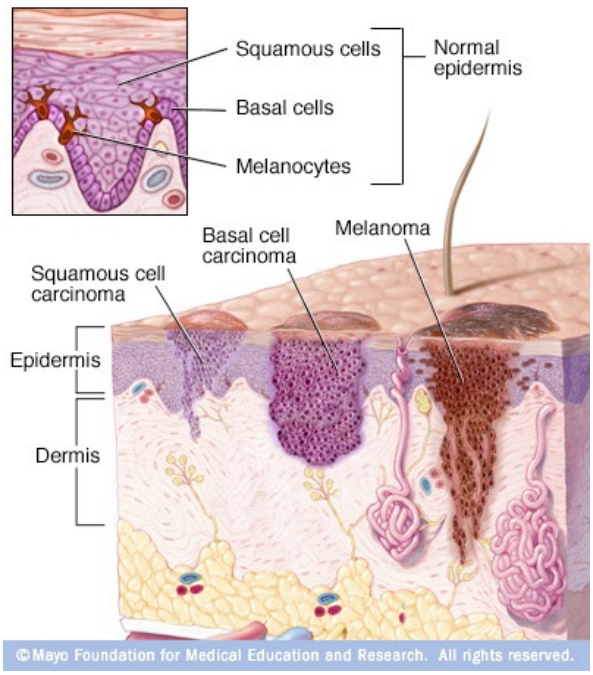
Today: Invasion & metastasis

Specific barrier:
Most cell types firmly adhere to their neighbors and to *extracullar matrix (ECM)*.
Those that manage to escape will face new environments to which they are not adapted.

Acquired capability:

Rewiring of multiple signaling circuits and remodeling of ECM facilitate cell survival, plasticity and detachment from primary tumors by reactivating specific developmental programs.

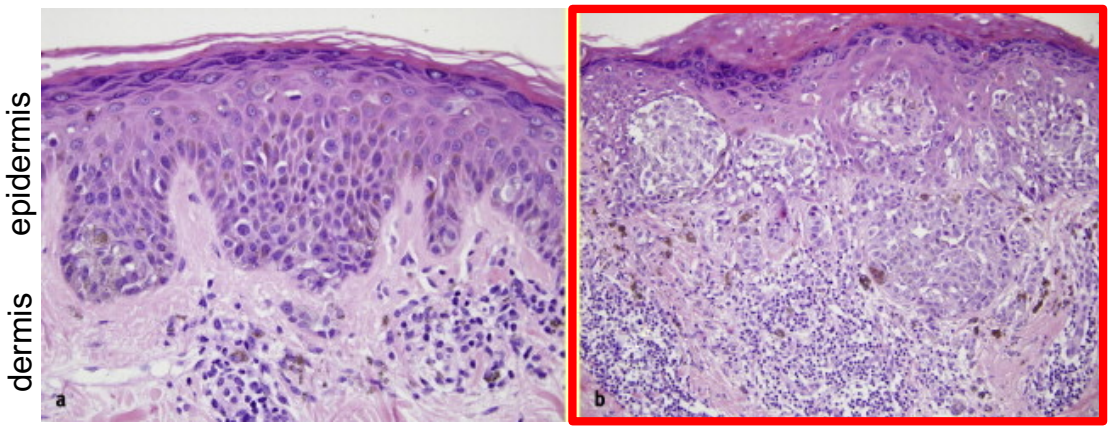
Not all cancer types are equally prone to metastasize



In skin only <4% SCC, and <0.1% of BCC metastasize, compared to up to 70% of melanoma

Some cancers almost always metastasize

- Radially growing melanomas rarely metastasize (left)
- By contrast, e.g. melanoma in vertical growth phase almost always spawn metastases



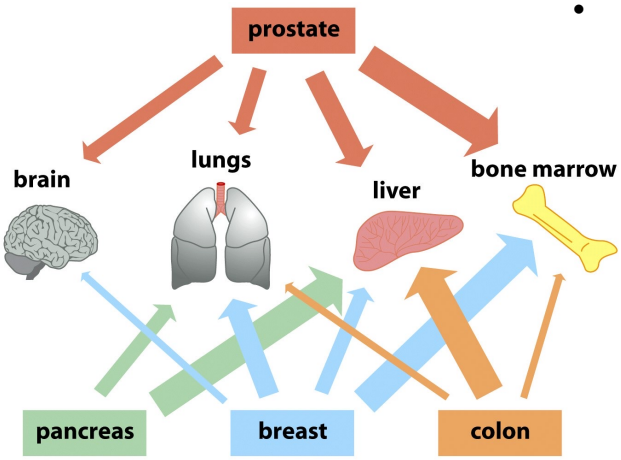
radial growth phase =
only few, sparse tumor cells in dermis compared to epidermis

vertical growth phase =
tumor mass in dermis exceeds that in epidermis ("iceberg")

S. Hamza 2010 Diagnost Histopathol 16:330 - 336

Organotropism

- Not all cancers disseminate to every organ
- Seed and soil hypothesis (Stephen Piaget, 1889): *"(When) a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial (congénère/*wesensverwandt*) soil"*
- e.g. a common neurectodermal origin might explain a tropism of melanoma towards brain



- however, it does not explain why "contralateral" spreading is rare, e.g. from one breast to the other

Figure 14.43 & 44 *The Biology of Cancer*

Metastasis by anatomical and mechanical routes

- James Ewing, 1928 (founder of MSKCC, New York):
  "The seed does not spread randomly."
- E.g. the lungs and liver get higher dose of 'seeds', because their blood capillaries are the first to trap blood-borne disseminating cells:

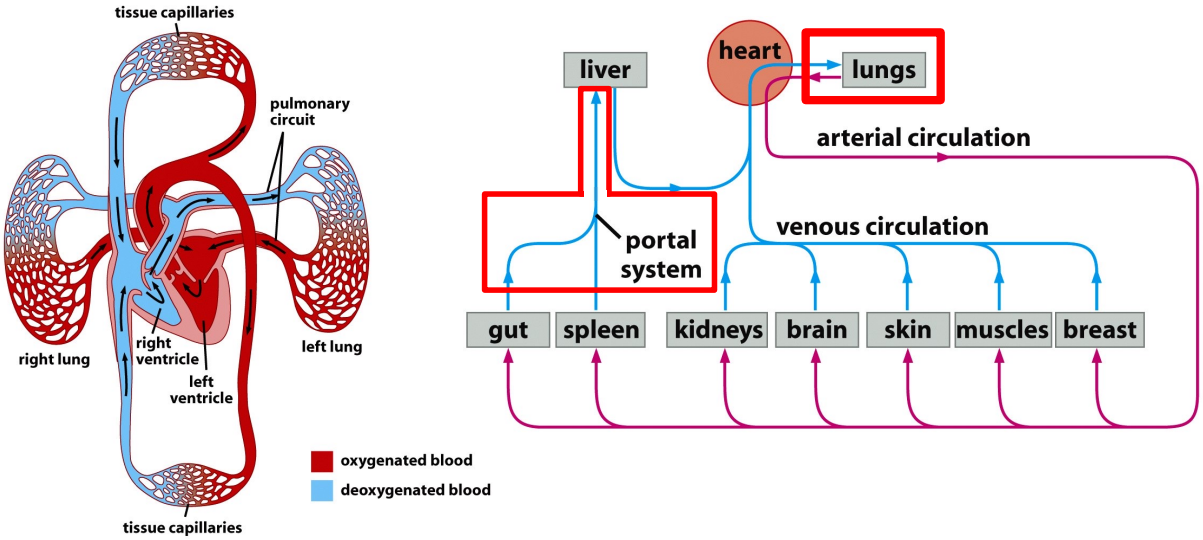
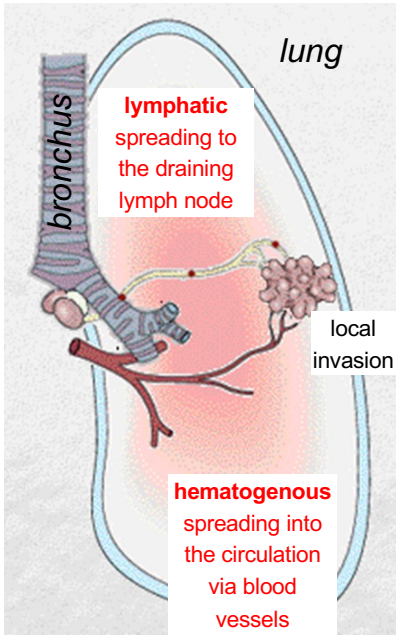
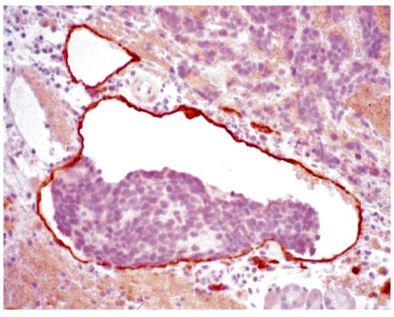


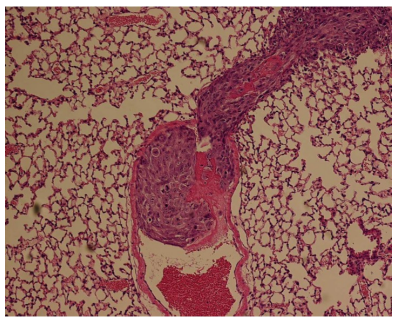
Figure 14.6 & 14.44 *The Biology of Cancer* (© Garland Science 2007)

Cancer cells also disseminate via lymphatics

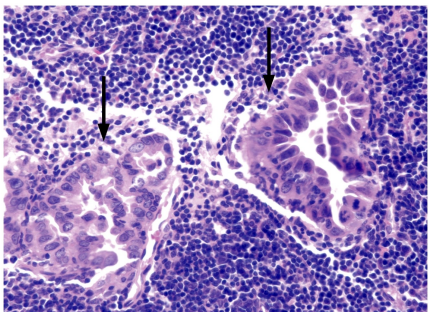


Mouse pancreatic cancer cells in a lymphatic vessel:



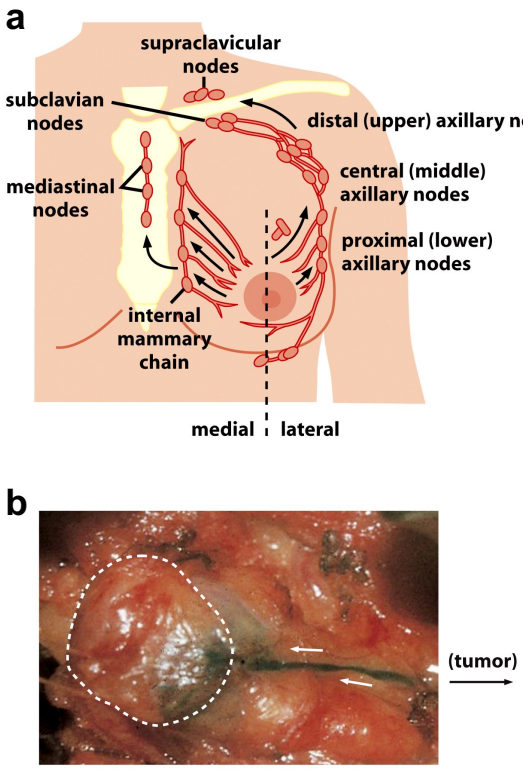


Lymph node with two lung adenocarcinoma micrometastases (one has a duct-like structure):



Lung cancer cells entering a blood vessel

Draining lymph nodes (example)



a. Lymphatics drain interstitial fluid from various sectors of the breast to region-specific lymph nodes

b. A dye injected into the tumor mass drains into its "sentinel" lymph node via the lymphatic duct

c. Diagnostic staining of cytokeratin (epithelial marker) can detect even small micrometastases in a draining lymph node:

Figure 14.42 *The Biology of Cancer*

Disseminating from lymphatics to blood vessels

- Spreading beyond lymph nodes can occur through *lymphatics that drain into the subclavian vein* near the heart:

