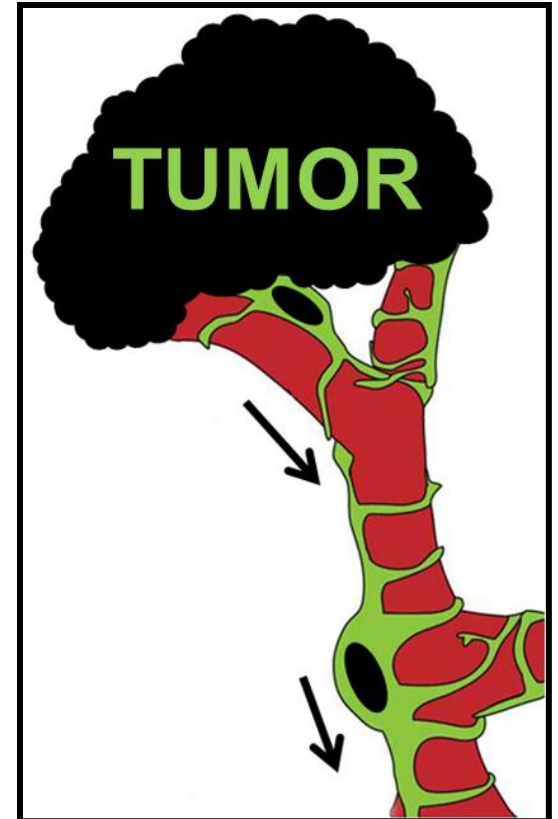


Angiotropic Extravascular Migratory Metastasis in Melanoma

Claire Lugassy and Raymond Barnhill

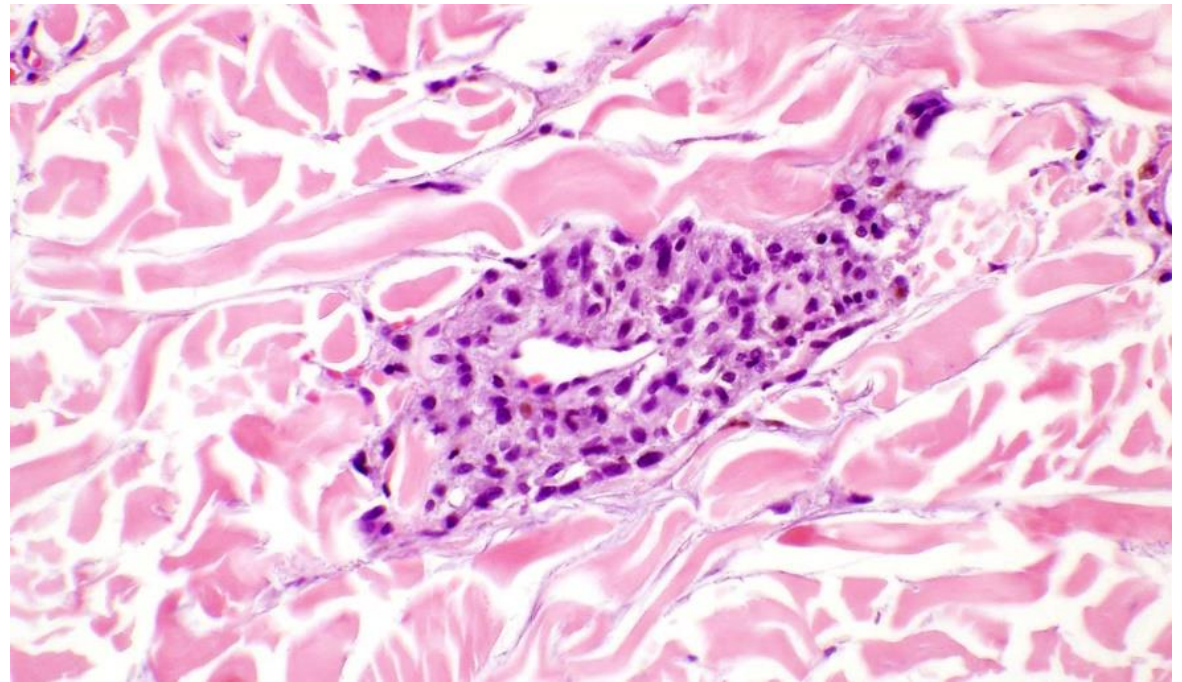
Angiotropism in Melanoma

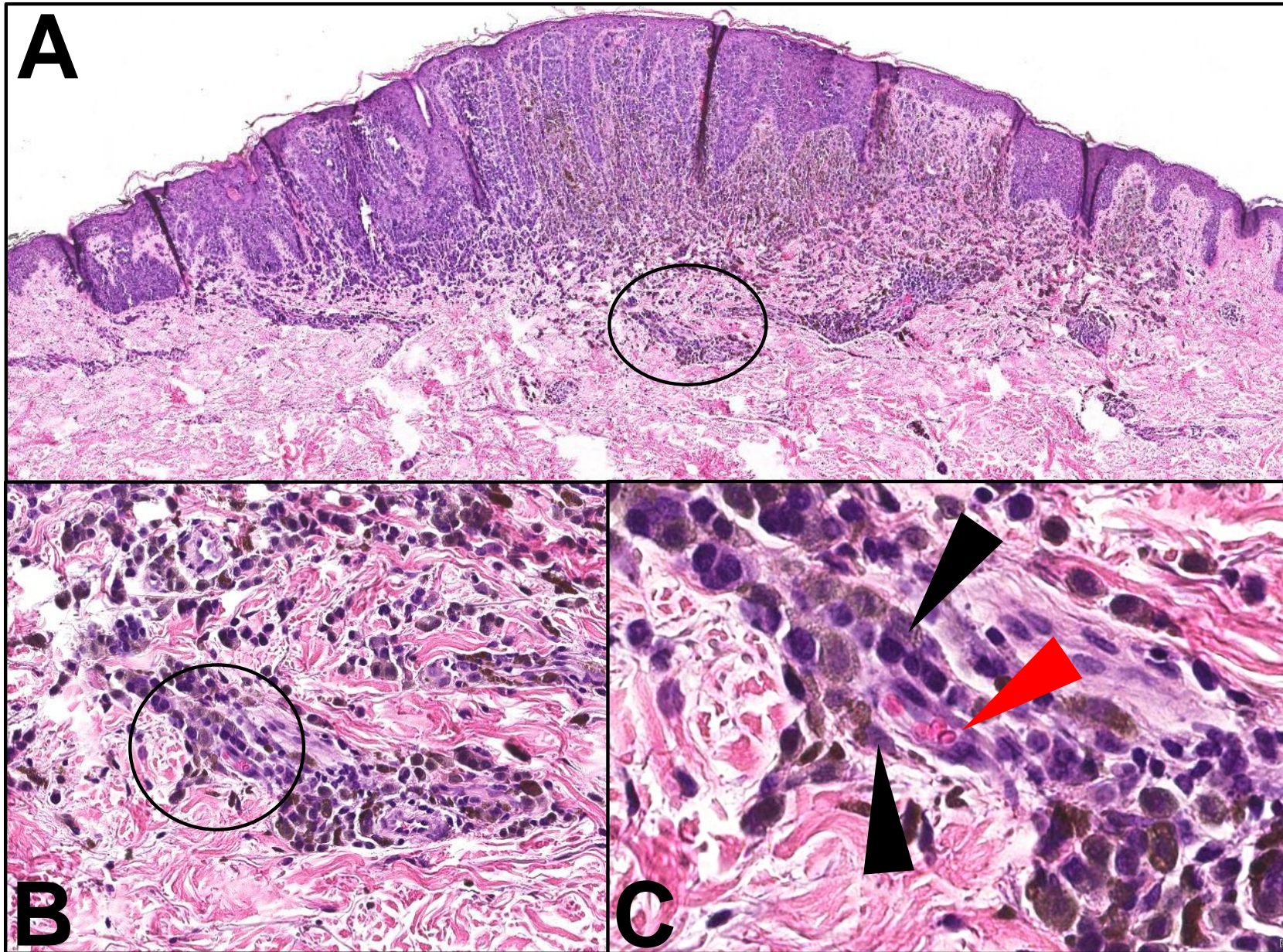
- Angiotropism is the presence of tumor cells arrayed along the external surfaces of vascular channels at the advancing front without intravasation
- Histopathologists utilizing standardized histological criteria can reliably and reproducibly identify angiotropism in human melanoma



Angiotropism in Cutaneous Melanoma: Histopathological image

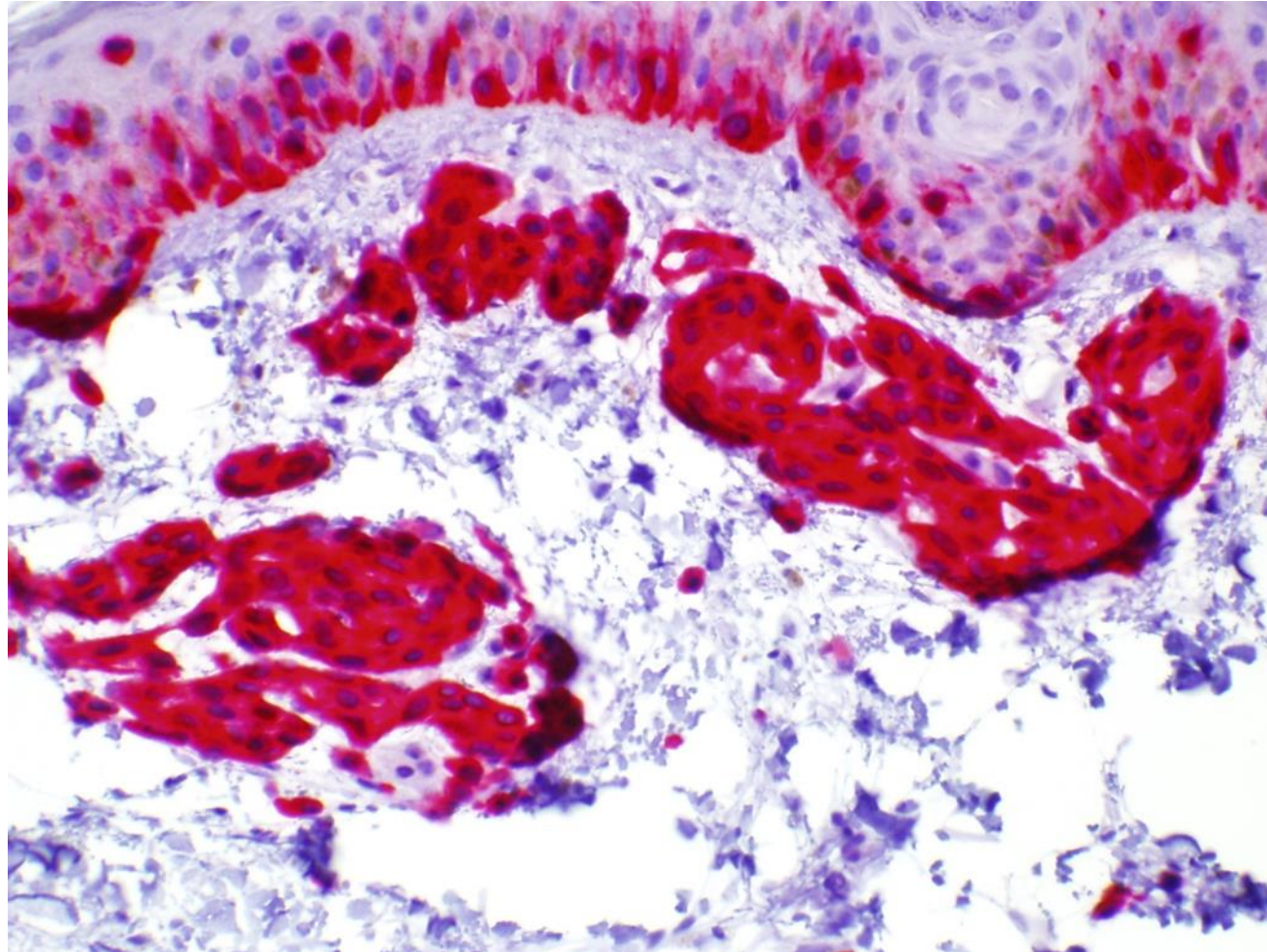
- Advancing front
- Melanoma cells disposed along microvessels
- Without intravascular invasion



