Metastasis and Angiogenesis

Advanced Topics in Cancer Biology

2009

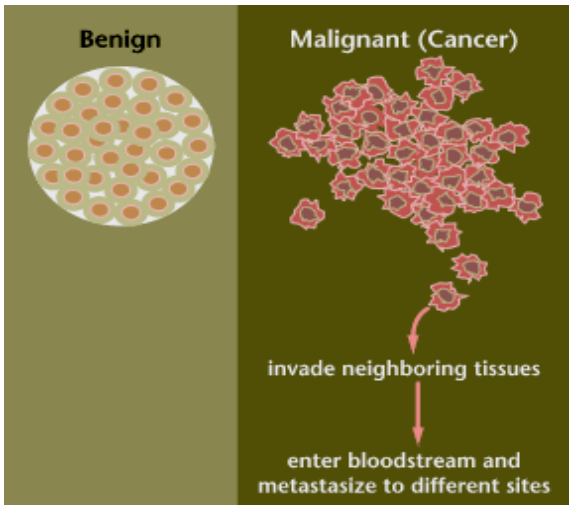
Sears

Outline

- General metastasis.
- Epithelial to mesenchymal transition.
- Linear versus parallel models of metastasis.
- Organ site specific metastasis.
- The metastatic niche.
- Angiogenesis.
- Anti-angiogenic therapy.

Malignant versus Benign Tumors

- Benign tumors generally do not spread by invasion or metastasis
- Malignant tumors are capable of spreading by invasion and metastasis – leading cause of cancer deaths



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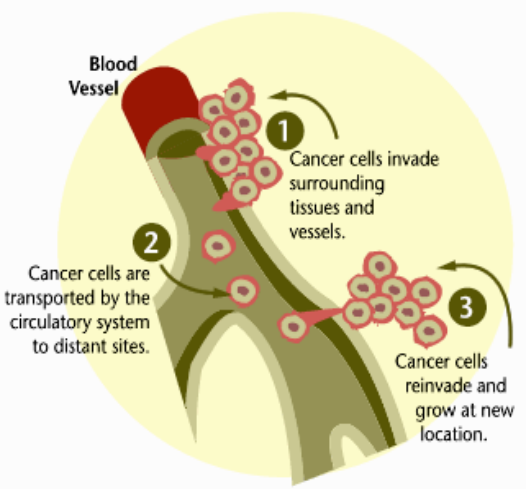
Invasion and Metastasis



- Abnormal cells proliferate and spread (metastasize) to other parts of the body
- **Invasion - direct migration and penetration into neighboring tissues**
- **Metastasis - cancer cells penetrate into lymphatic system and blood vessels**

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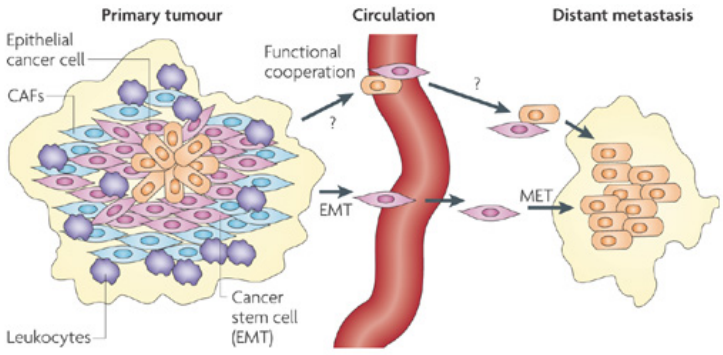
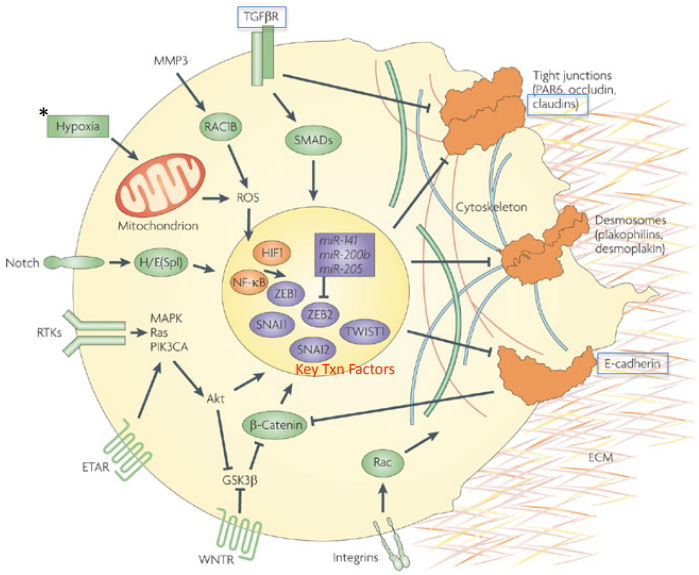
A Role for EMT in The Metastatic Process



The epithelial–mesenchymal transition (EMT) is triggered by a diverse set of stimuli including growth factor signalling, tumour–stromal cell interactions and hypoxia.
EMT has been shown to result in cancer cells with stem cell-like characteristics that have a propensity to invade surrounding tissue and display resistance to certain therapeutic interventions.
The mesenchymal–epithelial transition (MET) may have a role in the reversion of disseminated mesenchymal tumour cells to a more epithelial state in distant metastases.
microRNAs have been identified as a new class of EMT regulators, in part owing to their regulation of EMT-inducing transcription factors.

Polyak and Weinberg, 2009

Signaling networks regulating EMT



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Polyak and Weinberg, 2009

Expression and clinical relevance of selected EMT-associated genes

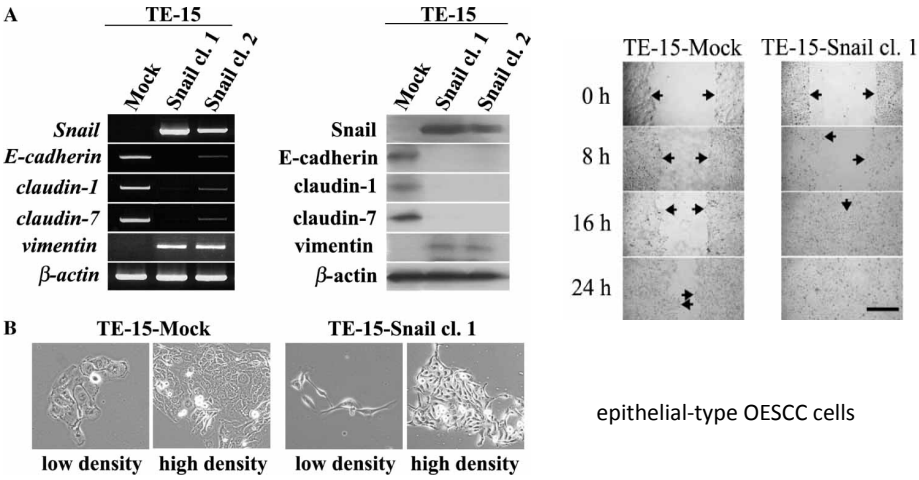
Gene	Cancer type	Tumour stage	Association with clinico-pathological features	Refs
miR-200 and *miR-205* family	Serous papillary ovarian cancer	FIGO stage III–IV	ND	46
miR-200 and *miR-205* family	Breast cancer	ND	ND	44
miR-335 gene signature	Breast cancer	Primary invasive tumours	Decreased metastasis-free survival	50
miR-10b	Breast cancer	Primary invasive tumours	Presence of metastasis	49
EMT markers	Breast cancer	Primary invasive tumours	Basal-like subtype	58
FOXC2	Breast cancer	Primary invasive tumours	Basal-like subtype	59
SNAI1	Breast cancer	Primary invasive tumours	Poor prognosis	83,85, 86
SNAI2	Breast cancer	Primary invasive tumours	Poor prognosis	83,85, 86
TWIST1	Breast cancer	Primary invasive tumours	Poor prognosis	86
ZEB2	Breast and ovarian cancer	Tumours of different stages	Poor prognosis	87
ZEB1	Uterine cancer	Primary invasive tumours	Aggressive tumour characteristics	83,85

