsible for $\text{CD11b}^+/\text{CD14}^+$ monocytes trafficking into tumor tissue and promoting angiogenesis [127]. The authors propose VCAM-1 and CS-1 fibronectin as potential ligands for monocytes in the tumor tissue, notwithstanding other groups could show that angiogenic tumor vessels are in a state of anergy with reduced VCAM-1 and ICAM-1 expression due to VEGF and bFGF stimulation [154–158]. Hence, CS-1 fibronectin seems presumably to be the VLA-4 ligand in the leaky tumor vessel sprouts. The implication of VLA-4 in the recruitment of myeloid cells to solid tumors and their implication in angiogenesis should not be confounded with the recruitment of $\text{CD11b}/\text{Gr1}^{\text{mid}}$ myeloid cells to the nascent metastatic niche mentioned in the previous section.

In a third fashion, VLA-4 expressed on proliferating ECs participates in blood vessel formation. For a proper neovascularization of healthy as well as tumor tissue, the interaction between ECs and mural cells seems to play a fundamental role [159, 160]. Several studies proposed an enmeshment of VLA-4 and VCAM-1 in this reciprocity, but the studies' foci were more related to soluble VCAM-1 and its chemotactic activity toward ECs under inflammatory conditions [161–164]. Varner highlighted a VLA-4 expression on proliferating but not on mature ECs due to VEGF-A stimulation [165]. VCAM-1 on

