

Review

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Extravasation of leukocytes in comparison to tumor cells

Carina Strell and Frank Entschladen*

Address: Institute of Immunology, Witten/Herdecke University, Stockumer Str. 10, 58448 Witten, Germany

Email: Carina Strell - Carina.strell@uni-wh.de; Frank Entschladen* - entschladen@uni-wh.de

* Corresponding author

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Abstract

The multi-step process of the emigration of cells from the blood stream through the vascular endothelium into the tissue has been termed extravasation. The extravasation of leukocytes is fairly well characterized down to the molecular level, and has been reviewed in several aspects. Comparatively little is known about the extravasation of tumor cells, which is part of the hematogenic metastasis formation. Although the steps of the process are basically the same in leukocytes and tumor cells, i.e. rolling, adhesion, transmigration (diapedesis), the molecules that are involved are different. A further important difference is that leukocyte interaction with the endothelium changes the endothelial integrity only temporarily, whereas tumor cell interaction leads to an irreversible damage of the endothelial architecture. Moreover, tumor cells utilize leukocytes for their extravasation as linkers to the endothelium. Thus, metastasis formation is indirectly susceptible to localization signals that are literally specific for the immune system. We herein compare the extravasation of leukocytes and tumor cells with regard to the involved receptors and the localization signals that direct the cells to certain organs and sites of the body.

Introduction

General steps of the extravasation process

The extravasation is a multi-step process of the emigration of cells from the blood stream into the tissue. The most prominent types of extravasating cells are leukocytes, i.e. T lymphocytes, natural killer (NK) cells, neutrophil granulocytes and monocytes. These cells have to leave the blood vessels in order to reach tissue sites of inflammation, infection or injury. But not only the emigrating cells, the vascular endothelium takes actively part in the extravasation process, too. It has to deliver certain localization signals, so that the leukocytes know where and when to emigrate. Therefore, the endothelium changes dynamically its architecture including receptor expression and cell-cell contacts. In contrast to the important and physiological extravasation of leukocytes, tumor cells are disseminated by the circulation through the body during

hematogenic metastasis development. Akin to leukocytes, tumor cells do not randomly leave the blood vessels, but are guided by certain signals that are also delivered by the vascular endothelium. Earliest observations on this have been documented more than hundred years ago by Paget, who regarded in his theory on breast cancer metastasis the cancer cells as a seed that can only grow at certain sites of the body, which he considered as the soil [1]. Balkwill and Mantovani worded the assumption that tumor cells might use chemokine gradients to spread around the body [2], therefore, tumor cells and leukocytes might use similar localization signals. The stromal cell-derived factor (SDF)-1 α is such a chemokine, which is expressed in organs that are the first destination of breast cancer metastases [3]. We have elaborated this hypothesis on localization signals: ligands to G protein-coupled receptors (GPCRs), which are chemokines and neurotransmitters as well, might

chemotactically guide tumor cells to their destination of metastasis formation [4].

The extravasation process is in principle divided into three sequential steps regardless the type of cells. In the first step, the cells slackly attach to the vascular endothelial cells. Due to this loose interaction, the extravasating cells are still pulled along with the blood stream, which results in a rolling motion of the cells on the vascular surface. In the second step, the cells tightly attach to the endothelial cells, whereas the receptors for this interaction are different from those involved in the rolling process. After attachment, the previously mostly spherical cells spread on the endothelium and actively transmigrate through the endothelial barrier. This third step of the extravasation process is called diapedesis.

In some descriptions of the extravasation process of leukocytes, two further differentiating steps are included: before the cells get in contact to the endothelium, they have to move to the fringe of the blood stream, which is termed 'margination'. There is an equilibrium between circulating and marginated leukocytes, whereas the ratio of these subpopulations changes under certain conditions [5]. At the margin of the blood vessel, the flow rate is much lower than in the centre, thus, the blood flow has a parabolic profile of variable steepness [6]. Therefore, the cells are slower at the fringe of the blood vessels, which relieves rolling and adhesion. A second additional step of the extravasation process is sometimes included between rolling and adhesion, where the leukocytes are activated by receptor-bound chemokines to attach tightly. This tight attachment is mediated by integrins, which are activated by chemokine signalling via the so-called 'inside-out' signalling. These mechanisms will be discussed in detail below together with the characterization of the rolling process, since these steps happen at the same time.

Types of endothelium

The structure of the vascular endothelium considerably varies between organs and tissues of the body (Tab. 1). This variability consequently results in differences of the function with regard to permeability, leukocyte trafficking and homeostasis [7]. In principle, the blood vessels are constituted of a continuous/non-fenestrated (skin, lung, heart, brain) endothelial monolayer, where all endothelial cells are closely and continuously connected to each other, a continuous/fenestrated (endocrine glands, intestinal and gastric mucosa, glomeruli) endothelial monolayer, where some minor gaps occur, especially at those places, where three cells meet each other, or a discontinuous/sinusoidal (liver) endothelial monolayer, with large gaps that expose the underlying extracellular matrix (ECM) [7]. Leukocytes generally leave the blood stream in the postcapillary venules of the skin, muscle and mesentery, whereas the extravasation process is different in lung, liver and lymph nodes. In lung and liver, the cells leave the blood stream in the microvasculature, in lymphoid organs in the high endothelial venules (HEV), which possess a certain structure [7].

The pulmonary circulation differs fundamentally from the systemic circulation [8]. The concentration of leukocytes in pulmonary capillary blood is 35 to 100 fold higher than in the large vessels of systemic circulation [9,10]. As written above, extravasation occurs predominantly at postcapillary venules in the systemic circulation, whereas in pulmonary circulation extravasation takes place in the microvasculature [11,12]. Pulmonary capillaries are too small to allow rolling. Furthermore, the involvement of selectins – an important group of adhesion molecules discussed in detail below – is not clear: there are selectin-dependent and -independent mechanisms described as well [8,10]. In the liver, the hepatic artery and portal vein drain into the hepatic sinusoids; hepatic sinusoidal endothelial cells are discontinuous (6 to 8 percent of endothelium surface are fenestrae, which directly expose parts of the extracellular matrix) [13]. Next

Table 1: Types of endothelium

| Tissue | Type of endothelium | Adhesion molecules | Site of extravasation |
|---------------------|-----------------------------|--|-------------------------------------|
| Lung | non-fenestrated, continuous | minor role of selectins | microvasculature |
| Liver | discontinuous, fenestrated | minor role of selectins, adhesion to exposed ECM | microvasculature |
| Lymph organs | HEV, plump und cuboidal | constitutive expression of L-selectin ligands | postcapillary venules, constitutive |
| Skin | non-fenestrated, continuous | adhesion molecules upregulated in case of inflammation | postcapillary venules |
| Blood/brain barrier | non-fenestrated, continuous | | no regular extravasation |

to the lung, the liver is the site in the body with the greatest part of leukocyte margination. As in the lung, the extravasation process lacks the rolling phase and endothelial cells do not express selectins, which are the predominant receptors for this process [13]. The HEV of the secondary lymph organs represent specialized postcapillary venules. A special form of leukocyte trafficking was found in these HEV of the secondary lymph organs, such as peripheral and mesenteric lymph nodes, Peyer's patches, appendix, and tonsils. Here, a constitutive transmigration of leukocytes takes place to enable leukocyte circulation between blood and lymphatic organs, in contrast to normal venules, where transmigration only occurs in case of inflammation [14,15]. Endothelial cells of the HEVs differ from their counterparts in other organs: the cells are plump or cuboidal instead of being flat [14,15].

Rolling Leukocytes

Since the endothelia differ in their structural and functional features as well as in the hemodynamic pattern, there are differences in the way to recruit leukocytes [16]. In brain microvasculature, where high shear forces and low adhesion molecule expression exist, rolling seems to play a minor role. It was found that platelets function as a bridge connecting leukocytes and endothelium to mediate adhesion [16].

Generally, rolling of leukocytes is mainly mediated by selectins, also named as lectin cell adhesion molecules, which are expressed on the surface of both endothelial cells and leukocytes (Fig. 1; Tab. 2). In contrast, tumor cells do not express selectins, but their respective ligands. The family of selectins comprises three members: the L-, P- and E-selectin (CD62L, P, E). All selectins are calcium-dependent single-chain transmembrane glycoproteins. Rolling of leukocytes on HEV is mainly mediated by L-selectin and its ligands. L-selectin ligands are different sulphated glycans, that are collectively known as peripheral lymph node addressin [17], and are constitutively expressed on HEV: GlyCAM-1 [18], CD34 [19], nepmucin [20], Sgp200 [21] and others. They can function as ligands for L-selectin when decorated with a specific carbohydrate structure known as 6-sulfo-sialyl Lewis X (sialic acid α 2-3Gal β 1-4 [Fruca α 1-3(sulfo-6)]GlcNAc β 1-R) [22]. This modification is mainly mediated by the HEV restricted N-acetylglucosamine-6-O-sulfotransferase (also known as GST-3 or LSST) [17]. LSST is constitutively expressed in HEV but recent studies have shown that it can be induced in other kinds of endothelium in case of inflammatory lesions, e.g. rheumatoid arthritis [23]. L-selectin was first defined as a homing receptor for lymphocytes, since it is a key regulator for the recruitment of naïve and central memory lymphocytes to the lymph node, a process that occurs constitutively. HEVs in mesenteric lymph nodes

also express the mucosal addressin cell adhesion molecules (MadCAM) as a ligand for L-selectin [23], in order to mediate rolling and adhesion via the α 4 β 7-integrin on leukocytes [7] (Fig. 1).