EMT, epithelial–mesenchymal transition; FOXC1, forkhead box protein C1; ND, not determined.

Polyak and Weinberg, 2009

Snail-associated epithelial-mesenchymal transition promotes oesophageal squamous cell carcinoma motility and progression

Y Usami 1 2, S Satake 1, F Nakayama 1, M Matsumoto 1, K Ohnuma 1, T Komori 2, S Semba 1, A Ito 1, H Yokozaki 1 *

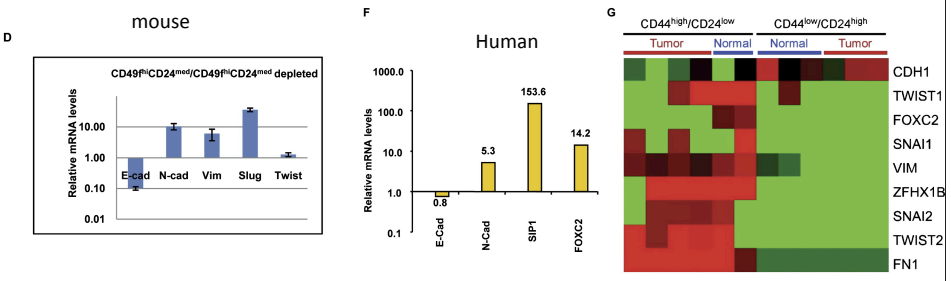


epithelial-type OESCC cells

2008 J. Pathology

The Epithelial-Mesenchymal Transition Generates Cells with Properties of Stem Cells

Sendurai A. Mani1,3,9,Go To Corresponding Author,,Wenjun Guo1,9,Mai-Jing Liao1,9,Elinor Ng. Eaton1,Ayyakkannu Ayyanan4,Alicia Y. Zhou1,2,Mary Brooks1,Ferenc Reinhard1,Cheng Cheng Zhang1,Michail Shipitsin5,6,Lauren L. Campbell5,7,Kornelia Polyak5,6,7,Cathrin Brisken4,Jing Yang8andRobert A. Weinberg1,2



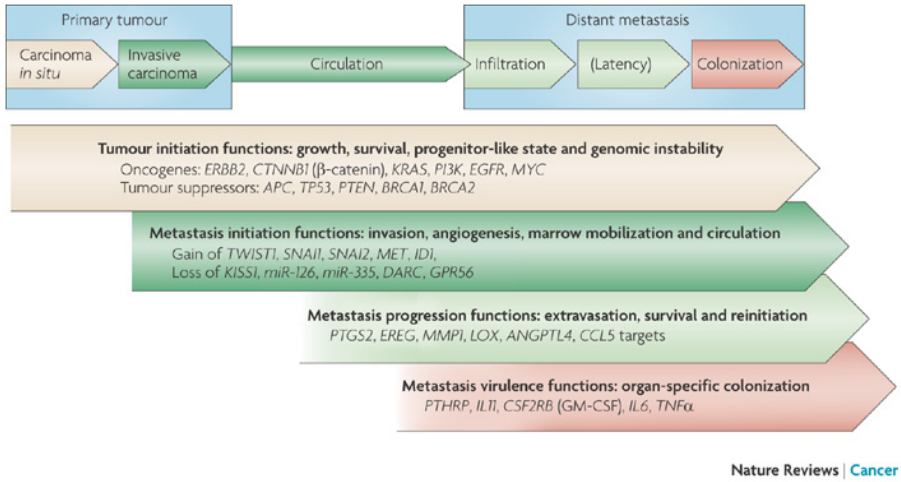
CDH1=E-cadherin

Cells Injected	Tumors Incidence/Number of Injections			
	1X 10^6	1 X10^5	1X 10^4	1X 10^3
HMLE-Vector-Ras	2/6	3/9	0/9	0/9
HMLE-Snail-Ras	6/6	9/9	9/9	6/9
HMLE-Twist-Ras	6/6	9/9	9/9	7/9

Table 2 Tumor Incidence of Transformed HMLEs Induced to Undergo EMT by Ectopic Expression of Snail or Twist and Then Injected into Host Mice in Limiting Dilutions

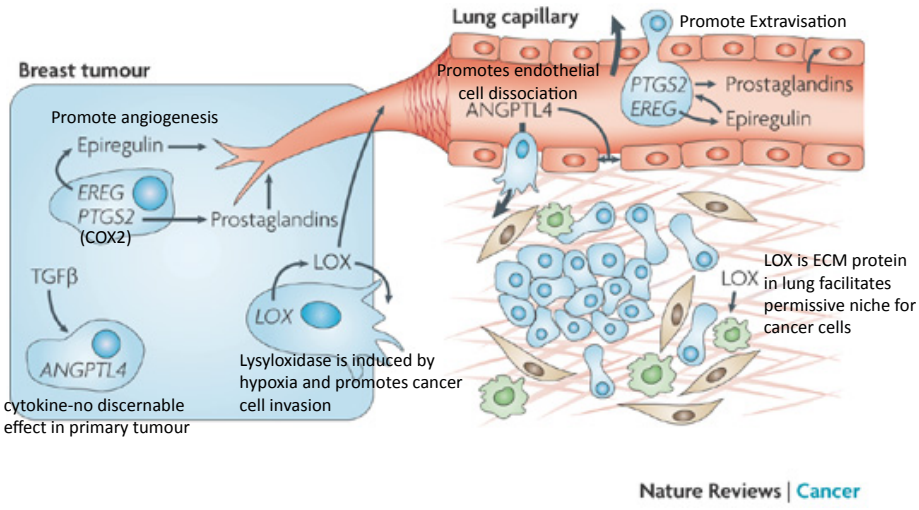
Cell, Volume 133, Issue 4, 704-715, 16 May 2008

Genes involved in metastatic steps, where is the selective pressure?