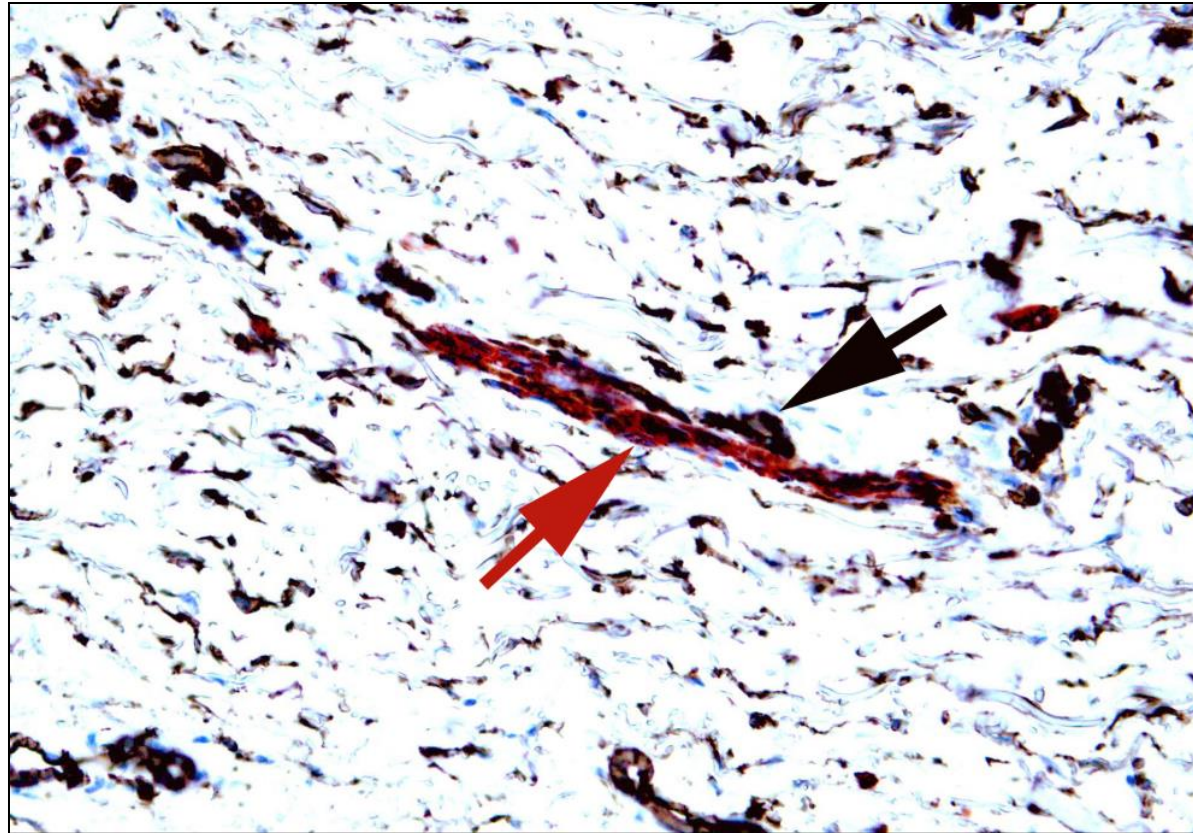


Angiotropism in primary cutaneous melanoma.

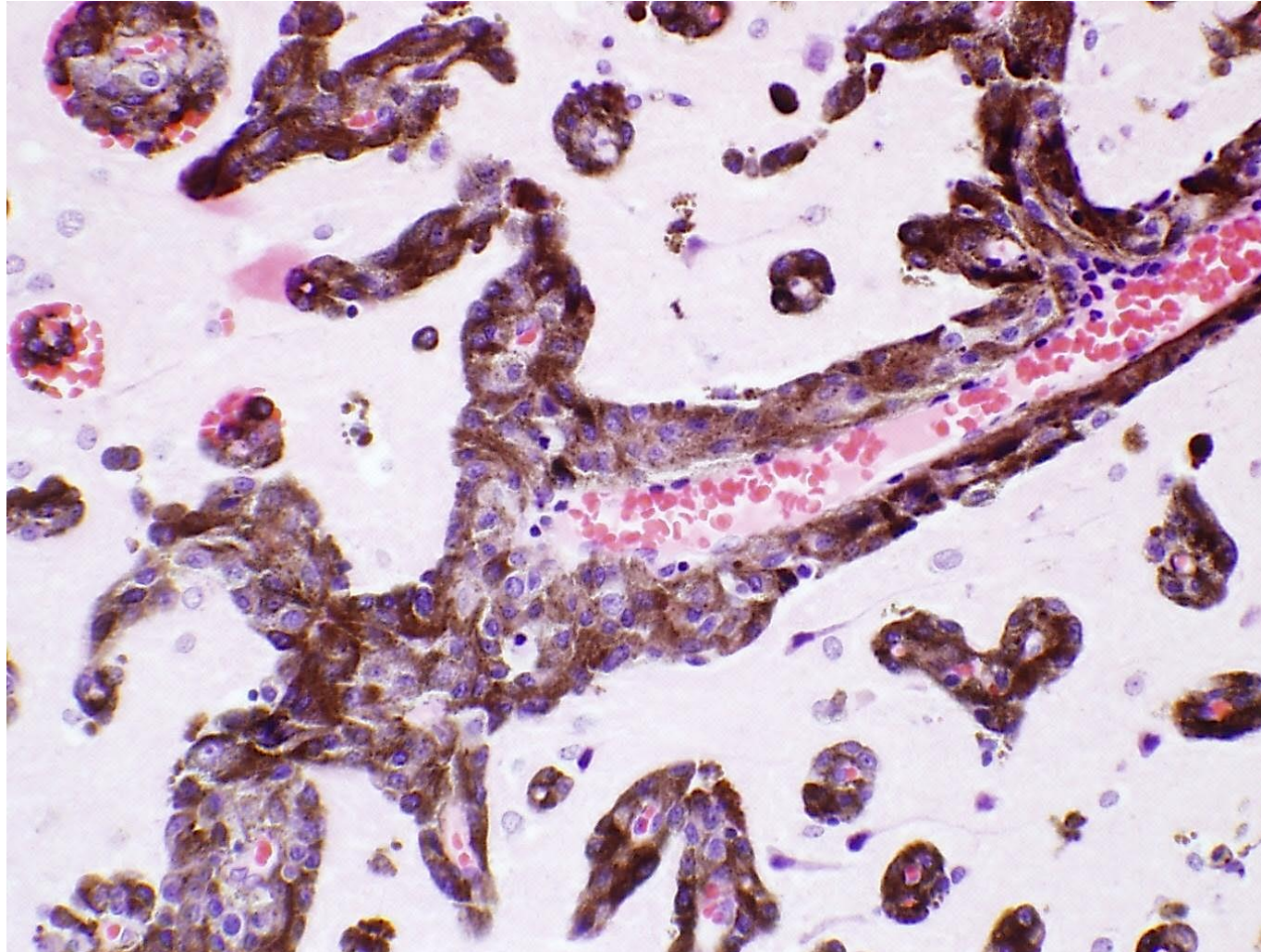
Immunohistochemistry with Melan-A



Double Immunohistochemistry Melan-A and CD31



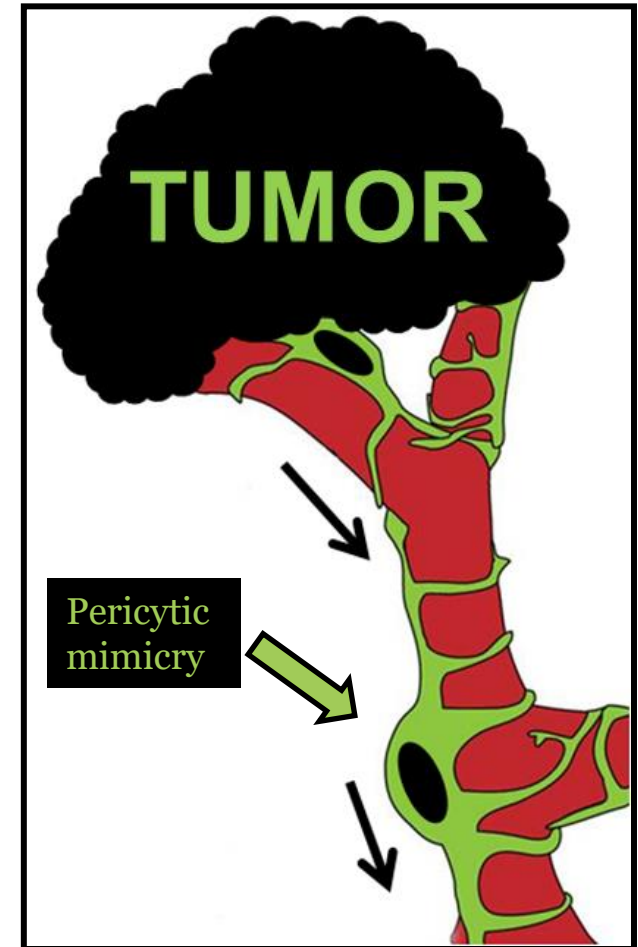
Angiotropism of Metastatic Melanoma Involving the Brain



*Barnhill RL, Benson PJ, Lugassy C.
Am J Dermatopathol. 2009;31:205-208.*

Pericytic Mimicry

- Angiotropic tumor cells compete with pericytes for spatial localization along abluminal vascular surfaces*
- Tumor cells spread and migrate along the external surfaces of vessels in a pericytic location*
- **Without intravasation**



*

•

•

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Lugassy et al. J Submicrosc Cytol Pathol. 1997 Jan;29(1):19-28.

Barnhill RL, Lugassy C. Pathology. 2004 Oct;36(5):485-90.

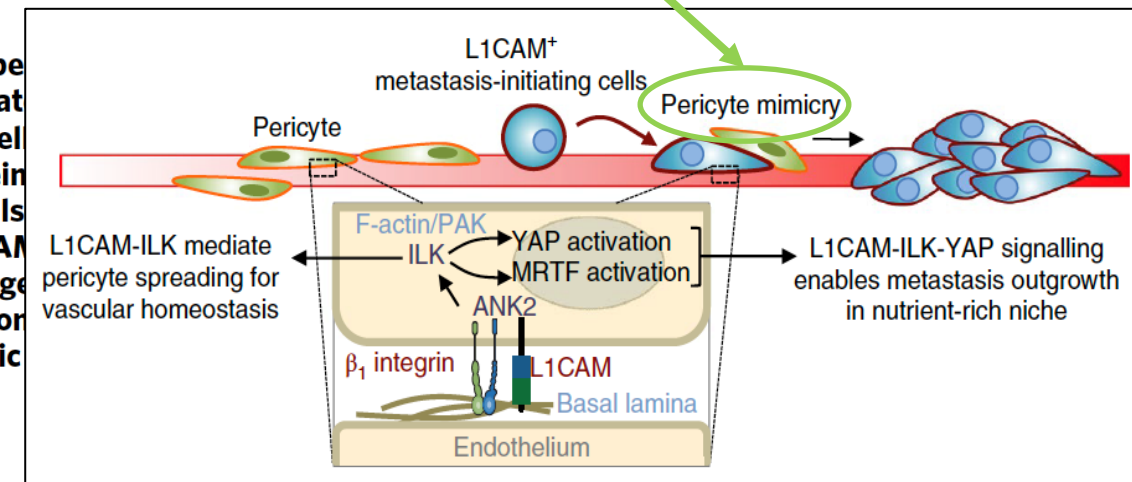
Lugassy et al. Pigment Cell Melanoma Res. 2013 Sep;26(5):746-54.