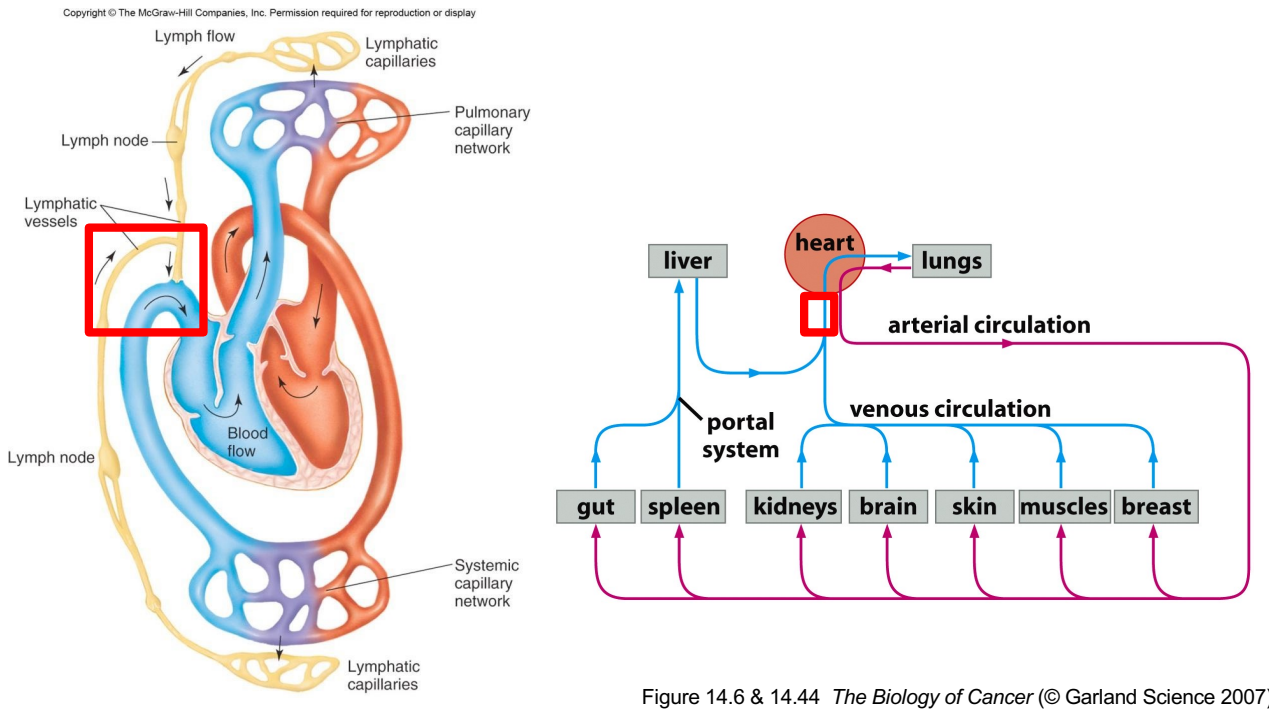


Figure 14.6 & 14.44 *The Biology of Cancer* (© Garland Science 2007)

Key questions

- So *how* do metastatic cells invade surrounding stroma and enter lymphatics and/or blood vessels?
- Thereafter, what *additional* changes are needed in cancer cells to successfully seed metastases?
- What are the *drivers* of metastasis, and does their elucidation offer a therapeutic window of opportunity?

CellPress

Cancer Cell
Review

Cancer Genome Evolutionary Trajectories in Metastasis

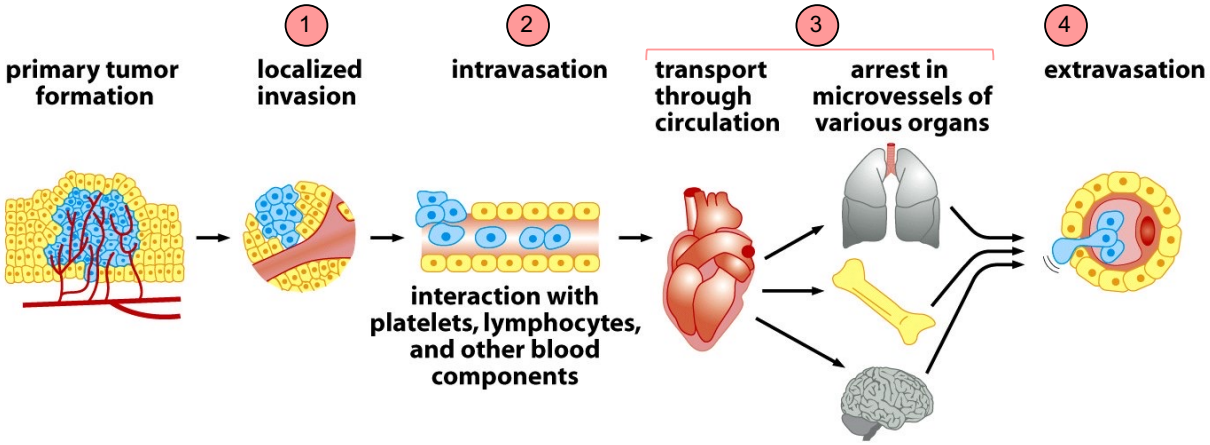
Nicolai J. Birkbak[1,2,*] and Nicholas McGranahan[3,4,*]

"...to this day we have been unable to identify any common (genetic) events that may act as gate-keepers facilitating metastatic potential."

→ Metastasis does *not* require additional mutations distinct from those of the primary tumor.
→ Instead, metastasis appears to be driven by epigenetic and/or reversible transcriptional alterations.

Birkbak & McGranahan (2020) Cancer Cell 37:8-19

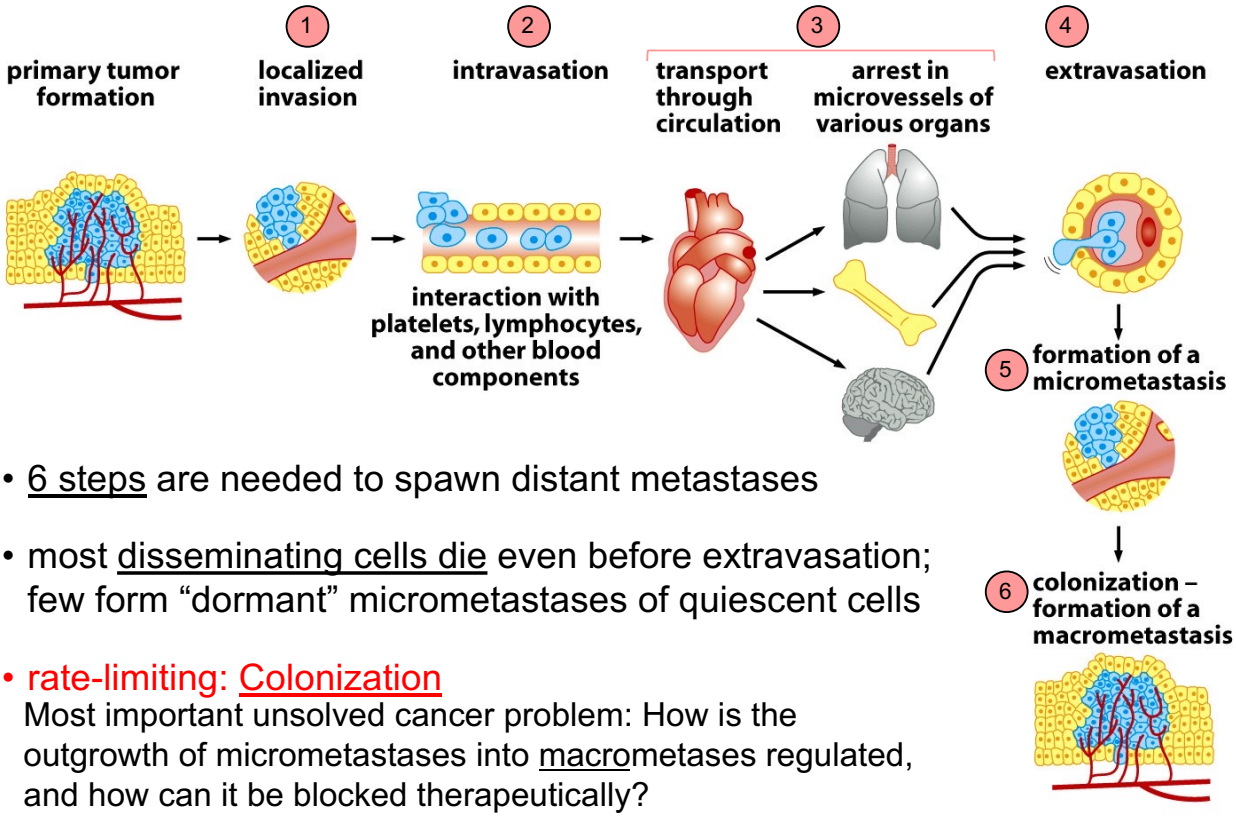
The ‘invasion-metastasis cascade’



- 6 steps are needed to spawn distant metastases
- most disseminating cells die even before extravasation; few form “dormant” micrometastases of quiescent cells

Figure 14.3 *The Biology of Cancer*

The ‘invasion-metastasis cascade’



- 6 steps are needed to spawn distant metastases
- most disseminating cells die even before extravasation; few form “dormant” micrometastases of quiescent cells
- rate-limiting: Colonization
  Most important unsolved cancer problem: How is the outgrowth of micrometastases into macrometases regulated, and how can it be blocked therapeutically?

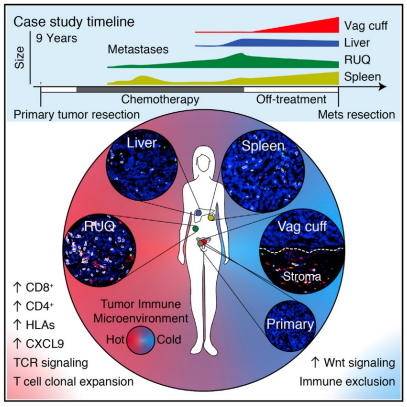
Figure 14.3 *The Biology of Cancer*

Article

Cell (2017)

Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient

Graphical Abstract



Authors
Alejandro Jiménez-Sánchez, Danish Memon, Stephane Pourpe, ..., Taha Merghoub, Alexandra Snyder, Martin L. Miller

Correspondence
snyderca@mskcc.org (A.S.), martin.miller@cruk.cam.ac.uk (M.L.M.)

In Brief
Distinct tumor immune microenvironments co-exist within a single individual and may help to explain the heterogeneous fates of metastatic lesions often observed post-therapy.

- The load of passenger mutations continues to increase in metastases
- As adaptive immunity co-evolves, it becomes *spatially non-uniform* among metastases:

→ Is this heterogeneity *responsible* for selecting a few metastatic outgrowths?

The Genomic and Immune Landscapes of Lethal Metastatic Breast Cancer PDF

Leticia De Mattos-Arruda, Stephen John Sammut et al.

Cell Reports, 27, 9, 5 2019

Outline

1. **Localized invasion**
   - **Cell motility and survival** mediated by integrin adhesion to ECM
   - **Breach of basal lamina:** Invadopodia and a protease cascade
   - **Cell-cell detachment:** Epithelial-mesenchymal transitions (EMT)
2. **Intravasation**
3. **Circulating tumor cells (CTCs)**
4. **Extravasation**
5. **Seeding of micrometastases**
6. **Colonization:** Mesenchymal-epithelial transition (MET) and its regulation by Id1

Outline

1. **Localized invasion**

   - **Cell motility and survival** mediated by integrin adhesion to ECM

What's that ?

