

The control of tumor vessels: what you would not expect from a neural adhesion molecule

Francesca Angiolini and Ugo Cavallaro*

Molecular Medicine Program; Department of Experimental Oncology; European Institute of Oncology; Milano, Italy

Keywords: L1 cell adhesion molecule; tumor vessels; angiogenesis; vascular normalization; IL6/JAK/STAT3 pathway

The neural adhesion molecule L1 is involved in development and plasticity of the nervous system. We recently reported aberrant expression of L1 in the vasculature of various human tumor types. Genetic and functional inactivation of endothelial L1 in a mouse tumor model resulted in decreased tumor angiogenesis and promoted vascular normalization. Thus, endothelial L1 might represent a novel therapeutic target for vessel-targeted treatments of solid tumors.

The pioneering work of Judah Folkman¹ paved the way for several studies that have conclusively established the essential role of neovascularization in the context of tumor progression. Indeed, solid tumors cannot grow beyond a small size without the formation of new blood vessels that supply oxygen and nutrients to neoplastic cells. In addition, tumor-associated vessels offer the main route for metastatic dissemination of cancer cells to distant organs.

These observations have provided a strong rationale to dissect the cellular and molecular mechanisms that govern tumor angiogenesis, aimed at designing therapeutic strategies to interfere with the process and therefore inhibit tumor growth and metastasis. So far, the main output of these efforts has been a series of compounds that target the vascular endothelial growth factor (VEGF) pathway, a major player in tumor neovascularization. The prototypic example of a VEGF-targeted drug is provided by the anti-VEGF antibody bevacizumab, which has entered clinical practice for different cancer types. In addition, several compounds that inactivate either VEGF itself or its receptors are under clinical evaluation.²

While VEGF-targeted therapy has proven efficacious in the inhibition of

tumor-associated angiogenesis, prolonging progression-free survival in patients with certain cancer types,³ its beneficial effects are often transient and modest. Furthermore, following the initial therapeutic response, tumors often develop mechanisms of resistance and/or evasion, resulting in disease recurrence and/or increased aggressiveness.⁴ These findings highlight the need to design novel therapeutic strategies to interfere with tumor angiogenesis, an objective that, in turn, strictly depends on a deeper knowledge of the molecular players and pathways involved in the formation and function of tumor vasculature.

Our group has observed that the immunoglobulin-like cell adhesion molecule L1 (also known as L1CAM or CD171), previously characterized as a neural Ig-CAM involved in brain development and plasticity,⁵ is aberrantly expressed in the vessels of different tumor types but almost absent from the normal vasculature.^{6,7} To investigate whether L1 plays a functional role in cancer vessels, we combined genetic ablation of L1 in mouse endothelium with an orthotopic syngeneic model of pancreatic carcinoma. Endothelial deficiency of L1 resulted in reduced tumor growth and metastasis and longer mouse survival, accompanied by reduced tumor angiogenesis and vascular

normalization.⁷ Similar results were obtained by treating tumor-bearing mice with a neutralizing anti-L1 antibody.⁷

Overall, this study provides the rationale to test inactivation of L1 as a novel antiangiogenic approach (Fig. 1). Of note, vascular expression of L1 is induced by different angiogenic and proinflammatory stimuli including VEGF-A, tumor necrosis factor- α , interferon- γ , and transforming growth factor- β 1 (our unpublished results and Ref. 6) and L1, in turn, stimulates the expression of several molecules that promote neovascularization, including VEGF-A, VEGF-C, delta-like ligand-4, and interleukin-6.⁷ Thus, it is conceivable that therapeutic inhibition of L1 would interfere with a broad spectrum of proangiogenic pathways that are commonly activated in the tumor microenvironment.