Pericyte-like spreading by disseminated cancer cells activates YAP and MRTF for metastatic colonization

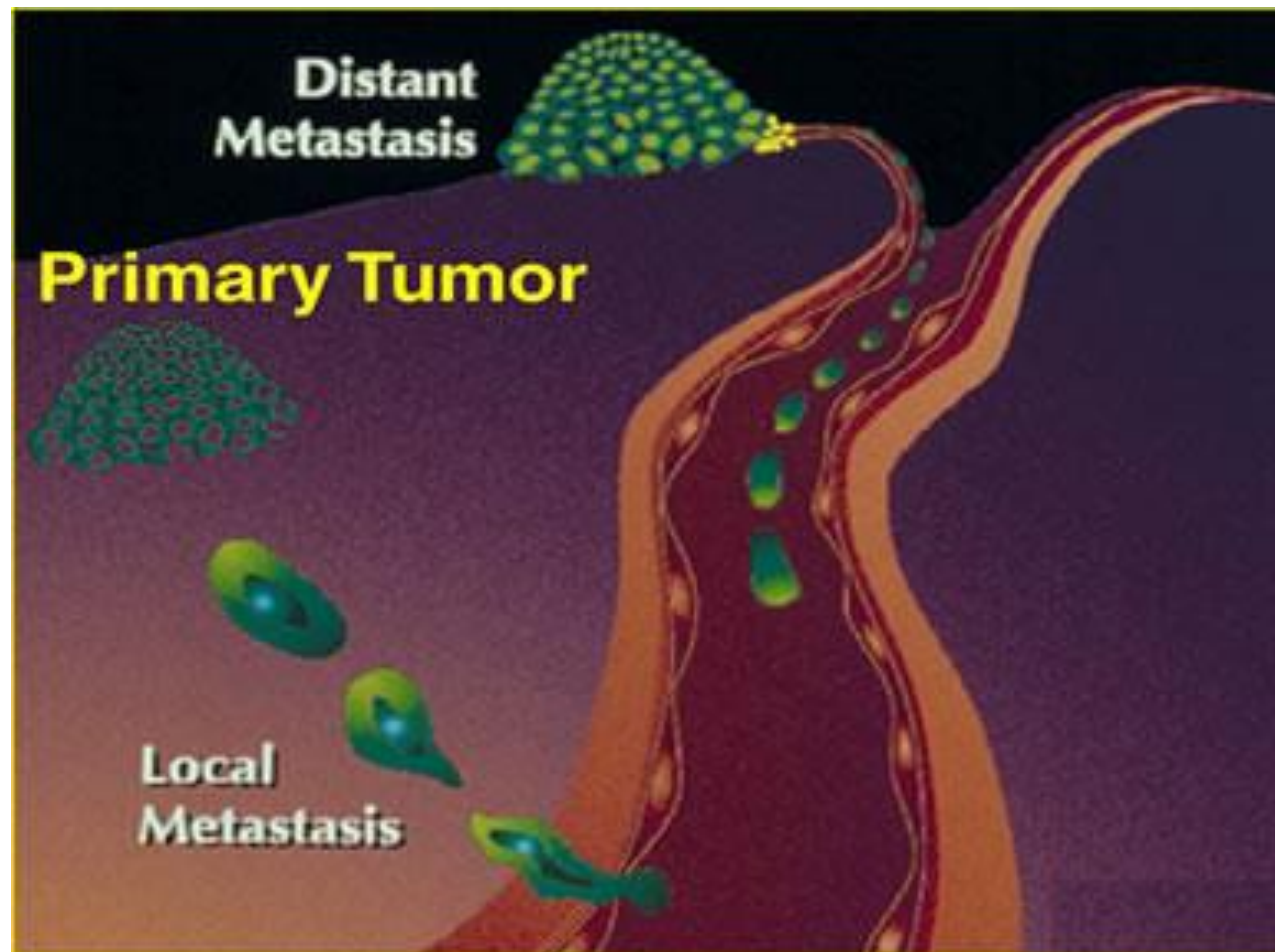
= *Pericytic Mimicry*

Ekrem Emrah Er¹, Manuel Valiente^{1,8,11}, Karuna Ganesh^{1,2,11}, Yilong Zou¹, Saloni Agrawal¹, Jing Hu¹, Bailey Griscom¹, Marc Rosenblum³, Adrienne Boire^{4,5}, Edi Brogi³, Filippo G. Giancotti^{1,9}, Melitta Schachner^{6,7}, Srinivas Malladi^{1,10} and Joan Massagué^{1*}

Metastatic seeding by disseminated cancer cells principally occurs in pericyte-like spreading. This spreading is robust enough to displace resident pericytes, which activates YAP by engaging β_1 integrin and ILK (integrin-linked kinase). L1CAM⁺ metastasis-initiating cells both immediately following their infiltration of target tissue and during pericyte-like spreading. Our results identify an important step in the initiation of metastatic colonization and provide an explanation for the widespread association of L1CAM with metastatic

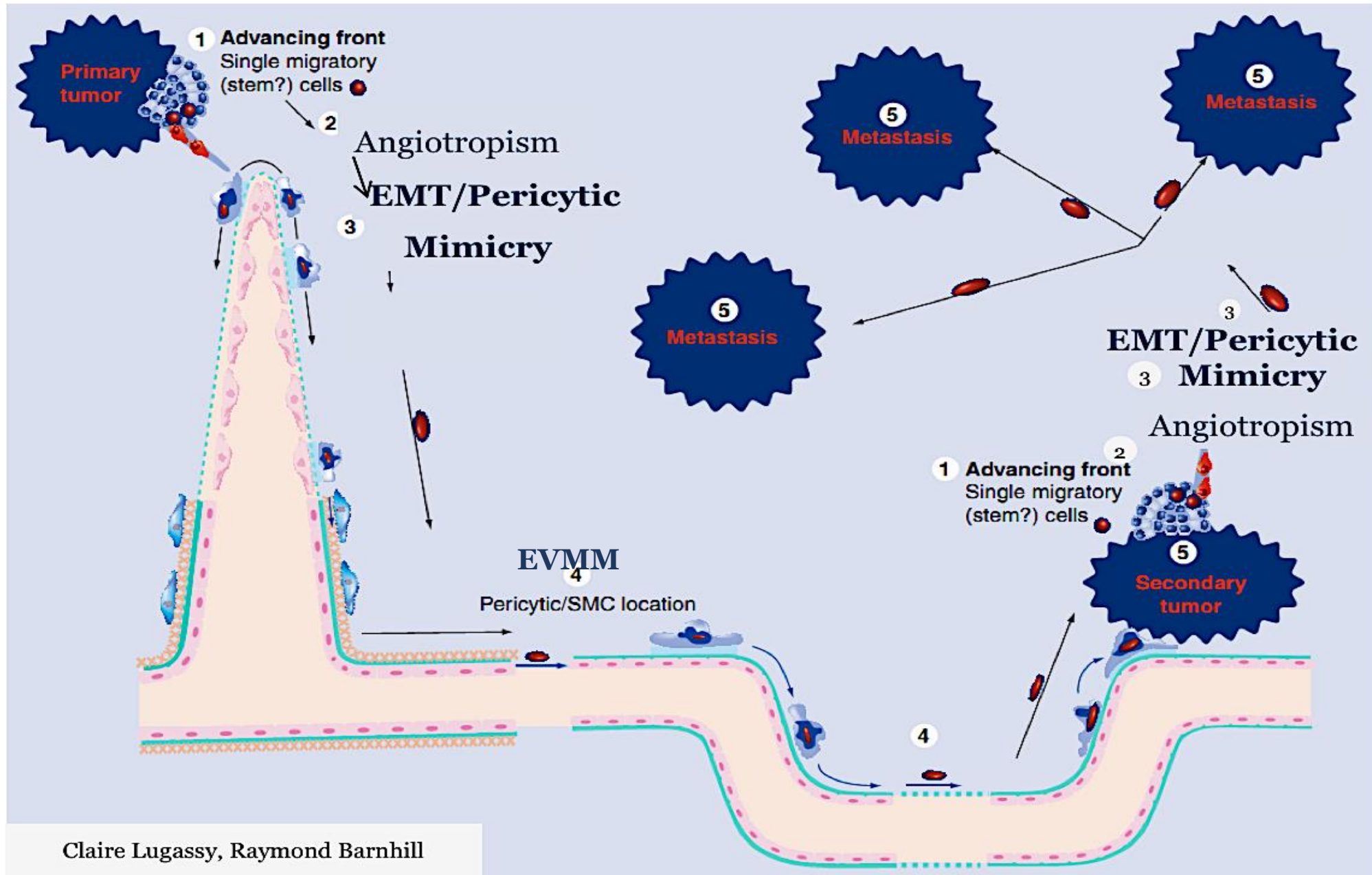


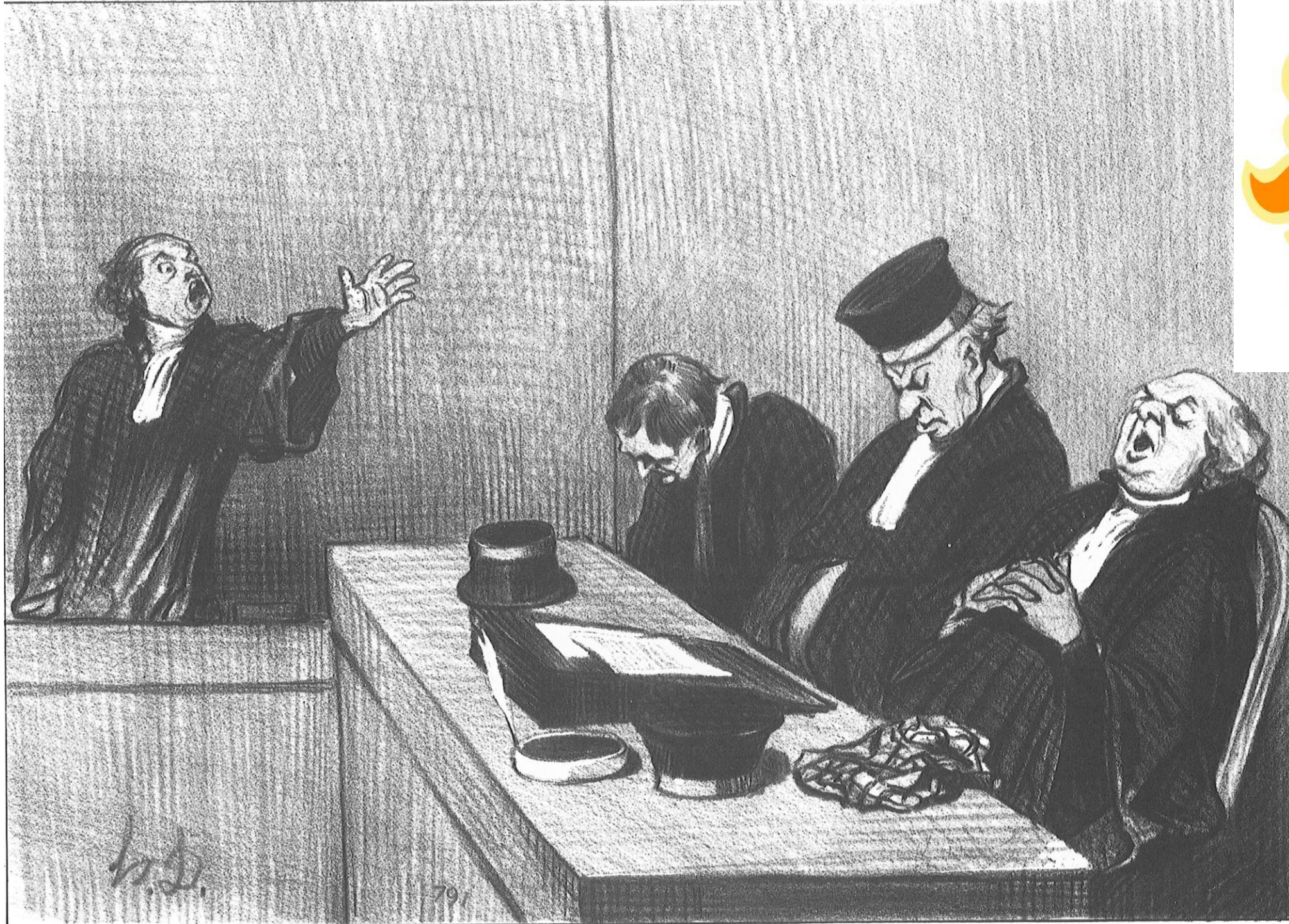
Since the end of the 19th century,
intravascular spread of cancer was the
only recognized mode of metastasis



For more than 25 years, we have questioned this statement and have developed the field of Angiotropic Extravascular Migratory Metastasis (Angiotropic EVMM)