Cell adhesion to ECM is mediated by integrins

Collagen fibers in the Extracellular Matrix (ECM) of rat cornea

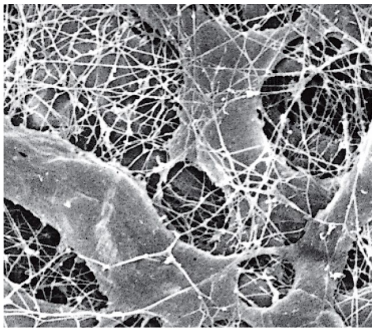
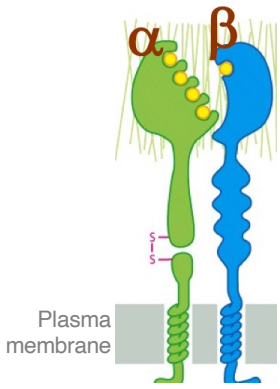


Figure 5-26 The Biology of Cancer (© Garland Science 2007)

Collagens
Fibronectin
Laminins
Hyaluronan
Proteoglycans



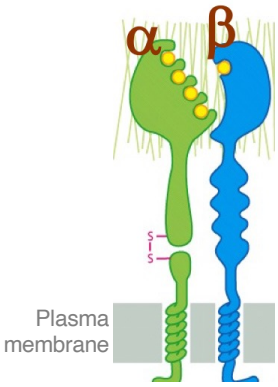
≥ 24 integrin distinct heterodimers:
=> great versatility (adhesion receptors for all occasions on any "terrain")

Functions:
Cell adhesion, shape, motility, survival

Table 5.4 Examples of integrins and their extracellular matrix ligands

Integrin	ECM ligand
α1β1	collagens, laminin
α1β1	vitronectin, fibronectin
αvβ3	vitronectin, fibrinogen, thrombospondin
α5β1	fibronectin
α6β1	laminin
α7β1	laminin
α2β3	fibrinogen

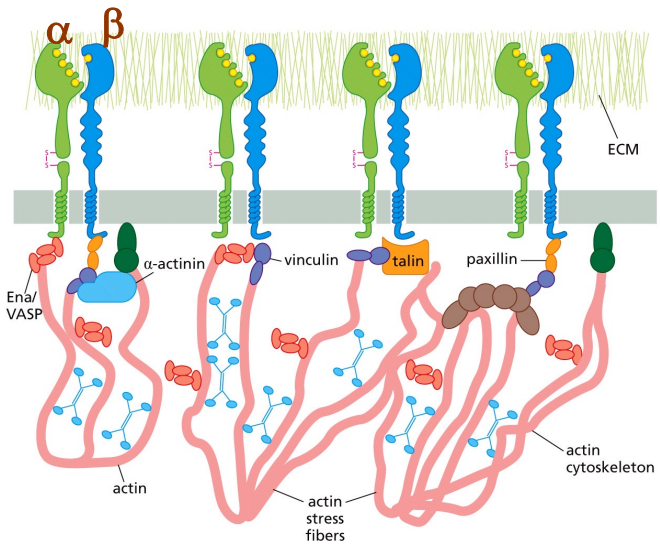
Cell adhesion to ECM is mediated by integrins



≥ 24 integrin distinct heterodimers:
=> great versatility (adhesion receptors for all occasions on any “terrain")

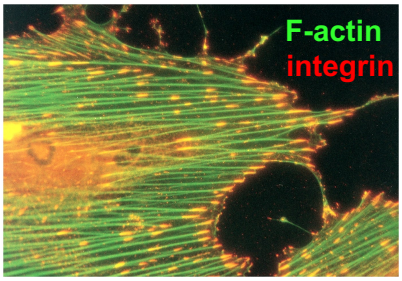
Functions:
Cell adhesion, shape, motility, survival

Integrin adhesion to ECM organizes the intracellular actin cytoskeleton



C-terminal domains
interact with various actin-binding proteins →

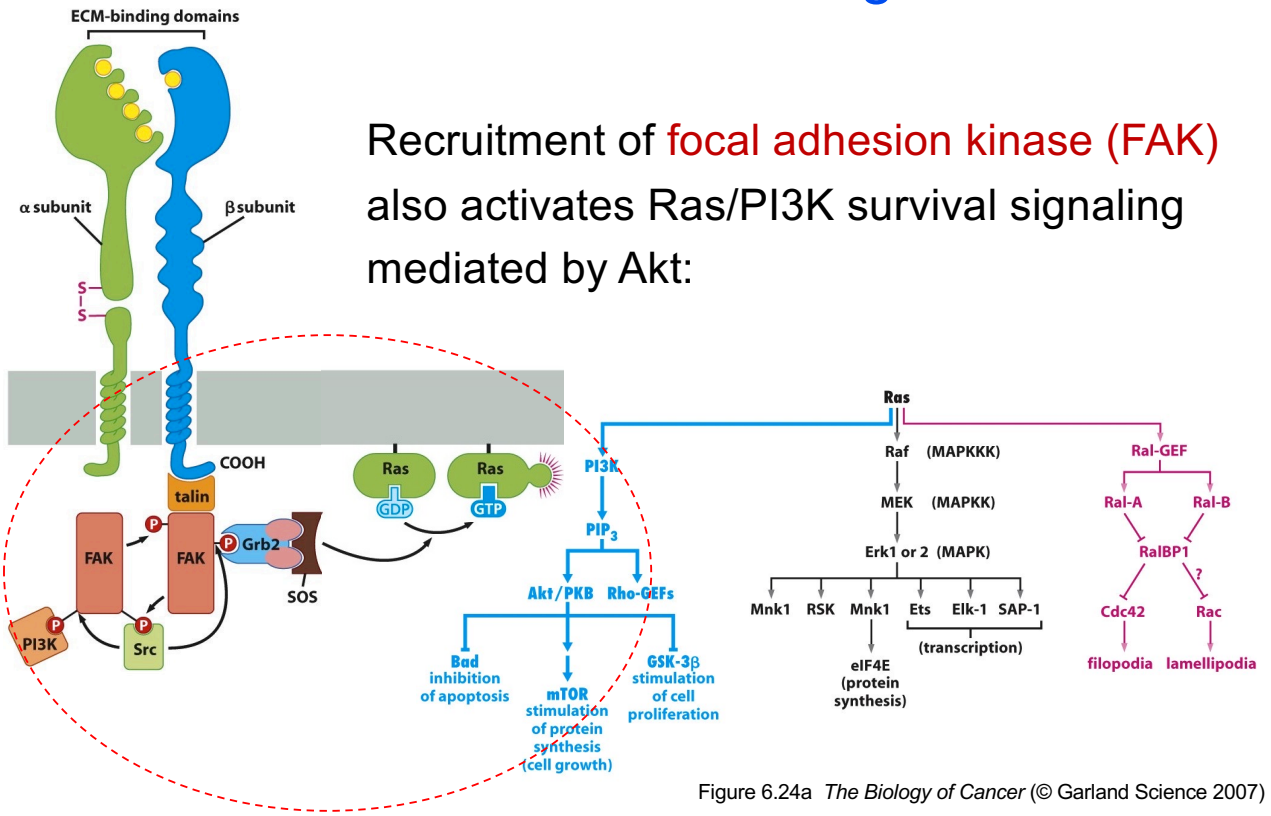
Integrins in focal adhesions
orient actin stress fibres:



- Cell-substrate adhesion → cell survival signals
- Cell locomotion
- Focal adhesion kinase (FAK): Stimulation of cell proliferation

Figure 5-28B

Adhesion to ECM via integrins activates essential cell survival signals



Recruitment of focal adhesion kinase (FAK) also activates Ras/PI3K survival signaling mediated by Akt:

Figure 6.24a *The Biology of Cancer* (© Garland Science 2007)

2019

cancers

MDPI

Review

Role of Focal Adhesion Kinase in Small-Cell Lung Cancer and Its Potential as a Therapeutic Target

Frank Aboubakar Nana [1,2], Marie Vanderputten [1] and Sebahat Ocak [1,3,*]

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2 Division of Pneumology, Cliniques Universitaires St-Luc, UCL, 1200 Brussels, Belgium
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Received: 15 September 2019; Accepted: 24 October 2019; Published: 29 October 2019

check for updates

2021

Research | Open Access | Published: 09 March 2021

Focal adhesion kinase inhibition synergizes with nab-paclitaxel to target pancreatic ductal adenocarcinoma

T. Y. S. Le Large, M. F. Bijlsma, B. El Hassouni, G. Mantini, T. Lagerweij, A. A. Henneman, N. Funel, B. Kok, T. V. Pham, R. de Haas, L. Morelli, J. C. Knol, S. R. Piersma, G. Kazemier, H. W. M. van Laarhoven, E. Giovannetti & C. R. Jimenez

Journal of Experimental & Clinical Cancer Research 40, Article number: 91 (2021) | Cite this article

573 Accesses | 3 Altmetric | Metrics

- anti-proliferative and anti-migratory effects.
- Combination with (nab-)paclitaxel had a synergistic effect on cell proliferation *in vitro* and reduced tumor growth *in vivo*.

Table 1. FAK inhibitors with anti-tumor activity in preclinical studies and clinical trials.

Name	Type	Specificity	Cancers Targeted	Study Phase	References
TAE-226 Novartis	Kinase inhibitor ATP competitive	FAK, IGF-IR, c-Met, Pyk2	Glioma, ovarian	Preclinical	[47,62]
PF-573,228 Pfizer	Kinase inhibitor ATP competitive	FAK	Prostate, breast	Preclinical	[48]
GSK2256098 GlaxoSmithKline	Kinase inhibitor ATP competitive Reversible	FAK, UGT1A1	Solid tumors (ovarian, pancreatic, meningioma, glioblastoma, malignant pleural mesothelioma)	Clinical: phase I & II	[34–36,44,49] NCT00996671, NCT02523014
NVP-TAC544	Kinase inhibitor ATP competitive	FAK	N/A	Preclinical	[50]
VS-4718 (PND-1186) Verastem	Kinase inhibitor ATP competitive Reversible	FAK, Pyk2	Solid tumors (pancreas, breast, ovarian), acute myeloid leukemia, B-cell acute lymphoblastic leukemia	Clinical: phase I	[51]
VS-6062 (PF-562271 and PF271) Verastem	Kinase inhibitor ATP competitive Reversible	FAK, CDK2/CyclinE, CDK3/CyclinE, CDK1/CyclinB, Pyk2	Prostate, pancreatic, head and neck	Clinical: phase I	[37,52]
VS-6063 (Defactinib) Verastem	Kinase inhibitor ATP competitive	FAK, Pyk2	NSCLC, pancreatic cancer, ovarian, malignant pleural mesothelioma, hematologic	Clinical: phase I/Ib & II	[38–40,45,53] NCT02758587 NCT02004028 NCT03875820 NCT03727880, NCT02943317, NCT02913716, NCT02465060, NCT02546531
1H-Pyrrolo(2,3-b) Merk Serono	Kinase inhibitor non-ATP competitive	Hinge region of FAK	N/A	Preclinical	[54]
C4 CureFAKtor Pharmaceuticals	Scaffold inhibitor	FAK /VEGFR-3	Neuroblastoma, pancreatic, breast	Preclinical	[55–57]
Compound R2 (Roslins) CureFAKtor Pharmaceuticals	Scaffold inhibitor	FAK, p53	Colon, reast	Preclinical	[58]
Y11 CureFAKtor Pharmaceuticals	Scaffold inhibitor	FAK Y397 site	Colon, breast	Preclinical	[59]
BI853520	ATP competitive inhibitor	FAK	Malignant pleural mesothelioma, non-hematologic malignancies	Preclinical, clinical	[42,43,60]

Outline

1. **Localized invasion**
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2. **Intravasation**
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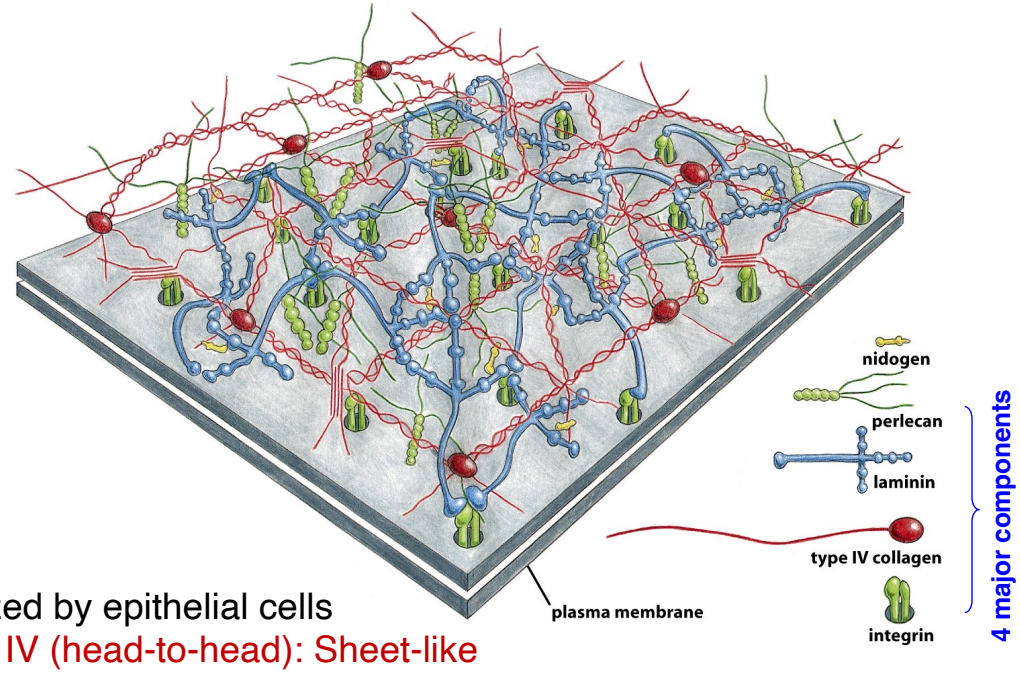
5. **Seeding of micrometastases**
6. **Colonization:** Mesenchymal-epithelial transition (MET) and its regulation by Id1