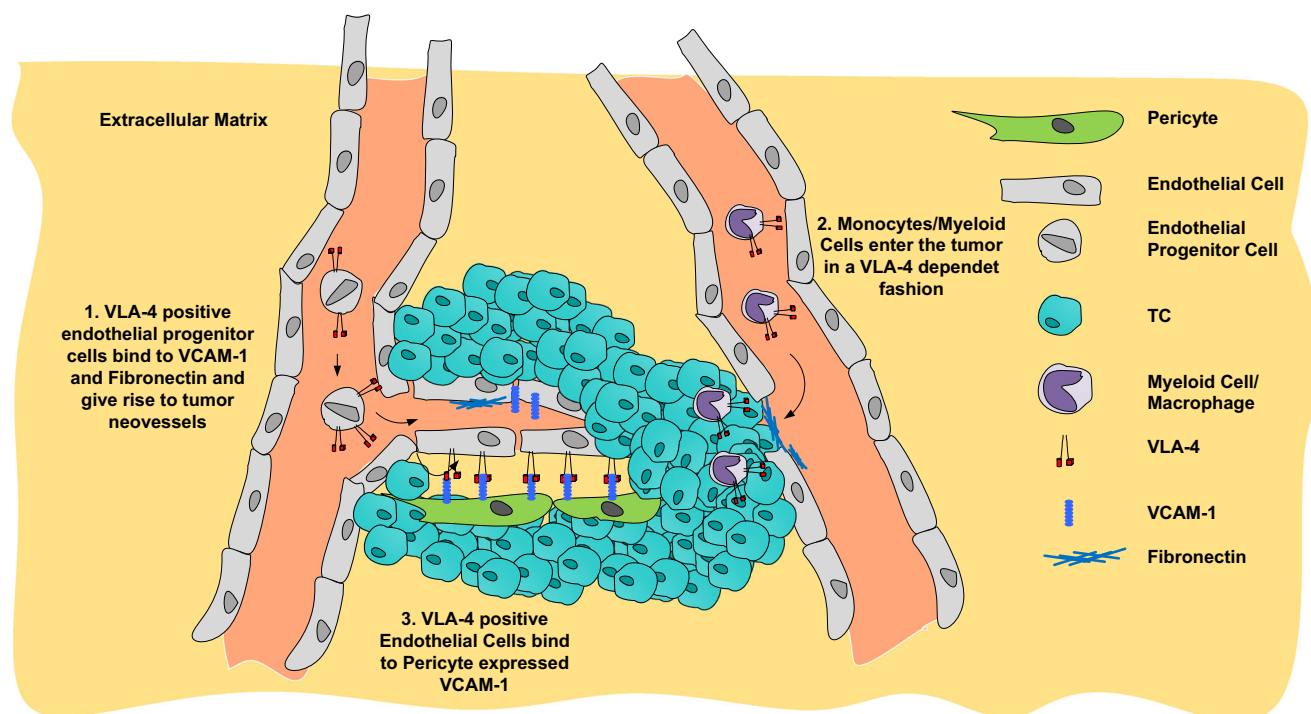


Fig. 3 Contribution of VLA-4 to angiogenesis. VLA-4 expressing endothelial progenitor cells bind to VCAM-1 and fibronectin in the tumor periphery, differentiate into endothelial cells, and are involved in the formation of new blood vessels in the tumor (1). Furthermore, monocytes or myeloid cells enter the tumor with the contribution of VLA-4 and

promote angiogenesis, immune suppression, and finally TC survival (2). VLA-4 is upregulated on proliferating endothelial cells, while VCAM-1 is expressed on pericytes surrounding newly formed vessels, but not on quiescent ones (3). Consequently, the VLA-4/VCAM-1 interplay contributes to a stable embedding of the blood vessels into the tissues

the contrary was expressed by pericytes surrounding the ECs of neovessels in human breast carcinomas but not on pericytes of quiescent vessels. VLA-4 and VCAM-1 mediated the adhesion between ECs and pericytes *in vitro* and *in vivo* and a blockade of VLA-4 induced EC and pericyte apoptosis. Thus, EC-pericyte interactions induced by VLA-4 and VCAM-1 apparently facilitate the survival of both cell types during angiogenesis. The inhibition of this interaction reduced angiogenesis and tumor growth *in vivo* [165].

The investigation of lymphangiogenesis has long time been difficult due to the lack of distinct markers for lymphatic vessels. Since 1999, the specific lymphatic vessels markers Lyve-1 (a member of the CD44 family of proteins), Prox-1 (a transcription factor), podoplanin (a membrane-based glycoprotein), and CD31 were identified and opened the field for detailed investigations [166–169]. Several integrins have been implicated in the development of the lymphatic system or lymphangiogenesis under inflammatory conditions. Integrin $\alpha_9\beta_1$ is required for a functional lymphatic development and lymphatic valve morphogenesis—mice deficient in $\alpha_9\beta_1$ frequently die from chylothorax—but $\alpha_9\beta_1$ is also important for VEGF-C-mediated lymphatic endothelial cell (LEC) motility, a process which is crucial for proper lymphangiogenesis [170–172]. Integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_5\beta_1$ have been investigated in their functional context of lymphangiogenesis of healing wounds and cornea inflammation [173–175]. VLA-4 in contrast was identified as a marker for proliferating LECs, whereas it is poorly expressed in normal lymphatic vessels. A strong VLA-4 upregulation could be detected in growing lymphatic vessels in tumor bearing mice or in mice treated with VEGF-C. Blocking VLA-4 either by mAbs, soluble VCAM-1, or knockdown approaches lead to an attenuated LEC migration and survival, mitigated adhesion to VCAM-1, reduced vessel branch point formation, and finally weakened lymphangiogenesis. Mice with VLA-4 knock-in mutations, in which VLA-4 is incapable to associate with intracellular paxillin and talin, revealed reduced lymphangiogenesis after tumor inoculations compared to wild-type mice [134, 135, 176, 177]. This confirms that VLA-4 signaling and not only VLA-4 expression is necessary for lymphangiogenesis. Later on, Varner and colleagues demonstrated that VEGF-C stimulates the PI3K α /Akt signaling pathway in LEC which in turn activates VLA-4 to conduct lymphangiogenesis [178, 179]. Independent from lymphangiogenesis, the abundantly VLA-4 equipped lymphatic microenvironment serves as a harbor and site of accumulation for VCAM-1 expressing tumor cells [92, 100, 101]. Thus, VLA-4 contributes to metastasis via the lymphatic system in two different ways: fostering lymphangiogenesis and offering a harbor for VCAM-1 positive TCs.