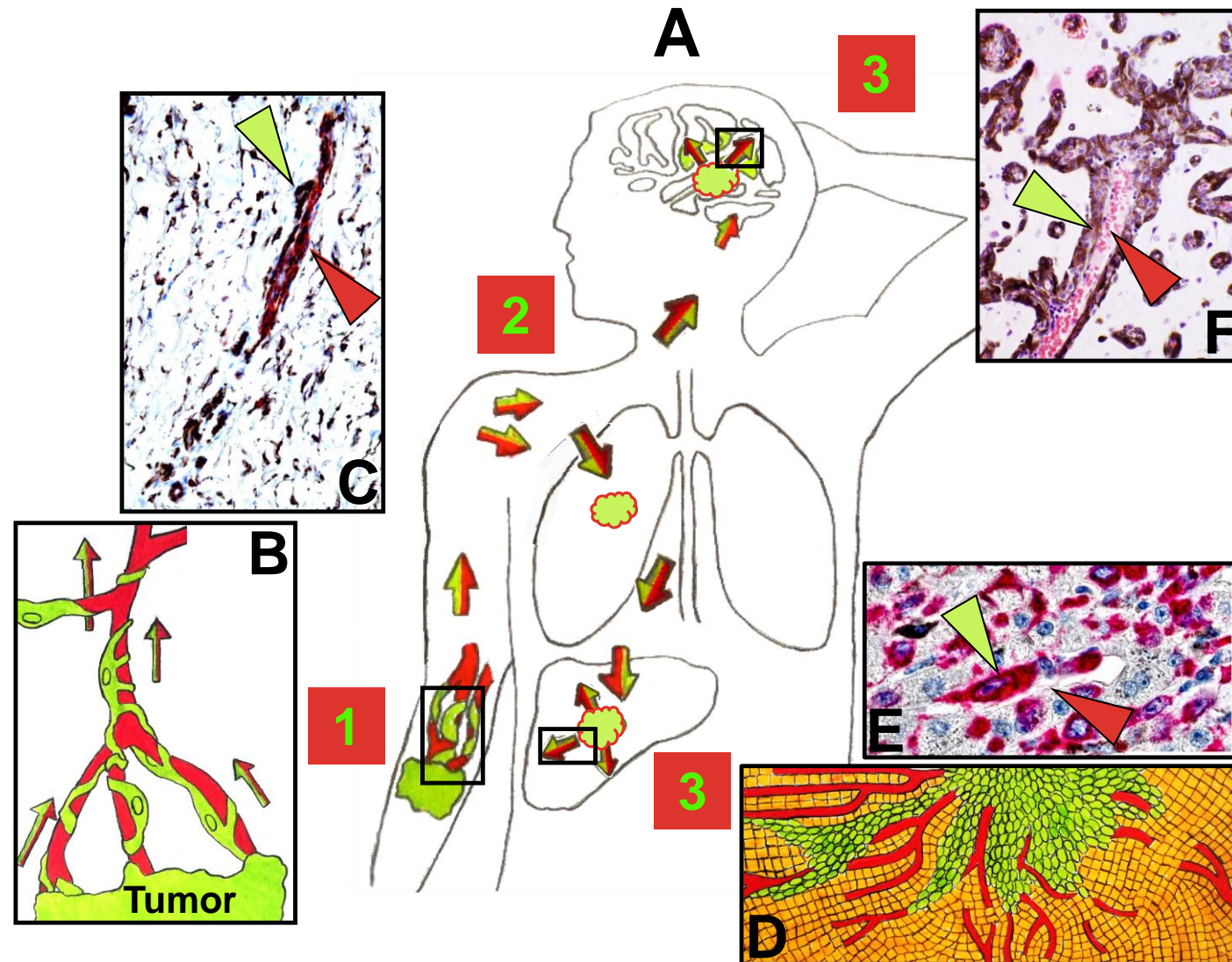
Angiotropic EVMM





Extravascular Migratory Metastasis (EVMM)

Melanoma cells spread to nearby and more distant sites



Angiotropism is a prognostic factor in Melanoma

2002

Angiotropism in Cutaneous Melanoma: A Prognostic Factor Strongly Predicting Risk for Metastasis. Barnhill R, Dy K, Lugassy C *J Invest Dermatol*;119:703-703.

....

2024

Can angiotropism and lymphovascular invasion refine the current cutaneous melanoma staging system? *J Cutan Pathol. Apr*;51(4):288-298.

From 2024

Ongoing Study: Review of a cohort of 1000 cases
InterMel Group

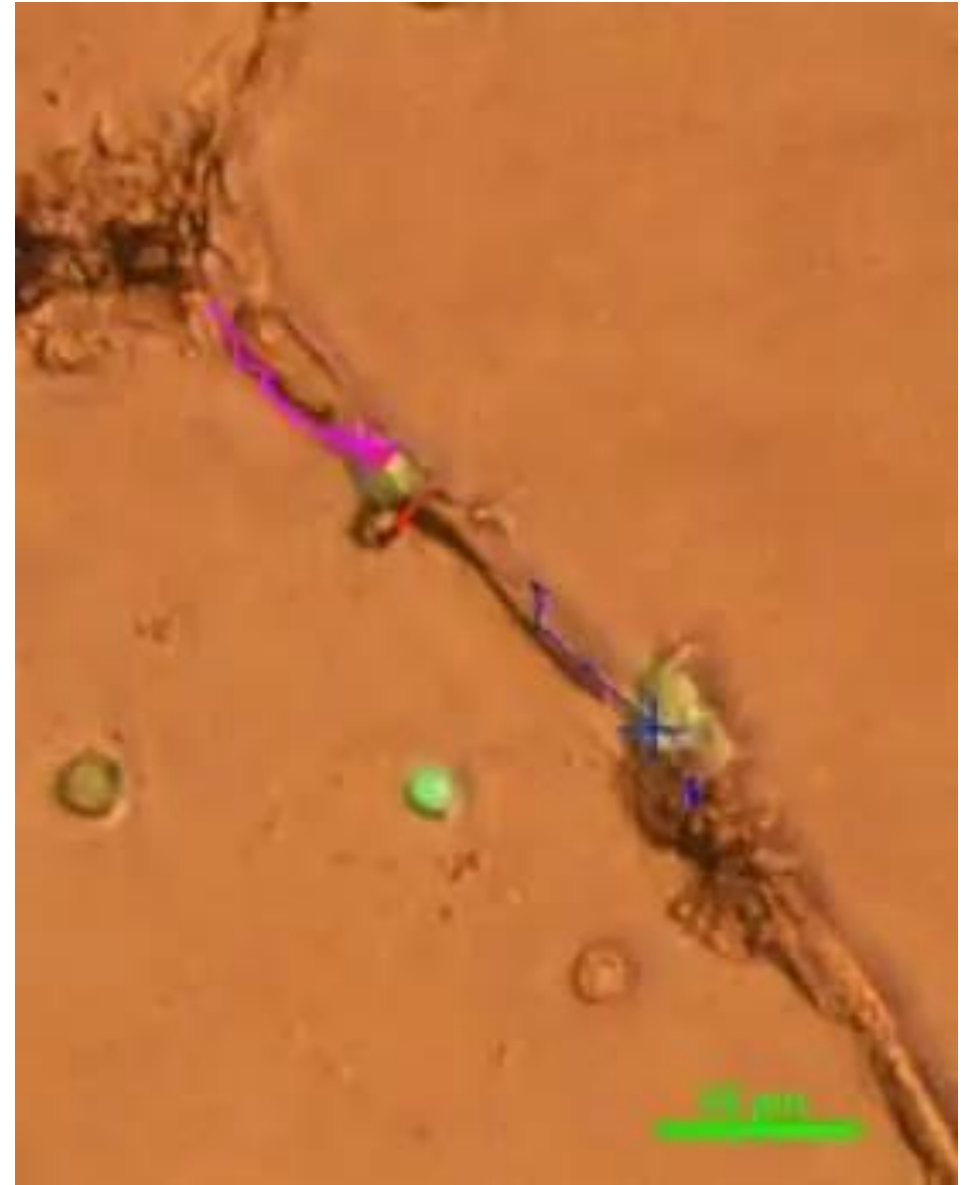
Angiotropism : a microscopic marker
of pericytic mimicry / EVMM

In vitro model of PM

Co-culture of endothelial tubules and GFP tumor cells

Lugassy C, Kleinman HK, Fernandez PM, Patierno SR, Webber MM, Ghanem G, Spatz A, Barnhill RL. Human melanoma cell migration along capillary-like structures in vitro: a new dynamic model for studying extravascular migratory metastasis. J Invest Dermatol. 2002 Sep;119(3):703-4.

Zadran S, McMickle R, Shackelford D, Kleinman H, Barnhill R, Lugassy C. Monitoring extra-vascular migratory metastasis (EVMM) of migrating cancer cells using an in vitro co-culture system. Protoc exch. 2013;22:2013.