The basal lamina (or "basement membrane") is the *specialized* extracellular matrix (ECM) of *epithelial* cells



- synthesized by epithelial cells
- Collagen IV (head-to-head): Sheet-like
- *permeable* for signaling molecules

Figure 13.6 *The Biology of Cancer*

Premalignant lesions do not breach the basement membrane

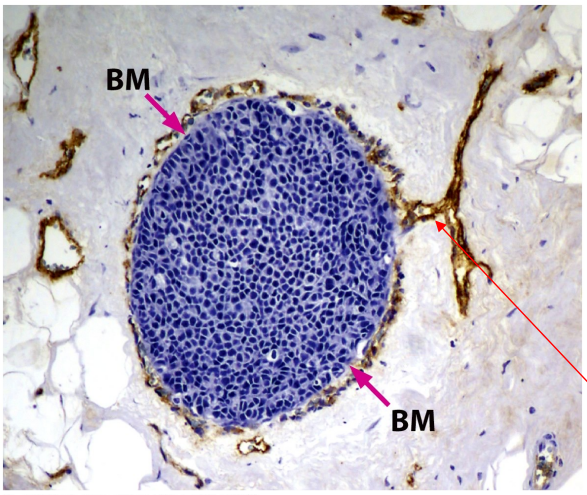
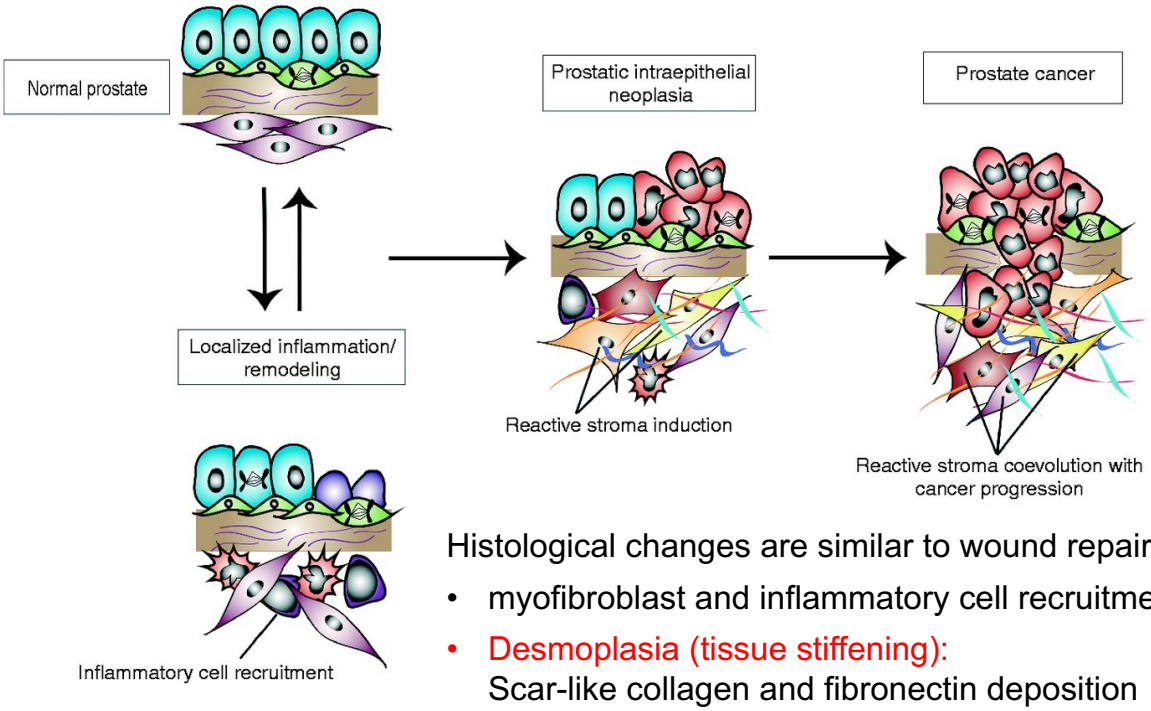


Figure 13-39a The Biology of Cancer (© Garland Science 2007)

Ductal in situ breast carcinoma (DCIS):

- non-invasive
- basement membrane (BM) = ECM of epithelial cells (collagen IV)
- BM acts as a barrier for cancer cells but is permeable e.g. for angiogenic factors:
  Recruitment of a blood vessel

①Localized invasion is facilitated by a 'reactive stroma'

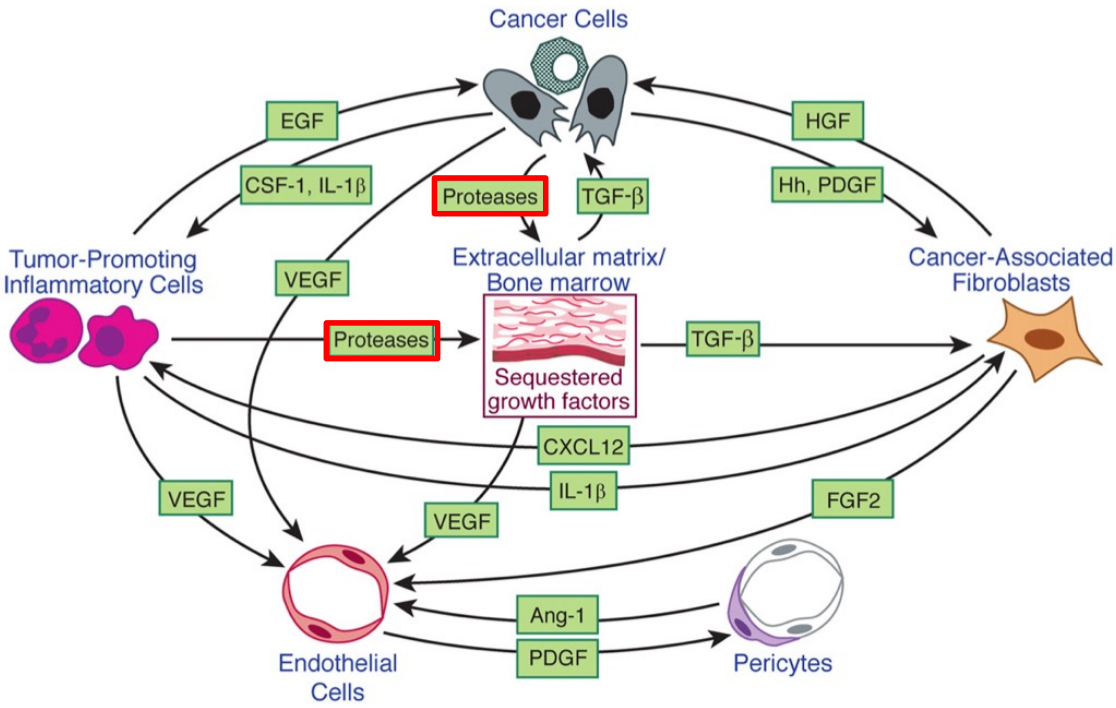


Histological changes are similar to wound repair:

- myofibroblast and inflammatory cell recruitment
- Desmoplasia (tissue stiffening):
  Scar-like collagen and fibronectin deposition

Barron & Rowley 2012, Endocr Relat Cancer

Molecular cross-talk with stromal cells includes proteases for ECM remodelling



Secreted proteases that degrade ECM components

Matrix metalloproteases (MMPs)

- secreted
- Zn^{2+}-dependent

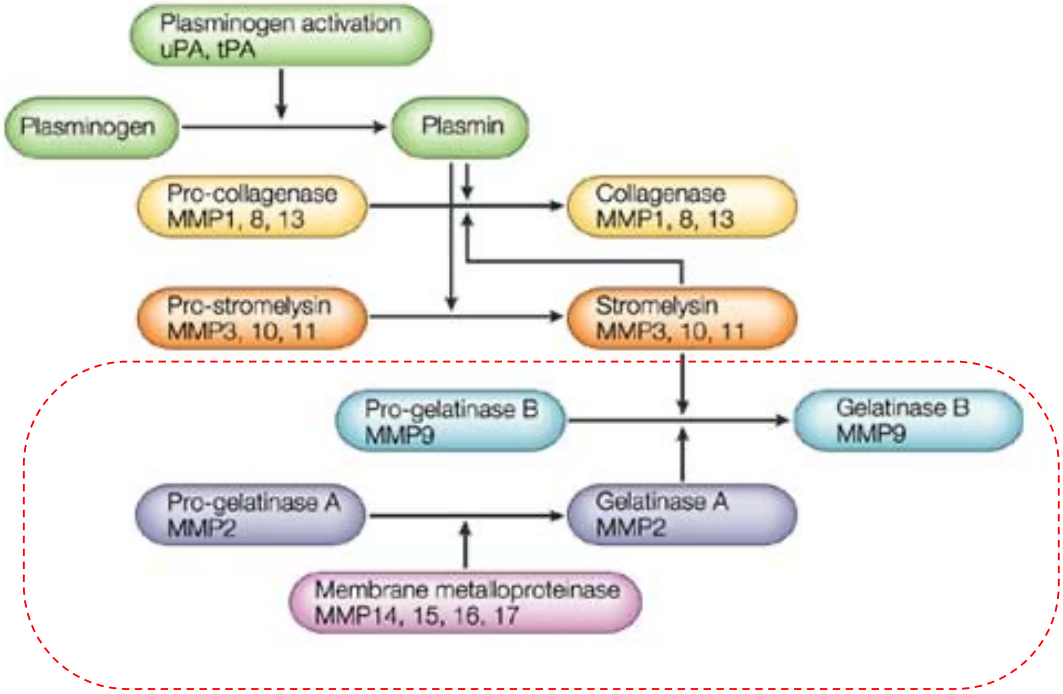
MMP9 and MMP2 (soluble):

- degrade collagen IV, laminins, fibronectins
- clear a path for invading tumor cells & vessels in desmoplastic stroma
- release ECM-bound growth factors

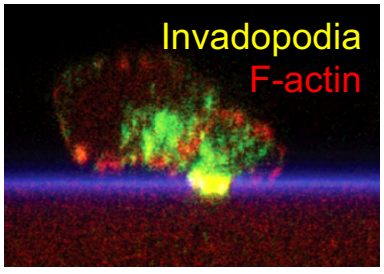
MMP14 (= MT-MMP1, membrane-type):

- mediates proteolytic maturation of pro-MMP2 zymogen
- enables so-called invadopodia to drill localized holes in ECM

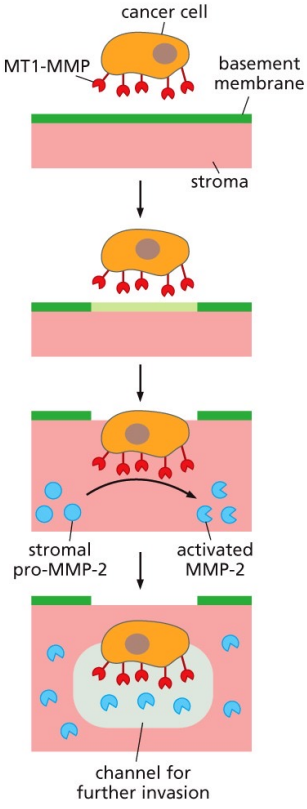
Regulation of ECM remodeling by protease cascades