In endothelium different to HEV, additionally P- and E-selectin regulate the rolling process of lymphocytes: P-selectin and L-selectin lead to rolling and initial localization of leukocytes and platelets, whereas E-selectin enhances the subsequent recruitment of leukocytes. P- and E-selectin both bind to PSGL-1 (P-selectin glycoprotein ligand-1), which is expressed on all blood lymphocytes, monocytes and neutrophils. P-selectin is preformed and stored in Weibel-Palade bodies of the endothelium and α -granules of platelets; exposure of endothelial cells to inflammatory cytokines, histamine, thrombin and other proinflammatory mediators leads to a rapid upregulation of P-selectin [24].

E-selectin is expressed within the first hours after exposure to inflammatory mediators, with the exception of the skin and bone microvessels, where it is constitutively expressed [25,26]. E-selectin ligands, e.g. CD43 and PSGL-1, are proteins that are decorated with sialyl Lewis X (sLeX) or sialyl Lewis A (sLeA). Decorated PSGL-1 is called cutaneous lymphocyte antigen (CLA), that regulates skin homing of activated T cell subtypes [25]. HCELL (hematopoietic cell E-/L-selectin ligand) is a glycoform of CD44 that is expressed natively only on stem cells. It has the highest affinity of all natively expressed E-selectin ligands [27].

Furthermore, we have identified N-cadherin as a molecule involved in the rolling process of neutrophil granulocytes on pulmonary microvasculature [28]. Cadherins are calcium-dependent homophilic cell-cell adhesion molecules in any solid tissue of the body [29]. The cadherin family comprises several members with a distinct tissue expression patterns. Classical cadherins are the epithelial (E-), neural (N-), placental (P-), retinal (R-) and vascular endothelial (VE-) cadherin [29]. As the name says, N-cadherin is expressed in neural but also in other mesenchymal and connective tissues [30]. Besides N-cadherin, only the vascular endothelial (VE)-cadherin has yet been identified to play a role in the extravasation process of leukocytes, since it is expressed in endothelial adherens junctions [31]. However, it is so far not clear, how leukocytes manage crossing of that barrier [32].

Tumor cells

Although tumor cells mimic mechanisms used by leukocytes, the adhesion molecules and ligands involved in tumor cell extravasation are somehow different suggesting additionally or alternatively non-leukocyte-like mechanisms [33]. Specific interactions between receptors of tumor cells and ligands expressed on endothelium may

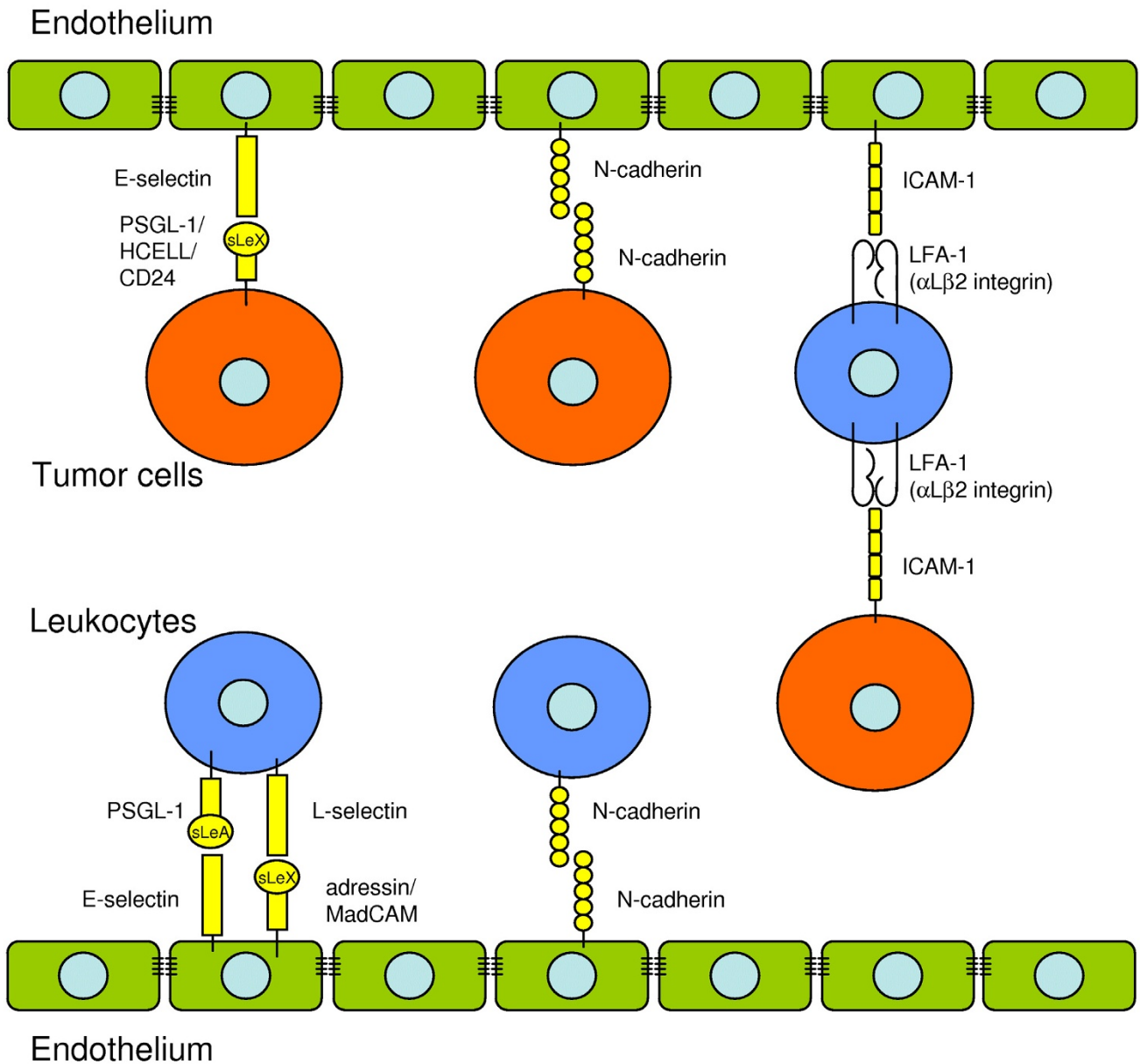


Figure 1
Receptors involved in the rolling of leukocytes and tumor cells. The endothelium is shown in green, leukocytes in blue and tumor cells in red.

explain the distinct tropism of cancer cell subsets [25]. Several groups found tumor cells to express ligands for E-selectin, which leads to rolling and extravasation at endothelial sites expressing E-selectin. As written above, these are sites of inflammation or vessels of the bones, where E-selectin is expressed constitutively. The expression of these ligands correlates with a high potential of bone metastasis: HCELL, which binds to L- and E-selectin, was found on LS174 colon carcinoma cells [34], as well as

further variants of CD44 that allow L-, E- and P-selectin interactions [35,36]. Hanley et al. [35] defined E-selectin mediating slow, P-selectin intermediate and L-selectin fast rolling of tumor cells. Furthermore, bone-metastatic prostate carcinoma cell lines were found to express PSGL-1 and the E-selectin ligand-1 [37]. CD24, when decorated with sLeX, also promotes hematogenic metastasis formation, but it is less efficient than PSGL-1 [38]. Recently, the carcinoembryonic antigen (CEA), which is expressed on

Table 2: Comparison of receptor-ligand pairs involved in the extravasation of tumor cells and leukocytes

| | Rolling | Adhesion | Diapedesis |
|--------------------|--|---|--|
| Tumor cells | PSGL-1 – E-selectin HCELL – E-selectin CD24 – E-selectin CEA – E/L-selectin N-cadherin – N-cadherin ICAM-1/LFA-1 mediated rolling via leukocytes | $\alpha 4\beta 1/\beta 7$ – VCAM CD44v6 – galectin-3 Lamp1/2 – galectin-3 $\alpha v\beta 3$ – LI-CAM | N-cadherin – N-cadherin Apoptotic mechanisms |
| Leukocytes | PSGL-1 – E-selectin HCELL – E-selectin CD24 – E-selectin L-selectin – peripheral lymph node addressin L-selectin – MadCAM N-cadherin – N-cadherin | LFA-1 – ICAM-1/2 Mac-1 – ICAM-1/2 LFA-1 – JAM-A Mac-1 – JAM-C VLA-1 – VCAM LI-CAM – VLA-5 glycosylated proteins – galectins | paracellular: JAM-C – JAM-B LFA-1 – JAM-A VLA-4 – JAM-B Mac-1 – JAM-C CD99 – CD99 PECAM-1 – PECAM-1 transcellular: LFA-1 – ICAM-1 PECAM-1 – PECAM-1 |

colon carcinoma cells, has been reported to act as a mediator for rolling on E- and L-selectin, too [39].

We have shown that N-cadherin mediates the rolling of MDA-MB-468 human breast carcinoma cells on pulmonary endothelium [28]. In addition, an other group has described N-cadherin to be involved in the transmigration process [40]. In conclusion, it seems that in the case of tumor cells, there is not such a clear difference between the receptors used for rolling, adhesion and diapedesis as it is for leukocytes.