SCIENTIFIC REPORTS

OPEN

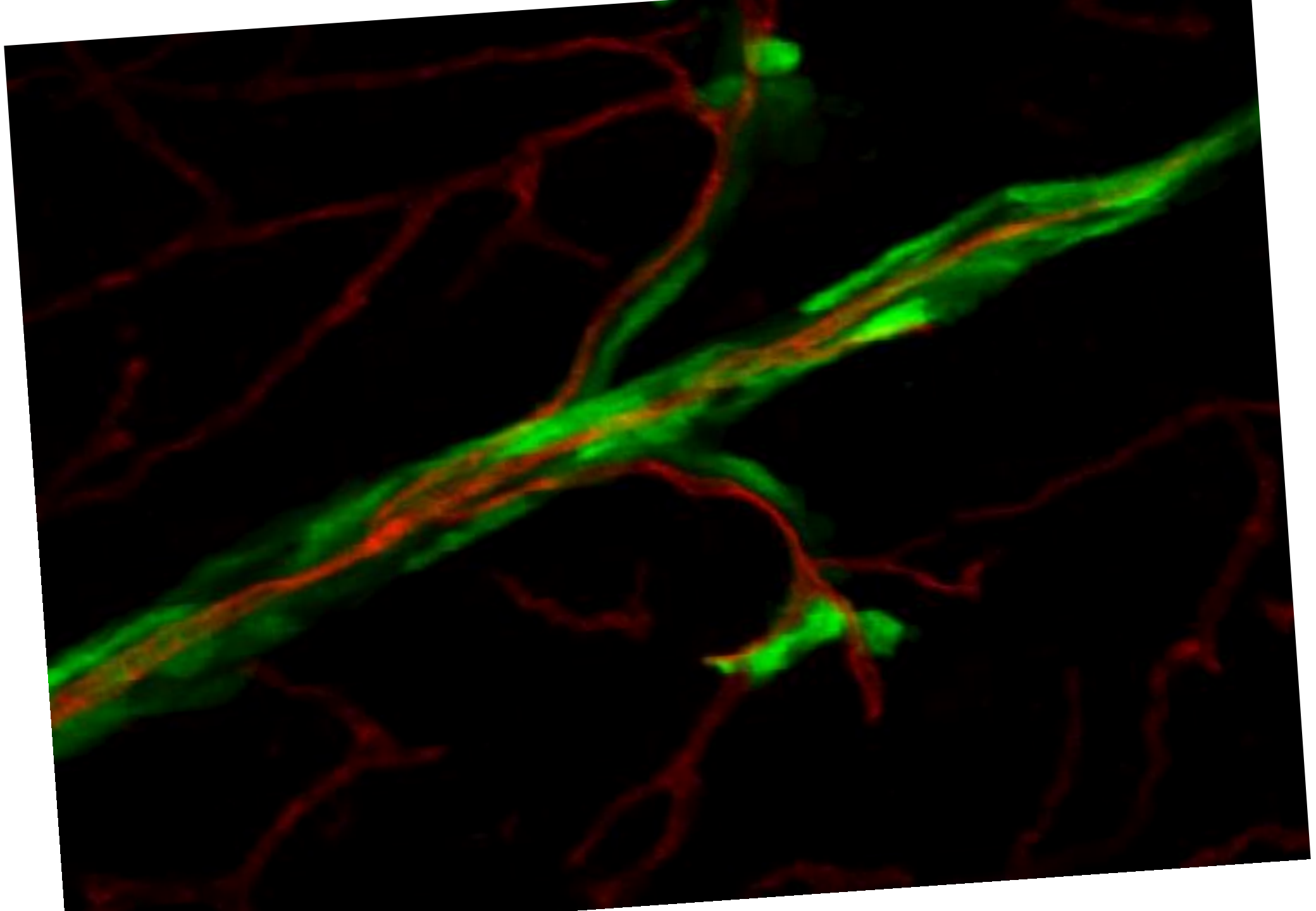
Received: 14 January 2016

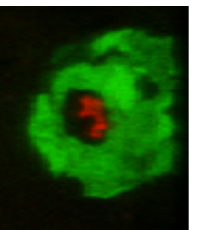
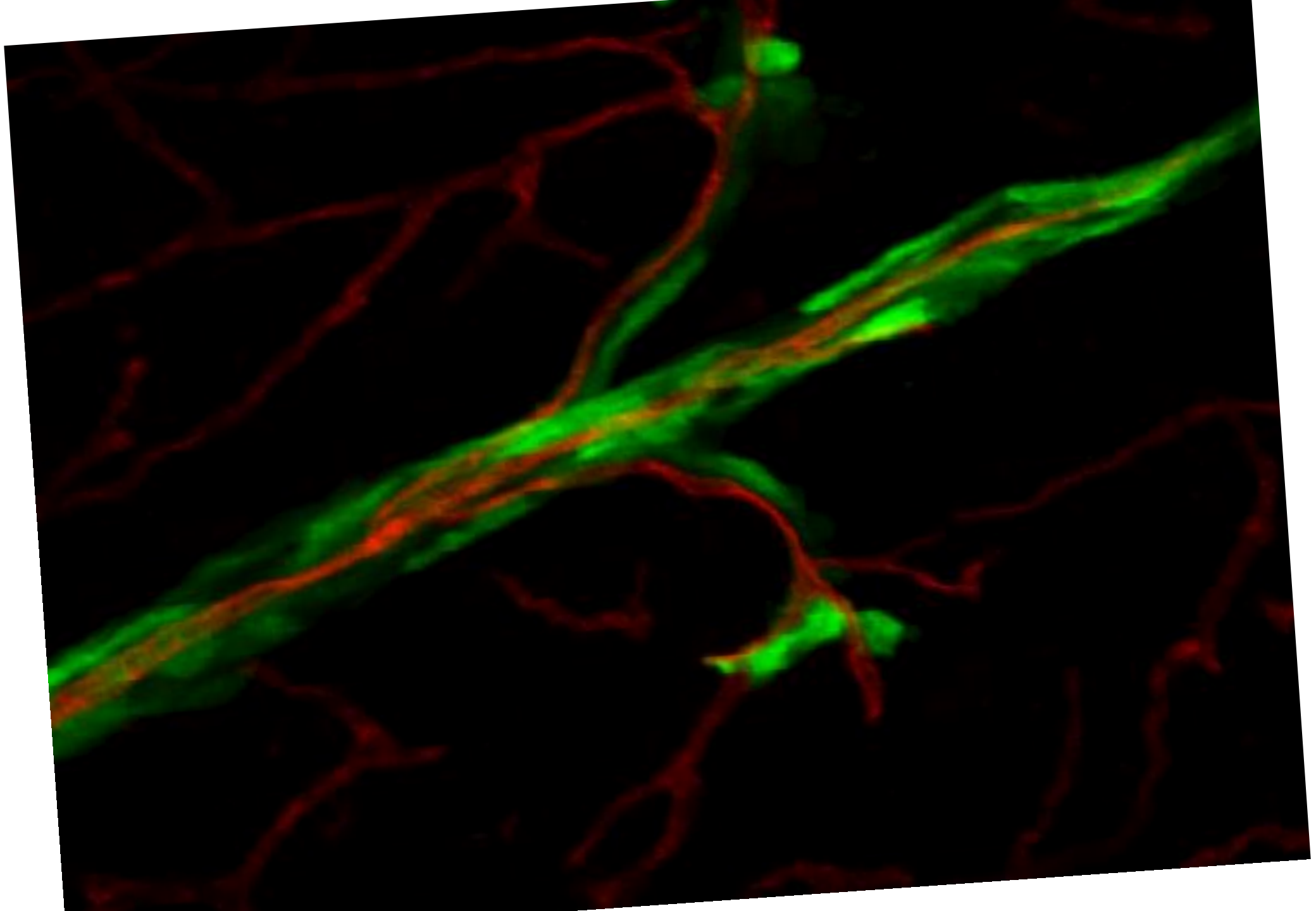
Accepted: 15 February 2016

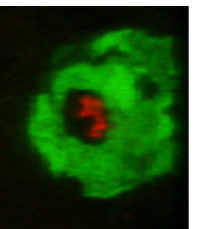
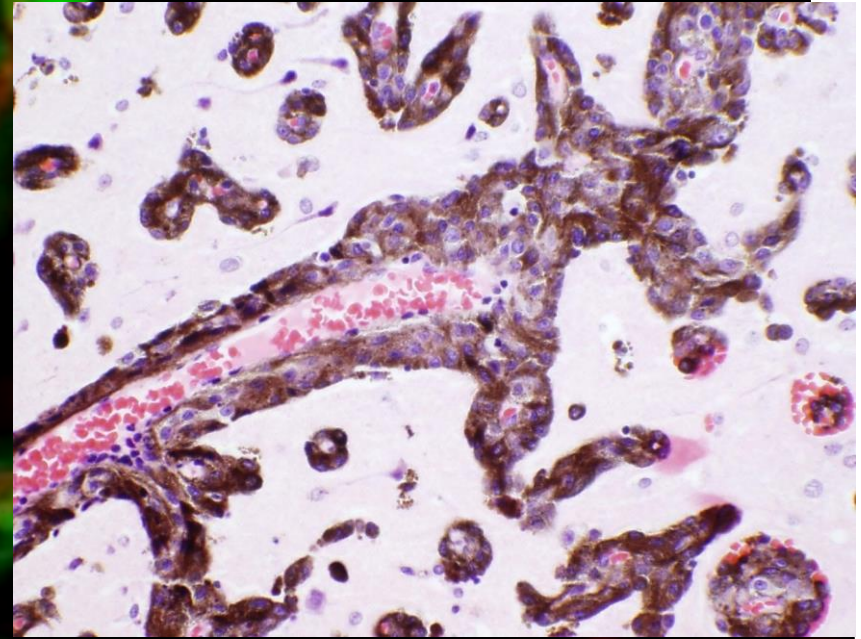
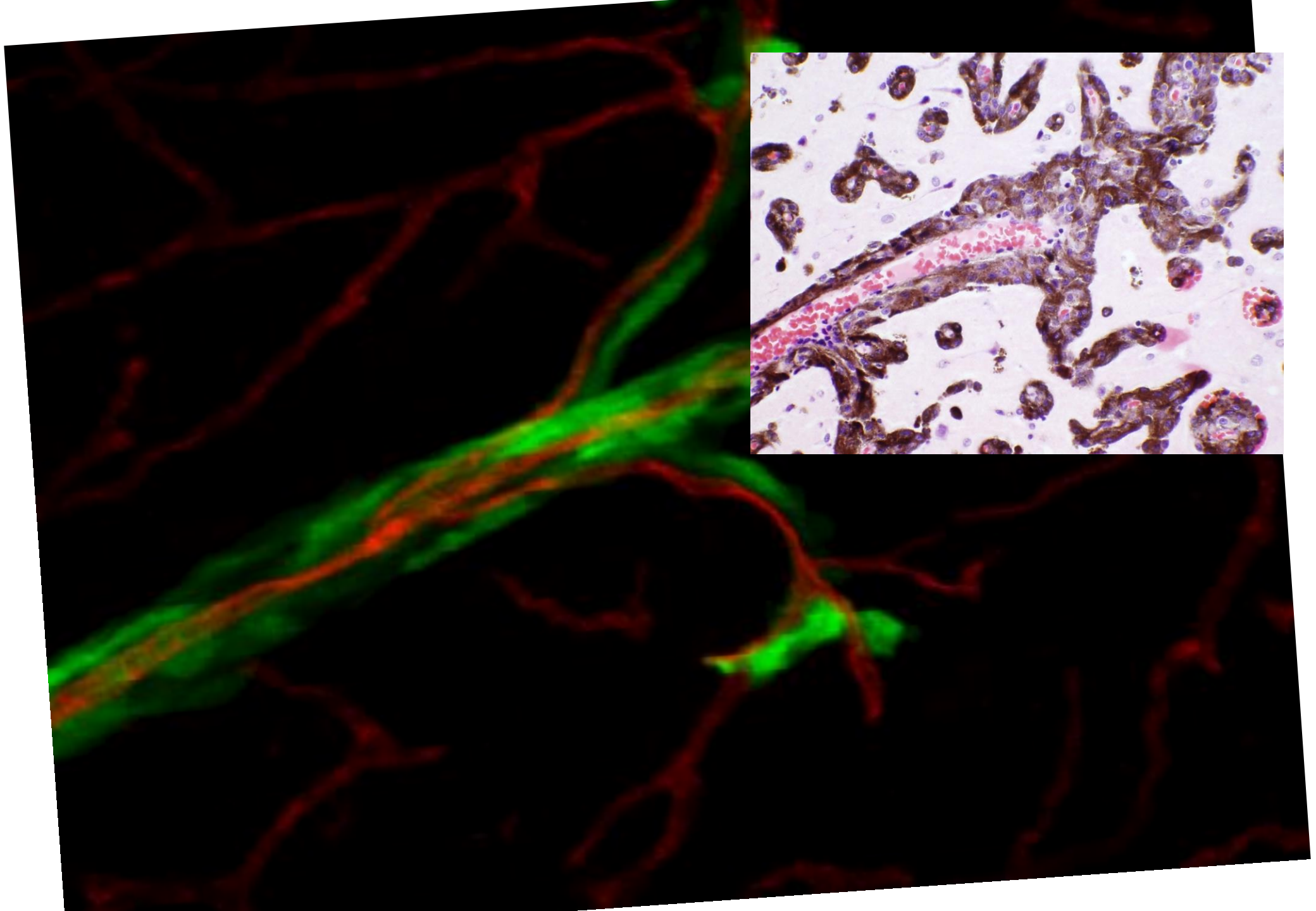
Published: 06 April 2016

Imaging of Angiotropism/Vascular Co-Option in a Murine Model of Brain Melanoma: Implications for Melanoma Progression along Extravascular Pathways

Laurent A. Bentolila^{1,2}, Roshini Prakash³, Daniela Mihic-Probst⁴, Madhuri Wadehra⁵, Hynda K. Kleinman⁶, Thomas S. Carmichael³, Bruno Péault^{7,8}, Raymond L. Barnhill⁹ & Claire Lugassy¹⁰







Zebrafish Model

www.nature.com/scientificreports

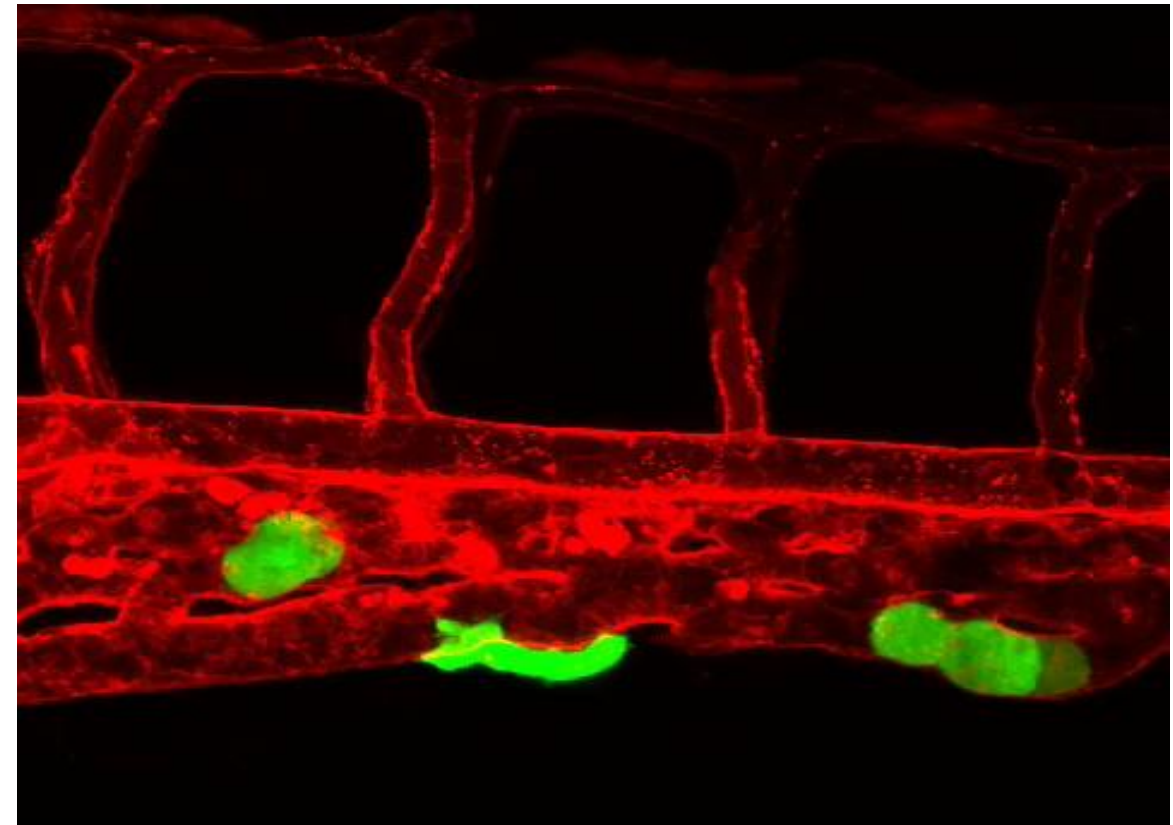
SCIENTIFIC REPORTS

OPEN Angiotropism and extravascular migratory metastasis in cutaneous and uveal melanoma progression in zebrafish model

Received: 26 September 2017

Ilia Fornabaio^{1,2,3}, Raymond L. Barnhill^{4,5}, Claire Lugassy², Laurent A. Bentolila^{6,7},
thalie Cassoux^{5,8}, Sergio Roman-Roman², Samar Alsafadi² & Filippo Del Bene^{1,2}

aneous melanoma is a highly aggressive cancer with a propensity for distant metastasis to various
ans. In contrast, melanoma arising in pigmented uveal layers of the eye metastasizes mostly in the
liver. The mechanisms of these metastases, which are ultimately resistant to therapy, are still unclear.
Metastasis via intravascular dissemination of tumour cells is widely accepted as a central paradigm.
However, we have previously described an alternative mode of tumour dissemination, extravascular
migratory metastasis, based on clinical and experimental data. This mechanism is characterised by
the interaction of cancer cells with the abluminal vascular surface, which defines angiotropism. Here,
we employed our 3D co-culture approach to monitor cutaneous and uveal human melanoma cells
dynamics in presence of vascular tubules. Using time-lapse microscopy, we evaluated angiotropism, the