Invadopodia locally concentrate MMP activities to drill pores in the ECM



← Layer of ECM protein (fibronectin)



RNAi of MT1-MMP in human fibrosarcoma cells grown in collagen I matrix (3D):

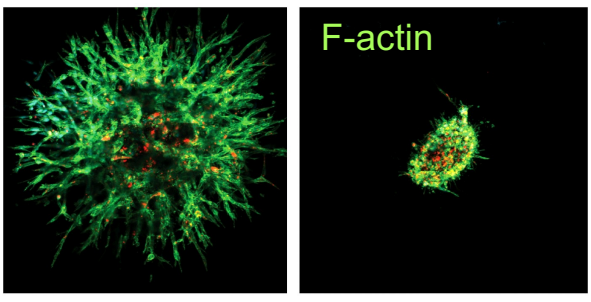
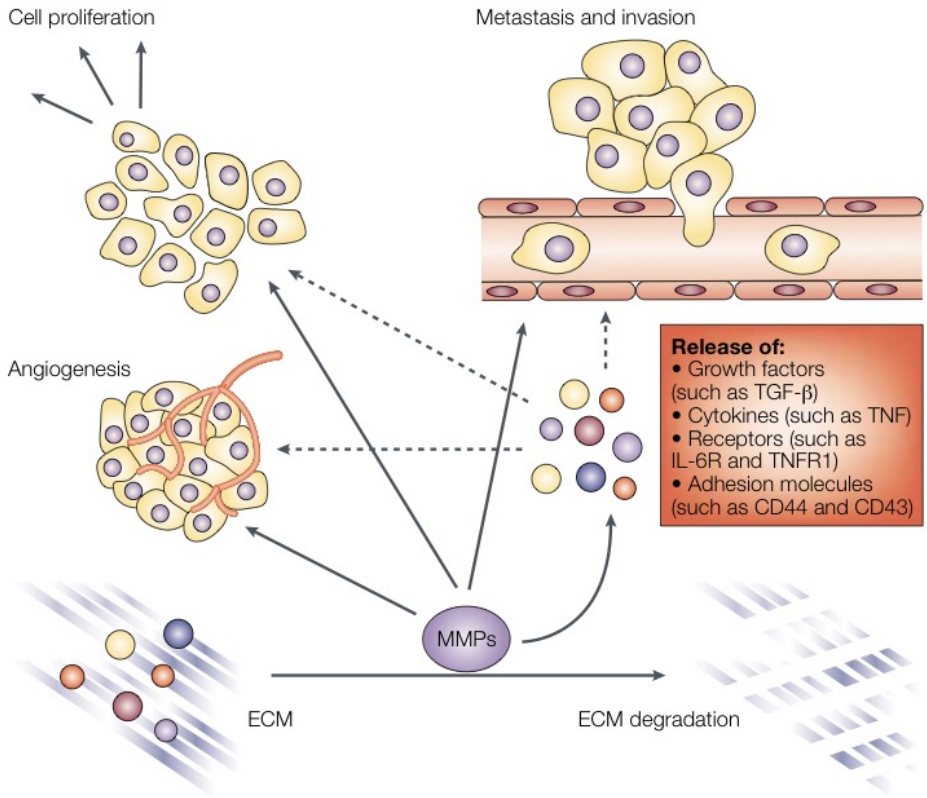


Figure 14.33-34 *The Biology of Cancer*

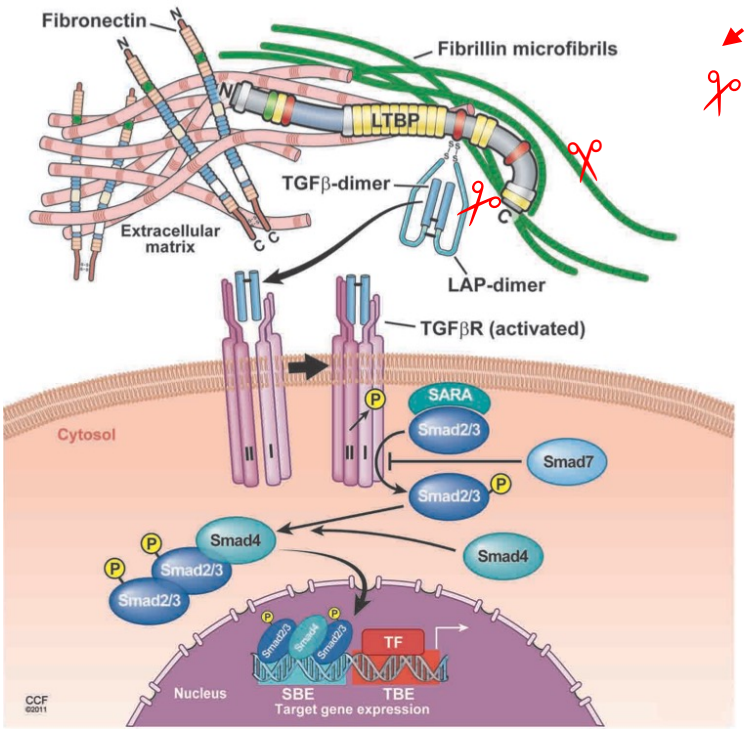
MMPs mediate several oncogenic effects

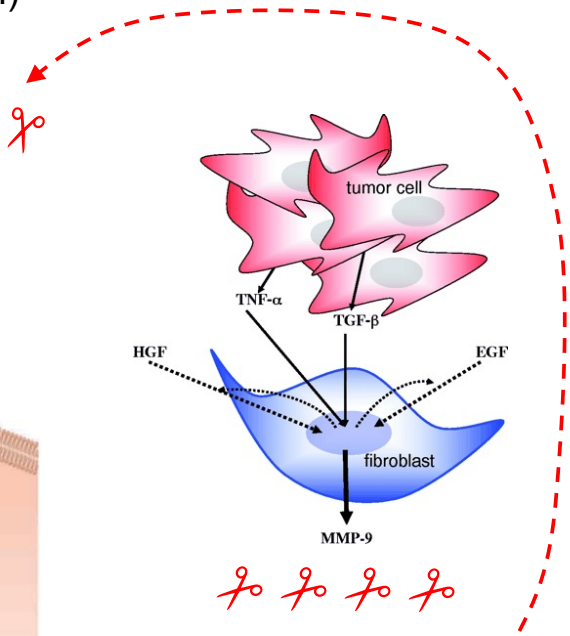


Rao 2003 Nat. Rev. Cancer 3:489-501

Activation of stored latent TGF-β in the ECM

LTBP: Latent TGF-β binding protein (ECM)



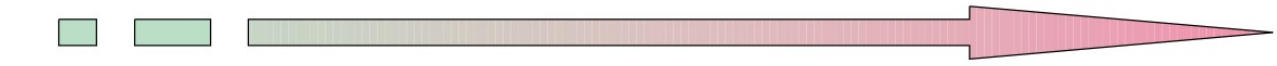


self-reinforcing loop of localized TGF-β release

Hayashi & Sakai 2012 Front. Physiol.

Change of TGF-β functions during tumor progression

	Tumor suppressor activities	Pro-oncogenic activities
Initiated cell target	Growth Inhibition Apoptosis Negative angiogenic regulator profile Maintenance of genomic stability Induction of replicative senescence Prevention of immortalization Maintenance of tissue architecture	Enhanced epithelial → mesenchymal transition Increased motility Increased invasiveness Increased colonization of bone (PTHrP secretion) Growth stimulation
Stromal target	Maintenance of tissue architecture?	Suppression of immune surveillance Increased angiogenesis



Normal epithelium

Suppressor activities dominate

Reduced or altered epithelial responsiveness to TGF-βs

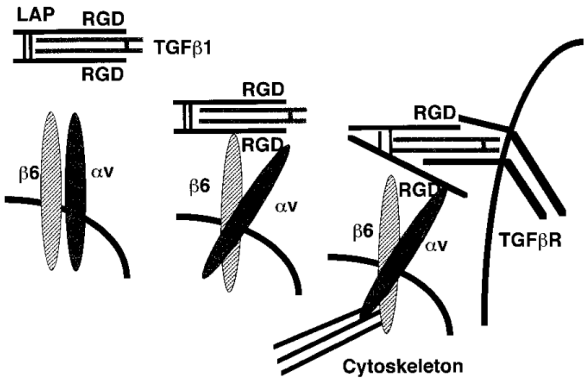
Increased TGF-β production or activation

Invasive metastatic cancer

Oncogenic activities dominate

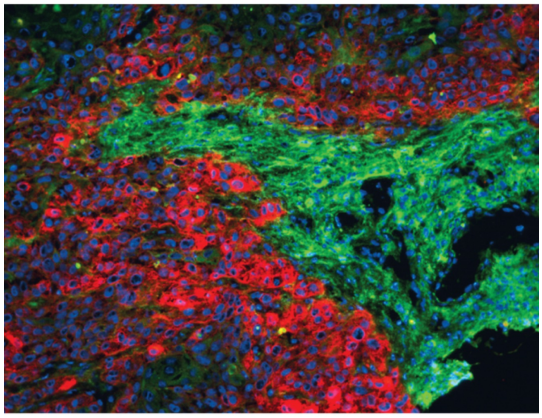
Wakefield & Roberts 2002 Curr Opin Genet Dev

Mechanical activation of stromal latent TGF-β by specific integrins expressed at the invasive front



Xenograft model of human pharyngeal carcinoma cells

TGF-β (stromal cells)

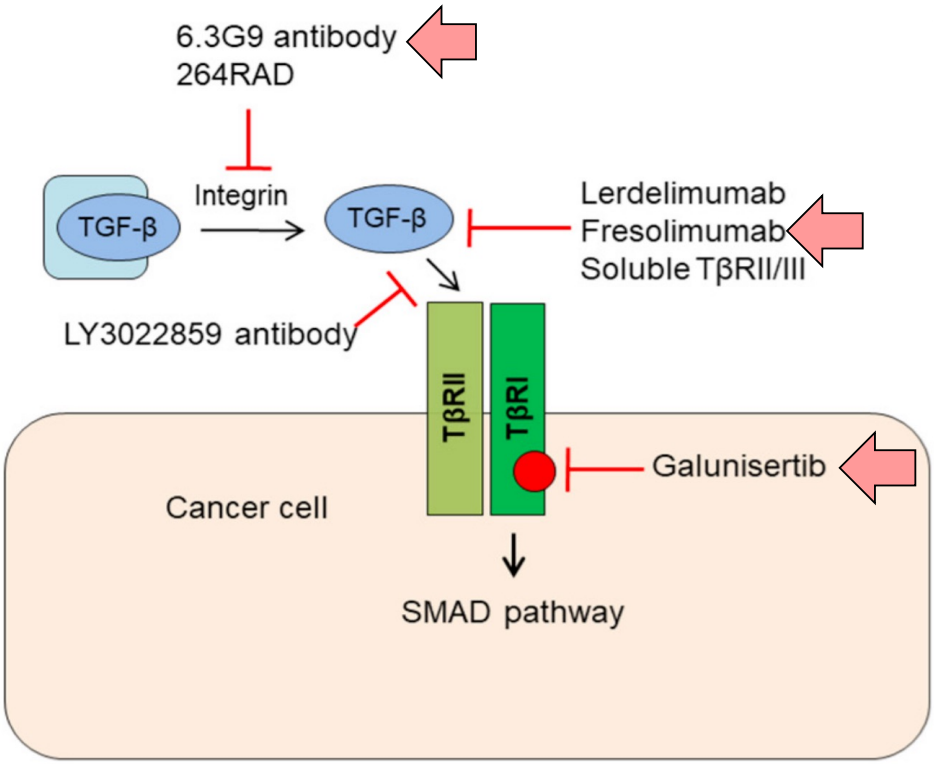


$\alpha_v\beta_6$ integrin (carcinoma cells)

Figure 14.13d The Biology of Cancer (2023)

Munger et al., Cell 96:319–328 (1999)