Nguyen, Bos and Massague, 2009

Dual functions of metastasis progression genes allow for selection in the primary tumour



Nguyen, Bos and Massague, 2009

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Two Fundamental Models of Metastasis

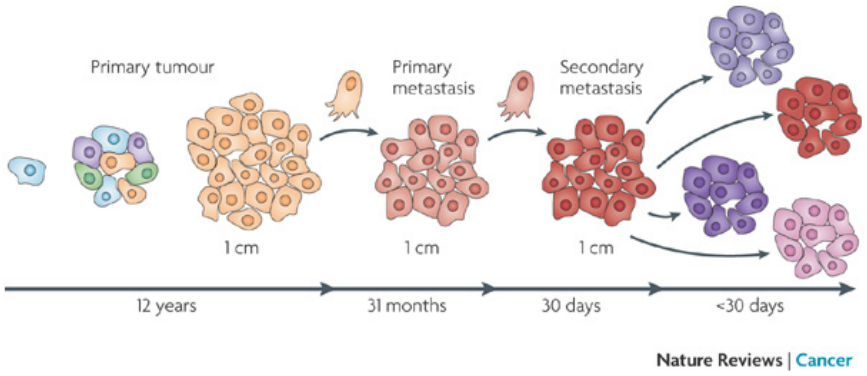
Linear Model of Metastasis

In this model, tumour ontogeny proceeds to full malignancy within the primary tumour microenvironment, after which tumour cell dissemination founds a metastasis. Therefore, the primary tumour prescribes the molecular characteristics of DTCs (Disseminated Tumour Cells) spread throughout the body.

Parallel Model of Metastasis

In this model, tumour cells depart the primary lesion before the acquisition of fully malignant phenotypes to undergo somatic progression and metastatic growth at a distant site. The proposition of early dissemination and divergent progression of primary tumours and DTCs towards metastasis questions the role of the primary tumour for therapy prediction.

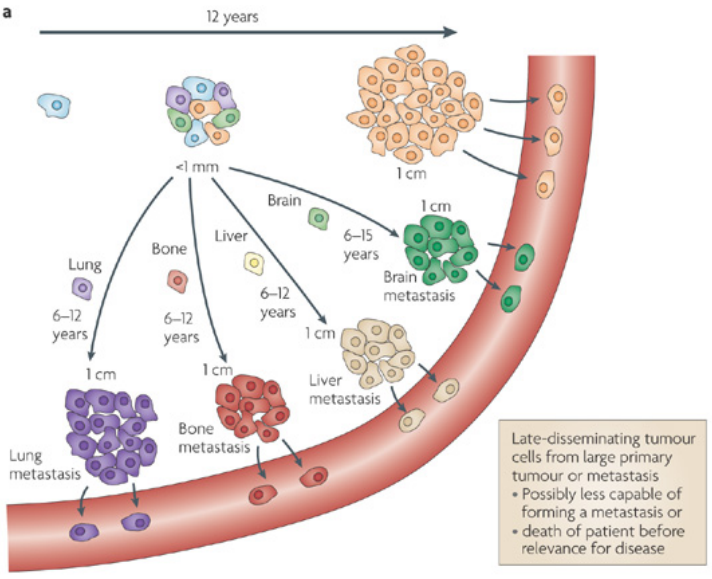
Linear progression model



Support for: Correlation with tumour size and frequency of metastasis.
Support against: average doubling time 157 days for breast cancer so 12 years to reach 1 CM, then another 6-12 years for first met to reach 1 CM, not consistent with evidence.

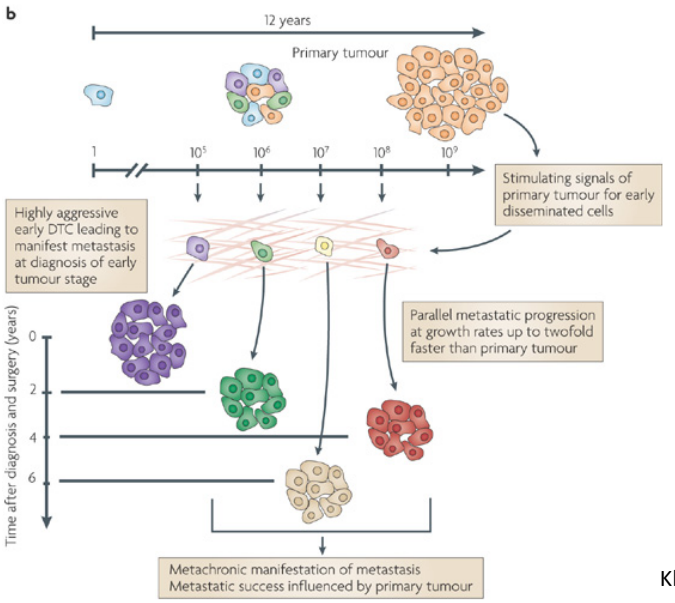
Klein, 2009

Parallel progression model



Klein, 2009

Parallel progression model-better fit with clinical data



Klein, 2009

Genetic evidence supporting the Parallel progression model

Seminal paper by Schlimok and Riethmuller detecting cytokeratin positive cells in bone marrow in patients with M0 and M1 breast cancer. Only 1-10 cells per 2 million bone marrow cells. Proc Natl Acad Sci U S A. 1987 Dec;84(23):8672-6

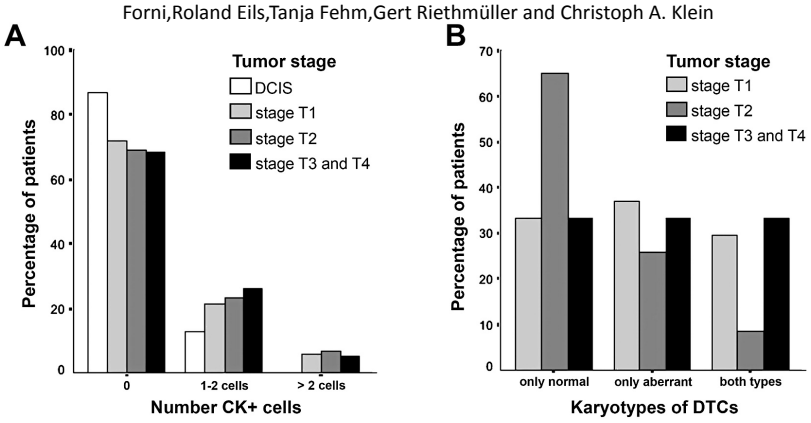
Advent of single cell CGH showed bone marrow DTCs had significantly fewer genetic aberrations than primary tumour cells. In BC 50% of cytokerratin-positive cells had normal karyograms whereas all matched primary tumour karyograms were abnormal. But while DTC were karyotypically normall they contained small deletions typical of BC indicating that they disseminated before genome-wide instability was acquired (Schardt et al., 2005 Cancer Cell 8:227)

ERBB2 amplification in DTCs did not correlate with amplification in primary tumour. Interestingly, ERBB2 amplification in primary tumour (T1,T2) did not correlate with reduced survival, but patients displaying a gain in ERBB2 in a single DTC from bone marrow or lymph node died within 23 months. (Stoecklein et al., 2008, Cancer Cell 13:441)

KRAS and P53 mutations show heterogeneity within and between primary colorectal carcinomas and matched metastases. (Albanese et al., 2004, Biochem. Biophys. Res. Commun. 325:784)