Molecular Studies using Microarray analysis in our co-Culture Model of Pericytic Mimicry

Cancer Microenvironment (2013) 6:19–29

DOI 10.1007/s12307-012-0128-5

ORIGINAL PAPER

Pilot Study on “Pericytic Mimicry” and Potential Embryonic/ Stem Cell Properties of Angiotropic Melanoma Cells Interacting with the Abluminal Vascular Surface

Claire Lugassy • Madhuri Wadehra • Xinmin Li •
Mirko Corselli • David Akhavan • Scott W. Binder •
Bruno Péault • Alistair J. Cochran • Paul S. Mischel •
Hynda K. Kleinman • Raymond L. Barnhill

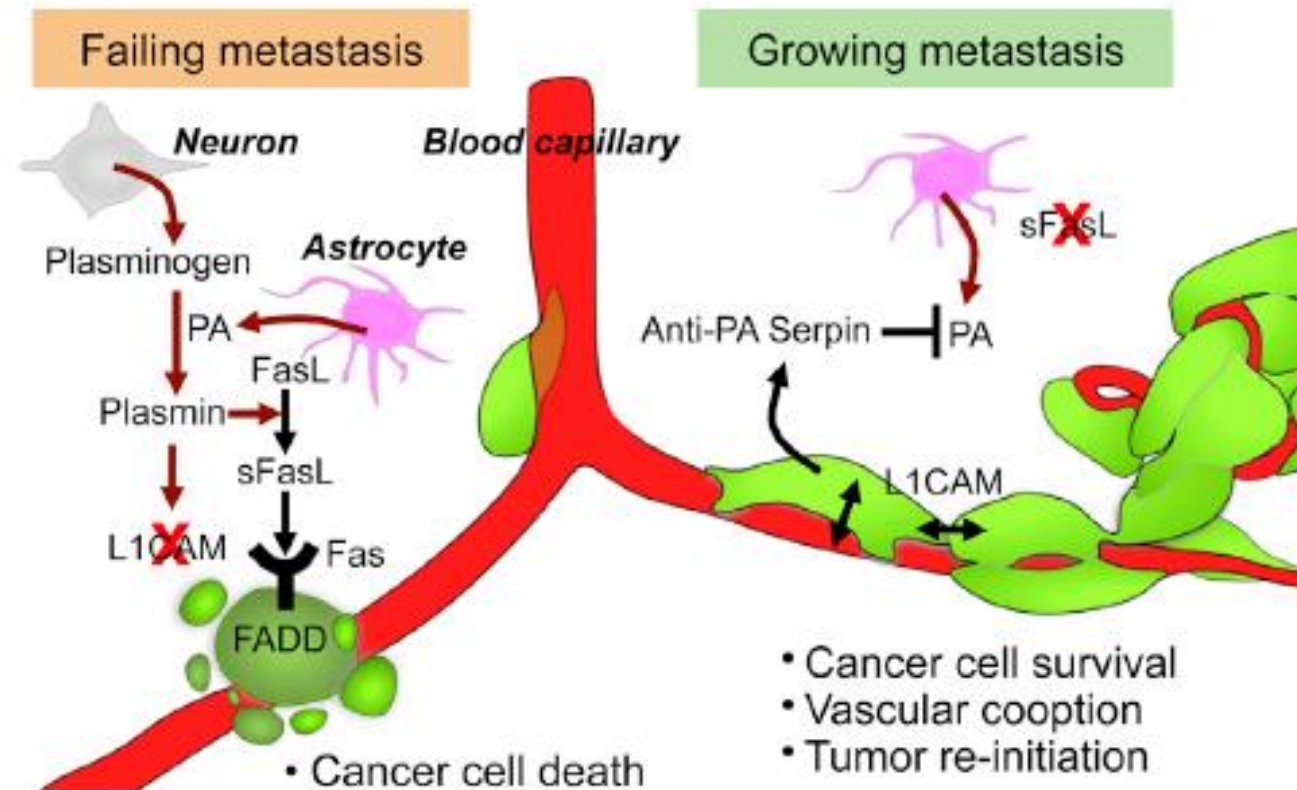
28 significantly differentially expressed genes Linked to tumor progression and embryogenesis

- Cell migration CCL2 , ICAM1, IL6, RGNEF, RANBP9, PDGFB
- Cancer progression CCL2 , ICAM1, SELE, TRAF1, IL6, **SERPINB2**
CXCL6, BLID, MALT1, UPF1, PLAA, RGNEF, ZXDC
- EMT CCL2 , IL6, ICAM1, PDGFB
- Embryonic/(cancer) stem cell properties CCL2, PDGFB, EVX1, CFDP1, RANBP9
- Pericytic recruitment PDGFB
- Inflammation CCL2, IL6, TRAF1, CXCL6, SELE, ICAM1,
SERPINB2, SLC7A2, C2CD4B, PDGFB

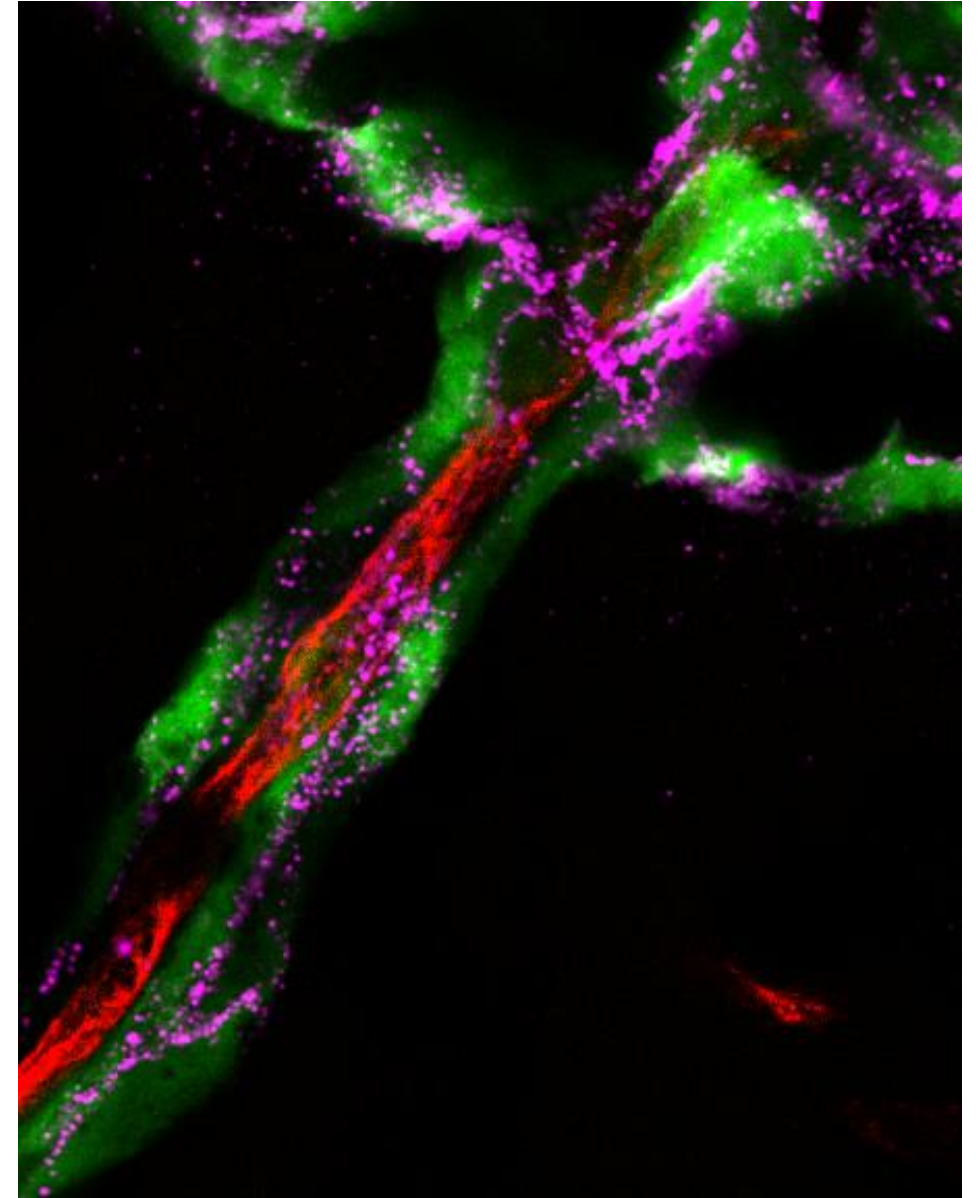
Serpins Promote Cancer Cell Survival and Vascular Co-Option in Brain Metastasis

Manuel Valiente,¹ Anna C. Obenauf,¹ Xin Jin,¹ Qing Chen,¹ Xiang H.-F. Zhang,^{1,8} Derek J. Lee,¹ Jamie E. Chaff,² Mark G. Kris,² Jason T. Huse,^{3,4} Edi Brogi,⁵ and Joan Massagué^{1,4,6,7,*}

Brain metastatic cells express high levels of anti-PA serpins, including **serpin B2**



**Serpin B2 expressed by
angiotropic melanoma cells
spreading along vessels in
our murine model of brain
melanoma**



Bentolila LA, Prakash R, Mihic-Probst D, Wadehra M, Kleinman HK, Carmichael TS, Péault B, Barnhill RL, Lugassy C.

Imaging of Angiotropism/Vascular Co-Option in a Murine Model of Brain Melanoma: Implications for Melanoma Progression along Extravascular Pathways. Sci Rep. 2016 Apr 6;6:23834.

28 significantly differentially expressed genes Linked to tumor progression and embryogenesis

- Cell migration CCL2 , ICAM1, IL6, RGNEF, RANBP9, PDGFB
- Cancer progression CCL2 , ICAM1, SELE, TRAF1, IL6, SERPINB2 , CXCL6, BLID, MALT1, UPF1, PLAA, RGNEF, ZXDC
- EMT CCL2 , IL6, ICAM1, PDGFB
- Embryonic/(cancer) stem cell properties CCL2, PDGFB, EVX1, CFDP1, RANBP9
- Pericytic recruitment PDGFB
- Inflammation CCL2, IL6, TRAF1, CXCL6, SELE, ICAM1, SERPINB2, SLC7A2, C2CD4B, PDGFB

Ultraviolet-radiation-induced inflammation promotes angiotropism and metastasis in melanoma

Tobias Bald¹, Thomas Quast², Jennifer Landsberg¹, Meri Rogava¹, Nicole Glodde¹, Dorys Lopez-Ramos¹, Judith Kohlmeyer¹, Stefanie Riesenberger³, Debby van den Boorn-Konijnenberg³, Cornelia Hömig-Hölzel³, Raphael Reuten⁴, Benjamin Schadow⁵, Heike Weighardt⁵, Daniela Wenzel⁶, Iris Helfrich⁷, Dirk Schadendorf⁷, Wilhelm Bloch⁸, Marco E. Bianchi⁹, Claire Lugassy¹⁰, Raymond L. Barnhill¹⁰, Manuel Koch⁴, Bernd K. Fleischmann⁶, Irmgard Förster⁵, Wolfgang Kastenmüller¹¹, Waldemar Kolanus², Michael Hölzel³, Evelyn Gaffal¹ & Thomas Tüting¹

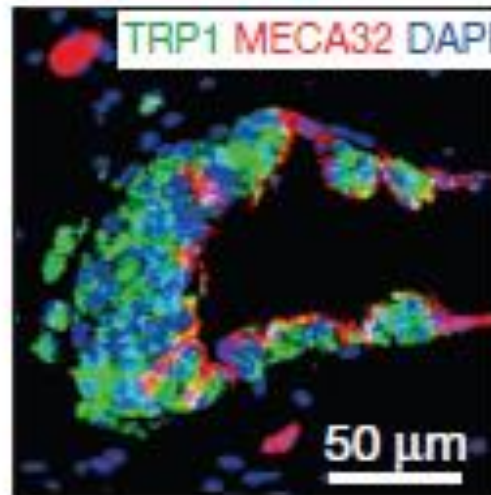
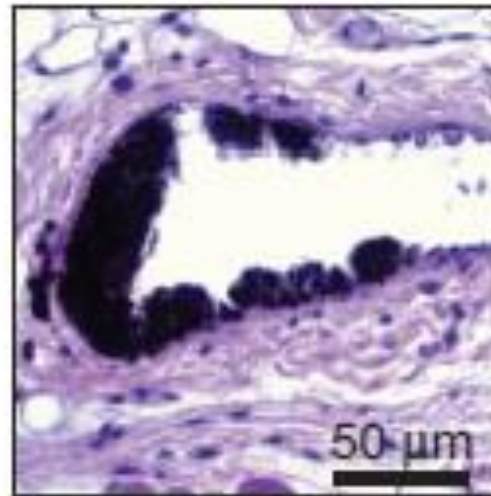
Intermittent intense ultraviolet (UV) exposure represents an important aetiological factor in the development of malignant melanoma¹. The ability of UV radiation to cause tumour-initiating DNA mutations in melanocytes is now firmly established², but how the microenvironmental effects of UV radiation^{3,4} influence melanoma pathogenesis is not fully understood. Here we report that repetitive UV exposure of primary cutaneous melanomas in a genetically engineered mouse model⁵ promotes metastatic progression, independent of its tumour-initiating effects. UV irradiation enhanced the expansion of tumour cells along abluminal blood vessel surfaces and increased the number of lung metastases. This effect depended on the recruitment and

due to an oncogenic CDK4(R24C) mutation in the germline cause the development of invasive and metastatic melanomas as seen in patients⁵.