Furthermore, leukocytes facilitate the contact between tumor cells and the endothelium. Leukocytes act as bridge- or linker-cells. We have described that neutrophil granulocytes promote the adhesion of MDA-MB-468 breast carcinoma cells to pulmonary endothelium. The tumor cells lack $\beta 2$ -integrins, which are the ligand for the intercellular adhesion molecule (ICAM)-1 on the endothelium. Instead, the tumor cells express ICAM-1 and adhere to neutrophil granulocytes, which then act as a linker connecting the tumor cells to the endothelium and thereby enable firm adhesion [28] (Fig. 1). The same mechanism has also been described for other blood cells and other molecules involved, e.g. the interaction of platelets and leukocytes with tumor cells bearing ligands for P-selectin [41-43].

Localization signals – role of GPCR ligands

Chemokines are ligands to GPCRs, which are known as signal substances that regulate the migratory activity of cells and chemotactically guide these cells. Chemokines are 8 to 12 kDa peptides with highly conserved structural elements. According to these elements they have been divided into four subgroups [44]. More than 50 chemok-

ines and 20 chemokine receptors have been characterized [45]. Chemokines are soluble proteins that are presented on endothelial cells by glycosaminoglycans. These receptors are not the specific chemokine-receptors, but prevent the chemokines from rapid removal by the blood flow [22,46]. The chemokine receptors on the leukocytes are specifically expressed in certain subpopulations [47]. Therefore, leukocyte subsets are selectively susceptible to various chemokines. The signal transduction of the chemokines receptors leads to changes of the affinity and avidity of integrins on leukocytes to their specific ligands expressed on the endothelium by the so-called 'inside-out' signalling [48]. This activation of integrins is due to the phosphorylation of the cytoplasmic domain of both chains of the heterodimeric integrins [49,50], which then leads to an integrin-mediated arrest of the cells on the endothelium [51], as will be discussed in detail below.

Chemokines are functionally divided into two groups, the inflammatory and the constitutive chemokines, for they are either guiding leukocytes to sites of inflammation or to other sites such as secondary lymph organs. For example, CCL17/TARC (thymus- and activation-regulated chemokine) functions as a skin-homing factor. It is presented on the skin microvasculature and induces the adhesion of CCR4 bearing CD4+ lymphocytes [52]. HEV express constitutively CCL21, which activates the leukocyte function-associated antigen (LFA)-1 by a G protein-mediated pathway. LFA-1 is the $\alpha \beta 2$ -integrin, on rolling CCR7 expressing lymphocytes. Consequently, this leads solely to the adhesion of these cells and no other leukocyte subsets transmigrate through the HEV [7,22,53]. SDF-1 α (CXCL12) is described as a very potent constitutive chemokine for the localization of lymphocytes, homing of stem cells to bone marrow and tumor cells [54]. SDF-

1α was the first chemokine identified to play a role in the localization of breast cancer metastases [3]. Meanwhile, it is generally accepted that similar to leukocytes, tumor cells use chemokines as guidance signals, too [55-57]. SDF-1 α plays also a role in B16 melanoma cell adhesion via integrins [58], and CX3CL1 (fractalkine) has been reported to guide prostate carcinoma cells to the bone marrow endothelium, which constitutively expresses this chemokine [59,60].

Besides chemokines, we have suggested that neurotransmitters play a role in the localization of metastases, too [4]. Like chemokines, neurotransmitters mostly bind to GPCRs. However, there is no common neurotransmitter receptor, which could present neurotransmitters on the endothelial surface. But neurotransmitters provide strong chemotactic signals for tumor cells [61,62], and might indirectly deliver localization signals by stimulating the release of such signals from the endothelial cells [63]. We have shown that the development of lymph node metastases of PC-3 prostate carcinoma cells in mice increases by norepinephrine treatment [64].

Adhesion

Leukocytes

Integrins are the main adhesion molecules of leukocytes. During the rolling step, integrins are activated, and then bind to their counterparts on the endothelial cells to mediate a tight attachment (or arrest) of the leukocytes. Integrins are single transmembrane cell adhesion molecules consisting of two non-covalently associated α and β chains. In vertebrates 18 α and 8 β subunits form 24 known $\alpha\beta$ dimers, whereas $\beta 2$ and $\beta 7$ integrins are exclusively expressed on leukocytes [65]. Chemokines are the most powerful physiological activators of integrin-mediated adhesion. Within less than a second they regulate integrin affinity and avidity in a cell-type specific manner by inside-out signalling [66,67]. This signalling induces integrins to undergo conformational changes from a bent low-affinity state to an extended intermediate- or high-affinity state resulting in the opening of the ligand-binding pocket [68]. However, chemokine-stimulated inside-out signalling induces conformational changes in only a fraction of integrins [51]. The intracellular signalling cascades leading from GPCR activation to integrin activation are still incompletely understood, since different cell types, species and integrins use different signalling steps. A linear signal-transduction pathway would be a simplification of the complex network between chemoattractant-binding and integrin-adhesion [69]. Key regulatory events are the phosphorylation of the cytoplasmic domains [49,50], as well as binding of talin [70].

The integrin receptor family is subdivided into 8 groups according to the β chains. The following groups are most widely studied: $\beta 1$ (CD29) = very late activation antigen (VLA) integrins, which mediate mainly cell-matrix interactions; $\beta 2$ (CD18) = leukocyte integrins, which mediate mainly cell-cell interactions; $\beta 3$ (CD61) with various adhesive functions, and $\beta 7$ integrins mediating cell-cell interactions (Tab. 2). Those integrins that are most important in leukocyte arrest belong to the $\beta 1$ and $\beta 2$ subfamilies [33,51]: the $\alpha L\beta 2$ (lymphocyte function-associated antigen (LFA)-1) and $\alpha M\beta 2$ (Mac-1) are both ligands for ICAM-1/-2 (Fig. 2), whereas $\alpha L\beta 2$ can also bind to the junctional adhesion molecule-A (JAM-A), and $\alpha M\beta 2$ to JAM-C on the endothelium, which will be explained in detail in the diapedesis section. The $\alpha 4\beta 1$ integrin VLA-4 is the ligand for the vascular cell adhesion molecule (VCAM)-1. Neutrophil granulocytes express VLA-4 only in case of sepsis [71]. ICAM-1/-2 (CD54/CD102) are expressed on surface of resting endothelium at low levels, but ICAM-1 becomes upregulated at sites of inflammation, e.g. in response to the tumor necrosis factor (TNF) α or interleukin (IL)-1 β [65]. VCAM-1 (CD106) is also upregulated in response to inflammatory mediators. VCAM-1 is described as a key molecule in monocyte recruitment to early lesions in experimental models of atherogenesis [72,73]. Thus, on the side of the endothelium, several members of the immunoglobulin (Ig) superfamily are involved in the adhesion of leukocytes [74]. Furthermore, integrins that are expressed on endothelial cells are involved in leukocyte adhesion, too. The integrins $\alpha 5\beta 1$ (VLA-5) and $\alpha v\beta 3$ on the endothelium bind to L1-CAM, which is a molecule of the Ig superfamily expressed on lymphocytes [33,75,76].

There is a growing body of evidence, that galectins are involved in this part of the extravasation. Galectins are defined by their ability to recognize β -galactose because of their conserved carbohydrate-recognition domains (CRDs) of about 130 amino acids. Fifteen mammalian galectins have been identified, which can be subdivided into three groups: those with one CRD, those with two CRDs, and chimera galectins with a tail of short tandem repeats fused to one single CRD [77,78]. Galectins can be secreted or, depending on the cell-type and activation state, be localized in the nucleus, the cytoplasm or the cell surface, where they interact with appropriately glycosylated proteins [79]. The most extensively studied function of galectins is their involvement in regulation of apoptosis (galectin-3 has anti-apoptotic function, galectin-1 is a pro-apoptotic receptor) [80,81], but during the past ten years it became evident that galectins are modulators of the immune response (pro- and anti-inflammatory functions) [82], as well as modulators of cell migration, adhesion and angiogenesis [78].

