Vascular co-option

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Vascular co-option in normal and transformed cells

Vascular co-option in cancer was initially described by Holash et al. [1] in gliomas and lung metastasis. The finding reported how tumors remain vascularized without the action of angiogenesis by establishing a physical interaction between cancer cells and pre(pre-existing)existing vessels. This process mimics a similar mechanism developed by a variety of normal cells inhabiting the perivascular niche [2] (Fig. 3.1). A key aspect of vascular co-option is that there is no angiogenesis involved, and thus its molecular regulation follows different mechanisms. Surprisingly, in spite of its potential for treating cancer, specially the disseminated disease, vascular co-option remains poorly understood possibly as the consequence of the intense research dedicated to angiogenesis [3]. However, antiangiogenic treatments have not reached the expectations initially predicted [4–9]. Studies devoted to understand the mechanisms of resistance to antiangiogenic therapy identified the involvement of vascular co-option. This finding confirmed the independence of their molecular regulation, and thus the importance of further characterize the less studied regulatory logic of vascular co-option as an emerging opportunity to impair cancer progression [7–9].

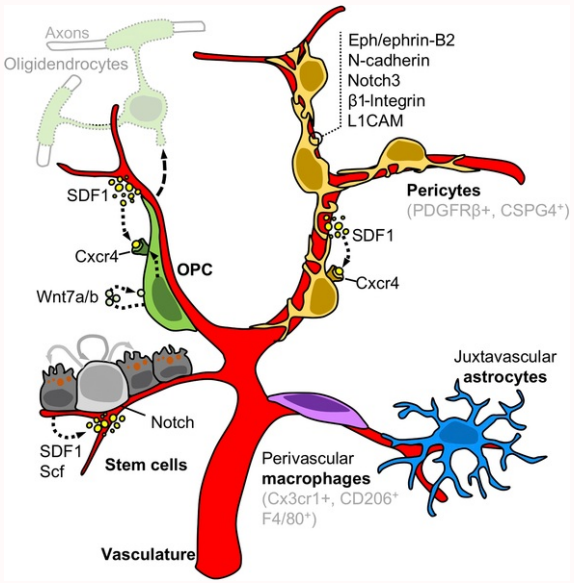

Figure 3.1 Vascular co-option in normal noncancer cells.

Different cell types have been demonstrated to interact with pre(pre-existing)existing vessels. The process of vascular co-option mediates their developmental program, favors their survival, maintains their self-renewal capacity, and could influence the

emergence of cell heterogeneity by inducing specific functional properties to co-opting cells. Different regulatory mechanisms in different co-opting cells are indicated. In gray markers used for defining the identity of the specific cell type is shown. *OPC*, oligodendrocyte precursor cell.

Vascular co-option in non(non-cancer)cancer cells

Noncancer cells interact with the vasculature in multiple diverse scenarios. During the development of nervous system, oligodendrocyte precursor cells (OPCs), which will differentiate into oligodendrocytes to

produce the myelin covering axons [10], migrate from their origin to their specific target areas using the endothelial surface of neighboring blood vessels [11,12]. This process is regulated by autocrine Wnt signaling. OPCs release Wnt7a and Wnt7b that induce the expression of the chemokine receptor Cxcr4 [12]. Cxcr4 binds to its ligand(introduce a comma) the chemokine SDF-1(introduce a comma) secreted by co-opted endothelial supporting the migration of OPCs along the vessels [12]. OPCs remain undifferentiated during the migration due to Wnt signaling [12,13] and only mature when they arrive at their final destination, which coincides with the detachment from endothelial cells as they decrease the expression of Wnt pathway and Cxcr4 [12] (Fig. 3.1).