In addition to its functional role in cancer vascularization, the surface expression of L1 on vascular endothelium represents another attractive feature with potentially relevant therapeutic implications. Indeed, antibody-mediated delivery of cytotoxic compounds (drugs, toxins, radioisotopes, etc.) to cell surface-associated epitopes remains an actively explored approach for targeted therapy,⁸ and the specific expression pattern of L1 implies

© Francesca Angiolini and Ugo Cavallaro

*Correspondence to: Ugo Cavallaro; Email: ugo.cavallaro@ieo.eu

Submitted: 10/27/2014; Revised: 10/27/2014; Accepted: 10/27/2014

<http://dx.doi.org/10.4161/23723556.2014.982045>

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

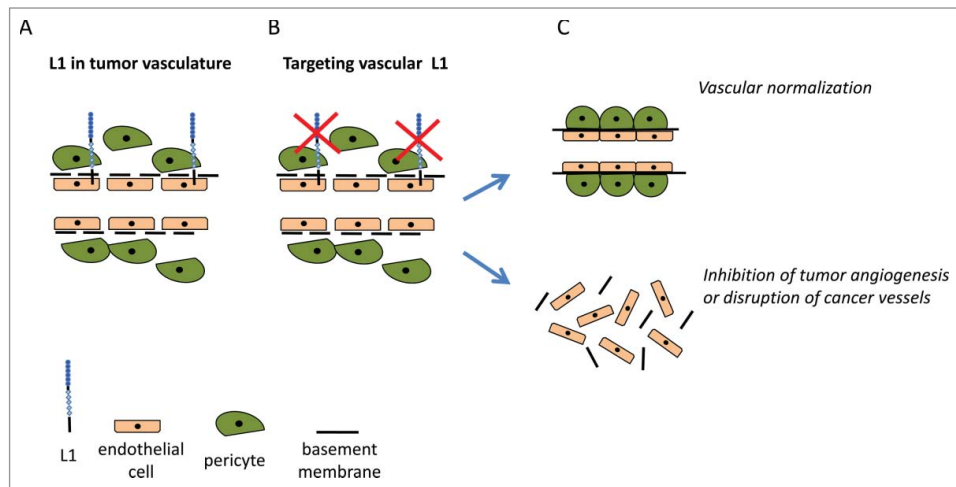


Figure 1. Vascular L1 as a therapeutic target for cancer therapy. The specific expression of L1 in tumor vessels results in altered endothelial junctions, deficient deposition of basement membrane, and poor pericyte coverage (A). Genetic ablation or the functional inactivation of L1 (B) prevents tumor angiogenesis and/or promotes vascular normalization (C). In addition, antibody-drug conjugates based on anti-L1 antibodies could lead to the selective disruption of tumor vessels (C, bottom).

that this strategy might result in the selective elimination of tumor vessels while sparing their normal counterpart (Fig. 1). Finally, it is noteworthy that L1 is frequently expressed in tumor cells themselves, where it exerts a promalignant function. In these tumor types, therefore, L1-targeted therapies would offer the dual advantage of disrupting cancer vessels and killing neoplastic cells, possibly resulting in a synergistic antitumor effect.

Unexpectedly, we found that inactivating endothelial L1, either genetically or via antibody-mediated neutralization, leads to vascular normalization in the tumor as shown by increased pericyte coverage and basement membrane deposition, resulting in reduced vessel permeability.⁷ It has long been proposed that vascular normalization, achieved by improving tumor perfusion, would facilitate the delivery of chemotherapies or targeted drugs into the tumor mass, thus enhancing the therapeutic response. This theory has received support from a number of preclinical and clinical studies.⁹ Our findings point to L1 neutralization as a novel strategy to induce vessel normalization, providing the rationale for testing

whether this increases the efficacy of anti-cancer treatments (Fig. 1).