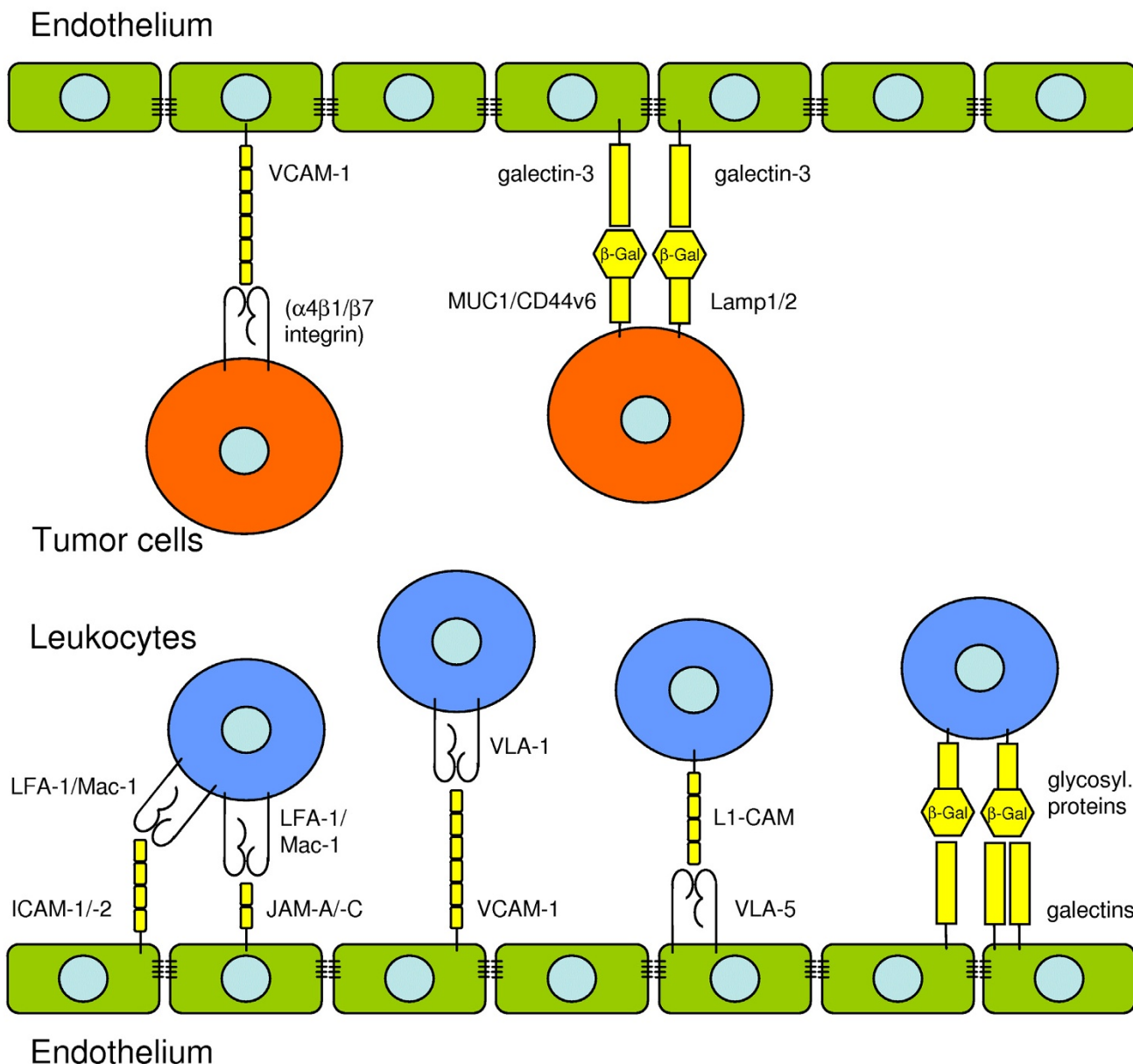


Figure 2
Receptors involved in the adhesion of leukocytes and tumor cells to endothelial cells. The endothelium is shown in green, leukocytes in blue and tumor cells in red.

Galectin-1 is a non-covalent homodimer of two single CDR molecules, expressed in cultured endothelia of different origin as human aorta, umbilical vein, pulmonary artery, and mouse liver, brain and lung [78]. The activation of endothelial cells, e.g. with lipopolysaccharides, or tumor-conditioned media results in a rapid increase of galectin-1 expression, and it is constitutively expressed in tumor-associated endothelial cells [83,84]. Galectin-1 has

anti-inflammatory properties. Peretti et al. observed that endogenous galectin-1 inhibits the adhesion of leukocytes to the endothelium, in the case of neutrophil granulocytes this was due to a suppression of Mac-1 upregulation [85-87]. Thus, galectin-1 seems more to function as a signaling molecule in the regulation of integrins than to act as a classical adhesion molecule. Accordingly, the upregulation of galectin-1 in tumor-associated endothelial cells is

postulated as a tumor escape mechanism from immune response [88].

The expression of galectin-3 on endothelial cells is reported in vitro in human umbilical vein and dermal microvasculature as well as mouse lung, brain and liver [78]. Galectin-3 is upregulated in endothelial cells of cancer tissue as found in vivo and in a murine tumor model of hepatocellular carcinoma [89]. Nieminen et al. recently found a role for galectin-3 in the $\beta 2$ integrin-independent adhesion of neutrophil granulocytes to lung endothelium in case of *Streptococcus* infection [90].

Galectin-9 was originally identified as a chemoattractant for eosinophil granulocytes [91]. Recently, it was identified as a receptor for the T_{helper1} lymphocyte-specific cell-surface molecule Tim-3, thus negatively regulating T_{helper1} lymphocyte responses [92]. Galectin-9 is expressed in various endothelial cells but so far mainly studied in the context of viral infections.

Tumor cells

As mentioned above, the expression of several integrins, especially of the $\beta 2$ subgroup, is restricted to leukocytes. Thus, tumor cells must use some different receptors or mechanisms for their adhesion to the endothelium, such as utilizing leukocytes as linker cells (Fig. 1). However, expression of the $\alpha 4$ integrin was demonstrated on many different human tumors and tumor cell lines, especially on melanoma and sarcoma. Together with the $\beta 1$ or $\beta 7$ integrin, the $\alpha 4$ integrin can act as a ligand for VCAM-1 and fibronectin. Animal models demonstrated, that the metastatic capacity of melanoma cells enhanced through $\alpha 4\beta 1$ integrin interactions with VCAM-1 [93,94]. Furthermore, expression of the $\alpha v\beta 3$ integrin has been associated with the metastatic potential of tumor cells, but it seems more to be involved in transmigration by interacting with L1-CAM, than in the adhesion process [95]. Thus, as already mentioned for the role of N-cadherin in tumor cell rolling and diapedesis, the receptor involvement in each step of the extravasation process in tumor cells is yet not as clearly defined as in leukocytes.

In contrast to integrins, galectins are a group of adhesion receptors that leukocytes share with tumor cells. In several experimental systems galectin-3 expression in cancer cells was associated with a metastatic phenotype [96]. During the last twenty years it became evident, that an altered glycosylation and exposure of glycoproteins by tumor cells may facilitate their adhesion to endothelial galectins [78]. The Thomsen-Friedenreich antigen (TA) is expressed on human breast and prostate carcinoma cell lines and serves as a ligand for galectin-3 expressed on endothelium, leading to increased tumor cell adhesion [97-99]. TA is a simple mucin-type disaccharide with β -galactose as a terminal

sugar (Gal $\beta 1$ -3GalNAc). It is expressed on the surface of T cell-lymphomas and most human carcinomas [100,101], so far two surface molecules are known to represent TA: MUC1 [102] and CD44v6 [103]. Furthermore, lysosomal-membrane-associated glycoproteins (Lamps) 1 and 2 are described as potential binding partners for galectin-3. Lamps are rarely found on the plasma membrane of normal cells, but there is an increased surface expression in tumor cells [104].

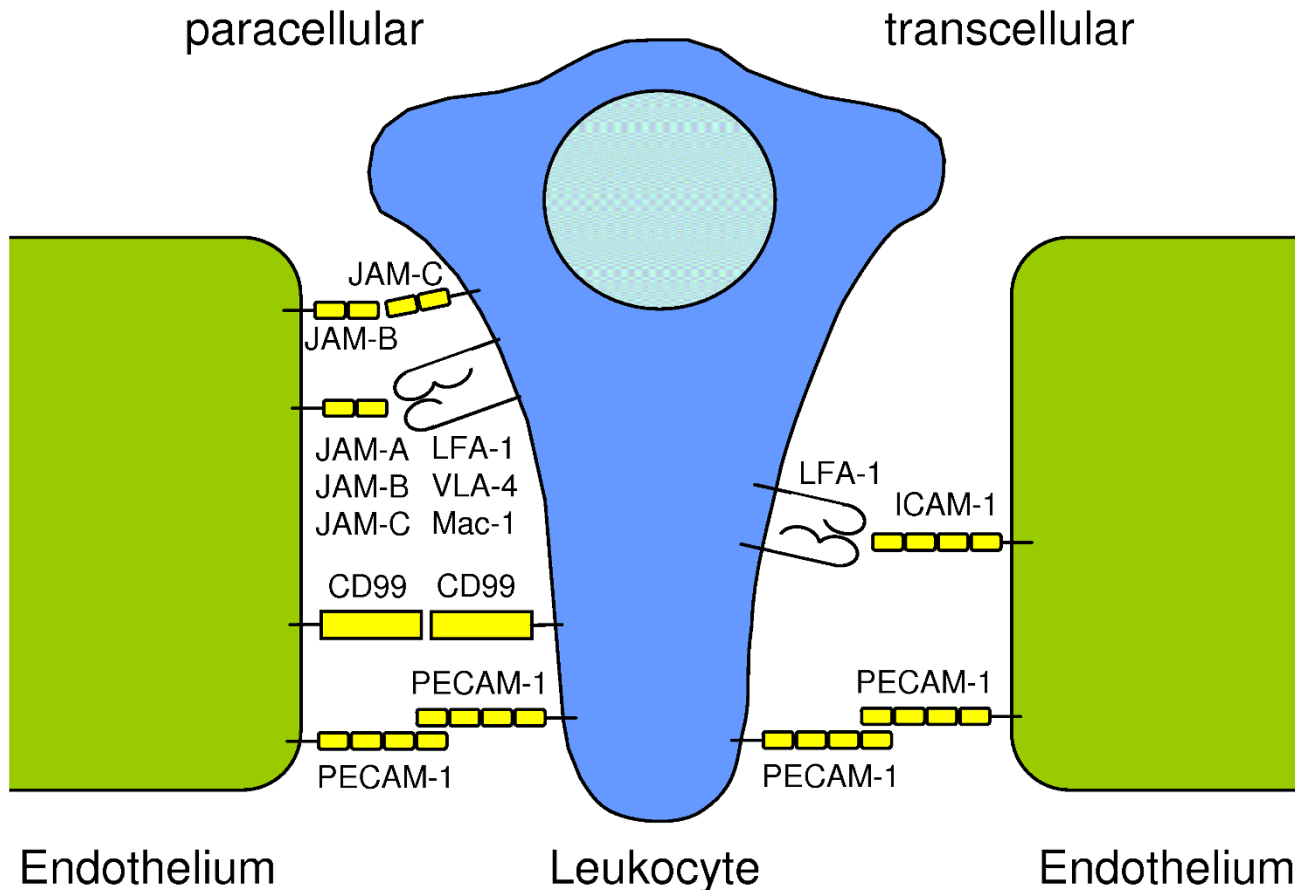
Clausse et al. observed that culture media, that were conditioned by incubation with the prostate carcinoma cell line PC-3, induced galectin-1 expression on the surface of endothelial cells. This resulted in an increased attachment of the tumor cells. The effect was inhibited by the addition of a galectin-1-specific antibody [83]. This stands in clear contrast to the above discussed role of galectin-1 in leukocyte adhesion: here, adhesion was decreased in case of galectin-1 expression.