However, the prototypical non(non-cancer)cancer cell performing vascular co-option is the pericyte. Pericytes, the mural cells surrounding endothelial cells of small blood vessels, are required for maintaining vessel stability [14]. In addition, pericytes regulate blood flow due to their contractibility properties and contribute to tissue regeneration given their mesenchymal stem cell properties [14,15]. Pericytes are specially relevant in the brain, where they are key for the homeostasis of the blood–brain barrier (BBB) regulating its maturation during development and then its permeability [14,15]. Not surprisingly, the brain has one of the highest pericyte coverage of the vascular network [14,15]. Pericytes interact with (the)endothelium using the same mechanism described in OPCs involving the SDF-1/Cxcr4 axis, which is responsible for pericyte recruitment and migration along the vessels [16]. In addition, the interaction between Jagged-1, from endothelial cells, and Notch3, from pericytes, is responsible for vessel stabilization [17]. Eph/ephrin-B2 signaling is also involved in this cell("cell-to-cell" instead of "cell-cell")–cell interaction [18,19]. Finally, the cell adhesion molecules N-cadherin [20], VCAM1, which binds to endothelial α4β1-integrin activating a downstream signaling influenced by the pericyte proteoglycan NG2 [21,22], and L1CAM, which reinforces the β1-integrin/ILK signaling pathway and mediates pericytes spreading and vessel homeostasis [2], are all known mediators of vascular co-option in pericytes (Fig. 3.1).

Other non(non-cancer)cancer cells that are known to reside at the perivascular space are stem cells. For instance, 95% of Hoxb5+, a recently discovered nuclear marker for hematopoietic stem cells, were detected attached to the endothelial cells in the bone marrow [23]. Similarly, neural stem cells are in direct contact with the special vasculature of the subventricular zone in the brain where the BBB lacks astrocytes endfeets and pericyte coverage [24]. The perivascular location provides stem cells preferential access to molecules from the blood such as nutrients, growth factors, and hormones; as well as angiocrine factors that regulate their differentiation, self-renewal proliferation, and/or migration capacities [24,25]. To mention a few molecular regulators of vascular co-option in stem cells, the SDF-1/Cxcr4 axis is also involved by inducing the expression of α6-integrin to bind to endothelial cell-derived laminin [26,27], and the stem cell ability to self-renew is promoted by Notch signaling [28–30] as well as the production of the stem cell factor(Change "stem cell factor" for "Stem Cell Factor (SCF)") [31] (Fig. 3.1).

Recent analyses on cell heterogeneity in brain macrophages and astrocytes have described cellular subtypes that have been mainly defined by their perivascular location [32,33]. Although the molecular nature of their interaction with the vessels is unknown, existing evidence suggests that this specific location could be a major contributor to their functional specialization, thus participating in the emergence of cell heterogeneity [33] (Fig. 3.1).

Vascular co-option in cancer cells

Vascular co-option has been described not only in experimental models but also in patients affected by glioma [1,34–36], melanoma [37–39], lung cancer [4,9,40–42], breast cancer [43–46], renal cancer [47], and liver cancer [7,8] both in the primary tumor [1,8,34–37,40] and in distant organs such as brain [2,38,40,43,44,46,48–54], lungs [1,2,9,37,42,44], bone [2,44,55], or liver [2,7] where metastatic cells disseminate (Fig. 3.2A).

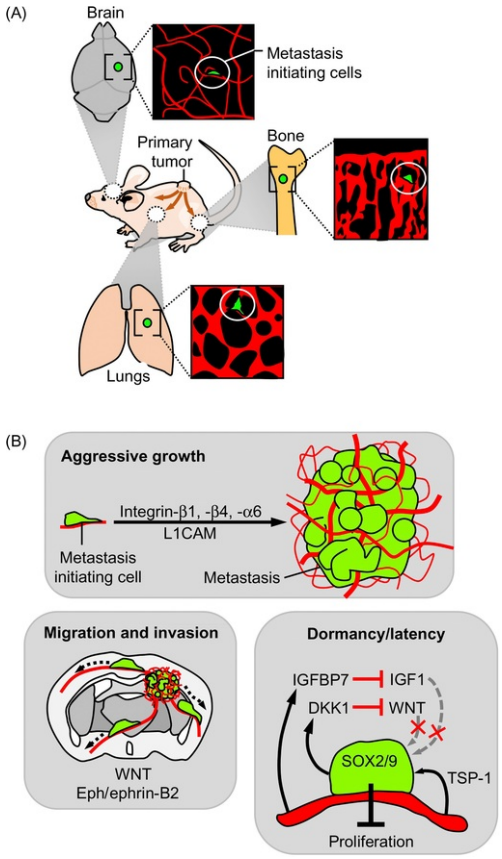

Figure 3.2 Implications of vascular co-option in cancer.