Rebhun et al. in contrast detected an enhanced VCAM-1 expression on lymphatic vessels of melanoma bearing mice and could show that VLA-4 expressing B16 cells have a high

affinity to these LECs. B16 cells with decreased VLA-4 expression displayed a reduced binding to LECs. The *in vivo* situation confirmed that cells with high VLA-4 levels share a higher tendency to establish metastatic foci in the lymph nodes than cell populations with poor VLA-4 expression [72]. These results have to be regarded carefully due to the fact that not only VLA-4 mediates cell adhesion but it is also capable to confer pro-survival signals in cells [180]. Accordingly, low VLA-4 surface levels could finally transmit pro-apoptotic signals in comparison to cells with upregulated VLA-4. Hence, the conclusion that VLA-4 fosters lymphatic metastasis merely by cell adhesion is possibly impermissible or too constricted.

5 Fusion of tumor cells with endothelial cells due to VLA-4 and VCAM-1 interaction

Fusion between cells of different entities is a well-known fact involved in reproduction and tissue formation and has also been used since the 1970s for the production of monoclonal antibodies [181].

Inflammatory conditions seem to be helpful to enhance heterotypic cell fusion [182–184]. In 2004, Mortensen et al. investigated the interaction between endothelial cells and breast cancer cells and observed a fusion of both to new hybrid cells, sharing properties of both parents [185]. Song and colleagues could reveal that the fusion between oral squamous cell carcinoma cells and ECs (HUVEC) depends on a VCAM-1/VLA-4 interaction. The activation of endothelial cells with TNF α augmented VCAM-1 expression and facilitated the subsequent fusion with VLA-4-positive carcinoma cells, whereas a blockade of VCAM-1 or VLA-4 with mAbs reduced cell fusion. These findings assign VCAM-1 a completely new mechanism in tumor cell fusion and metastasis [186].

6 VLA-4 mediated effects on chemoresistance

The development of chemoresistance is one of the worst complications in cancer treatment. Despite a primary response to chemotherapy, cancer patients finally become unresponsive to a broad quantity of cytostatic drugs. One mechanism of chemoresistance is cell adhesion-mediated drug resistance (CAM-DR) first described by Durand and Sutherland, who investigated irradiation effects on single Chinese hamster cells compared to agglomerates. They exhibited an enhanced repair of sublethal radiation damages in cells grown and irradiated in agglomerates [187]. Since that time, a lot of studies have been conducted revealing that integrin-dependent cell-cell or cell-ECM contacts mediate survival signals to various types of cancer cells and thus induce multiple resistance phenomena [188–192].

A small population of TCs surviving the initial chemotherapy, called minimal residual disease (MRD), are the predominant obstacle for a successful cancer treatment and a long-term survival [193, 194]. CAM-DR seems to account partially for MRD, and especially, VLA-4 appears to be involved in CAM-DR in multiple myeloma, lung, breast, and ovarian cancer [195–201]. Whereas in first studies, the transient VLA-4-mediated ECM binding and shielding effects in the matrix have been exposed correlating increased VLA-4 expression levels with a drug-resistant phenotype [196, 202, 203]; later studies were dedicated to reveal the signaling pathways in TCs leading to survival during chemotherapeutic drug application. Most findings are exemplarily summarized in Fig. 4.