Diapedesis

Leukocytes transmigrate through the endothelium without irreversibly impairing its integrity. There are two routes that the cells can use and it is not clear, to which part each of these ways is involved in the diapedesis overall. The leukocytes can either move between the endothelial cells, which is termed the paracellular or junctional route, or they can migrate through an endothelial cell, which is termed the transcellular route. Which way the leukocytes take, obviously depends on several circumstances. Leukocytes subpopulations prefer different routes, and the decision seems to depend on further factors, e.g. the duration of endothelium activation with cytokines [105].

Paracellular route

When leukocytes transmigrate between two endothelial cells, they have to pass tight junctions and adherens junctions. Therefore, the connection between the endothelial cells has to be temporarily abrogated and substituted by the corresponding binding molecule on the leukocyte surface. The platelet/endothelial cell adhesion molecule-1 (PECAM-1; CD31) is one of these molecules (Fig. 3). It is a member of the Ig superfamily, homophilic binding, and expressed on vascular endothelial cells as well as leukocytes. It is one of the most important adhesion molecules for the diapedesis of leukocytes. Blockade of this receptor inhibits the transmigration of neutrophil granulocytes [106], monocytes [106,107], and NK cells [108]. In contrast, CD31 is dispensable for the transmigration of lymphocytes [109]. However, there are reports showing that PECAM-1 has to be regarded not only as an adhesion receptor but also as a signalling receptor [110].

**Figure 3**

Transmigration of leukocytes through endothelium. Receptors involved in the paracellular or junctional route are shown on the left side, receptors involved in the transcellular route are shown on the right side. The endothelial cells are shown in green, the leukocytes in blue.

CD99 and the related molecule CD99L2 are further adhesion molecules in the transmigration of leukocytes. CD99 is involved in monocyte, neutrophil granulocyte and lymphocyte diapedesis [111-113], but CD99L2 is not relevant for lymphocyte diapedesis to inflamed tissue [112]. Like PECAM-1, it is expressed on both leukocytes and endothelial cells, and it has homophilic binding properties [105,110].

A third group of receptors that have been identified to play a role in the paracellular diapedesis of leukocytes are the junctional adhesion molecules [114]. JAMs belong to the Ig superfamily having two extracellular Ig domains. To date, three members are known, designated as JAM-A to -C (or JAM-1 to -3), whereas JAM-A is best characterized for its role in leukocyte diapedesis. Initially, JAM-A has been characterized as a homophilic adhesion receptor of tight

junctions on epithelial and endothelial cells [105,115]. Furthermore, JAM-A binds to LFA-1 (α L β 2 integrin) [116]. JAM-B binds to VLA-4 (α 4 β 1 integrin), and JAM-C binds to Mac-1, (α M β 2 integrin). All of the integrins are expressed on leukocytes and consequently, endothelial JAMs are supposed to play a crucial role in leukocyte diapedesis [105,110,117]. It is worth of note, that JAM-C is also expressed on leukocytes and interacts with JAM-B on endothelial cells [118]. The endothelial selective adhesion molecule (ESAM) is a receptor related to JAMs. However, its expression is restricted to endothelial cells and activated platelets, no binding partner or counter-receptor on leukocytes has been identified so far [110].

Transcellular diapedesis

As compared to the paracellular route, much less is known about the transcellular diapedesis. This way of extravasa-

tion seems to be less used but much faster than the paracellular transmigration, whereas the part and kind of leukocytes using this route varies obviously in dependence on the type of endothelium [51,105]. ICAM-1 on the endothelial cells is best characterized to play an important role in the transcellular migration. ICAM-1 binds to the integrin LFA-1 on leukocytes. ICAM-1 is redistributed to caveolae-rich regions, where vesiculo-vacuolar organelles form intracellular channels [51]. Besides ICAM-1, PECAM-1 has been identified to be involved in this extravasation route, and in general, the assumption has been made that the same molecules that mediate paracellular migration are mediators for the transcellular migration, too [117].

When the extravasating cells have successfully passed the cellular barrier, they face a further hurdle. This is the basement membrane, to which the leukocytes in fenestrated or discontinuous endothelia, e.g. of the liver, can also attach directly via integrin receptors to matrix components. In order to cross this barrier, leukocytes express matrix-metalloproteinases (MMPs), e.g. T lymphocytes express gelatinases A and B, which are two MMPs that digest the major basal lamina constituents collagen types IV and V [119]. However, it is not clear, if MMPs only play a role in the extravasation process or whether they contribute to the migration of leukocytes through tissue [120].

Cancer cell diapedesis

In contrast to leukocytes, tumor cells are supposed not to leave the endothelium intact after diapedesis. This is understandable just from the bare size of the cells. Tumor cells are much larger than leukocytes and it would be difficult to squeeze between endothelial cells without any damage. One of the few reports on the mechanisms of tumor cell diapedesis aims on the influence of N-cadherin [40], which we have identified to mediate rolling of tumor cells, too [28].

Consequently, a new model completely skips the transmigration process: metastatic tumor cells attach to the endothelium of pulmonary capillaries. Transmigrated cells were cleared quickly by the immune cells of the lung, whereas early tumor colonies were entirely attached to endothelium on the intravascular side, as investigated by intact organ epifluorescence microscopy in mice [121]. However, we have observed in an in vitro model, that T24 bladder carcinoma cells transmigrate through a monolayer of human umbilical vein endothelial cells (HUVECs). During transmigration, the endothelium is irreversibly damaged [122], which is probably due to induction of apoptosis by the loss of cell-cell contacts [123]. Such an endothelial retraction is currently the favored model for tumor cell diapedesis [33].

In conclusion, the first two steps of the extravasation of tumor cells and leukocytes, rolling and adhesion, seem to have similarities with regards to the mechanisms and receptors involved. In contrast, the third step, the diapedesis, is fundamentally different between these two cell types, whereas there is somehow a lack of knowledge with regard to tumor cells. But it seems that the function of leukocytes as guardians of the body is reflected in a cautious manipulation of the endothelial cells. Tumor cells on the contrary display a destructive behaviour.

List of abbreviations

CEA: carcinoembryonic antigen; CLA: cutaneous lymphocyte antigen; CRD: carbohydrate-recognition domains; ECM: extracellular matrix; GPCR: G protein-coupled receptor; HCELL: hematopoietic cell E-/L-selectin ligand; HEV: high endothelial venules; HUVEC: human umbilical vein endothelial cells; ICAM: intercellular adhesion molecule; Ig: immunoglobuline; IL: interleukin; JAM: junctional adhesion molecule; LFA: leukocyte function-associated antigen; MadCAM: mucosal addressin cell adhesion molecule; MMP: matrix-metalloproteinase; NK cells: natural killer cells; PECAM: platelet/endothelial cell adhesion molecule; PSGL: P-selectin glycoprotein ligand; SDF: stromal cell-derived factor; TA: Thomsen-Friedenreich antigen; TARC: thymus- and activation-regulated chemokines; TNF: tumor necrosis factor; VCAM: vascular cell adhesion molecule; VLA: very late activation antigen.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Both authors together wrote this manuscript.

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References

1. Paget S: **The distribution of secondary growths in cancer of the breast.** *Lancet* 1889, **133(3421)**:571-573.
2. Balkwill F, Mantovani A: **Inflammation and cancer: back to Virchow?** *Lancet* 2001, **357(9255)**:539-545.
3. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: **Involvement of chemokine receptors in breast cancer metastasis.** *Nature* 2001, **410(6824)**:50-56.
4. Entschladen F, Drell TL, Lang K, Joseph J, Zaenker KS: **Tumour-cell migration, invasion, and metastasis: navigation by neurotransmitters.** *Lancet Oncol* 2004, **5(4)**:254-258.
5. Iadocicco K, Monteiro LH, Chaui-Berlinck JG: **A theoretical model for estimating the margination constant of leukocytes.** *BMC Physiol* 2002, **2(3)**.
6. Osada T, Radegran G: **Alterations in the rheological flow profile in conduit femoral artery during rhythmic thigh muscle contractions in humans.** *Jpn J Physiol* 2005, **55(1)**:19-28.
7. Aird WC: **Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms.** *Circ Res* 2007, **100(2)**:158-173.

