Query Details Back to Main Page

1. Figures 1 and 2 were received; however, no citation was provided in the manuscript. Please check and confirm the inserted citation of Figures 1 and 2 are correct. If not, please suggest an alternative citation. Please note that figures and tables should be cited in ascending numerical order in the text. and should be inside the main body of the text.

There is no figure 2. It was replaced when I answered the reviewers by the new version, which is now Figure 1.
I have added a citation for Figure 1 in the text

2. Kindly confirm the processed Figure 2 is correct and provide its caption which is missing.

Figure 2 was eliminated in the correction in response to editors. THERE IS ONLY ONE FIGURE, which is figure 1. Please remove figure 2 from the final version

3. Keywords has been taken from the submissive system. Kindly check and confirm.

I have checked and they are ok with the exception that I request to remove "Molecular regulation"

Vascular co-option in brain metastasis

Pedro García-Gómez, [1]

Email pggomez@cnio.es

Manuel Valiente, [1]✉

Email mvaliente@cnio.es

[1] Brain Metastasis Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain

Received: 18 July 2019 / Accepted: 26 October 2019

Abstract

Vascular co-option by brain metastasis-initiating cells has been

demonstrated as a critical step in organ colonization. The physical interaction between the cancer cell and the endothelial cell is mediated by integrins and L1CAM and could be involved in aggressive growth but also latency and immune evasion. The key involvement of vascular co-option in brain metastasis has created an emerging field that aims to identify critical targets as well as effective inhibitors with the goal of preventing brain metastases.

Keywords

Brain metastasis
Capillaries
Breast cancer
Lung cancer
Melanoma
Vascular co-option
Molecular regulation (please remove this keyword)

Vascular co-option

Holash et al. [1] reported that gliomas and metastatic cells in the lungs interact physically with preexisting vessels without any sign of angiogenesis and termed this cell-to-cell interaction vascular co-option. Further work has demonstrated that, although not specific to the brain, metastatic cells in this organ are highly dependent on vascular co-option to sustain tumor progression, especially during the initial stages of colonization after they completed extravasation. In fact, extravasated cancer cells do not move away from capillaries but they instead remain physically in contact with the abluminal side of the vessel. This cell-to-cell interaction is defined as vascular co-option, and has been proposed as a during hallmark metastasis initiation [2, 3, 4, 5, 6, 7]. Since vascular co-option does not involve major structural modifications of blood vessels in contrast to angiogenesis, the underlying regulatory mechanisms of both phenomena are different. The massive attention and research efforts devoted to angiogenesis in the past have not generated sufficient clinical benefits [8, 9, 10, 11, 12, 13]. The inefficacy reported on the use of anti-angiogenic drugs might have different explanations, for instance, the generation of a hypoxic environment, which is known to increase

invasiveness and resistance to therapy. In fact, vascular co-option has been proposed to underlie resistance to anti-angiogenic therapy [10, 11, 12]. In addition, the vasculature associated with brain metastasis has a higher proportion of mature and preexisting vessels in comparison with their matched primary tumors [14], which might explain the particular importance of vascular co-option in this organ.

Vascular co-option in brain metastasis

Vascular co-option is the first step in brain colonization after cancer cells cross the blood–brain barrier (BBB). Extravasated cancer cells remain in the perivascular niche interacting with the basal lamina of capillaries [2, 3, 5, 6, 15]. This interaction with the basal lamina of the vessels allows metastatic cells to get access to the rich matrix of the basal lamina in addition to a preferential location to obtain nutrients and oxygen and other factors produced by endothelial cells (angiocrine factors). All these benefits might be key to sustain the viability during the demanding initial moments of organ colonization and to facilitate the re-initiation of cancer cell proliferation [16, 17, 18, 19, 20, 21]. A detailed description of the vascular co-option process was performed following intracarotid injection of breast and melanoma cancer cells using detailed immunofluorescence and immunohistochemistry to track them through confocal imaging applied to fixed tissues during obtained during the initial steps of brain colonization [5]. Although the authors reported the existence of differences between tumor types, once metastatic cells completed extravasation 5 to 7 days post-injection, cancer cells lined up along capillaries forming elongated clusters of metastatic cells [5]. Although notorious changes in the brain microenvironment correlate with the presence of metastatic cells, such as those reported in astrocytes and microglia, they do not involve changes to capillaries, at least at the morphological level [5] (Figs. 1, 2).