(A) Once they arrive to a secondary organ, disseminated metastasis-initiating cells remain attached to the abluminal side of vessels after extravasation performing vascular co-option. This process has been described in multiple organs targeted by metastasis and shown to be independent of the primary tumor type. (B) Vascular co-option favors many aspects that are highly relevant for metastasis such as the development of aggressive growth, the ability to invade locally, and the ability to remain alive but dormant over long periods of time.

In primary tumors, cancer cells in close contact with vessels receive and have access to oxygen, nutrients, and other factors produced by endothelial cells (angiocrine factors) that support their viability and growth [56,57]. This is particularly important to cancer stem cells (CSCs), which can maintain their properties by getting access to secreted angiocrine factors such as vascular endothelial growth factor (VEGF), which promotes their survival [58,59], Notch ligands like Jagged-1, which activates CSCs nurturing their self-renewal capacity, chemoresistance, and tumorigenicity [60,61]. As the primary tumor develops, the ability of cancer cells to co-opt the vessels initiates the process by which few of them will get access to systemic circulation upon intravasation, thus starting the metastatic cascade [37].

The interaction between cancer cells and vessels is resumed once metastatic cells reach the capillary network of distal organs after they disseminate from the primary tumor through systemic circulation. Interestingly, those tumor cells that extravasate do not abandon the perivascular niche but remain closely attached to the abluminal side of the vessels performing vascular co-option [50,53]. The initial observation of this process in lung metastasis [1] has been extended to multiorgan metastases, establishing vascular co-option as a hallmark of metastasis-initiating cells [2,38,41,46,50,52,53]. Besides the benefits described in the primary tumor [50,52,56,57] (Remove refs 50 and 52), there

are additional implications of vascular co-option involving several cancer hallmarks [62] such as aggressive growth, immune evasion, latency, and resistance to therapy [9,35,37,44,49,55,63,64](add ref 8) that are especially relevant in metastasis.

Molecular regulation of vascular co-option

Given that vascular co-option has been linked to several cancer hallmarks, its molecular characterization is key to develop novel anticancer strategies, which might have relevant implications to prevent the development of metastasis.

Molecular regulation of cell adhesion and proliferation in vascular co-option

Immediately after crossing the vascular barrier of any given secondary organ, metastasis-initiating cells remain attached to the abluminal side of the vessels using preexisting capillaries [2,46,50,53]. This interaction is dependent on adhesion molecules including integrins and L1CAM [50,52].

β1-Integrin mediates the interaction between the cancer cell and different components of the basal lamina of capillaries including collagen I and IV, fibronectin, vitronectin, and laminin [52]. Activation of β1-integrin in the cancer cells by basal lamina components initiates a signaling cascade involving FAK-dependent ERK1/2 phosphorylation, which supports the proliferation of metastatic cells in secondary organs upon arrival [52] (Fig. 3.2B). In addition to favor growth, this molecular mechanism is also important for protecting not aggressive dormant cancer cells, which might drive relapses later on [55]. This scenario is very relevant in breast cancer, which could develop a significant period of time between the removal of the primary tumor until metastases manifest clinically. This clinical scenario is explained by the ability of disseminated tumor cells (DTCs) to enter in a dormant state in the secondary organ until they found favorable conditions to grow [65,66]. DTCs in a dormant state have been shown to remain attached to the perivascular niche [44] being protected from chemotherapy in a β1-integrin and $\alpha_v\beta_3$ integrin-dependent manner [55]. β1-Integrin is key for the formation of filopodium-like protrusions that allow cancer cells to established cell–cell interactions with components of the extracellular matrix of basal lamina [67]. The underlying molecular regulation of these protrusions involves Rif/mDia2 actin machinery and the ILK/β-parvin/cofilin signaling pathway that signals through the FAK–ERK pathway [67]. Thus vascular co-option benefit cancer cells in a β1-integrin-dependent manner by granting access to the components of the basal lamina of co-opted vessels. In addition, cancer cells might also benefit by the influence of β1-integrin on the production of angiocrine factors in endothelial cells. This process has been shown to be dependent on β1-integrin, given its ability to couple growth with blood flow leading to the activation of mechanotransduction processes that induces the secretion of these factors [56].