8. Wagner JG, Roth RA: **Neutrophil migration mechanisms, with an emphasis on the pulmonary vasculature.** *Pharmacol Rev* 2000, **52(3)**:349-374.
9. Doerschuk CM, Allard MF, Martin BA, MacKenzie A, Autor AP, Hogg JC: **Marginated pool of neutrophils in rabbit lungs.** *J Appl Physiol* 1987, **63(5)**:1806-1815.
10. Doerschuk CM, Beyers N, Coxson HO, Wiggs B, Hogg JC: **Comparison of neutrophil and capillary diameters and their relation to neutrophil sequestration in the lung.** *J Appl Physiol* 1993, **74(6)**:3040-3045.
11. Doerschuk CM, Allard MF, Hogg JC: **Neutrophil kinetics in rabbits during infusion of zymosan-activated plasma.** *J Appl Physiol* 1989, **67(1)**:88-95.
12. Downey GP, Worthen GS, Henson PM, Hyde DM: **Neutrophil sequestration and migration in localized pulmonary inflammation. Capillary localization and migration across the interalveolar septum.** *Am Rev Respir Dis* 1993, **147(1)**:168-176.
13. Aird WC: **Phenotypic heterogeneity of the endothelium: II. Representative vascular beds.** *Circ Res* 2007, **100(2)**:174-190.
14. Girard JP, Springer TA: **High endothelial venules (HEVs): specialized endothelium for lymphocyte migration.** *Immunol Today* 1995, **16(9)**:449-457.
15. Miyasaka M, Tanaka T: **Lymphocyte trafficking across high endothelial venules: dogmas and enigmas.** *Nat Rev Immunol* 2004, **4(5)**:360-370.
16. Liu L, Kubes P: **Molecular mechanisms of leukocyte recruitment: organ-specific mechanisms of action.** *Thromb Haemost* 2003, **89(2)**:213-220.
17. Yang J, Rosen SD, Bendele P, Hemmerich S: **Induction of PNA and N-acetylglucosamine 6-O-sulfotransferases I and 2 in mouse collagen-induced arthritis.** *BMC Immunol* 2006, **7(12)**:.
18. Lasky LA, Singer MS, Dowbenko D, Imai Y, Henzel WJ, Grimley C, Fennie C, Gillett N, Watson SR, Rosen SD: **An endothelial ligand for L-selectin is a novel mucin-like molecule.** *Cell* 1992, **69(6)**:927-938.
19. Baumhuter S, Singer MS, Henzel W, Hemmerich S, Renz M, Rosen SD, Lasky LA: **Binding of L-selectin to the vascular sialomucin CD34.** *Science* 1993, **262(5132)**:436-438.
20. Umamoto E, Tanaka T, Kanda H, Jin S, Tohya K, Otani K, Matsutani T, Matsumoto M, Ebisuno Y, Jang MH, Fukuda M, Hirata T, Miyasaka M: **Nepmucin, a novel HEV sialomucin, mediates L-selectin-dependent lymphocyte rolling and promotes lymphocyte adhesion under flow.** *J Exp Med* 2006, **203(6)**:1603-1614.
21. Hemmerich S, Butcher EC, Rosen SD: **Sulfation-dependent recognition of high endothelial venules (HEV)-ligands by L-selectin and MECA 79, and adhesion-blocking monoclonal antibody.** *J Exp Med* 1994, **180(6)**:2219-2226.
22. Kawashima H: **Roles of sulfated glycans in lymphocyte homing.** *Biol Pharm Bull* 2006, **29(12)**:2343-2349.
23. Rosen SD: **Endothelial ligands for L-selectin: from lymphocyte recirculation to allograft rejection.** *Am J Pathol* 1999, **155(4)**:1013-1020.
24. Elangbam CS, Qualls CW Jr, Dahlgren RR: **Cell adhesion molecules – update.** *Vet Pathol* 1997, **34(1)**:61-73.
25. Barthel SR, Gavino JD, Descheny L, Dimitroff CJ: **Targeting selectins and selectin ligands in inflammation and cancer.** *Expert Opin Ther Targets* 2007, **11(11)**:1473-1491.
26. Schweitzer KM, Drager AM, Valk P van der, Thijsen SF, Zevenbergen A, Theijssmeijer AP, Schoot CE van der, Langenhuijsen MM: **Constitutive expression of E-selectin and vascular cell adhesion molecule-1 on endothelial cells of hematopoietic tissues.** *Am J Pathol* 1996, **148(1)**:165-175.
27. Sackstein R: **The bone marrow is akin to skin: HCELL and the biology of hematopoietic stem cell homing.** *J Invest Dermatol Symp Proc* 2004, **9(3)**:215-223.
28. Strell C, Lang K, Niggemann B, Zaenker KS, Entschladen F: **Surface molecules regulating rolling and adhesion to endothelium of neutrophil granulocytes and MDA-MB-468 breast carcinoma cells and their interaction.** *Cell Mol Life Sci* 2007, **64(24)**:3306-3316.
29. Ivanov DB, Philippova MP, Tkachuk VA: **Structure and functions of classical cadherins.** *Biochemistry (Mosc)* 2001, **66(10)**:1174-1186.
30. Christofori G: **Changing neighbours, changing behaviour: cell adhesion molecule-mediated signalling during tumour progression.** *EMBO J* 2003, **22(10)**:2318-2323.
31. Garrido-Urbani S, Bradfield PF, Lee BP, Imhof BA: **Vascular and epithelial junctions: a barrier for leucocyte migration.** *Biochem Soc Trans* 2008, **36(Pt 2)**:203-211.
32. Su WH, Chen HI, Jen CJ: **Differential movements of VE-cadherin and PECAM-1 during transmigration of polymorphonuclear leukocytes through human umbilical vein endothelium.** *Blood* 2002, **100(10)**:3597-3603.
33. Miles FL, Pruitt FL, van Golen KL, Cooper CR: **Stepping out of the flow: capillary extravasation in cancer metastasis.** *Clin Exp Metastasis* 2008, **25(4)**:305-324.
34. Burdick MM, Chu JT, Godar S, Sackstein R: **HCELL is the major E- and L-selectin ligand expressed on LS174T colon carcinoma cells.** *J Biol Chem* 2006, **281(20)**:13899-13905.
35. Hanley WD, Napier SL, Burdick MM, Schnaar RL, Sackstein R, Konstantopoulos K: **Variant isoforms of CD44 are P- and L-selectin ligands on colon carcinoma cells.** *Faseb J* 2006, **20(2)**:337-339.
36. Napier SL, Healy ZR, Schnaar RL, Konstantopoulos K: **Selectin ligand expression regulates the initial vascular interactions of colon carcinoma cells: the roles of CD44v and alternative sialofucosylated selectin ligands.** *J Biol Chem* 2007, **282(6)**:3433-3441.
37. Dimitroff CJ, Descheny L, Trujillo N, Kim R, Nguyen V, Huang W, Pienta KJ, Kutok JL, Rubin MA: **Identification of leukocyte E-selectin ligands, P-selectin glycoprotein ligand-1 and E-selectin ligand-1, on human metastatic prostate tumor cells.** *Cancer Res* 2005, **65(13)**:5750-5760.
38. Aigner S, Ramos CL, Hafezi-Moghadam A, Lawrence MB, Friederichs J, Altevogt P, Ley K: **CD24 mediates rolling of breast carcinoma cells on P-selectin.** *Faseb J* 1998, **12(12)**:1241-1251.
39. Thomas SN, Zhu F, Schnaar RL, Alves CS, Konstantopoulos K: **Carcinoma embryonic antigen and CD44 variant isoforms cooperate to mediate colon carcinoma cell adhesion to E- and L-selectin in shear flow.** *J Biol Chem* 2008, **283(23)**:15647-15655.
40. Qi J, Chen N, Wang J, Siu CH: **Transendothelial migration of melanoma cells involves N-cadherin-mediated adhesion and activation of the beta-catenin signaling pathway.** *Mol Biol Cell* 2005, **16(9)**:4386-4397.
41. Borsig L, Wong R, Feramisco J, Nadeau DR, Varki NM, Varki A: **Heparin and cancer revisited: mechanistic connections involving platelets, P-selectin, carcinoma mucins, and tumor metastasis.** *Proc Natl Acad Sci USA* 2001, **98(6)**:3352-3357.
42. Kim YJ, Borsig L, Han HL, Varki NM, Varki A: **Distinct selectin ligands on colon carcinoma mucins can mediate pathological interactions among platelets, leukocytes, and endothelium.** *Am J Pathol* 1999, **155(2)**:461-472.
43. Laubli H, Stevenson JL, Varki A, Varki NM, Borsig L: **L-selectin facilitation of metastasis involves temporal induction of Fut7-dependent ligands at sites of tumor cell arrest.** *Cancer Res* 2006, **66(3)**:1536-1542.
44. Luster AD: **Chemokines – chemotactic cytokines that mediate inflammation.** *N Engl J Med* 1998, **338(7)**:436-445.
45. Baggiolini M, Dewald B, Moser B: **Human chemokines: an update.** *Annu Rev Immunol* 1997, **15**:675-705.
46. Tanaka Y, Adams DH, Shaw S: **Proteoglycans on endothelial cells present adhesion-inducing cytokines to leukocytes.** *Immunol Today* 1993, **14(3)**:111-115.
47. Entschladen F, Lang K, Durchdewald M, Niggemann B, Zaenker KS: **Characteristics of leukocyte migration.** *Int J Med Biol Front* 2007, **12(3/4)**:241-256.
48. McEver RP, Zhu C: **A catch to integrin activation.** *Nat Immunol* 2007, **8(10)**:1035-1037.
49. Dib K: **BETA 2 integrin signaling in leukocytes.** *Front Biosci* 2000, **5**:D438-451.
50. Fagerholm SC, Hilden TJ, Nurmi SM, Gahmberg CG: **Specific integrin alpha and beta chain phosphorylations regulate LFA-1 activation through affinity-dependent and -independent mechanisms.** *J Cell Biol* 2005, **171(4)**:705-715.
51. Ley K, Laudanna C, Cybulsky MI, Nourshargh S: **Getting to the site of inflammation: the leukocyte adhesion cascade updated.** *Nat Rev Immunol* 2007, **7(9)**:678-689.
52. Campbell JJ, O'Connell DJ, Wurbel MA: **Cutting Edge: Chemokine receptor CCR4 is necessary for antigen-driven cutaneous accumulation of CD4 T cells under physiological conditions.** *J Immunol* 2007, **178(6)**:3358-3362.
53. Debes GF, Arnold CN, Young AJ, Krautwald S, Lipp M, Hay JB, Butcher EC: **Chemokine receptor CCR7 required for T lym-**