Fig. 1

Schema of the process of vascular co-option and its molecular regulation in brain metastasis-initiating cells. **a** A mouse previously inoculated with brain metastatic cells is analyzed before the developlopment of macrometastases . In the brain, metastatic cells are found at the very early stages of colonization. A schema depicts a time frame of the process of vascular co-option after extravasation. Three steps could be differentiated including

adhesion (1), spreading (2), and perivascular proliferation (3). Specific molecules involved in each step are depicted. Blood vessels are depicted in red and cancer cells are in green. A cross-section is provided in (2) to emphasize the contact between the cancer cells and the basal lamina of the vessel. *Cc* cancer cell, *ec* endothelial cell, *bl* basal lamina. **b** Brain metastatic cells could remain latent or quiescent or re-initiate growth, all of which are associated with vessels. Main mechanisms described are depicted. Transcription factors Sox2 and Sox9 have been shown to be required for avoiding the attack of the immune system during latency and reactivation of proliferation since they downregulate NK ligands

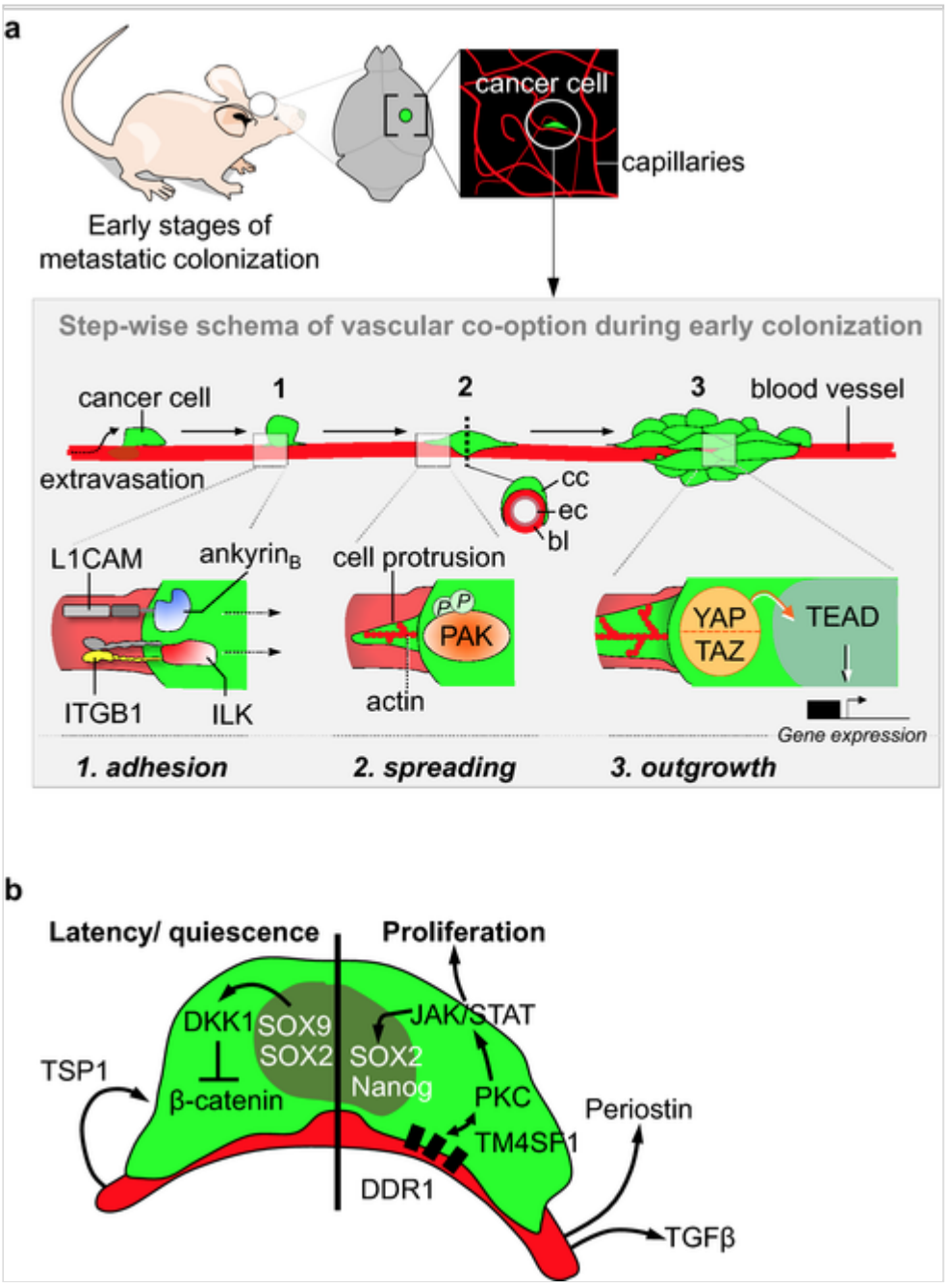
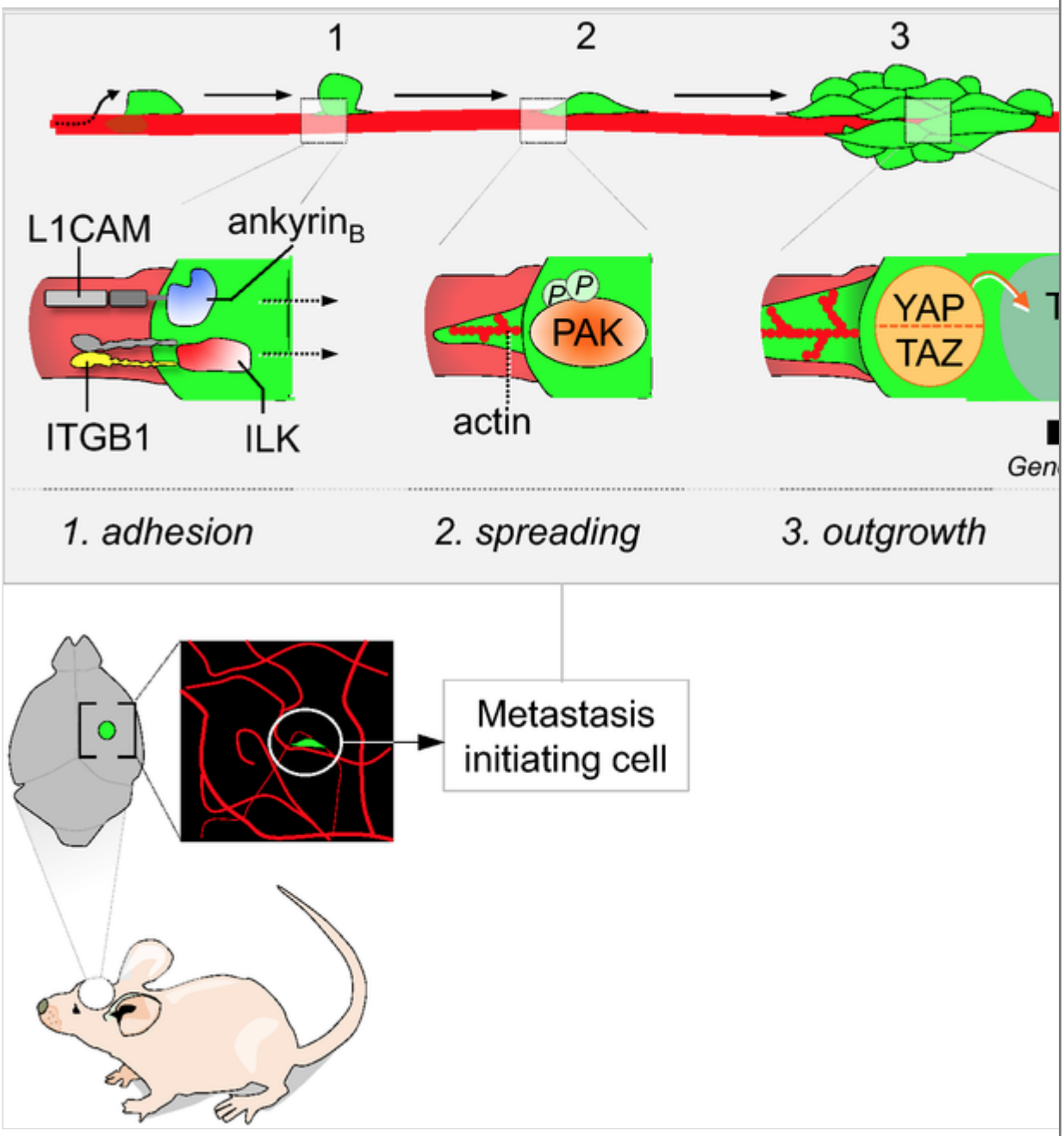