Additional integrins have been described to participate in vascular co-option, which suggests certain plasticity within the communication between cancer cells and the endothelium. For instance, acute lymphoblastic leukemia cancer cells metastasize to the central nervous system without the need to cross the BBB, given their ability to exploit arachnoid veins by α6-integrin, which allows them to interact with laminin

[68]. Binding of β4-integrin from cancer cells to basal lamina components has been shown to activate ErbB2 by the ability of the integrin cytoplasmic domain to activate receptor tyrosine kinases (RTKs). This signaling pathway that links vascular co-option to the activation of RTKs stimulates the production of secreted molecules, such as VEGF, that will modify endothelial cells to favor tumor growth [43].

Besides integrins, additional cell adhesion molecules are involved in vascular co-option. L1CAM, which is expressed during the development of the nervous system [69], becomes deregulated in cancer and correlates with poor prognosis in many tumor types [47,70]. This cell adhesion molecule was initially found to be enriched at the interface between tumor and endothelial cell in brain metastasis [50] and later involved multiorgan metastases from different primary tumors [2]. Loss of function of L1CAM in cancer cells mimics the phenotype of targeting β1-integrin, which involves the inability of cancer cells to spread on co-opted vessels thus blocking their proliferation and consequently

preventing the development of macrometastasis [50]. L1CAM promotes β1-integrin signaling by increasing PAK1/2 phosphorylation in an ILK-dependent manner. PAK1/2 phosphorylation promotes the formation of actin filaments in an ankirin2-dependent manner, which allows the formation of cell protrusions required for cell spreading on vessels [2], which might alternatively involve Arp2/3 [2]. The induction of cancer cell spreading along the preexisting vessels activates YAP pathway, which induces a gene expression signature responsible for reactivating the proliferative program when metastatic cells initiate the colonization of a new organ [2]. Thus (add a comma) targeting the integrin–L1CAM signaling pathway in cancer cells impairs the initial stages of metastasis colonization and prevents the development of macrometastases as has been shown in different experimental cancer models including lung, breast, skin, colorectal, and renal cancer as well as lymphoma [2,50,52,55].

Although the process of vascular co-option involves the cancer cell and the endothelial cell, it could be influenced by additional cells of the microenvironment induced by inflammation. This has been demonstrated in melanoma, a type of skin cancer highly linked to UV radiation, where vascular co-option has been shown to be dependent on HMGB1–TLR4–dependent inflammation [37]. Excessive UV light induces the secretion of the danger-associated molecular pattern HMGB1 from damaged melanocytes. HMGB1 is detected by neutrophils through the TLR4 receptor producing an inflammatory response that favors the ability of melanoma cells to co-opt the vessels at the primary tumor and then disseminate systemically [37]. The enhanced co-option of cancer cells seems to be dependent on the TNF released by the neutrophils during the inflammatory response [37].

Molecular regulation of migration and invasion linked to vascular co-option

The process of migration is highly relevant in cancer. Dissemination of cancer cells out of the primary tumor is a necessary step for the development of systemic cancer and (Change "and" for "but") also when dissemination is local it (change "when dissemination is local it" for "local dissemination") can have important clinical implications. For instance, intracerebral invasion of glioma cells is a marker of poor prognosis [71]. Vascular co-option is highly relevant in the ability of cancer cells to migrate in the brain. Glioma cells exploit ephrin signaling to move along pre (pre-existing) existing capillaries [35]. Normally, Eph/ephrin signaling constrains the migration of incipient premalignant lesions [72]; however, as the tumor evolves and increases its aggressiveness this initial antitumor mechanism gets rewired. Invasive cancer cells upregulate ephrin-B2 levels to activate Eph forward signaling through homotypic cell–cell interactions that support migration [35] (Fig. 3.2B).