Systemic Spread Is an Early Step in Breast Cancer

Yves Hüsemann,Jochen B. Geigl,Falk Schubert,Piero Musiani,Manfred Meyer,Elke Burghart,Guido Forni,Roland Eils,Tanja Fehm,Gert Riethmüller and Christoph A. Klein

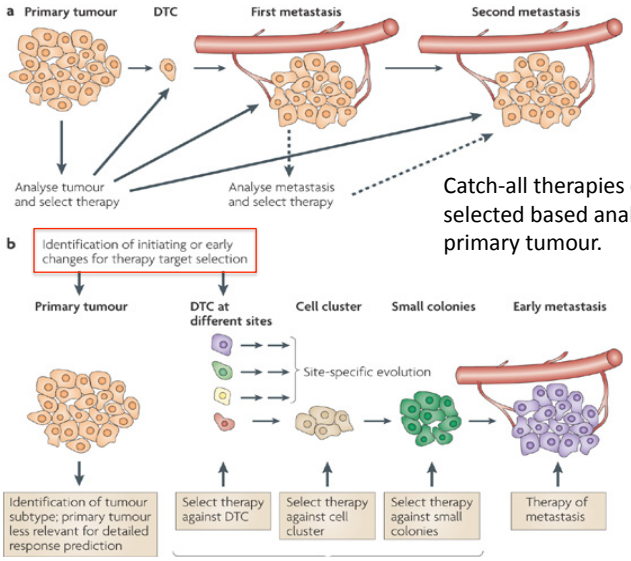


A. Number of detected CK+ cells per 2 X10^6 bone marrow cells in patients with different tumor stages (DCIS, n = 39; T1, n = 328; T2, n = 202; and T3/4, n = 38).There was no association between tumor stage and the presence of disseminated cells, and specifically, the finding of CK+ cells in patients with ductal carcinoma in situ (DCIS; 13%) andT1-stage patients (22%) was statistically not different (p=0.093, Pearson's chi-square test).

B. 105 single CK+ cells isolated from 56 patients showed no significant difference between patients with small and large, indicating that the well-known association of large tumor size and development of manifest metastasis is not explained by an increased frequency of genetically progressed cancer cells in bone marrow

Cancer Cell, 13, 58-68, 2008

Linear and Parallel progression models- consequences for therapy



Catch-all therapies can be selected based analysis of the primary tumour.

Klein, 2009

Predicting responses to therapies will require the molecular characterization of DTCs.

Questioning metastasis from metastasis

- A cascade model previously argued that targeting the first metastasis might prevent additional metastasis. (Weiss et al., 1988, J Cancer Res. Clin. Oncol. 114:605)
- Median time for 50% of BC patients with M0 disease and surgery to develop a single met is the same as for multiple mets.(Klein and Holzel, 2006, Cell Cycle 5:1788)
- Halsted's theory of continuous cancer spread by lymphatic dissemination (1907) is the basis for surgeon's removal of 10-12 lymph nodes in patients with a positive sentinel node.
- However, three randomized trials showed no effect of removal of clinically negative nodes on distant metastasis or survival. (Rudenstam et al., 2006, J. Clin. Oncol. 24:337)

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Metastasis is not random

- Seed and soil hypothesis
  - 1889: Stephen Paget analyzed autopsy records of 735 women with breast cancer
  - Metastasis to distant sites was not due to chance
  - Certain tumor cells (the "seed") has an affinity for the milieu (the "soil") of certain organs. Metastases resulted when the seed and soil were compatible
- Regional metastases can be attributed to anatomic and mechanical factors but distant organ metastases is specific
  - 1964: Sugarbaker
  - Lymphatic drainage to regional lymph nodes
  - Organ-specific metastases: breast, prostate, and lung cancer metastasize to the bone, while colorectal cancer metastasized to the liver and lymph nodes

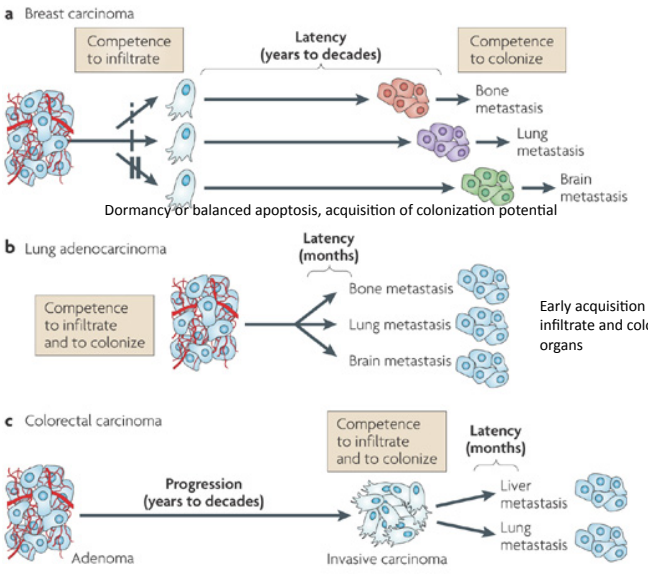
Typical sites of metastatic relapse for solid tumours

Tumour type	Principal sites of metastasis
Breast	Bone, lungs, liver and brain
Lung adenocarcinoma	Brain, bones, adrenal gland and liver
Skin melanoma	Lungs, brain, skin and liver
Colorectal	Liver and lungs
Pancreatic	Liver and lungs
Prostate	Bones
Sarcoma	Lungs
Uveal melanoma	Liver

•Kinetics of metastasis is based on the temporal gap between organ *infiltration* and *colonization* producing a period of metastatic *latency*.
•Dramatically different latency for different cancer types: For example, adeoncarcinomas of the breast and lung relapse in a similar range of organs. However BC recurrence take years or decades while lung cancers establish distant metastases within months of diagnosis.

Nguyen, Bos and Massague, 2009

Temporal course of metastasis



Dormancy or balanced apoptosis, acquisition of colonization potential

Early acquisition of potential to infiltrate and colonize other organs

acquisition of infiltration and colonization potential

Nguyen, Bos and Massague, 2009

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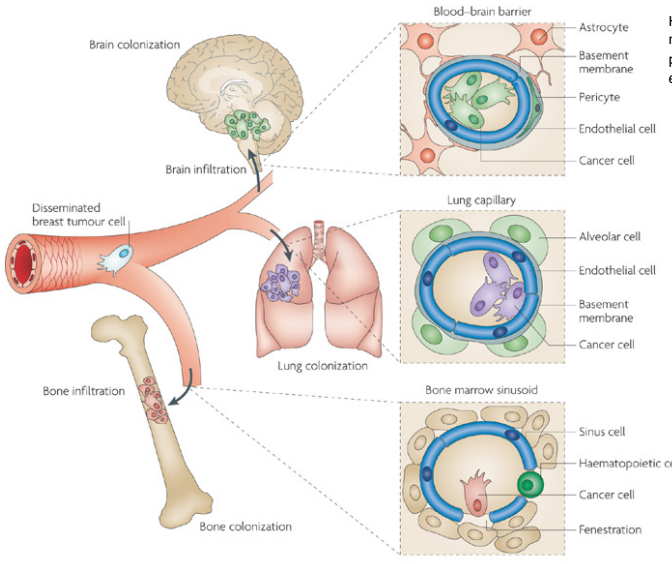
Organ-specific barriers to metastatic infiltration



Hardest. Basement membrane and astrocyte foot processess inhibit extravisation.