Fig. 2 (please remove Fig2 from the final version, this was replaced by figure 1 shown above)

.



AQ1

AQ2

More recently, a more sophisticated approach based on multiphoton laser-scanning video-microscopy was applied to experimental lung and melanoma brain metastasis to characterize the early stages of colonization in vivo [2]. The authors were able to describe the essential steps of brain

metastatic colonization that must be accomplished by metastasis-initiating cells in order to generate macrometastases. The steps include intravascular arrest at microvessels, extravasation, vascular co-option, and perivascular growth [2]. Interestingly, differences were observed in the way cancer cells interact with vessels depending on the primary tumor type they originated from. Melanoma cells grew via vascular co-option whereas lung cancer cells switch to angiogenesis-dependent growth [2]. Of note, additional lung cancer experimental brain metastasis models have been shown to be dependent on vascular co-option by several groups [3, 4]. In vivo video-microscopy demonstrated that in the absence of a close physical contact with blood vessels, cancer cells are unable to grow in the brain [2]. However, this research also showed that the mere contact with the abluminal side of brain capillaries does not grant the ability to grow since non-proliferating dormant metastatic cells were found performing vascular co-option and migrating along preexisting vessels. This suggests that vascular co-option also supports the viability of dormant metastatic cells [2].

AQ3

The process of vascular co-option is additionally required during local invasion within the brain. This has been analyzed after intracranial injection of melanoma cells by measuring cancer cell migration out of the lesion core [6]. Melanoma cells at invasive fronts traveled long distances up to 581 μm in 4 weeks in contact with the abluminal side of preexisting vessels [6]. This observation was also validated in human brain metastasis samples [6].

Molecular regulation of vascular co-option

Vascular co-option has been linked to several cancer hallmarks, which emphasize its central role in the process of metastasis. Consequently, the molecular characterization of vascular co-option is key to understand the biology of this interaction that might help to develop novel therapies aiming at blocking brain metastasis from the very initial moment of colonization.

Molecular regulation of cell adhesion, proliferation, and invasion

Metastatic cells attach to the abluminal side of preexisting vessels as the

first step following extravasation in the brain [2, 3, 5, 6]. This interaction is dependent on different adhesion molecules including integrins and L1CAM [3, 15].