The acquisition of migratory properties in glioma cells could also be linked to the cell of origin [36]. Glioma cells with an Olig2+ oligodendrocyte precursor origin, which are cells performing vascular co-option during their normal development [12], are more prone to invade using the preexisting vessels (add ref 36). Glioma cells hijack the developmental pathway used by OPC to migrate, which involves the secretion of Wnt7b (Fig. 3.1). Besides the poorer prognosis of infiltrative gliomas, the fact that vascular co-option maintains the structure of the BBB better than the disruption induced in the process of angiogenesis could have important implications for treatment [73]. In spite of being highly sensitive to Wnt inhibitors such as porcupine, Olig2+ glioma cells are more protected from chemotherapies that do not cross the BBB than Olig2− cells. In contrast, the infiltration of immune cells will be higher in angiogenic Olig2− tumors than Olig2+ gliomas. This will define the type of immunophenotype (change "immunophenotyped" for "immunophenotype") d that these tumor types will develop [36], which might have important implications in emerging strategies using the immune system to target cancer cells.

Molecular regulation of dormancy, latency, and awakening linked to vascular co-option

As slow-proliferative normal stem cells located at the perivascular niche do, cancer cells could develop a state of quiescence associated with vessels. This state could be induced by molecules produced by endothelial cells or derived from the intrinsic properties of cancer cells. Although several programs have been described, it is not known whether they are interconnected.

Thrombospondin-1 (TSP-1) was initially described as a molecule secreted by endothelial cells capable of preventing tumor growth due to

the inhibition of angiogenesis [74,75]. Additionally, in brain, bone, and lung metastasis, co-opted endothelial cells producing TSP-1 induce dormancy on co-opting cancer cells through unknown mechanism (Fig. 3.2).

Complementarily, endothelial cells undergoing sprouting angiogenesis downregulate TSP-1 and increase TGFβ1,(Remove this comma) and periostin that promotes tumor cell growth [44,76].

Additional programs of latency described in metastatic cells at the perivascular space depend on the presence of the transcription factors SOX2 and SOX9, which induce the expression of the Wnt inhibitor DKK1. Cancer cell–secreted DKK1 prevents Wnt-dependent influence on B-catenin, thus blocking its potential positive influence on proliferation [49]. Thus when DKK1 is targeted in cancer cells, metastatic cells transit out of dormancy and resume proliferation. However, cancer cells forced to leave the latent state are rapidly eliminated by host innate defenses due to the induction of NK-cells ligands expression derived from loss of SOX expression and gain of Wnt signaling [49] (Fig. 3.2). Consequently, the switch from dormant/latent state to a productive proliferative state requires additional regulatory mechanisms. Metastatic cells from breast cancer in the lung, bone, and brain use TM4SF1 to switch from dormancy to aggressive growth [63]. This phenotypic switch in vascular co-opting cells is initiated by the upregulation of TM4SF1, which couples the collagen I receptor DDR1 to PKCα that activates the JAK–STAT pathway, which effectively drives proliferation of metastatic cells [63]. Interestingly, this non(non-canonical)canonical TM4SF1 signaling pathway also enhances CSC properties inducing SOX2 and NANOG, which reinforces the link of CSC traits and metastatic reactivation [63]. Additional mechanisms developed by cancer cells to escape from the proliferative-checkpoint imposed at the perivascular niche have been described. By inducing the secretion of FGF4, indolent tumor cells co-opting blood vessels are able to activate the FGFR1 in endothelial cells, which drives ETS2-dependent downregulation of IGFBP7 expression(add ref 64). Consequently, co-opting cancer cells start to receive increased amounts of IGF1 due to the reduction of extracellular IGFBP7, which was previously sequestering the growth factor and preventing its strong effect on proliferation [64] (Fig. 3.2). IGF1 not only promotes tumor aggressiveness but also makes cancer cells chemoresistant [64].