Inhibitors of TGF-β signaling in clinical trials



Huynh et al. 2019, Biomolecules 9: 2019-11-25

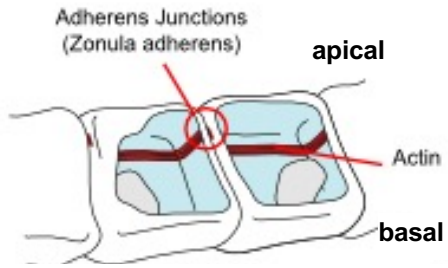
Outline

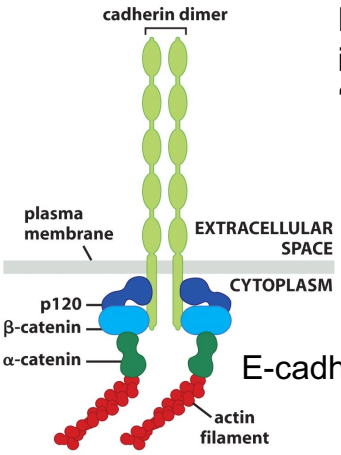
1. **Localized invasion**
   - **Cell motility and survival** mediated by integrin adhesion to ECM
   - **Breach of basal lamina:** Invadopodia and a protease cascade
   - **Cell-cell detachment:** Epithelial-mesenchymal transitions (EMT)
2. **Intravasation**
3. **Circulating tumor cells (CTCs)**
4. **Extravasation**
5. **Seeding of micrometastases**
6. **Colonization:** Mesenchymal-epithelial transition (MET) and its regulation by Id1

Normal epithelial cells tightly adhere to each other

Adherens junctions (AJs):

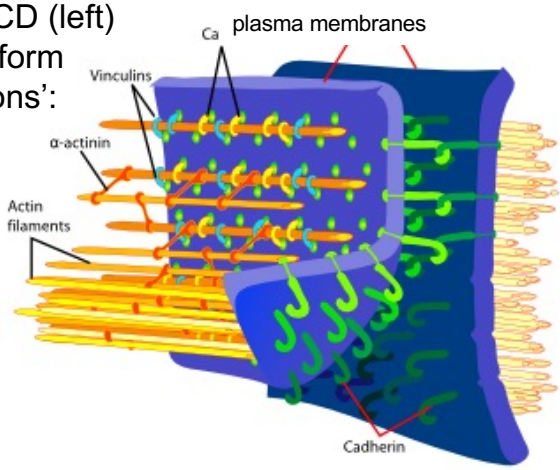
- glue epithelial cells to each other
- inhibit protein diffusion from basolateral to apical membrane
- assembled by **E-cadherin** (below)





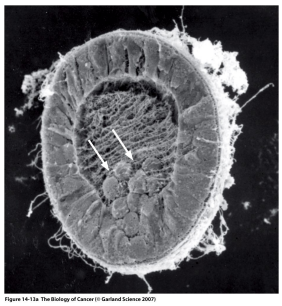
Dimers of E-cadh ECD (left) in presence of Ca^{2+} form 'homotypic interactions':

E-cadh ICD binds F-actin

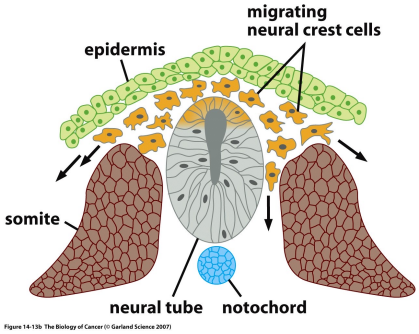


Adherens junctions are reversible

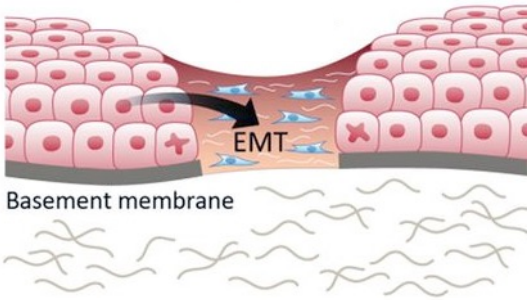
Gastrulation
(e.g. sea urchin)



Neural crest formation
(vertebrates)

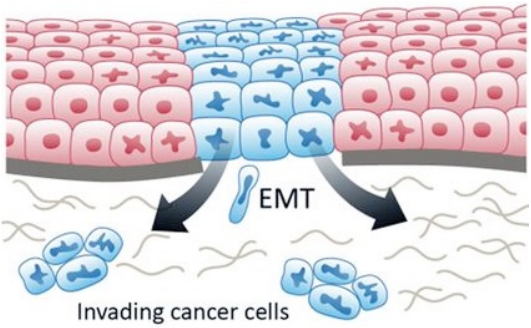


Wound healing

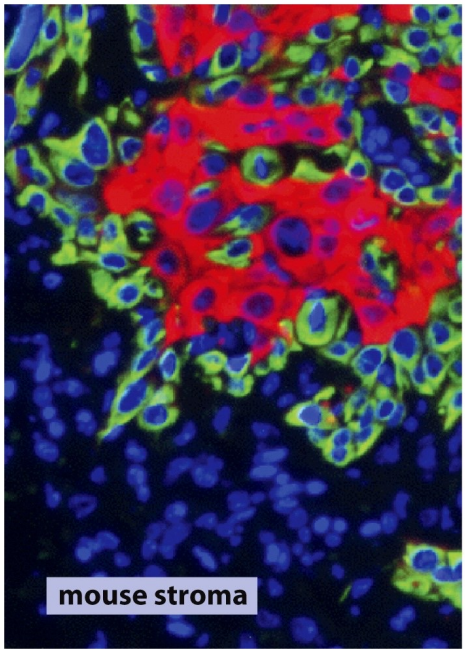


- Ability to dissolve AJs is essential for epithelial-mesenchymal transitions (EMT) during <u>development</u> and in <u>wound healing</u> => EMT must be <u>regulated</u>
- An EMT program in epithelial cancers can promote invasion (and other hallmarks)

Cancer cell invasion



Intratumoral cell heterogeneity: Signs of EMT at the *invasive front*



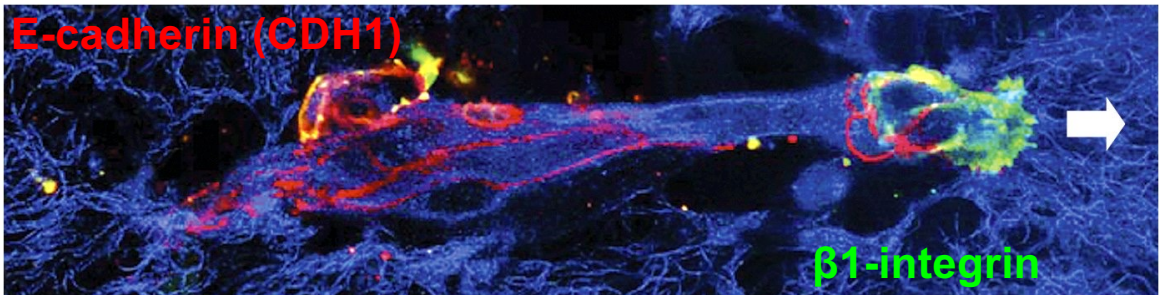
Xenografts of experimentally transformed human MECs were stained with human-specific antibodies:

- A cytokeratin (red) marks the center of the tumor mass
- By contrast, the mesenchymal marker vimentin (green) is found at the tumor edge contacting the stromal cells (blue nuclei) of the immunodeficient host

→ Does EMT of a subset of tumor cells make them (more) invasive?

Figure 14.17B *The Biology of Cancer* (© Garland Science 2014)

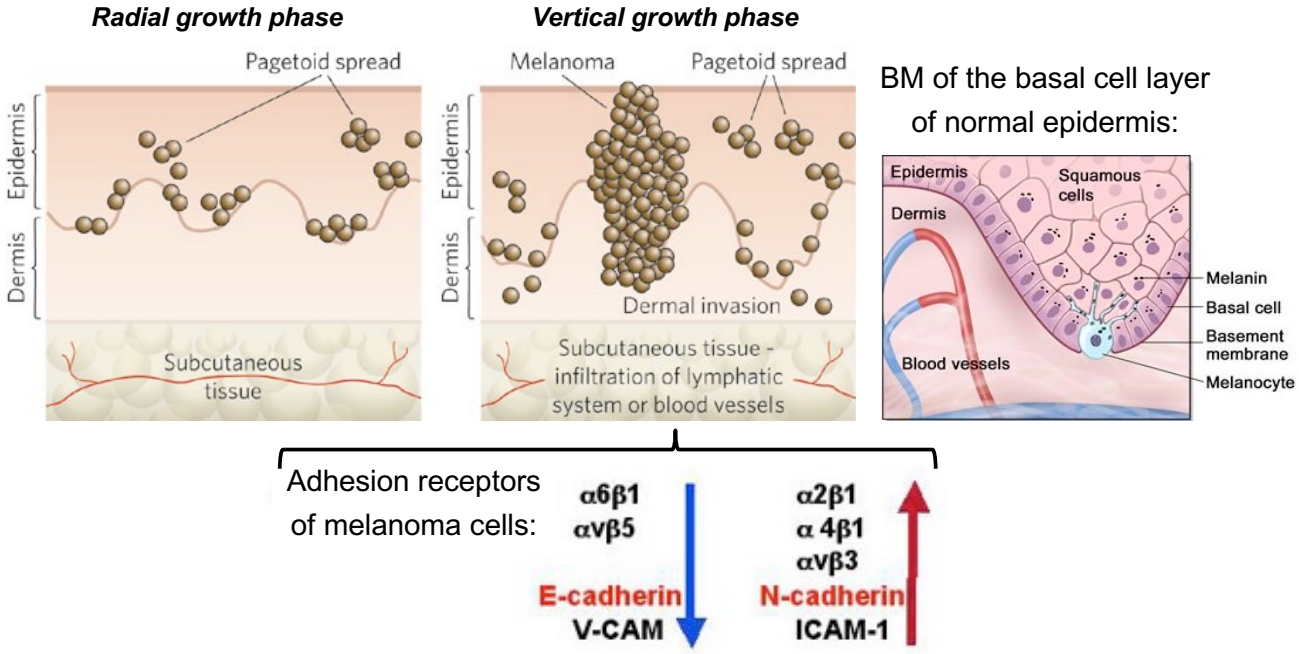
Most common pattern of invasion involves an "invasive front" of small *clusters* of cells



Cohort of 5-10 melanoma cells moving to the right (arrow):

- large gaps in collagen (blue) reflect proteolytic degradation
- heterogenous expression of adhesion receptors (here: β1-integrin)
- some invading cells lack E-cadherin, which mediates adhesion to other E-cadherin expressing cells

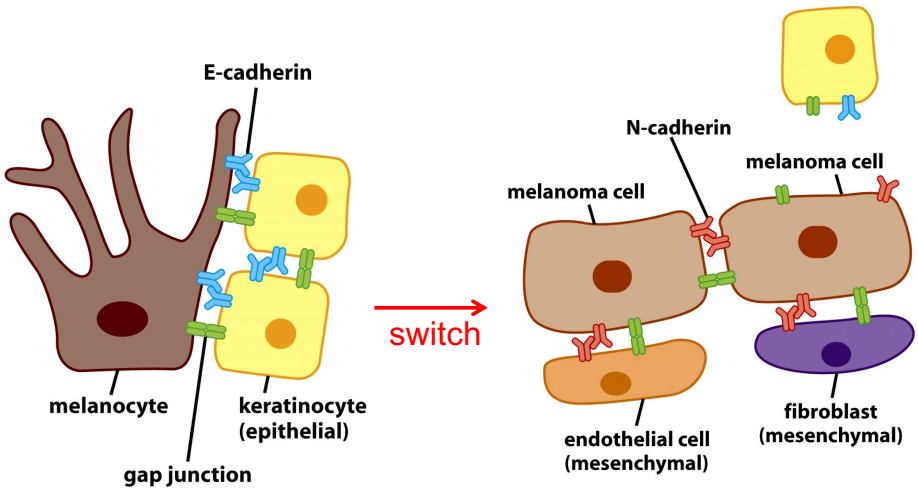
A pseudo-EMT promotes melanoma invasion



- Dermal invasion after breakdown of the basement membrane (BM) enables the vertical growth phase that leads to metastasis