β1-integrin on the metastatic cells plays a key role in governing the interaction with different components of the basal lamina of brain capillaries including fibronectin, laminin, vitronectin, and collagen I and IV [15]. Intracardiac and intracranial injection of breast cancer and melanoma cell lines engineered with loss function of β1-integrin proved that both adhesion to the basal lamina of vessels and proliferation of cancer cells in the brain were severely disrupted [15]. The interaction of cancer cells with the basal lamina of co-opted capillaries activates β1-integrin, which leads to FAK activation and ERK1/2 phosphorylation that regulates the proliferative phenotype of co-opting cancer cells [15]. Additional integrins have been involved in vascular co-option. For instance, β4-integrin from breast cancer cells has been shown to bind to components of the basal lamina to activate receptor tyrosine kinases (RTK) such as ErbB2 [22]. Activation of this RTK was shown to induce VEGF production, thus influencing endothelial cells to favor tumor growth and to potentiate cancer cell adhesion to the vessels [22]. α6-integrin has been shown to play a similar role in acute lymphoblastic leukemia (ALL). However, in this case, cancer cells do not cross the blood–brain barrier (BBB), but instead migrate through arachnoid vessels connecting the skull or vertebral bone marrow with the subarachnoid space. α6-integrin mediates the interaction with laminin-rich basal lamina of the blood vessels demonstrating a critical function of vascular co-option in ALL [23]. Since the expression of α6-integrin in leukemia cells is regulated by PI3K, targeting it prevented their ability to enter in the meningeal space [23].

In addition to integrins, other adhesion molecules are important in vascular co-option. L1CAM, a molecule critical for nervous system development [24] and able to bind to different receptors on endothelial cells and axons for neuronal guidance including itself in a homophilic interaction, integrins, ErbB receptors, and FGF receptors [25, 26, 27], was found to be equally important in the interaction between cancer cells and endothelial cells in brain metastasis [3]. Using loss of function approaches for L1CAM in lung and breast cancer cells, the authors reported an

impaired ability of metastatic cells to spread along the vessels. This morphological phenotype was shown to have a major impact on the ability of cancer cells to proliferate and thus prevented the generation of macrometastases [3]. This initial finding was subsequently expanded to additional experimental models representing different primary tumors metastatic to the brain but also to other organs [4]. These experiments demonstrated that vascular co-option is a key mechanism for the initiation of multi-organ metastasis in a broad number of solid tumors. At the molecular level, the role of L1CAM in vascular co-option consists of promoting β1-integrin signaling, which leads to PAK1/2 phosphorylation and Arp2/3 activation in an ILK-dependent manner [4]. Activation of the L1CAM-integrin pathway generates the induction of cell protrusions on cancer cells that underlie cell spreading along the endothelium [4]. When cancer cells are spread along the abluminal side of the vessels, the mechanosensitive YAP pathway becomes activated initiating a gene expression program involving the induction of the cell cycle that resumes cancer cell proliferation by metastases [4] (Fig1a).

Therefore, pharmacological strategies targeting integrins and/or L1CAM and their downstream signaling could potentially abrogate the initial stages of brain colonization preventing the formation of macrometastases [3, 4, 15].

Molecular regulation of dormancy, latency, and awakening

Vascular co-option has been also demonstrated to be important for dormant or quiescent cells [2, 5]. Some mediators of the crosstalk between endothelial cells and this special state of cancer cells have been discovered.

Thrombospondin-1 (TSP-1) is produced by endothelial cells that are associated with quiescent breast cancer cells in the brain. TSP-1 favors the non-proliferative state of metastatic cells by unknown mechanisms [28]. In fact, downregulation of TSP-1 and increased production of periostin and TGFβ1 seems to underlie the switch towards the activation of the pro-angiogenic program in endothelial cells, which correlates with the activation of tumor growth [28]. Additional mechanisms regulating dormancy involve the expression of the transcription factors SOX2 and

SOX9 in cancer cells [29]. Both transcription factors induce the secretion of the Wnt inhibitor DKK1 in co-opting metastatic cells. DKK1 impairs β-catenin signaling thus preventing this proliferative stimulus [29]. Consequently, targeting DKK1 in cancer cells forced transiting cancer cells out of dormancy. However, activation of β-catenin signaling also reduced SOX-mediated repression of NK-ligands, making cancer cells discoverable to innate defenses that immediately eliminate them [29]. Accordingly, additional regulatory mechanisms are needed to protect vascular co-opting metastatic cells when switching from a dormant to a proliferative state. Recent findings have started to clarify the underlying molecular regulation [30]. Latent vascular co-opting cancer cells could induce the expression of *TM4SF1*, which couples DDR1, a collagen I receptor, to PKCα thus activating the JAK-STAT pathway [30]. This signaling pathway can induce proliferation of co-opting cancer cells simultaneously to the induction of SOX2 and NANOG, thus avoiding the recognition of the immune system. In addition, this pathway reinforces the association between stem cells, which frequently reside at perivascular niches, and metastasis outgrowth [30] (Fig1b).