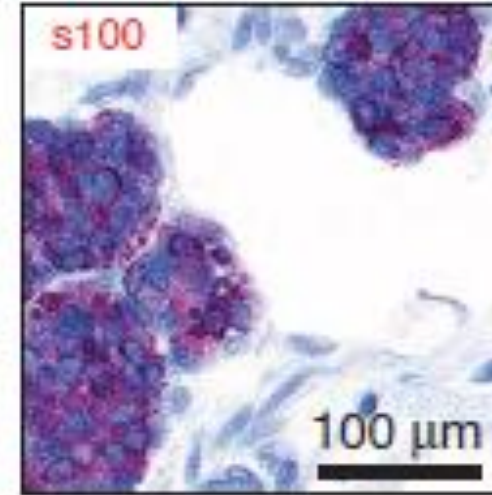
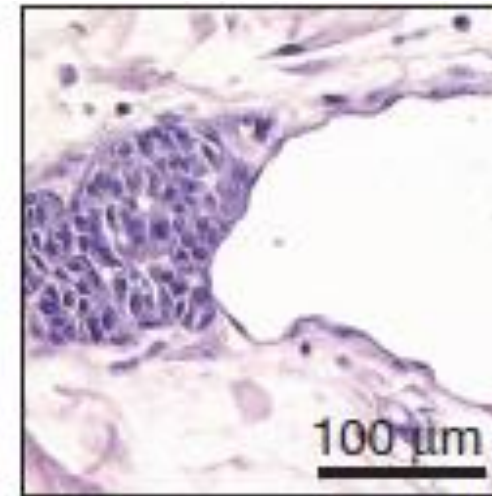
After two erythral doses of UV light, the skin of adult HGF-CDK4(R24C) mice showed a prominent inflammatory infiltrate consisting predominantly of CD11b⁺Ly6C⁺Ly6G⁺ neutrophils and, to a lesser extent, of CD11b⁺Ly6C⁺Ly6G⁻ inflammatory monocytes and CD11b⁺Ly6C⁻Ly6G⁻ macrophages along with reactive proliferation of epidermal keratinocytes and peripheral blood neutrophilia (Extended Data Fig. 1a–c). Exposure of HGF-CDK4(R24C) mice to UV twice weekly for 6 weeks led to accumulation of melanocytes predominantly in the upper dermis along epidermal basement membranes (Extended



Macroscopically visible melanoma cell expansion along dermal blood vessels (arrows)



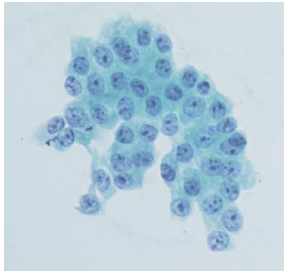
Corresponding haematoxylin and eosin (H&E)-stained section and immuno-fluorescence analysis.



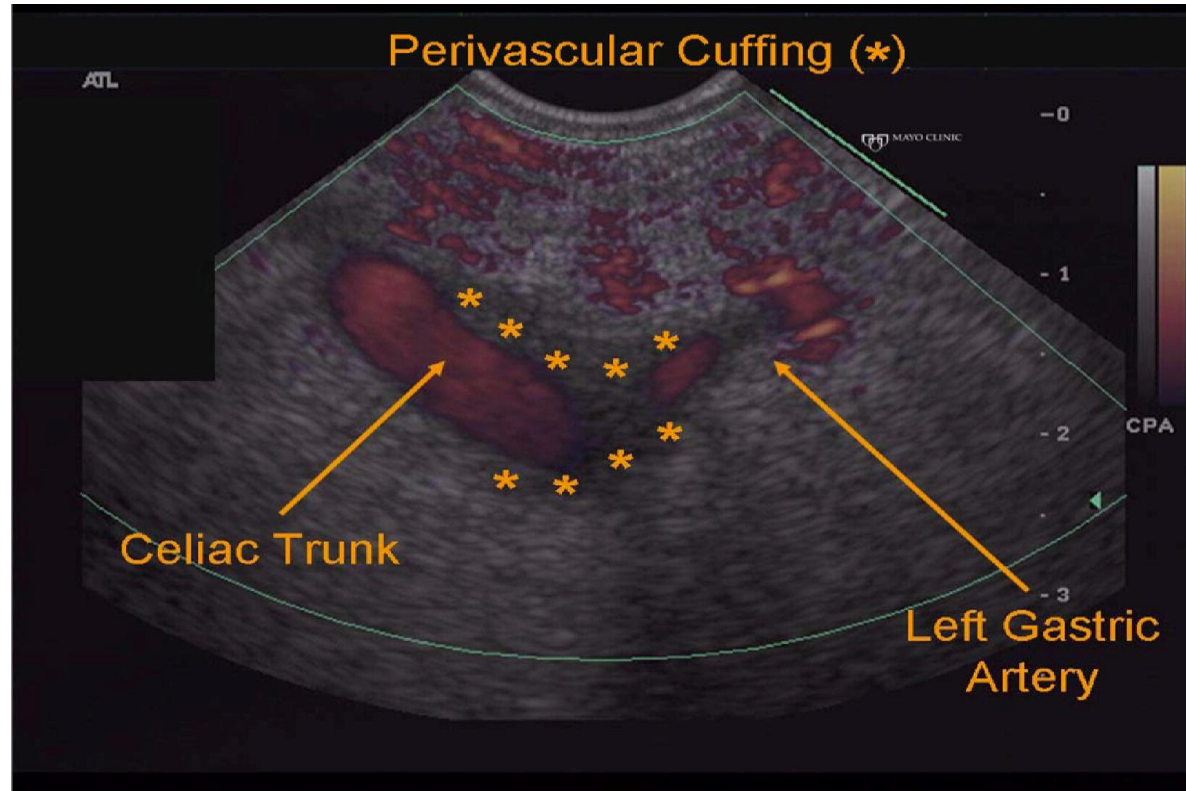
Angiotropic growth in a human melanoma.

Endoscopic ultrasound detection of extravascular migratory metastasis from a remotely located pancreatic cancer

Apparently without metastases



Confirmed by fine-needle aspiration



Tumor cells can migrate via perivascular cuffing to distant sites along blood vessels to form extravascular migratory metastases (EVMM).

- 23. Lugassy C, Zadran S, Bentolila LA, et al. Angiotropism, pericytic mimicry and extravascular migratory metastasis in melanoma: an alternative to intravascular cancer dissemination. *Cancer Microenviron* 2014;7:139-152.
- 25. Bamhill RL, Lugassy C. Angiotropic malignant melanoma and extravascular migratory metastasis: description of 36 cases with emphasis on a new mechanism of tumour spread. *Pathology* 2004;36:485-490.
- 26. Lugassy C, Bamhill RL, Christensen L. Melanoma and extravascular migratory metastasis. *J Cutan Pathol* 2000;27:481.
- 30. Lugassy C, Peault B, Wadehra M, et al. Could pericytic mimicry represent another type of melanoma cell plasticity with embryonic properties? *Pigment Cell Melanoma Res* 2013;26:746-754.

EVMM =
Negative Prognostic Factor

1. Endoscopic ultrasound fine-needle aspiration detection of extravascular migratory metastasis from a remotely located pancreatic cancer. *Clin Gastroenterol Hepatol*. 2009
2. Diagnostic Accuracy, and Effects of Endoscopic Ultrasound Fine-Needle Aspiration on Detection of Extravascular Migratory Metastases. *Clin Gastroenterol Hepatol*. 2019

APRIL 2019



APRIL 2024



ANALYZE OF SPEED OF TUMOR CELLS IN VIVO INTRAVITAL IMAGING

Table 1

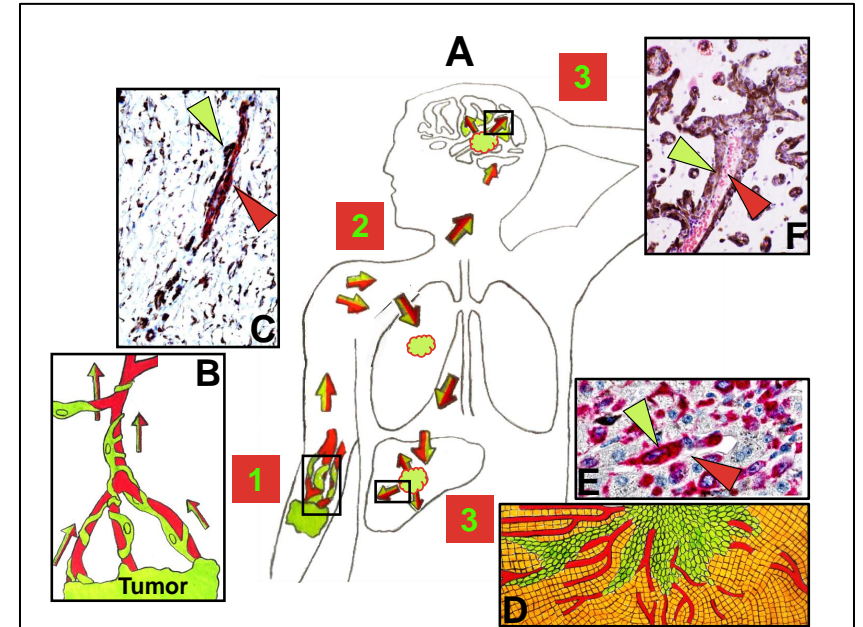
Migration modes and dynamics observed by intravital imaging.

Cell type	Fraction of motile cells	Migration mode	Speed	Reference
A375M2 human melanoma		70% amoeboid-like (single-cell; with and without blebs)	0.5–10 $\mu\text{m}/\text{min}$ amoeboid-like: $\sim 3 \mu\text{m}/\text{min}$ mesenchymal: $\sim 1 \mu\text{m}/\text{min}$	[6]
A375 human melanoma	1–5%	30% mesenchymal 55% amoeboid-like (single cell; without blebs) 10% amoeboid-like (single cell; with blebs) 35% mesenchymal	1–5 $\mu\text{m}/\text{min}$	[49,50]

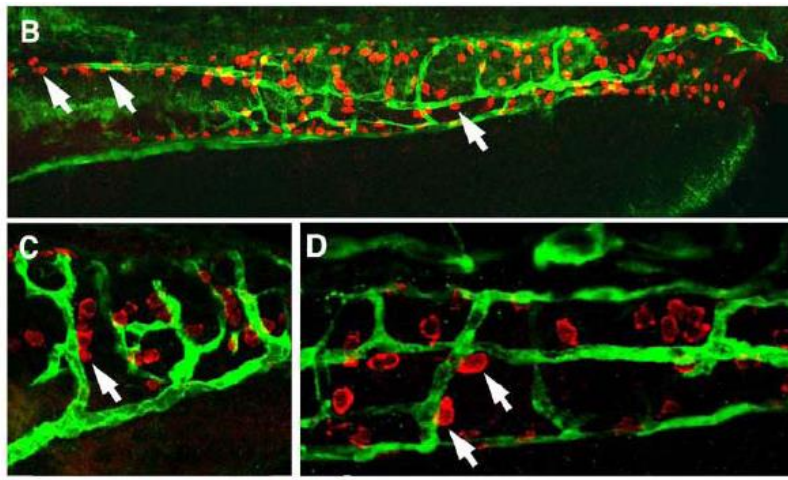
Distances travelled by Melanoma Cells
26 to 525 cm per year

Tumor cells may spread to nearby and distant sites via EVMM

- Such speeds are compatible with the time intervals between the recognition of the primary cancer and the formation of metastases (months, years)
- Pericytic Mimicry/EVMM (slow processess) may be an alternative to dormancy