Harder. Basement membrane and adjacent alveolar cells inhibit extravisation. Require specific mediators of trans-endothelial migration.

Easiest. Fenestrated to facilitate traffic of hematopoietic cells. Liver also.

Nguyen, Bos and Massague, 2009

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Organ Specific metastasis of Breast Cancer Cells

MDA-MB-231 Breast Cancer Cell Line
(established as the total outgrowth of cells derived from a pleural effusion of a patient who relapsed years after removal of the primary tumor)

↓

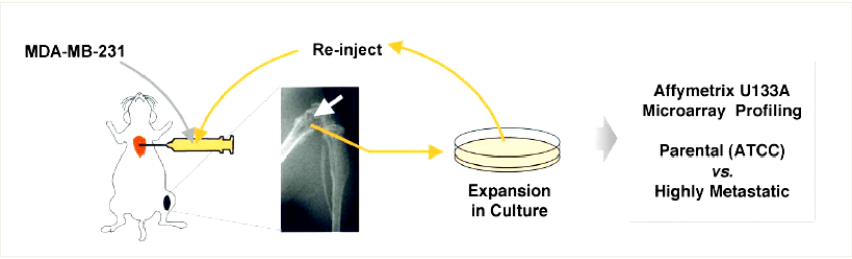
Isolate Single Clonal Populations (SCPs)

↓

Introduce Luciferase Bioluminescent Marker and GFP Fluorescence Marker

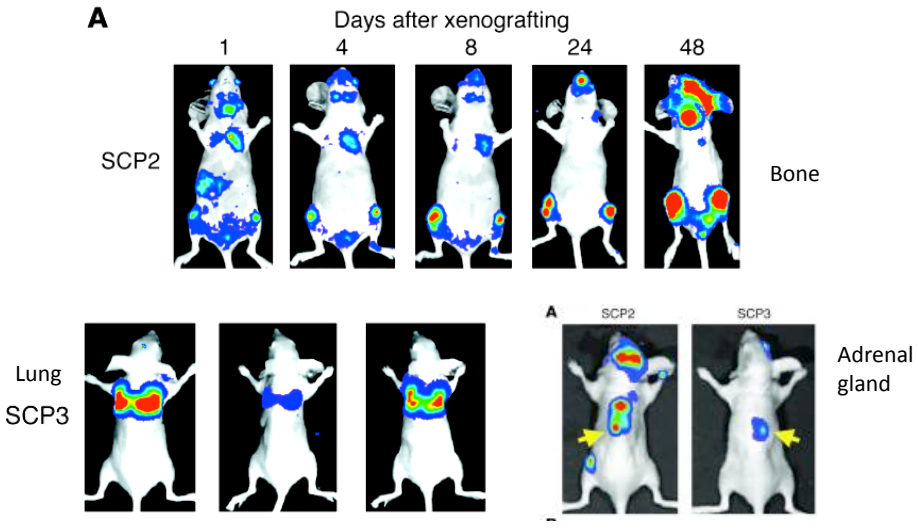
↓

Introduce into Nude Mice by intracardiac Injection



Minn, A. J. et al. J. Clin. Invest. 2005;115:44-55

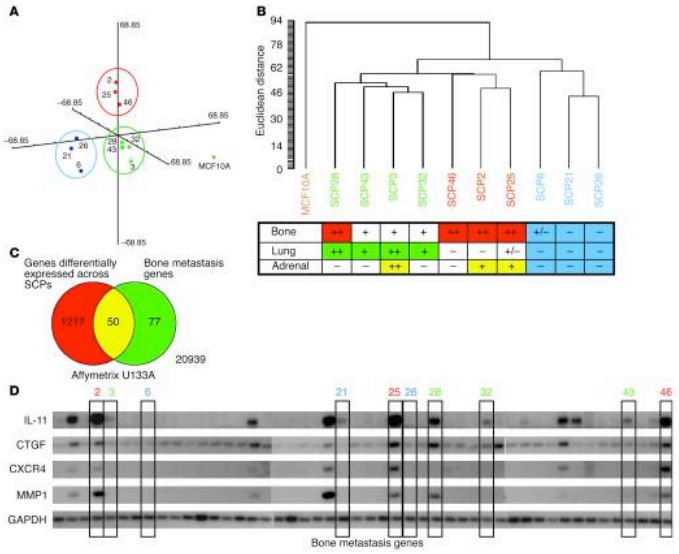
SCPs exhibit different abilities to metastasize to bone or lung



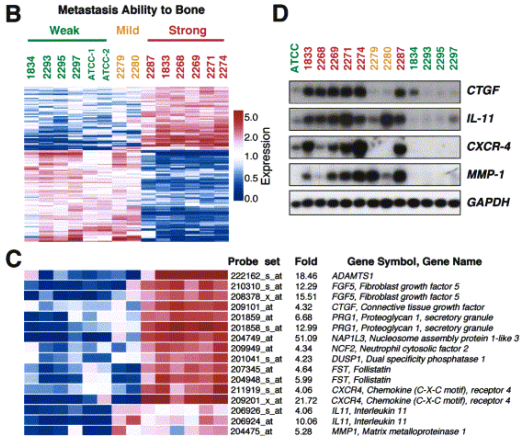
Minn, A. J. et al. J. Clin. Invest. 2005;115:44-55

Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors

Andy J. Minn, Yibin Kang, Inna Serganova, Gaorav P. Gupta, Dilip D. Giri, Mikhail Doubrovin, Vladimir Ponomarev, William L. Gerald, Ronald Blasberg, and Joan Massagué

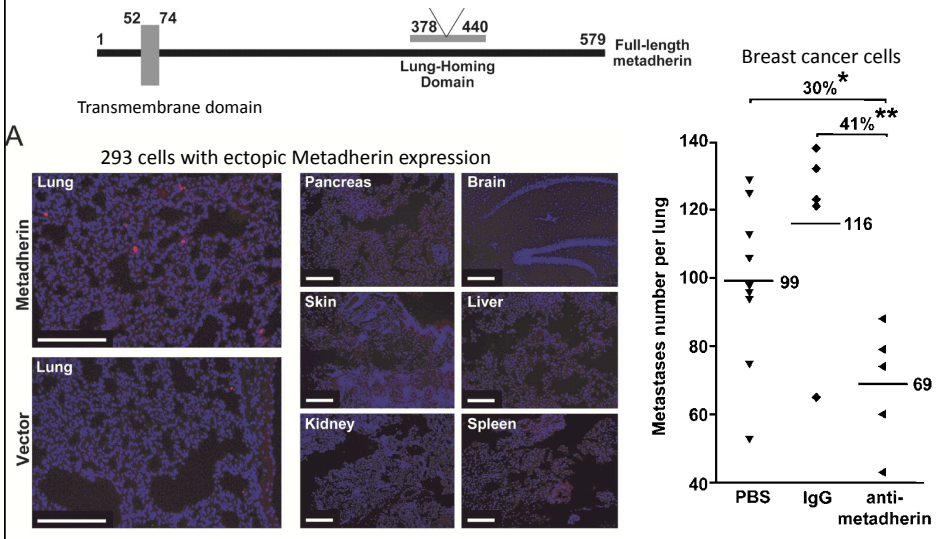


Genes that mediate metastasis to the Bone



CXCR4 – bone homing chemokine receptor
CTGF – connective tissue growth factor
IL-11 – activator of osteoclast differentiation
(mediators of bone resorption in bone metastases)
MMP1 – matrix metalloproteinase/collagenase, promotes osteolysis by cleaving a specific peptide bond in the collagen of bone matrix
OPN – osteopontin (consistently overexpressed in metastatic cells)