Vascular co-option in human brain metastasis

The potential relevance of vascular co-option derived from experimental models has been validated in human brain metastasis samples. The clinical evidence of vascular co-option in brain metastasis is being considered as a potential source of biomarkers and novel targets that might help to improve the efficacy of treatments against disseminated cancer cells [6, 14, 31].

Brain metastasis from different primary origins was evaluated by the presence of any evidence of local invasion. Various patterns were reported including one compatible with vascular co-option. This co-optive growth pattern was especially relevant in brain metastasis coming from melanoma and non-small-cell lung cancer (NSCLC) [31]. Moreover, a 63.2% increase in mature vessels compared to neo-angiogenic vessels was found when comparing NSCLC brain metastasis to primary tumors of the lung, which favors the possibility that secondary brain tumors rely more on vascular co-option than angiogenesis, in contrast to the opposite situation that would apply to the primary tumor [14]. This data suggest that anti-

angiogenic drugs might not be effective impairing metastasis in the brain, as has been suggested by failures in several clinical trials [8, 32, 33, 34].

Perspective

Given the limited benefits of targeting angiogenesis, increasing attention is being gained by alternative mechanisms by which cancer cells interact with blood vessels. Among them vascular co-option has been proved to be key for metastasis initiation. As such, it has the potential to become a novel target for those therapeutic strategies aiming to block the very early steps of organ colonization, not only in the brain but elsewhere. However, pharmacological strategies are yet to be developed for this purpose since available blocking antibodies against known molecular mediators are not the best positioned reagents to be used in the brain. In spite of this current limitation, increasing knowledge on the molecular regulation of vascular co-option will surely provide novel pharmacological strategies that could be exploited in relevant pre-clinical models. In this sense, it is important to notice that the contribution of vascular co-option to the advanced stages of colonization is still unknown and must be studied with appropriate experimental designs. For instance, the presence of invasive fronts in a percentage of established brain metastasis could correlate with more an aggressive behavior or increased rates of relapse after neurosurgery, which should be addressed given the obvious clinical importance. In addition, defining the components of the basal lamina that might provide benefits to co-opting metastasis-initiating cells remain to be defined. This research could provide important biological insights regarding the different behaviors associated with co-opting cancer cells (i.e., aggressive versus quiescent cancer cells). Finally, vascular co-option could provide novel biomarkers in the future given the privileged access of metastasis-associated secreted molecules to systemic circulation. These biomarkers, which will be compatible with non-invasive liquid biopsies, could, in the future, guide clinicians to detect emerging metastasis in the brain and elsewhere. Such a possibility will favor the efficacy of current and future therapies with the final aim of preventing metastases.

Publisher's Note

Acknowledgements

Research in the Brain Metastasis Group is supported by MINECO-Retos SAF2017-89643-R (M.V.), Bristol-Myers Squibb-Melanoma Research Alliance Young Investigator Award (498103) (M.V.), Beug Foundation's Prize for Metastasis Research (M.V.), Fundación Ramón Areces (CIVP19S8163) (M.V.), Worldwide Cancer Research (19-0177) (M.V.), H2020-FETOPEN (828972) (M.V.), CLIP Award Cancer Research Institute (54545) (M.V.), and AECC Coordinated Translational Groups 2017 (GCTRA16015SEOA) (M.V.). P.G-G. is the recipient of La Caixa Foundation (ID100010434) and European Union´s Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement (No. 713673) PhD Program Fellowship (LCF/BQ /IN17/11620028). M.V. is a Ramón y Cajal Investigator (RYC-2013-13365) and EMBO YIP (4053).