Lazy (dormant)
pericytic mimicry



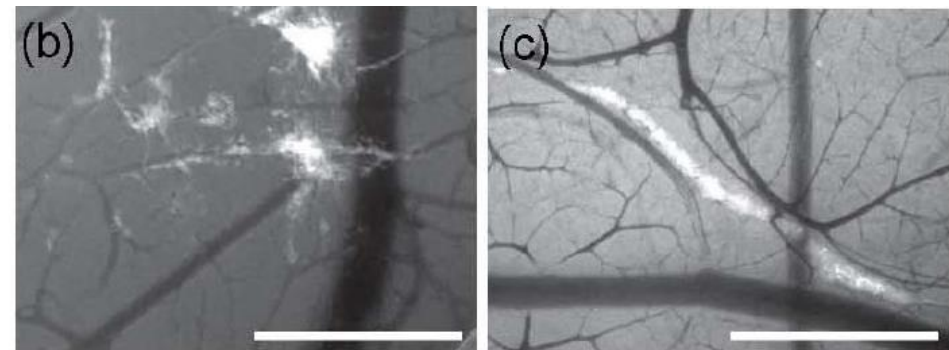
N. Nagy et al. / *Developmental Biology* 330 (2009) 263–272

Neural Crest Migration



Angiotropic EVMM is probably an embryogenesis-derived program

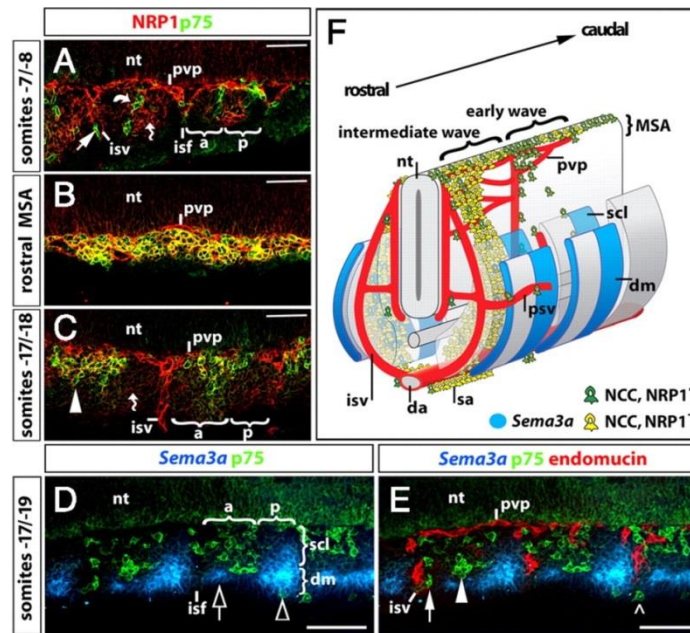
EVMM



Lugassy et al. *Br J Dermatol.* 2007;157(4):780-2.

EVMM and Neural Crest Stem Cell Migration

- Melanocytes are derived from the neural crest
- Neural crest stem cells migrate along vascular channels during a part of their journey



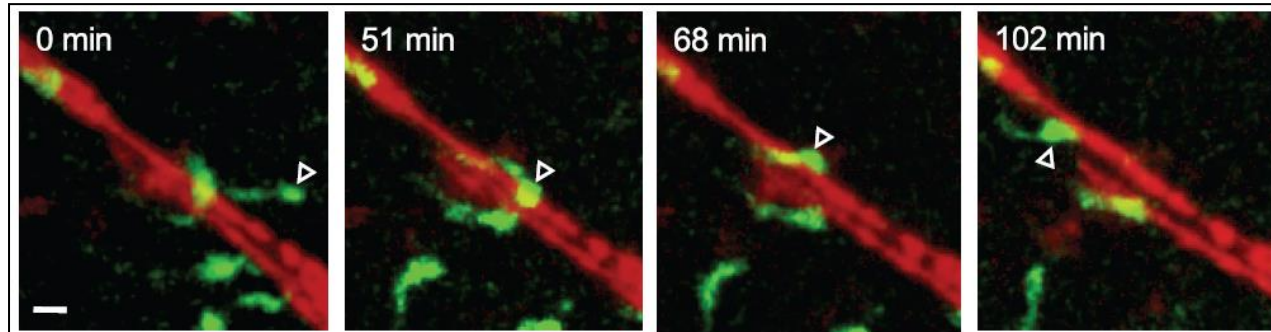
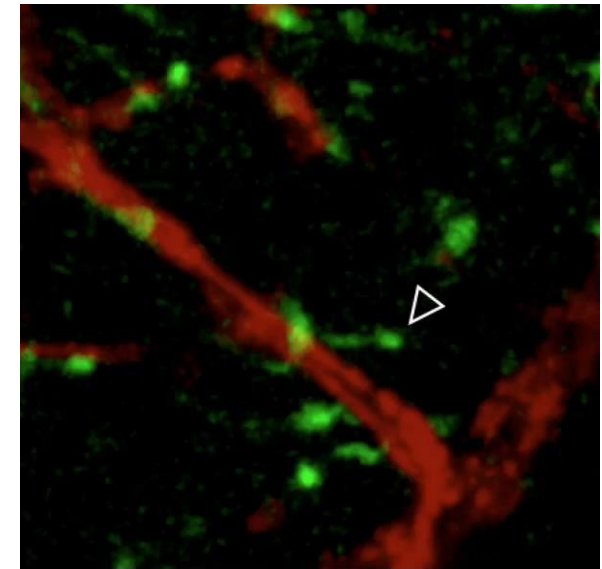
*Schwarz Q. et.al. PNAS
2009;106:6164-6169*

NEURODEVELOPMENT

Oligodendrocyte precursors migrate along vasculature in the developing nervous system

Hui-Hsin Tsai,^{1*} Jianqin Niu,^{1*} Roeben Munji,² Dimitrios Davalos,³ Junlei Chang,⁴ Haijing Zhang,^{4,5,6,7} An-Chi Tien,¹ Calvin J. Kuo,⁴ Jonah R. Chan,⁸ Richard Daneman,² Stephen P. J. Fancy^{1,8,9,10†}

Oligodendrocytes myelinate axons in the central nervous system and develop from oligodendrocyte precursor cells (OPCs) that must first migrate extensively during brain and spinal cord development. We show that OPCs require the vasculature as a physical substrate for migration. We observed that OPCs of the embryonic mouse brain and spinal cord, as well as the human cortex, emerge from progenitor domains and associate with the abluminal endothelial surface of nearby blood vessels. Migrating OPCs crawl along and jump between vessels. OPC migration in vivo was disrupted in mice with defective vascular architecture but was normal in mice lacking pericytes. Thus, physical interactions with the vascular endothelium are required for OPC migration. We identify Wnt-Cxcr4 (chemokine receptor 4) signaling in regulation of OPC-endothelial interactions and propose that this signaling coordinates OPC migration with differentiation.



REVIEW PAPER



Angiotropism, pericytic mimicry and extravascular migratory metastasis: an embryogenesis-derived program of tumor spread

Claire Lugassy¹ · Hynda K. Kleinman² · Peter B. Vermeulen^{3,4} · Raymond L. Barnhill^{1,5}

Received: 30 August 2019 / Accepted: 29 October 2019
© Springer Nature B.V. 2019

Abstract

Intravascular dissemination of tumor cells is the accepted mechanism of cancer metastasis. However, the phenomenon of angiotropism, pericyte mimicry (PM), and extravascular migratory metastasis (EVMM) has questioned the concept that tumor cells metastasize exclusively via circulation within vascular channels. This new paradigm of cancer spread and metastasis suggests that metastatic cells employ embryonic mechanisms for attachment to the abluminal surfaces of blood vessels (angiotropism) and spread via continuous migration, competing with and replacing pericytes, i.e., pericyte mimicry (PM). This is an entirely extravascular phenomenon (i.e., extravascular migratory metastasis or EVMM) without entry (intravasation) into vascular channels. PM and EVMM have mainly been studied in melanoma but also occur in other cancer types. PM and EVMM appear to be a reversion to an embryogenesis-derived program. There are many analogies between embryogenesis and cancer progression, including the important role of laminins, epithelial–mesenchymal transition, and the re-activation of embryonic signals by cancer cells. Furthermore, there is no circulation of blood during the first trimester of embryogenesis, despite the fact that there is extensive migration of cells to distant sites and formation of organs and tissues during this period. Embryonic migration therefore is a continuous extravascular migration as are PM and EVMM, supporting the concept that these embryonic migratory events appear to recur abnormally during the metastatic process. Finally, the perivascular location of tumor cells intrinsically links PM to vascular co-option. Taken together, these two new paradigms may greatly influence the development of new effective therapeutics for metastasis. In particular, targeting embryonic factors linked to migration that are detected during cancer metastasis may be particularly relevant to PM/EVMM.

Metastasis-Initiating Cells and Ecosystems



Joan Massagué¹ and Karuna Ganesh^{2,3}

ABSTRACT

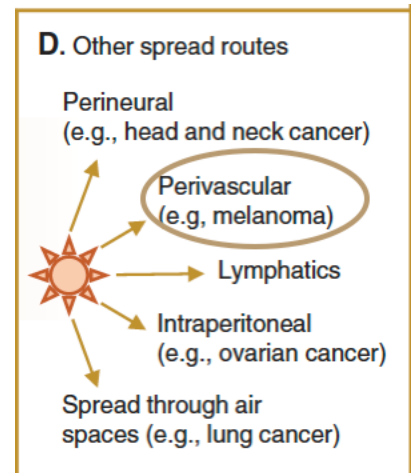
Metastasis is initiated and sustained through therapy by cancer cells with stem-like and immune-evasive properties, termed metastasis-initiating cells (MIC). Recent progress suggests that MICs result from the adoption of a normal regenerative progenitor phenotype by malignant cells, a phenotype with intrinsic programs to survive the stresses of the metastatic process,

Extravascular Spread

The metastatic spread of some tumors occurs largely or at least partly through routes that obviate the need to enter and exit the circulation (Fig. 2D).

Melanomas and other cancers can spread without entering and exiting the circulation by engaging in extravascular migratory metastasis, a process of migration over the abluminal surface of vessels that is common during embryogenesis⁽⁶⁰⁾

D. Beyond hematogenous dissemination, cancer cells originating in certain primary tumors can also reach distant organs via alternative routes.



60. Lugassy C, Kleinman HK, Vermeulen PB, Barnhill RL. Angiotropism, pericytic mimicry and extravascular migratory metastasis: an embryogenesis-derived program of tumor spread. *Angiogenesis* 2020;23:27–41.

¹Cancer Biology and Genetics Program, Sloan Kettering Institute, New York, New York. ²Molecular Pharmacology Program, Sloan Kettering Institute, New York, New York. ³Department of Medicine, Memorial Hospital, Memorial Sloan Kettering Cancer Center, New York, New York.

EDITORIAL



The vascular outsiders

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A recent perspective on vessel co-option and angiotropic extravascular migratory metastasis by Lugassy et al. suggests cancers use both mechanisms sequentially during tumour growth and spread.

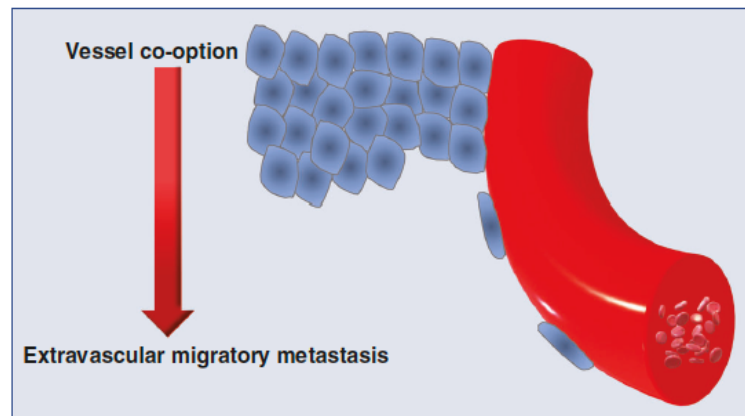
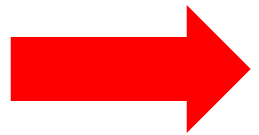


Fig. 1 Sequential tumour growth and spread by vessel co-option and extravascular migratory metastasis. Schematic of a tumour growing towards and around an existing blood vessel during vessel co-option, followed by metastatic escape of cells from the tumour which migrate along the external vessel wall during extravascular migratory metastasis.

The challenge now will be to design and deliver effective therapeutic approaches that limit the ability of tumours to use both strategies of vessel recruitment. A greater appreciation and understanding of extravascular routes of metastatic dissemination will also expand the possibilities to limit cancer spread. The work by Lugassy et al. provides new models for tumour growth and metastasis, which will ultimately uncover further avenues of research and opportunities to inhibit cancer.

Future Perspectives

Angiotropic EVMM is characterized by cancer cells migrating in vascular niches



Conferring Therapeutic Resistance



New Targets for Melanoma Treatment