- phocyte exit from peripheral tissues. *Nat Immunol* 2005, **6(9)**:889-894.
54. Broxmeyer HE: **Chemokines in hematopoiesis.** *Curr Opin Hematol* 2008, **15(1)**:49-58.
 55. Balkwill F: **Cancer and the chemokine network.** *Nat Rev Cancer* 2004, **4(7)**:540-550.
 56. Kakinuma T, Hwang ST: **Chemokines, chemokine receptors, and cancer metastasis.** *J Leukoc Biol* 2006, **79(4)**:639-651.
 57. Vandercappellen J, Van Damme J, Struyf S: **The role of CXC chemokines and their receptors in cancer.** *Cancer Lett* 2008, **267(2)**:226-244.
 58. Cardones AR, Murakami T, Hwang ST: **CXCR4 enhances adhesion of B16 tumor cells to endothelial cells in vitro and in vivo via beta(1) integrin.** *Cancer Res* 2003, **63(20)**:6751-6757.
 59. Jamieson WL, Shimizu S, D'Ambrosio JA, Meucci O, Fatatis A: **CX3CR1 is expressed by prostate epithelial cells and androgens regulate the levels of CX3CL1/fractalkine in the bone marrow: potential role in prostate cancer bone tropism.** *Cancer Res* 2008, **68(6)**:1715-1722.
 60. Shulby SA, Dolloff NG, Stearns ME, Meucci O, Fatatis A: **CX3CR1-fractalkine expression regulates cellular mechanisms involved in adhesion, migration, and survival of human prostate cancer cells.** *Cancer Res* 2004, **64(14)**:4693-4698.
 61. Drell TL, Joseph J, Lang K, Niggemann B, Zaenker KS, Entschladen F: **Effects of neurotransmitters on the chemokinesis and chemotaxis of MDA-MB-468 human breast carcinoma cells.** *Breast Cancer Res Treat* 2003, **80(1)**:63-70.
 62. Entschladen F, Palm D, Niggemann B, Zaenker KS: **The cancer's nervous tooth: Considering the neuronal crosstalk within tumors.** *Semin Cancer Biol* 2008, **18(3)**:171-175.
 63. Kapper S, Beck G, Riedel S, Prem K, Haak M, Woude FJ van der, Yard BA: **Modulation of chemokine production and expression of adhesion molecules in renal tubular epithelial and endothelial cells by catecholamines.** *Transplantation* 2002, **74(2)**:253-260.
 64. Palm D, Lang K, Niggemann B, Drell TL, Masur K, Zaenker KS, Entschladen F: **The norepinephrine-driven metastasis development of PC-3 human prostate cancer cells in BALB/c nude mice is inhibited by beta-blockers.** *Int J Cancer* 2006, **118(11)**:2744-2749.
 65. Luo BH, Carman CV, Springer TA: **Structural basis of integrin regulation and signaling.** *Annu Rev Immunol* 2007, **25**:619-647.
 66. Constantin G, Majeed M, Giagulli C, Piccio L, Kim JY, Butcher EC, Laudanna C: **Chemokines trigger immediate beta2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow.** *Immunity* 2000, **13(6)**:759-769.
 67. Laudanna C, Kim JY, Constantin G, Butcher E: **Rapid leukocyte integrin activation by chemokines.** *Immunity* 2002, **18**:37-46.
 68. Arnaut MA, Mahalingam B, Xiong JP: **Integrin structure, allostery, and bidirectional signaling.** *Annu Rev Cell Dev Biol* 2005, **21**:381-410.
 69. Kinashi T: **Intracellular signalling controlling integrin activation in lymphocytes.** *Nat Rev Immunol* 2005, **5(7)**:546-559.
 70. Wegener KL, Campbell ID: **Transmembrane and cytoplasmic domains in integrin activation and protein-protein interactions (review).** *Mol Membr Biol* 2008, **25(5)**:376-387.
 71. Ibbotson GC, Doig C, Kaur J, Gill V, Ostrovsky L, Fairhead T, Kubes P: **Functional alpha4 integrin: a newly identified pathway of neutrophil recruitment in critically ill septic patients.** *Nat Med* 2001, **7(4)**:465-470.
 72. Cybulsky MI, Gimbrone MA Jr: **Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis.** *Science* 1991, **251(4995)**:788-791.
 73. Cybulsky MI, Iiyama K, Li H, Zhu S, Chen M, Iiyama M, Davis V, Gutierrez-Ramos JC, Connelly PW, Milstone DS: **A major role for VCAM-1, but not ICAM-1, in early atherosclerosis.** *J Clin Invest* 2001, **107(10)**:1255-1262.
 74. Rao RM, Yang L, Garcia-Cardena G, Luscinskas FW: **Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall.** *Circ Res* 2007, **101(3)**:234-247.
 75. Montgomery AM, Becker JC, Siu CH, Lemmon VP, Cheresh DA, Pancook JD, Zhao X, Reisfeld RA: **Human neural cell adhesion molecule LI and rat homologue NILE are ligands for integrin alpha v beta 3.** *J Cell Biol* 1996, **132(3)**:475-485.
 76. Ruppert M, Aigner S, Hubbe M, Yagita H, Altevogt P: **The LI adhesion molecule is a cellular ligand for VLA-5.** *J Cell Biol* 1995, **131(6 Pt 2)**:1881-1891.
 77. Liu FT, Rabinovich GA: **Galectins as modulators of tumour progression.** *Nat Rev Cancer* 2005, **5(1)**:29-41.
 78. Thijssen VL, Poirier F, Baum LG, Griffioen AW: **Galectins in the tumor endothelium: opportunities for combined cancer therapy.** *Blood* 2007, **110(8)**:2819-2827.
 79. Hughes RC: **Galectins as modulators of cell adhesion.** *Biochimie* 2001, **83(7)**:667-676.
 80. Hoyer KK, Pang M, Gui D, Shintaku IP, Kuwabara I, Liu FT, Said JW, Baum LG, Teitel MA: **An anti-apoptotic role for galectin-3 in diffuse large B-cell lymphomas.** *Am J Pathol* 2004, **164(3)**:893-902.
 81. Stillman BN, Hsu DK, Pang M, Brewer CF, Johnson P, Liu FT, Baum LG: **Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death.** *J Immunol* 2006, **176(2)**:778-789.
 82. Rabinovich GA, Toscano MA, Illarregui JM, Rubinstein N: **Shedding light on the immunomodulatory properties of galectins: novel regulators of innate and adaptive immune responses.** *Glycoconj J* 2004, **19(7-9)**:565-573.
 83. Clausse N, Brule F van den, Waltregny D, Garnier F, Castronovo V: **Galectin-1 expression in prostate tumor-associated capillary endothelial cells is increased by prostate carcinoma cells and modulates heterotypic cell-cell adhesion.** *Angiogenesis* 1999, **3(4)**:317-325.
 84. Lotan R, Belloni PN, Tressler RJ, Lotan D, Xu XC, Nicolson GL: **Expression of galectins in microvessel endothelial cells and their involvement in tumour cell adhesion.** *Glycoconj J* 1994, **11(5)**:462-468.
 85. Cooper D, Norling LV, Perretti M: **Novel insights into the inhibitory effects of Galectin-1 on neutrophil recruitment under flow.** *J Leukoc Biol* 2008, **83(6)**:1459-1466.
 86. La M, Cao TV, Cerchiaro G, Chilton K, Hirabayashi J, Kasai K, Oliani SM, Chernajovsky Y, Perretti M: **A novel biological activity for galectin-1: inhibition of leukocyte-endothelial cell interactions in experimental inflammation.** *Am J Pathol* 2003, **163(4)**:1505-1515.
 87. Norling LV, Sampaio AL, Cooper D, Perretti M: **Inhibitory control of endothelial galectin-1 on in vitro and in vivo lymphocyte trafficking.** *Faseb J* 2008, **22(3)**:682-690.
 88. Rabinovich GA: **Galectin-1 as a potential cancer target.** *Br J Cancer* 2005, **92(7)**:1188-1192.
 89. Ryschich E, Lizdenis P, Itrich C, Benner A, Stahl S, Hamann A, Schmidt J, Knolle P, Arnold B, Hammerling GJ, Ganss R: **Molecular fingerprinting and autocrine growth regulation of endothelial cells in a murine model of hepatocellular carcinoma.** *Cancer Res* 2006, **66(1)**:198-211.
 90. Nieminen J, St-Pierre C, Bhaumik P, Poirier F, Sato S: **Role of galectin-3 in leukocyte recruitment in a murine model of lung infection by Streptococcus pneumoniae.** *J Immunol* 2008, **180(4)**:2466-2473.
 91. Matsumoto R, Matsumoto H, Seki M, Hata M, Asano Y, Kanegasaki S, Stevens RL, Hirashima M: **Human ecalectin, a variant of human galectin-9, is a novel eosinophil chemoattractant produced by T lymphocytes.** *J Biol Chem* 1998, **273(27)**:16976-16984.
 92. Zhu C, Anderson AC, Schubart A, Xiong H, Imitola J, Khoury SJ, Zheng XX, Strom TB, Kuchroo VK: **The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity.** *Nat Immunol* 2005, **6(12)**:1245-1252.
 93. Garofalo A, Chirivì RG, Foglieni C, Pigott R, Mortarini R, Martin-Padura I, Anichini A, Gearing AJ, Sanchez-Madrid F, Dejana E, et al.: **Involvement of the very late antigen 4 integrin on melanoma in interleukin 1-augmented experimental metastases.** *Cancer Res* 1995, **55(2)**:414-419.
 94. Okahara H, Yagita H, Miyake K, Okumura K: **Involvement of very late activation antigen 4 (VLA-4) and vascular cell adhesion molecule 1 (VCAM-1) in tumor necrosis factor alpha enhancement of experimental metastasis.** *Cancer Res* 1994, **54(12)**:3233-3236.
 95. Voura EB, Ramjeesingh RA, Montgomery AM, Siu CH: **Involvement of integrin alpha(v)beta(3) and cell adhesion molecule LI in transendothelial migration of melanoma cells.** *Mol Biol Cell* 2001, **12(9)**:2699-2710.