Matsunaga et al. elucidated the phosphatidylinositol-3-kinase (PI-3K)/Akt/Bcl-2 pathway to assign resistance to apoptosis to cytarabine [195]. St Croix detected an upregulation of the cyclin-dependent kinase inhibitor p27Kip1 due to the three-dimensional culturing of human and mouse carcinoma cell lines [194, 204]. The involvement of a focal adhesion kinase (FAK) phosphorylation upon VLA-4 activation in the suppression of apoptosis is described controversially and not yet completely clarified. Fukai among others reported on an

anti-apoptotic effect of phosphorylated FAK by Bcl-2 upregulation and p53 suppression [205, 206], whereas Damiano et al. could not detect an augmented tyrosine FAK phosphorylation due to fibronectin binding in a panel of myeloma cell lines [207]. Higashimoto unveiled a downregulation of Fas death receptor ligand by the adhesion of eosinophilic cells to fibronectin [208]. Further investigators focused on the Bcl-2 family of proteins involved in cell survival due to fibronectin ligation. De la Fuente detected an increased Bcl-2/Bax ratio in chronic lymphocytic leukemia cells [209], whereas Damiano could not prove changes in the expression of either pro- (Bcl-w, Mcl-1, Bik, Bak, Bad) or anti-apoptotic (Bcl-2, Bcl-XL) proteins in myeloma cells [196, 207]. Konopleva revealed an upregulation of Bcl-2 and Bcl-XL and an augmented survival in acute myeloid leukemia (AML) cells due to coculturing with stromal cells (Fig. 4) [210]. Further potential mechanisms leading to anti-apoptotic effects due to integrin ligation are reviewed excellently elsewhere [199].

Recently, Liu and coworkers created Jurkat cells which were originally deficient for the α_4 VLA-4 chain and inserted a truncated α_4 cytoplasmic motif connected to a carrier epitope in these cells. Cells carrying this membrane-proximal motif exhibited an adhesion-independent resistance, which

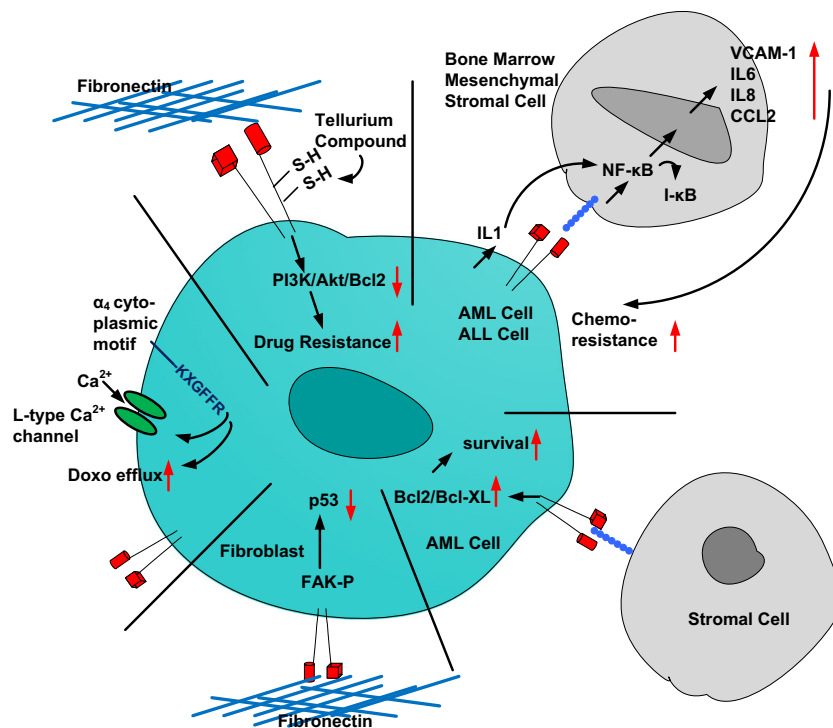


Fig. 4 VLA-4 promotes TC chemoresistance and survival. A plenty of mechanisms in different cells have been described by which VLA-4 maintains chemoprotection and survival. For blood-borne tumors like AML or ALL cells, a VLA-4-dependent interaction with bone marrow stromal cells confers chemoresistance to TCs by NF- κ B activation in the stromal cells and subsequent secretion of several signaling molecules (IL8, IL6, and CCL2). A VLA-4-mediated upregulation of Bcl-2 and Bcl-XL in AML cells was responsible for an augmented survival when