Preclinical applications to target vascular co-option: prevention of metastasis

Genetic targeting of cancer cell adhesion molecules L1CAM and β1-integrin has probed the potential of developing anti(anti-metastatic)metastatic pharmacological strategies on the basis of vascular co-option. Both spontaneous and induced metastases from lung cancer, breast cancer, renal

cancer,(remove the comma) and melanoma potentially affecting the bone, lungs, brain, and liver were dramatically reduced after targeting any of these cell adhesion molecules [2,39,50,52]. Not only metastases but also difficult-to-treat and life-threatening gliomas were highly sensitive to a combination therapy based on an antiangiogenic drug (bevacizumab) and a β1-integrin antagonist (OS2966) when applied to xenografts [77]. A similar combination therapy based on capecitabine, an antiangiogenic drug (B20-4.1.1), and the genetic inhibition of the Arp2/3 complex, required for vascular co-option, was superior to anti(anti-angiogenesis)angiogenesis monotherapy when applied to experimental breast cancer models that develop spontaneous liver metastasis [7]. Additional successful therapeutic strategies targeted Ephrin-B2 using blocking antibodies in experimental glioblastoma models [35].

Besides blocking vascular co-option to target metastasis, impairing the support provided by the perivascular niche has been shown to reduce the increased resistance to chemotherapies. For instance, bone metastasis were sensitized to doxorubicin and taxol by using blocking antibodies against β1 and $\alpha_V\beta_3$ [55]. Consequently, using therapeutic strategies targeting vascular co-option might be useful to reduce resistant cells at the perivascular niche that will drive relapse later on. Similarly, cells that might escape surgery because they have migrated out of the tumor core is a frequent cause of relapse in gliomas. Targeting cancer cells co-opting vessels by combining Wnt inhibitors LGK974 or XAV939 with the standard of care temozolomide increased the control of Olig2+ gliomas demonstrating the value of this adjuvant therapy [36].

One important caveat of targeting vascular co-option as an anti(anti-cancer)cancer strategy is the potential toxic effects of targeting normal cells that require a perivascular location (Fig. 3.1). Although several examples of normal cells developing vascular co-option are specific to development (i.e., L1CAM for axonal growth), others must be considered (i.e., pericytes or adult stem cells) since damaging them will have important implications. Consequently, besides genetic strategies that have been a valuable proof-of-concept to demonstrate the enormous potential of targeting vascular co-option as a novel anti(anti-metastasis)metastasis strategy, more pharmacological strategies are needed specially directed against aspects of vascular co-option specific for cancer cells.

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Further reading (These refs could be removed)

Li F., Lan Y., Wang Y., Wang J., Yang G., Meng F., et al., Endothelial Smad4 maintains cerebrovascular integrity by activating N-cadherin through cooperation with notch, *Dev Cell [Internet]* **20** (3), 2011, 291–302, Available from: <http://www.sciencedirect.com/science/article/pii/S1534580711000396>.

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Abstract

Vascular co-option is an alternative mechanism to interact with blood vessels used by normal cells and hijacked by cancer cells. Functional experiments using in vivo models have demonstrated that co-option o pre(pre-existing)existing capillary networks is a critical step for the initiation of multiorgan metastases from different cancer types. Thus targeting this process pharmacologically is a very promising strategy to preven metastases. Vascular co-option supports the viability of disseminated tumor cells, even when they remain dormant. Additionally, targeting vascular co-option could be a valuable strategy to challenge the emergence o resistance to more conventional therapies.

Keywords: Vascular co-option; capillaries; metastasis; dormancy; immunosuppression; aggressiveness; adhesion; stemness; angiocrine factors

Queries and Answers

Query: The city and country names have been inserted for the affiliation. Please check, and correct if necessary.

Answer: Correct

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Answer: We have added refs and remove others