The cadherin switch rearranges cell attachments

- Left: Normal melanocytes are attached to (epithelial) keratinocytes by homotypic interactions mediated by E-cadherin (CDH1)

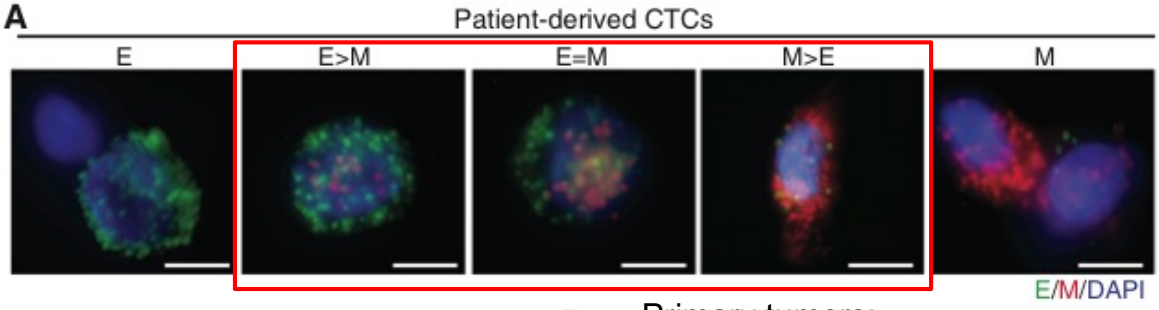


- Right: A switch from E-cadherin (epithelial) to N-cadherin (neural) expression enables invasive melanoma cells to leave keratinocytes and instead adhere to stromal cells and to each other

Figure 14.16 *The Biology of Cancer* (© Garland Science 2007)

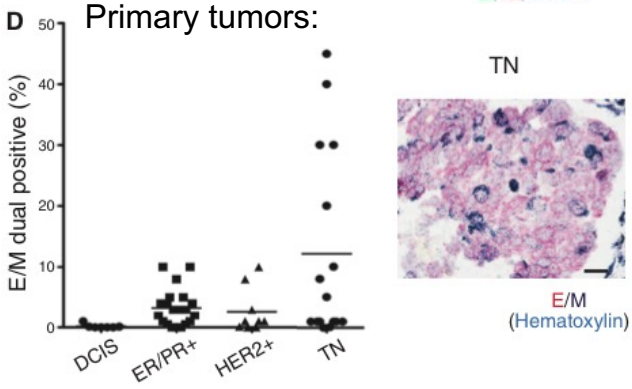
Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition
Min Yu *et al.*
Science **339**, 580 (2013);
DOI: 10.1126/science.1228522

Microfluidic capture of circulating tumor cells (CTC) from human breast cancers using epithelial- and tumor-specific antibodies → dual-color **RNA**–ISH (quantitative):



Epithelial (E) markers:
- keratins (KRT) 5, 7, 8, 18, and 19
- EpCAM (epithelial cell adhesion mol.)
- CDH1 (E-cadherin)

Mesenchymal (M) markers
- FN1 (fibronectin 1)
- CDH2 (N-cadherin)
- SERPINE1/PAI1

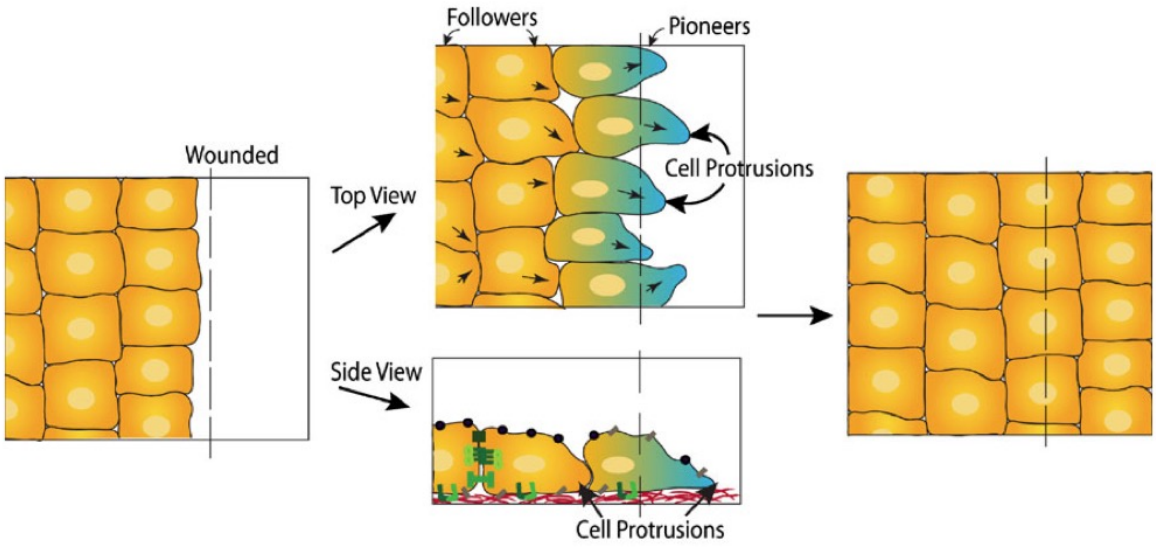


Partial EMT in circulating tumor cells (CTC) in human breast cancer

Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition
Min Yu *et al.*
Science **339**, 580 (2013);
DOI: 10.1126/science.1228522

- Confirmed that a human cancer spawns circulating tumor cells (CTC) with mesenchymal character
- The proportion of mesenchymal CTCs dynamically changed in response to drug treatments
- The extent of EMT correlated with poor prognosis

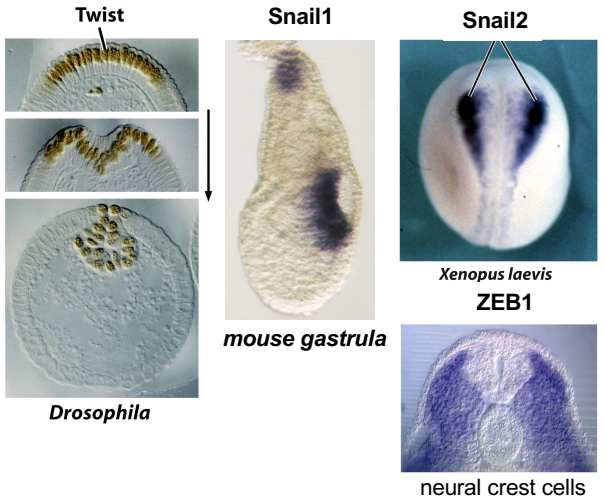
Incomplete EMT is reminiscent of collective cell migration in wound healing

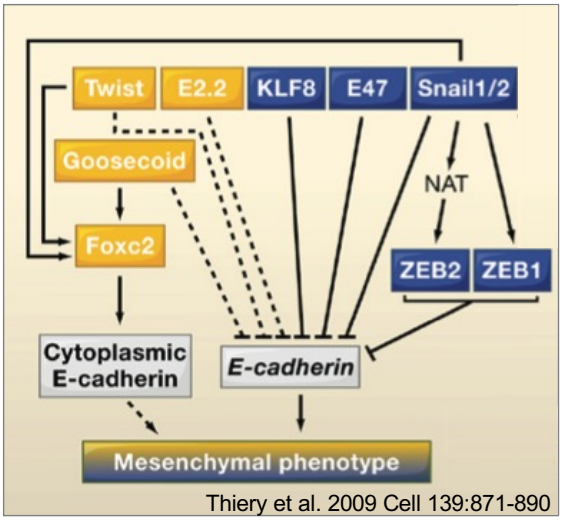


- Sheet movement: Pioneer cells undergo **a *partial* EMT**
- The incompleteness of EMT facilitates **re-epithelialization**

Micalizzi et al. 2010 J Mammary Gland Biol Neoplasia 15:117–134

Transcriptional repressors of E-cadherin

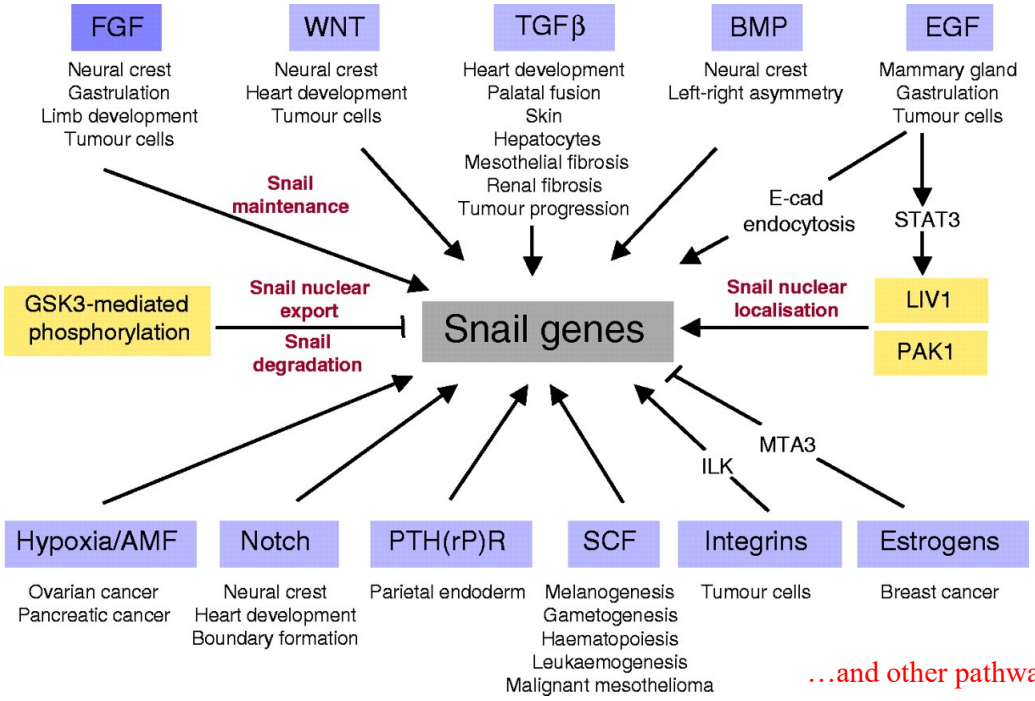




Thiery et al. 2009 Cell 139:871-890

- **Twist** acts in part indirectly by inducing retention of E-cadherin in the cytoplasm
- **Snail**, ZEB and KLF8 (ZNFs), and E47 (bHLH family) directly repress the E-cadherin promoter (and other AJ proteins)

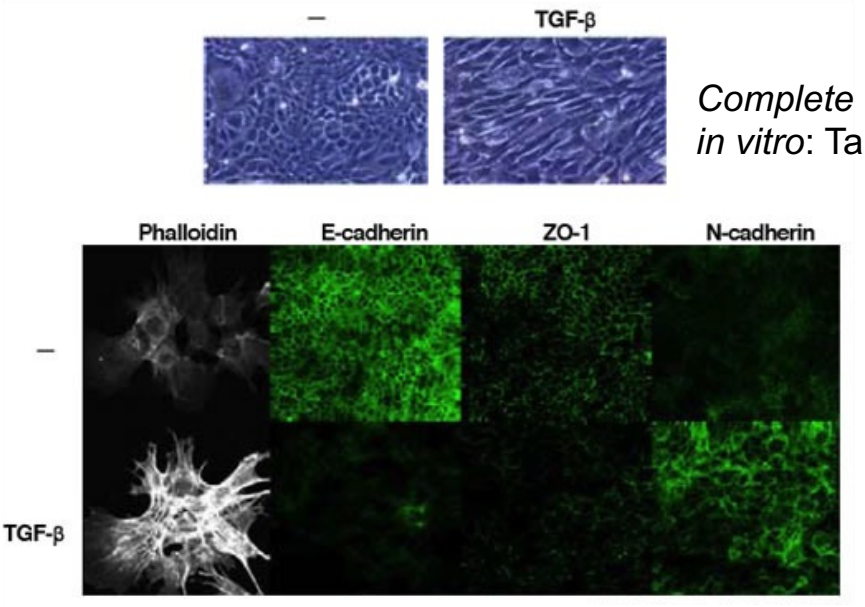
TGF-β and other EMT-inducing factors induce the Snail genes



…and other pathways !