TC were cocultured with stromal cells. In fibroblasts, a VLA-4 ligation to fibronectin in turn phosphorylated FAK and led to a p53 suppression. In Jurkat cells, the α_4 cytoplasmic tail was revealed to foster extracellular Ca^{2+} influx via L-type Ca^{2+} channels and doxorubicin efflux apparently responsible for the observed chemoresistance. A redox modulation of adjacent thiols in the exofacial domain of VLA-4 in AML cells by a tellurium compound restored chemosensitivity by mitigated Akt phosphorylation

did not require the β_1 -integrin as heterodimeric pair. Concomitantly, the authors detected an increased influx of extracellular Ca^{2+} by L-type Ca^{2+} channels, while a blockade of these channels restored chemosensitivity. Furthermore, these cells displayed similar levels of phosphorylated Akt like cells with wt α_4 and also a binding of calreticulin to the inserted peptide motif was unchanged [211]. A resulting enhanced doxorubicin efflux out of the cells could serve as a functional reason for chemoresistance.

Layani-Bazar introduced a nontoxic tellurium compound, which restored chemosensitivity in AML cells by the redox modulation of adjacent thiols in the exofacial domain of VLA-4 [212]. Due to the treatment of AML cells with the tellurium compound, a decreased Akt phosphorylation after fibronectin ligation was exhibited. In contrast to the former mentioned study, the extracellular part of VLA-4 appears to be the key for an augmented chemoresistance.

Jacamo and colleagues could unveil a VLA-4/VCAM-1-dependent cross-talk between acute lymphoblastic leukemia (ALL) or AML cells with bone marrow mesenchymal stromal cells conferring a protection from chemotherapy-induced apoptosis [213]. Instead of focussing on alterations in the ALL or AML cells due to stromal cell interactions, they aimed on changes in bone marrow mesenchymal stromal cells and found a reciprocal NF- κ B activation playing a pivotal role in chemoresistance. The gene expression analysis of stromal cells revealed an upregulation of VCAM-1, IL8, IL6, and CCL2 due to NF- κ B nuclear translocation, whereas a NF- κ B blockade reduced leukemia burden *in vivo*. The authors suggested a combination of soluble factor-mediated signals (IL1) and cell adhesion-mediated signals via the VLA-4/VCAM-1 axis as the key for ALL or AML cells to surmount chemotherapy.

Concluding, the VLA-4 ligation to fibronectin or VCAM-1 seems to confer chemoresistance to multiple tumor entities, whereas the exact mechanisms and molecular segments responsible for the observed sensitivity are far from being clear.

The application of VLA-4 inhibitors together with blunted cytostatic drugs could be an option to efficiently circumvent microenvironment-mediated chemoresistance and multidrug resistance.

7 Implications for a therapeutic VLA-4 targeting

VLA-4 is actively involved in various processes of cancer development and metastasis at different stages of disease. Consequently, VLA-4 appears as an attractive target for therapeutic approaches, or in term of diagnostic information. Concerning diagnosis, the VLA-4 subunit CD49d has been identified as a prognostic marker in chronic lymphocytic leukemia whereupon high CD49d expression correlated with

significantly shorter treatment-free and overall patients' survival than low CD49d levels [214–216].