Metadherin, a cell surface protein in breast tumors that mediates lung metastasis



Brown and Ruoslahti, 2004, Cancer Cell, 5, 365-374

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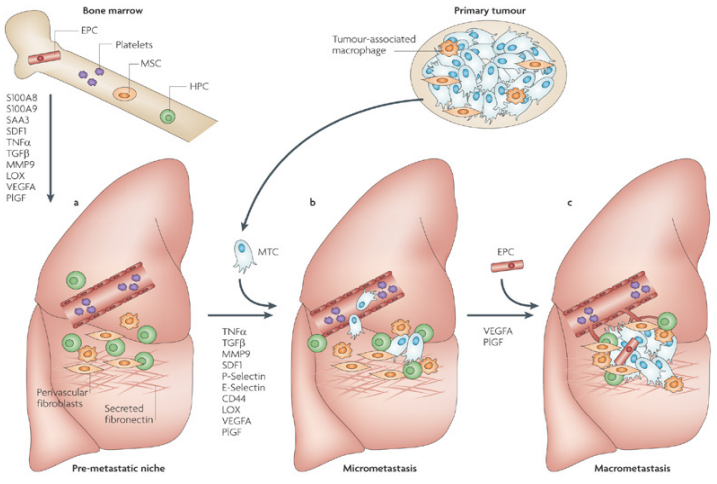
Bone marrow-derived cells (BMDCs) foster tumorigenesis and metastasis

- Haematopoietic progenitor cells are implicated as initiators of the pre-metastatic niche and are involved in angiogenesis. Mature monocyte and macrophage cells and neutrophils secrete chemokines and matrix-degrading enzymes that modulate the local microenvironment and mediate the chemoattraction of other inflammatory cells to the pre-metastatic niche.
- Endothelial progenitor cells are mobilized from the bone marrow during angiogenesis. It has been suggested that recruitment of endothelial progenitor cells instigates the micrometastatic to macrometastatic switch.
- Mesenchymal stem cells give rise to fibroblasts, which are important components of the tumour stroma. They may also directly interact with tumour cells to enhance their metastatic phenotype

Evolution of a metastatic niche – involves a tumour-permisive immunological or inflammatory microenvironment

EPC: endothelial progenitor cells; MSC: mesenchymal stem cells; HPC: haematopoietic progenitor cells; MTC: metastatic tumor cell

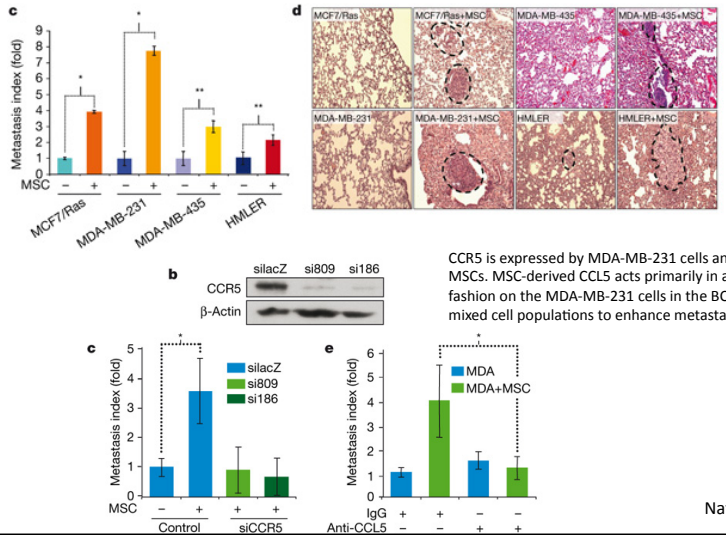


Before arrival of primary tumor cells. Resulting from systemic effects of factors secreted by primary tumor cells.

Psaila and Lyden, 2009

Mesenchymal stem cells within tumour stroma promote breast cancer metastasis

Antoine E. Karnoub, Ajeeta B. Dash, Annie P. Vo, Andrew Sullivan, Mary W. Brooks, George W. Bell, Andrea L. Richardson, Kornelia Polyak, Ross Tubo & Robert A. Weinberg



CCR5 is expressed by MDA-MB-231 cells and not by MSCs. MSC-derived CCL5 acts primarily in a paracrine fashion on the MDA-MB-231 cells in the BCC and MSC mixed cell populations to enhance metastasis.

Nature 2007

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Angiogenic Switch - Hypothesis

Expansion of a tumor mass beyond the initial microscopic size of a non-angiogenic tumor is dependent on the recruitment of its own vascular supply, by angiogenesis and/or blood vessel cooption. The ability of a tumor to progress from a non-angiogenic to angiogenic phenotype is central to the progression of cancer and is termed the "angiogenic switch".

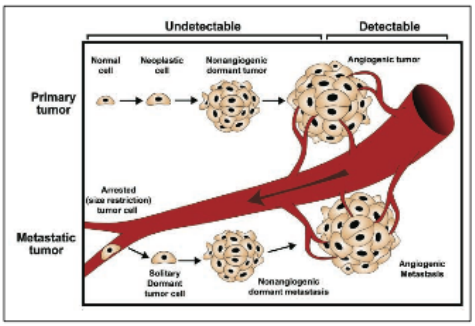


Figure 1. Dr. Judah Folkman (1933–2008).

History of Angiogenesis Research

- 1970's – Hypothesis of Folkman that tumor growth depends on angiogenesis
- 1980's- Identification of vascular growth factors – Proof of concept in animal models
- 1990's – Clinical Trials of angiogenic inhibitors – Early clinical failures - monotherapy
- 2004- FDA approval of bevacizumab for metastatic colorectal CA
- 2007- Bevacizumab + irinotecan efficacious for glioblastoma