References

1. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D et al (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284(5422):1994–1998

2. Kienast Y, Von Baumgarten L, Fuhrmann M, Klinkert WEF, Goldbrunner R, Herms J et al (2010) Real-time imaging reveals the single steps of brain metastasis formation. Nat Med 16(1):116

3. Valiente M, Obenauf AC, Jin X, Chen Q, Zhang XH-F, Lee DJ et al (2014) Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell 156(5):1002–1016

4. Er EE, Valiente M, Ganesh K, Zou Y, Agrawal S, Hu J et al (2018) Pericyte-like spreading by disseminated cancer cells activates YAP and MRTF for metastatic colonization. Nat Cell Biol 20:966

5. Lorger M, Felding-Habermann B (2010) Capturing changes in the brain microenvironment during initial steps of breast cancer brain metastasis. Am J Pathol 176(6):2958–2971

6. Bentolila LA, Prakash R, Mihic-Probst D, Wadehra M, Kleinman HK, Carmichael TS et al (2016) Imaging of angiotropism/vascular co-option in a murine model of brain melanoma: implications for melanoma progression along extravascular pathways. Sci Rep 6:23834

7. Nguyen DX, Chiang AC, Zhang XH-F, Kim JY, Kris MG, Ladanyi M et al (2009) WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. Cell 138(1):51–62

8. Ilhan-Mutlu A, Osswald M, Liao Y, Gömmel M, Reck M, Miles D et al (2016) Bevacizumab prevents brain metastases formation in lung adenocarcinoma. Mol Cancer Ther 15:702–710

9. Ilhan-Mutlu A, Siehs C, Berghoff AS, Ricken G, Widhalm G, Wagner L et al (2016) Expression profiling of angiogenesis-related genes in brain metastases of lung cancer and melanoma. Tumor Biol 37(1):1173–1182

10. Frentzas S, Simoneau E, Bridgeman VL, Vermeulen PB, Foo S, Kostaras E et al (2016) Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. Nat Med 22:1294. https://doi.org/10.1038/nm.4197

11. Reynolds AR, Bar-Zion A, Lee CR, Kuczynski EA, Foster FS, Daley F et al (2016) Co-option of Liver vessels and not sprouting angiogenesis drives acquired Sorafenib resistance in hepatocellular carcinoma. JNCI J Natl Cancer Inst. https://doi.org/10.1093/jnci/djw030

12. Bridgeman VL, Vermeulen PB, Foo S, Bilecz A, Daley F, Kostaras E et al (2017) Vessel co-option is common in human lung metastases and mediates resistance to anti-angiogenic therapy in preclinical lung metastasis models. J Pathol 241(3):362–374. https://doi.org/10.1002/path.4845

13. Yano S, Shinohara H, Herbst RS, Kuniyasu H, Bucana CD, Ellis LM et al (2000) Expression of vascular endothelial growth factor is necessary but not sufficient for production and growth of brain metastasis. Cancer Res 60(17):4959–4967

14. Jubb AM, Cesario A, Ferguson M, Congedo MT, Gatter KC, Lococo F et al (2011) Vascular phenotypes in primary non-small cell lung carcinomas and matched brain metastases. Br J Cancer 104:1877. https://doi.org/10.1038/bjc.2011.147

15. Carbonell WS, Ansorge O, Sibson N, Muschel R (2009) The vascular basement membrane as “soil” in brain metastasis. PLoS ONE 4(6):e5857

16. Lorenz L, Axnick J, Buschmann T, Henning C, Urner S, Fang S et al (2018) Mechanosensing by β1 integrin induces angiocrine signals for liver growth and survival. Nature 562(7725):128–132. https://doi.org/10.1038/s41586-018-0522-3

17. Rafii S, Butler JM, Ding B-S (2016) Angiocrine functions of organ-specific endothelial cells. Nature 529:316. https://doi.org /10.1038/nature17040