Mechanistically, the pleiotropic role of L1 in the cancer vasculature entails control of transcriptional activity in endothelial cells, which underlies the L1-dependent modulation of key gene networks and signaling pathways, such as the IL6-JAK-STAT3 axis. In particular, endothelial L1 induces the expression of both IL6 and IL6-receptor- α , resulting in the constitutive activation of signal transducer and activator of transcription 3 (STAT3), and blocking this pathway dramatically reduces L1-dependent endothelial cell proliferation and migration.⁷ The IL6-JAK-STAT3 cascade has been implicated in tumor angiogenesis, thus emerging as a prominent effector of L1-induced neovascularization. In addition, transcriptomic data (corroborated by morphological and functional findings in L1-deficient tumor vessels) indicated that L1 induces the endothelial-to-mesenchymal transition,⁷ a process that has been implicated in cancer progression¹⁰ and, therefore, is likely to contribute to the promalignant function of L1. Of course, given the large number of genes that are regulated by L1 in

endothelial cells, the relative impact of other networks/pathways on the tumor vasculature remains to be defined. Likewise, future studies should elucidate at the molecular level how L1 exerts its control on gene transcription, clarifying for example whether this entails a direct effect on specific promoters or rather depends on other intracellular effectors.

On one hand, the novel role of L1 in cancer-associated vessels sets the stage for identification of additional pathways and players involved in tumor angiogenesis and vascular normalization. On the other hand, these findings open new perspectives for the design of alternative therapeutic strategies aimed at selective targeting of the tumor vasculature.

Funding

Research in the authors' laboratory is supported by funding from the Association for International Cancer Research, the Associazione Italiana Ricerca sul Cancro, Fondazione Cariplo, Fondazione Telethon, and the Italian Ministry of Health.

References

1. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972; 175:409-16; PMID:5077799; <http://dx.doi.org/10.1097/0000658-197203000-00014>
2. Tugues S, Koch S, Gualandi L, Li X, Claesson-Welsh L. Vascular endothelial growth factors and receptors: anti-angiogenic therapy in the treatment of cancer. *Mol Aspects Med* 2011; 32:88-111; PMID:21565214; <http://dx.doi.org/10.1016/j.mam.2011.04.004>
3. Moutziou G, Pentheroudakis G, Carmeliet P. Bevacizumab and micrometastases: revisiting the preclinical and clinical rollercoaster. *Pharmacol Ther* 2014; 141:117-24; PMID:24076268; <http://dx.doi.org/10.1016/j.pharmthera.2013.09.003>

4. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. *Cancer Cell* 2009; 15:220-31; PMID:19249680; <http://dx.doi.org/10.1016/j.ccr.2009.01.027>
5. Rathjen FG, Schachner M. Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. *EMBO J* 1984; 3:1-10; PMID:6368220
6. Maddaluno L, Verbrugge SE, Martinoli C, Matteoli G, Chiavelli A, Zeng Y, Williams ED, Rescigno M, Cavallaro U. The adhesion molecule L1 regulates trans-endothelial migration and trafficking of dendritic cells. *J Exp Med* 2009; 206:623-35; PMID:19273627; <http://dx.doi.org/10.1084/jem.20081211>
7. Magrini E, Villa A, Angiolini F, Doni A, Mazzarol G, Rudini N, Maddaluno L, Komuta M, Topal B, Prenen H, et al. Endothelial deficiency of L1 reduces tumor angiogenesis and promotes vessel normalization. *J Clin Invest* 2014; 124:4335-50; PMID:25157817; <http://dx.doi.org/10.1172/JCI70683>
8. Leal M, Sapra P, Hurvitz SA, Senter P, Wahl A, Schutten M, Shah DK, Haddish-Berhane N, Kabbarah O. Antibody-drug conjugates: an emerging modality for the treatment of cancer. *Ann N Y Acad Sci* 2014; 1321:41-54; PMID:25123209; <http://dx.doi.org/10.1111/nyas.12499>
9. Goel S, Wong AHK, Jain RK. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease. *Cold Spring Harb Perspect Med* 2012; 2:2:a006486; PMID:22393532; <http://dx.doi.org/10.1101/cshperspect.a006486>
10. Potenta S, Zeisberg E, Kalluri R. The role of endothelial-to-mesenchymal transition in cancer progression. *Br J Cancer* 2008; 99:1375-9; PMID:18797460; <http://dx.doi.org/10.1038/sj.bjc.6604662>