Tumor Angiogenesis

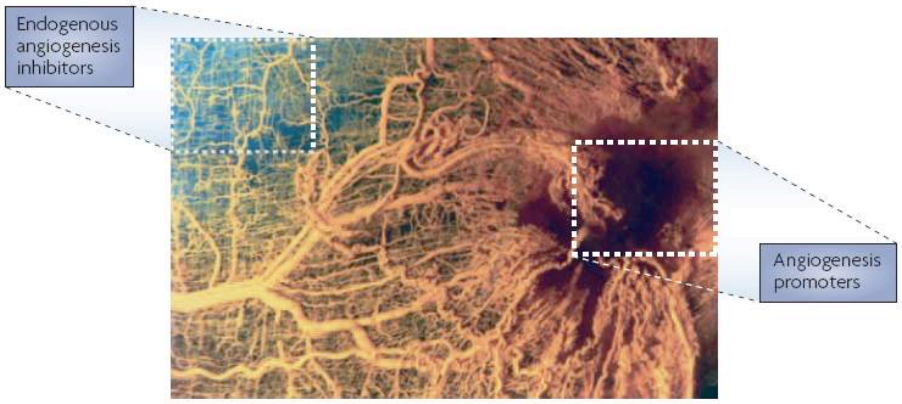


Figure 2 | Angiogenesis in rat sarcoma. In this micrograph, blood vessels grow towards a sarcoma (dark area at right) in rat muscle. This contrasts with the normal grid-like pattern of blood vessels that appears at the upper left. (Courtesy of L. Heuser and R. Ackland, University of Louisville, USA)

Folkman J, Nature Drug Discovery 6:274, 2007

Escape from tumor cell dormancy requires the angiogenic switch

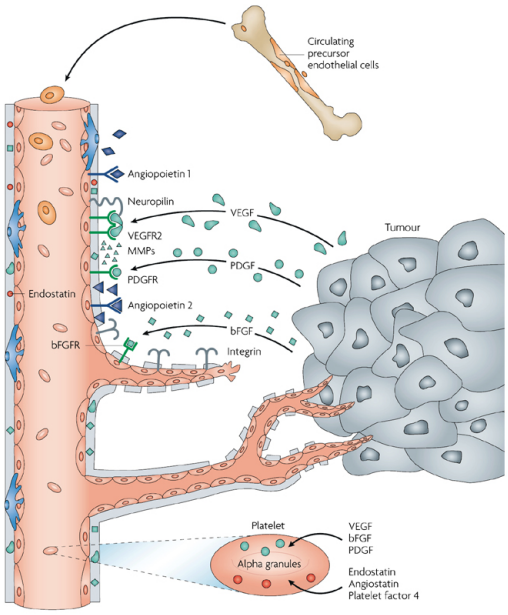
Table 1 **Human tumor cell lines that spontaneously switch to the angiogenic phenotype after a prolonged dormancy period in immunocompromised mice**

Human cell line	Cancer type	Dormancy period	Spontaneous angiogenic switch (% of tumors that switch to the angiogenic phenotype)	Reference
MDA-MB-436	Breast	4 months	~80%	Naumov et al., JNCI 2006
KHOS-24OS	Osteosarcoma	8 months	~20%	Naumov et al., JNCI 2006
T98G	Brain	8 months	~60%	Naumov et al., JNCI 2006
SAOS-2	Osteosarcoma	12 months	~10–15%	Udagawa et al., FASEB 2002 and unpublished results
ST-2	Gastric	>8 months	<3%	Udagawa et al., FASEB 2002
MG-63	Osteosarcoma	12 months	~5%	Udagawa et al., FASEB 2002 and unpublished results
MDA-MB-436-A1*	Breast	8 months	~20%	Naumov et al., JNCI 2006
SW 872*	Liposarcoma	4 months	>95%	Almog et al., FASEB 2006

*Single cell clone-derived tumor cell populations.

During dormancy cells: (1) remain harmless to the host until they switch to the angiogenic phenotype (i.e., may be harmless for 1 year or more, which is half the life-span of a mouse); (2) express equal or more antiangiogenic (i.e., thrombospondin-1) compared to angiogenic (i.e., VEGF, bFGF) proteins; (3) grow to approximately 1 mm in diameter or less in vivo, at which time further expansion ceases; (4) show active tumor cell proliferation in mice (balanced by apoptosis), and remain metabolically active during the dormancy period.

Naumov, Akslen and Judah Folkman, 2006 Cell Cycle



Folkman J, Nature Drug Discovery 6:274, 2007 Nature Reviews | Drug Discovery

•Angiopoietin 1 (ANGPT1) maintains normal blood vessels. Tumour cells secrete ANGPT2, which competes for binding to the endothelial TIE2 receptor. ANGPT2 increases the degradation of vascular basement membrane and migration of endothelial cells, therefore facilitating sprout formation.

•Vascular endothelial growth factor (VEGF) is secreted by tumour cells. It is the most common of at least six other pro-angiogenic proteins from tumours. Others include Platelet-derived growth factor (PDGF) and Basic fibroblast growth factor (bFGF; also known as FGF2). Endostatin is anti-mitogenic.

•Integrins facilitate endothelial cell binding to extracellular matrix and promote cell viability. Pro-angiogenic proteins upregulate endothelial integrins to sustain endothelial cell viability during the intermittant detachments required for migration.

•New endothelial cells are also recruited as precursor bone-marrow-derived endothelial cells.

•Some angiogenic regulatory proteins (both pro- and anti-angiogenic) are scavenged by platelets, stored in alpha granules and released within the tumour vasculature.

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- Anti-angiogenic therapy.

Current Angiogenic Inhibitors in Clinical Use and Clinical Trials

- Bevacizumab (Avastin™)
- Sunitinib (Sutent™)
- Sorafenib (Nexavar™)
- Cederanib (Recentin™ - AZD- 2171)
- Cilengitide
- VEGF-Trap

Many others in development

Different Mechanism of Action of 3 FDA-Approved Drugs

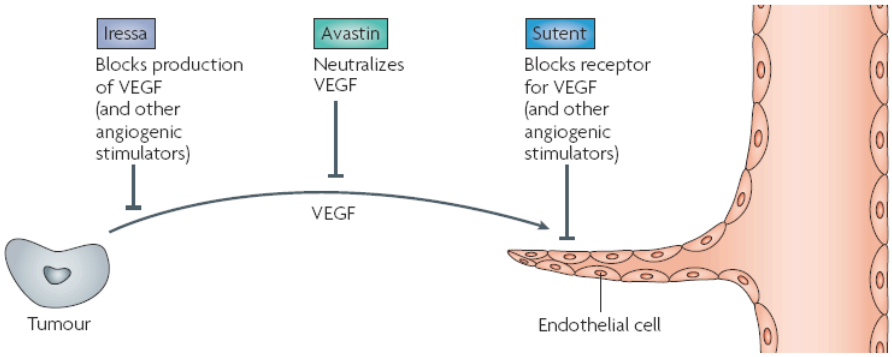


Figure 6 | **Three general mechanisms of angiogenesis inhibitors currently approved by the FDA.**