18. Lu J, Ye X, Fan F, Xia L, Bhattacharya R, Bellister S et al (2013) Endothelial cells promote the colorectal cancer stem cell phenotype through a soluble form of jagged-1. Cancer Cell 23(2):171–185

19. Wieland E, Rodriguez-Vita J, Liebler SS, Mogler C, Moll I, Herberich SE et al (2017) Endothelial Notch1 activity facilitates metastasis. Cancer Cell 31(3):355–367

20. Beck B, Driessens G, Goossens S, Youssef KK, Kuchnio A, Caauwe A et al (2011) A vascular niche and a VEGF–Nrp1 loop regulate the initiation and stemness of skin tumours. Nature 478(7369):399

21. Cao Z, Ding B-S, Guo P, Lee SB, Butler JM, Casey SC et al (2014) Angiocrine factors deployed by tumor vascular niche induce B cell lymphoma invasiveness and chemoresistance. Cancer Cell 25(3):350–365

22. Fan J, Cai B, Zeng M, Hao Y, Giancotti FG, Fu BM (2011) Integrin β4 signaling promotes mammary tumor cell adhesion to brain microvascular endothelium by inducing ErbB2-mediated secretion of

VEGF. Ann Biomed Eng 39(8):2223–2241

23. Yao H, Price TT, Cantelli G, Ngo B, Warner MJ, Olivere L et al (2018) Leukaemia hijacks a neural mechanism to invade the central nervous system. Nature 560(7716):55–60. https://doi.org/10.1038/s41586-018-0342-5

24. Wiencken-Barger AE, Mavity-Hudson J, Bartsch U, Schachner M, Casagrande VA (2004) The role of L1 in axon pathfinding and fasciculation. Cereb Cortex 14(2):121–131

25. Felding-Habermann B, Silletti S, Mei F, Siu C-H, Yip PM, Brooks PC et al (1997) A single immunoglobulin-like domain of the human neural cell adhesion molecule L1 supports adhesion by multiple vascular and platelet integrins. J Cell Biol 139(6):1567–1581

26. Donier E, Gomez-Sanchez JA, Grijota-Martinez C, Lakomá J, Baars S, Garcia-Alonso L et al (2012) L1CAM binds ErbB receptors through Ig-like domains coupling cell adhesion and neuregulin signalling. PLoS ONE 7(7):e40674

27. Kulahin N, Li S, Hinsby A, Kiselyov V, Berezin V, Bock E (2008) Fibronectin type III (FN3) modules of the neuronal cell adhesion molecule L1 interact directly with the fibroblast growth factor (FGF) receptor. Mol Cell Neurosci 37(3):528–536

28. Ghajar CM, Peinado H, Mori H, Matei IR, Evason KJ, Brazier H et al (2013) The perivascular niche regulates breast tumour dormancy. Nat Cell Biol 15(7):807

29. Malladi S, Macalinao DG, Jin X, He L, Basnet H, Zou Y et al (2016) Metastatic latency and immune evasion through autocrine inhibition of WNT. Cell 165(1):45–60

30. Gao H, Chakraborty G, Zhang Z, Akalay I, Gadiya M, Gao Y et al (2016) Multi-organ site metastatic reactivation mediated by non-canonical discoidin domain receptor 1 signaling. Cell 166(1):47–62

31. Berghoff AS, Rajky O, Winkler F, Bartsch R, Furtner J,

Hainfellner JA et al (2013) Invasion patterns in brain metastases of solid cancers. Neuro Oncol 15(12):1664–1672

32. Soria J-C, Mauguen A, Reck M, Sandler AB, Saijo N, Johnson DH et al (2012) Systematic review and meta-analysis of randomised, phase II/III trials adding bevacizumab to platinum-based chemotherapy as first-line treatment in patients with advanced non-small-cell lung cancer. Ann Oncol 24(1):20–30

33. Reck M, von Pawel J, Zatloukal P, Ramlau R, Gorbounova V, Hirsh V et al (2009) Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non–small-cell lung cancer: AVAiL. J Clin Oncol 27(8):1227–1234

34. Jain RK (2014) Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. Cancer Cell 26(5):605–622