However, concerning VLA-4-related treatment regimes, the utilization of VLA-4 inhibitors has not yet been established in clinical cancer practice. To pre-estimate the potential usefulness of therapeutic interventions aiming on VLA-4, one should take into account that VLA-4 is a versatile receptor on different hematopoietic cells contributing to the immunological balance. The blocking of VLA-4 induces immunosuppression which is an approved clinical principle for the treatment of chronic pathological inflammations using natalizumab, a mAb targeting VLA-4 in the course of relapsing-remitting multiple sclerosis and inflammatory bowel disease [217–220]. In several studies, natalizumab reduced the rate of clinical relapse in patients with multiple sclerosis and the risk of progression of disability [221, 222]. The impact on the immune system by this treatment became evident by serious adverse effects, e.g., the rare event of a multifocal leukoencephalopathy, a progressive and usually fatal complication by the activation of the polyoma JC virus in the immunosuppressed organisms [223]. However, considering a risk stratification for multifocal leukoencephalopathy onset upon natalizumab therapy, this antibody is a worthwhile contribution to therapy and confirms the clinical value of targeting VLA-4 [224, 225]. The impact of natalizumab on the immune status of a patient might also induce adverse effects for cancer development. In some subjects treated with natalizumab, a transformation of nevi to melanomas has been observed, which is in line with the findings that VLA-4 promotes homotypic interactions between melanoma cells, and a blockade of this interplay fosters melanoma spread and progression [80, 83, 226, 227].

Despite the nonexistence of clinical experience with VLA-4 inhibition for cancer treatment, a number of reports exist in the preclinical field, which confirms that an interference with VLA-4 functionality is beneficial to attenuate different processes of cancer development or growth. In a multiple myeloma mouse model, natalizumab or another anti-VLA-4 mAb, respectively, reduced multiple myeloma growth, VEGF secretion, angiogenesis, myeloma cell burden in blood and spleen, and finally prevented osteolysis of the bone marrow [197, 228, 229]. A clinical study on the efficiency of natalizumab in subjects with relapsed or refractory multiple myeloma has been terminated [230].

A recent report by Scalici et al. demonstrated that a VLA-4 blocking antibody is able to overcome the carboplatin resistance of ovarian cancer when co-administered with the cytostatic drug in a mouse model [201]. A VLA-4 blockade, next to other integrins involved in the CAM-DR phenomenon, appears as an extremely promising and upcoming field to foster the efficiency of a chemotherapeutic treatment.

The inhibition of VLA-4, e.g., by antibodies, has been reported in earlier studies of this field to directly affect the

metastatic spread of melanoma cells in mouse models, obviously by interference with the melanoma/EC binding [68–71].

Beside VLA-4 antibodies, a multitude of small-molecule VLA-4 antagonists has been developed and partly included in clinical trials aiming on the therapy of asthma, multiple sclerosis, inflammatory bowel disease, and stem cell mobilization in combination with CXCR4 antagonists and G-CSF [231, 232]. Small-molecule inhibitors can roughly be classified in phenylalanine-based and urea-based structures [232–234]. One of these small-molecule inhibitors has been applied to demonstrate the use of VLA-4 inhibition, together with IL-1 and SDF-1 blockade in terms of reduced accumulation of tumor-associated macrophages to interfere with the promotion of angiogenic effects [235].

Heparin and low molecular weight heparin (LMWH) have been reported as potential inhibitors of VLA-4 functionality. A recent report demonstrated that the experimental metastasis of B16F10 melanoma cells in mice is strongly attenuated by a VLA-4 blocking function of a LMWH [236]. Therefore, the multiple findings on antimetastatic efficiency of heparin can, in dependence on tumor entity and model, partly be attributed to an impact on the various VLA-4 effects.

Concluding, the application of VLA-4 inhibitors in the course of cancer disease seems very plausible due to the general participation of VLA-4 in metastasis and cancer progression. However, cancer patients are routinely treated with cytostatic drugs, but this is frequently accompanied by a therapy limiting immune suppression. Thus, a further inhibition of the immune surveillance by blocking VLA-4 has to be regarded critically for a potential therapeutic approach. Nevertheless, the promises of targeting VLA-4 to sensitively affect certain processes in cancer development are worthwhile to consider in clinics, and the perspectives will further increase with growing insights into the underlying molecular mechanisms.

Conflict of interest The authors declare that they have no conflict of interest.

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