96. Nangia-Makker P, Sarvis R, Visscher DW, Bailey-Penrod J, Raz A, Sarkar FH: **Galectin-3 and LI retrotransposons in human breast carcinomas.** *Breast Cancer Res Treat* 1998, **49(2)**:171-183.
97. Glinsky VV, Glinsky GV, Rittenhouse-Olson K, Huflejt ME, Glinskii OV, Deutscher SL, Quinn TP: **The role of Thomsen-Friedenreich antigen in adhesion of human breast and prostate cancer cells to the endothelium.** *Cancer Res* 2001, **61(12)**:4851-4857.
98. Glinsky VV, Huflejt ME, Glinsky GV, Deutscher SL, Quinn TP: **Effects of Thomsen-Friedenreich antigen-specific peptide P-30 on beta-galactoside-mediated homotypic aggregation and adhesion to the endothelium of MDA-MB-435 human breast carcinoma cells.** *Cancer Res* 2000, **60(10)**:2584-2588.
99. Khaldoynidi SK, Glinsky VV, Sikora L, Glinskii AB, Mossine VV, Quinn TP, Glinsky GV, Sriramarao P: **MDA-MB-435 human breast carcinoma cell homo- and heterotypic adhesion under flow conditions is mediated in part by Thomsen-Friedenreich antigen-galectin-3 interactions.** *J Biol Chem* 2003, **278(6)**:4127-4134.
100. Fonseca I, Costa Rosa J, Felix A, Therkildsen MH, Mandel U, Soares J: **Simple mucin-type carbohydrate antigens (T, Tn and sialosyl-Tn) in mucoepidermoid carcinoma of the salivary glands.** *Histopathology* 1994, **25(6)**:537-543.
101. Springer GF, Desai PR, Ghazizadeh M, Tegtmeier H: **T/Tn pancreatic carcinoma autoantigens: fundamental, diagnostic, and prognostic aspects.** *Cancer Detect Prev* 1995, **19(2)**:173-182.
102. Yu LG, Andrews N, Zhao Q, McKean D, Williams JF, Connor LJ, Gerasimenko OV, Hilkens J, Hirabayashi J, Kasai K, Rhodes JM: **Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion.** *J Biol Chem* 2007, **282(1)**:773-781.
103. Yu LG: **The oncofetal Thomsen-Friedenreich carbohydrate antigen in cancer progression.** *Glycoconj J* 2007, **24(8)**:411-420.
104. Sarafian V, Jadot M, Foidart JM, Letesson JJ, Brule F Van den, Castrovovo V, Wattiaux R, Coninck SW: **Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells.** *Int J Cancer* 1998, **75(1)**:105-111.
105. Petri B, Bixel MG: **Molecular events during leukocyte diapedesis.** *Febs J* 2006, **273(19)**:4399-4407.
106. Muller WA: **The role of PECAM-1 (CD31) in leukocyte emigration: studies in vitro and in vivo.** *J Leukoc Biol* 1995, **57(4)**:523-528.
107. Muller WA, Weigl SA, Deng X, Phillips DM: **PECAM-1 is required for transendothelial migration of leukocytes.** *J Exp Med* 1993, **178(2)**:449-460.
108. Berman ME, Xie Y, Muller WA: **Roles of platelet/endothelial cell adhesion molecule-1 (PECAM-1, CD31) in natural killer cell transendothelial migration and beta 2 integrin activation.** *J Immunol* 1996, **156(4)**:1515-1524.
109. Bird IN, Spragg JH, Ager A, Matthews N: **Studies of lymphocyte transendothelial migration: analysis of migrated cell phenotypes with regard to CD31 (PECAM-1), CD45RA and CD45RO.** *Immunology* 1993, **80(4)**:553-560.
110. Vestweber D: **Adhesion and signaling molecules controlling the transmigration of leukocytes through endothelium.** *Immunol Rev* 2007, **218**:178-196.
111. Lou O, Alcaide P, Luscinskas FW, Muller WA: **CD99 is a key mediator of the transendothelial migration of neutrophils.** *J Immunol* 2007, **178(2)**:1136-1143.
112. Bixel MG, Petri B, Khandoga AG, Khandoga A, Wolburg-Buchholz K, Wolburg H, Marz S, Krombach F, Vestweber D: **A CD99-related antigen on endothelial cells mediates neutrophil but not lymphocyte extravasation in vivo.** *Blood* 2007, **109(12)**:5327-5336.
113. Schenkel AR, Mamdouh Z, Chen X, Liebman RM, Muller WA: **CD99 plays a major role in the migration of monocytes through endothelial junctions.** *Nat Immunol* 2002, **3(2)**:143-150.
114. Ebnat K, Suzuki A, Ohno S, Vestweber D: **Junctional adhesion molecules (JAMs): more molecules with dual functions?** *J Cell Sci* 2004, **117(Pt 1)**:19-29.
115. Martin-Padura I, Lostaglio S, Schneemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruco L, Villa A, Simmons D, Dejana E: **Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration.** *J Cell Biol* 1998, **142(1)**:117-127.
116. Ostermann G, Weber KS, Zerneck A, Schroder A, Weber C: **JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes.** *Nat Immunol* 2002, **3(2)**:151-158.
117. Engelhardt B, Wolburg H: **Mini-review: Transendothelial migration of leukocytes: through the front door or around the side of the house?** *Eur J Immunol* 2004, **34(11)**:2955-2963.
118. Arrate MP, Rodriguez JM, Tran TM, Brock TA, Cunningham SA: **Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2 counter-receptor.** *J Biol Chem* 2001, **276(49)**:45826-45832.
119. Leppert D, Waubant E, Galardy R, Bunnett NW, Hauser SL: **T cell gelatinases mediate basement membrane transmigration in vitro.** *J Immunol* 1995, **154(9)**:4379-4389.
120. Friedl P, Weigelin B: **Interstitial leukocyte migration and immune function.** *Nat Immunol* 2008, **9(9)**:960-969.
121. Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ: **Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis.** *Nat Med* 2000, **6(1)**:100-102.
122. Heyder C, Gloria-Maercker E, Entschladen F, Hatzmann WW, Niggemann B, Zanker KS, Dittmar T: **Realtime visualization of tumor cell/endothelial cell interactions during transmigration across the endothelial barrier.** *J Cancer Res Clin Oncol* 2002, **128(10)**:533-538.
123. Brandt B, Heyder C, Gloria-Maercker E, Hatzmann WW, Rotger A, Kemming D, Zanker KS, Entschladen F, Dittmar T: **3D-extravasation model - selection of highly motile and metastatic cancer cells.** *Semin Cancer Biol* 2005, **15(5)**:387-395.

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