Folkman J, Nature Drug Discovery 6:274, 2007

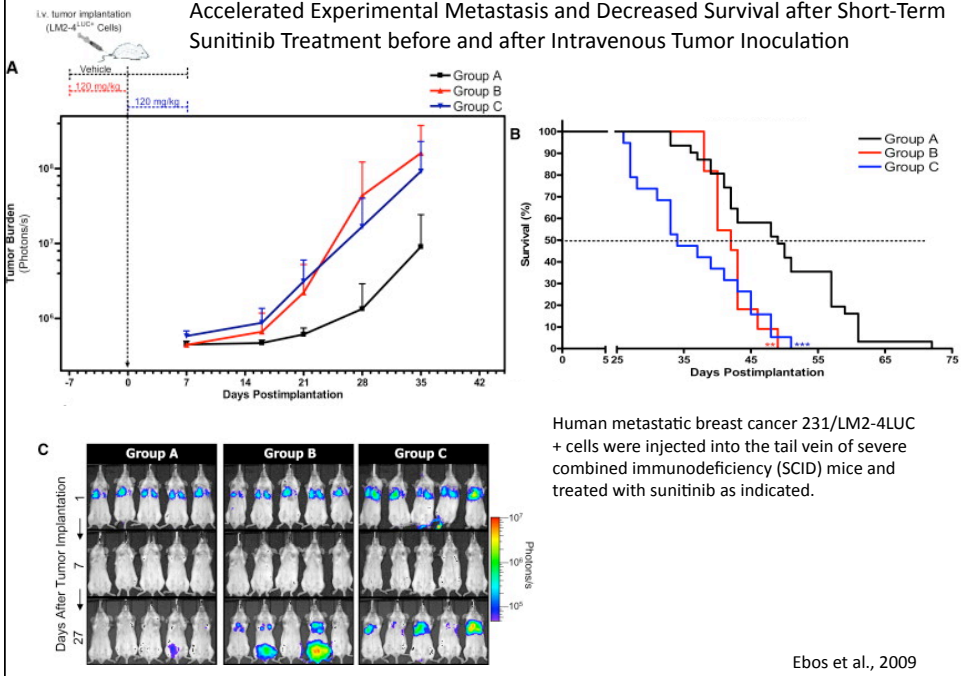
Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis

Marta Pàez-Ribes, Elizabeth Allen, James Hudock, Takaaki Takeda, Hiroaki Okuyama, Francesc Viñals, Masahiro Inoue, Gabriele Bergers, Douglas Hanahan, Oriol Casanovas

Accelerated Metastasis after Short-Term Treatment with a Potent Inhibitor of Tumor Angiogenesis

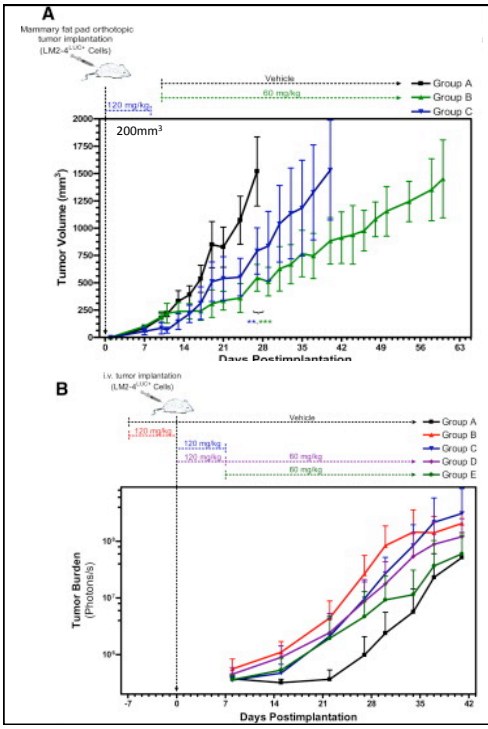
John M.L. Ebos, Christina R. Lee, William Cruz-Munoz, Georg A. Bjarnason, James G. Christensen, Robert S. Kerbel

Accelerated Experimental Metastasis and Decreased Survival after Short-Term Sunitinib Treatment before and after Intravenous Tumor Inoculation

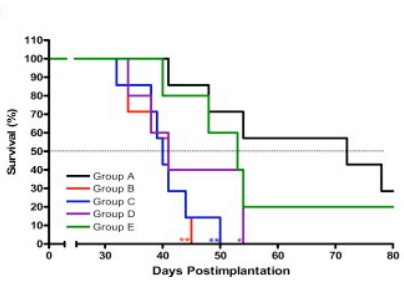


Human metastatic breast cancer 231/LM2-4LUC + cells were injected into the tail vein of severe combined immunodeficiency (SCID) mice and treated with sunitinib as indicated.

Ebos et al., 2009



Opposing Efficacies of Short-term and Sustained Sunitinib Treatment in Primary and Metastatic Disease

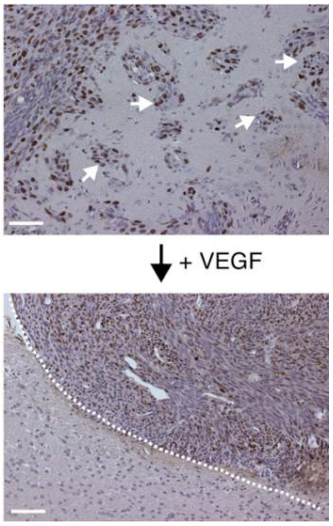


231/LM2-4LUC+ cells were implanted into the mammary fat pad of nu/nu mice and treated with Sunitinib as indicated.

Ebos et al., 2009

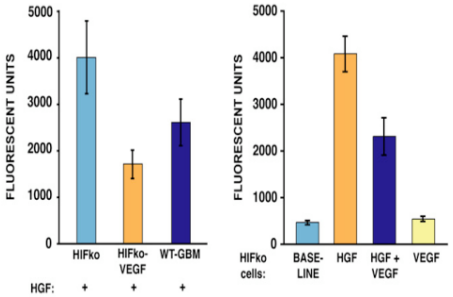
VEGF as a negative regulator of glioma invasion

HIFko GBM



perivascular invasion

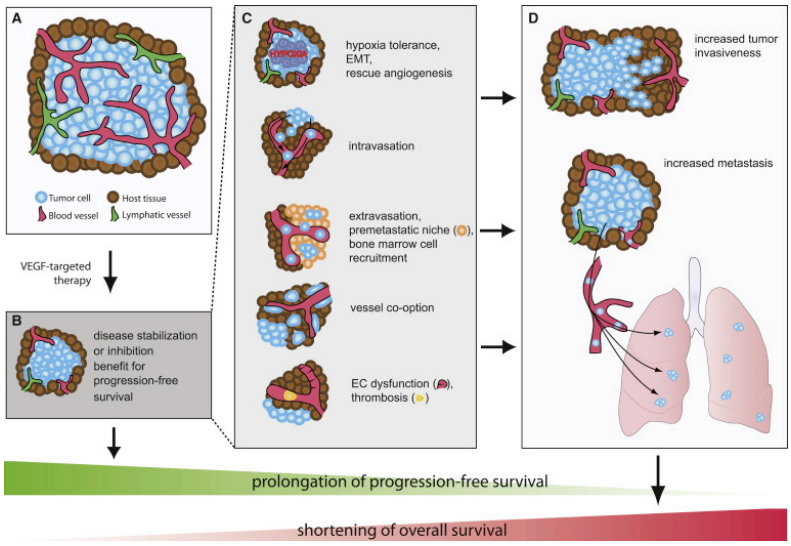
Boyden Chamber invasion assay



HIFko have reduced VEGF and reduced vascularization, but increased perivasculature invasion, this is likely a mechanism for the tumor cells to evade hypoxia.

Du R et al., *Cancer Cell* 13:206-220, 2008.

Increased tumor invasiveness and metastasis evoked by VEGF inhibitors



Loges et al., 2009, Cancer Cell

The End!