Barrallo-Gimeno and Nieto, 2005

Induction of EMT by TGF-β in cultured cells

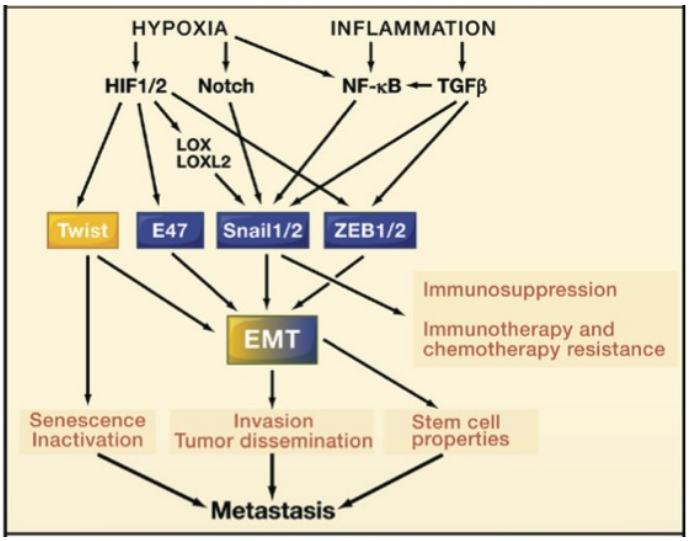


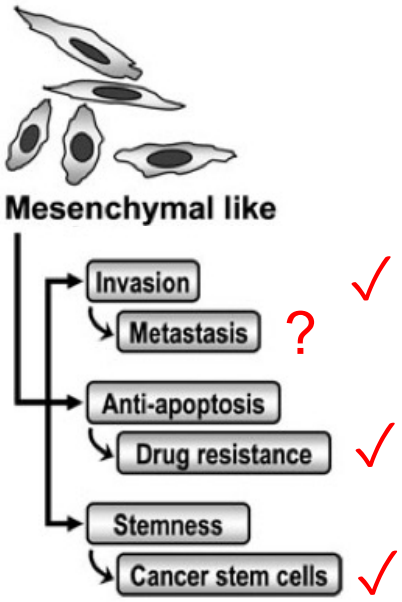
Complete EMT induced by TGF-β *in vitro*: Takes several days !

- Dissolution of AJs (Ecad)
- Dissolution of Tight Junctions (ZO1)
- Cell shape change (spindle shape)
- Cadherin switch (Ncad)

EMT promotes *several* cancer hallmarks

To induce EMT *in vivo,* TGF-β signaling synergizes with other factors, depending on the conditions (hypoxia, inflammation, ...)



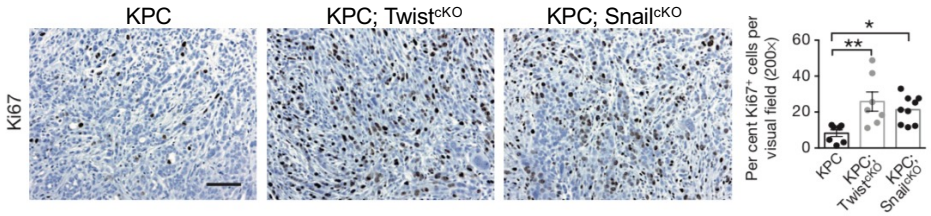


Thiery et al. 2009 Cell 139:871-890; Shih & Yang 2011 Carcinogenesis 32:1299–1304

Twist and Snail promote chemotherapy resistance

Pancreatic ductal adenocarcinoma (PDAC) model:

LSL-**K**ras^{G12D}; **p**53$^{R172H/+}$; Pdx1-**C**re (KPC mice)



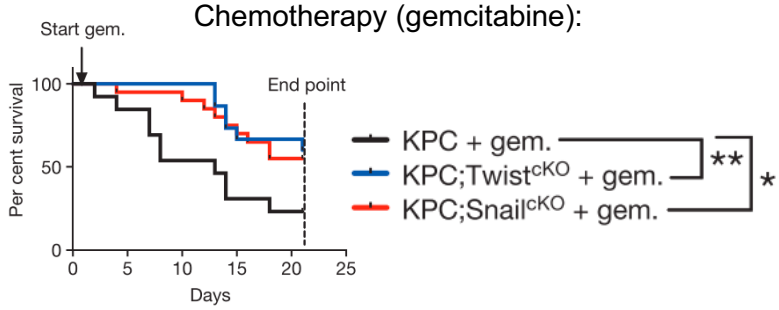
Proliferation

T

Twist, Snail1

Drug resistance

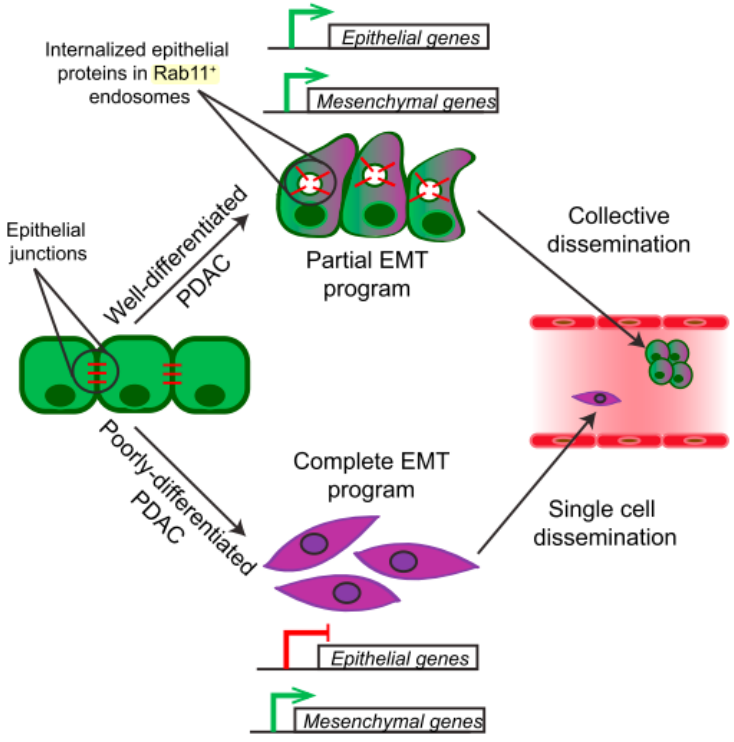
Chemotherapy (gemcitabine):



However, deletion of *Twist* or *Snail* alone did not inhibit KPC metastasis: Why not?

Zheng et al. (2015) Nature 527:525-530

Partial EMT in PDAC, breast and colon cancer



8/11 KPC tumors:

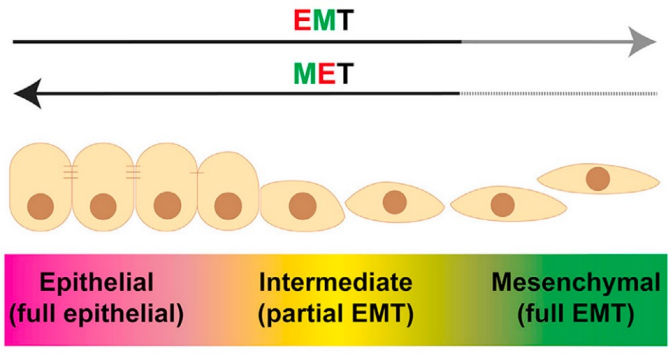
- *Post*-transcriptional downregulation of Ecad: No need for Snail.
- *Collective* dissemination

3/11 KPC tumors:

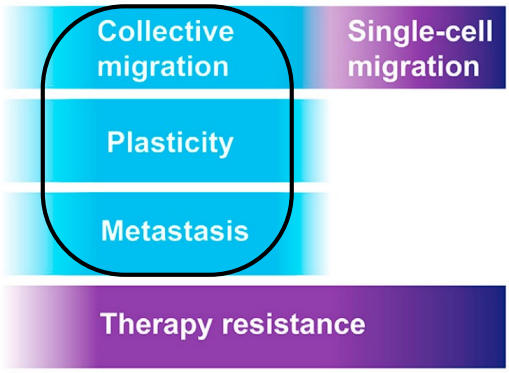
- *transcriptional* Ecad repression by Snail
- Single cell dissemination

Aiello et al. 2018 Dev Cell

Not all EMT states equally favor metastasis



Only a subset of cells within a given tumor !



Lüönd et al., Dev Cell 56:3203-3221.e11, 2021

1 Step 1: Localized invasion (summary)

- Integrins in focal adhesions mediate survival and motility
- Integrins of invadopodia activate MMPs to drill pores for motile cells and remodel the extracellular matrix to release bound growth factors
- TGF-β activities shift from tumor-suppressive to oncogenic signaling that increases invasion and EMT
- EMT (cadherin switch) facilitates cancer cell-cell detachment
- Complete and partial EMT states seem to have distinct functions in the tumor models analyzed so far

Outline

1. **Localized invasion**
   - **Breach of basal lamina:** Invadopodia and the proteolytic cascase
   - **Cell motility and survival:** integrin-mediated adhesion to ECM
   - **Cell-cell attachment:** Epithelial-mesenchymal transitions (EMT)
2. **Intravasation**
3. **Circulating tumor cells (CTCs)**
4. **Extravasation**
5. **Seeding of micrometastases**
6. **Colonization:** Mesenchymal-epithelial transition (MET) and its regulation by Id1

2 Intravasation is facilitated by stromal cells

- Invasive cancer cells must pass the endothelial lining and its basal membrane to intravasate
- Aided by neutrophils and tumor-associated macrophages (TAM)
- Cancer cells attract TAMs by releasing cytokines e.g. CSF-1
- TAMs in turn provide e.g. EGF and enhance CSF-1 release (feedback)
- Cooperation enhances intra-vasation

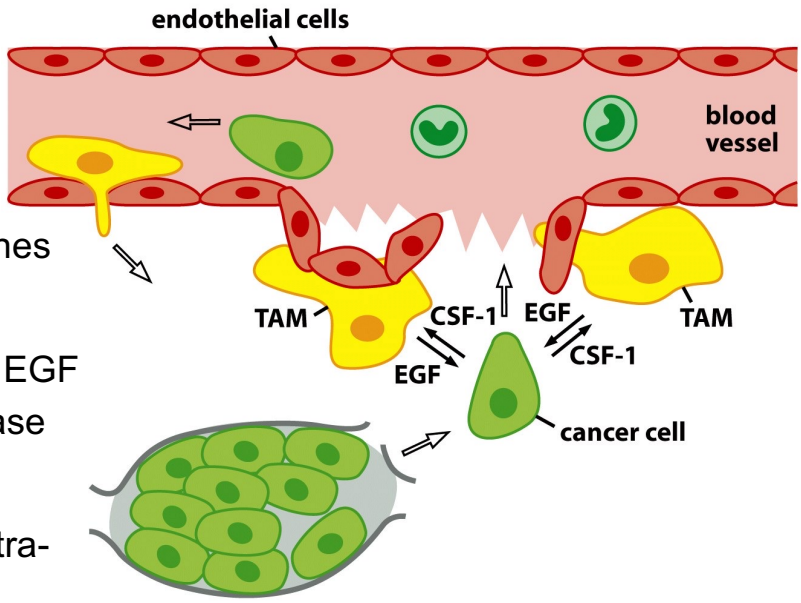
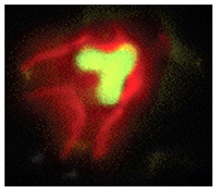
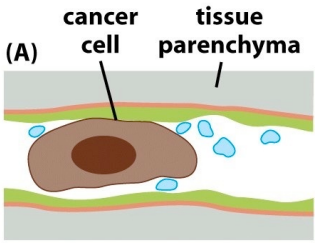


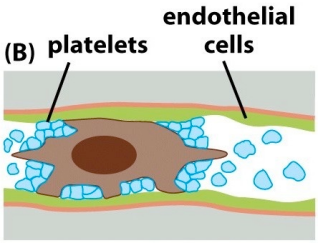
Figure 14.22c *The Biology of Cancer* (© Garland Science 2014)

3 Transport: Circulating tumor cells (CTC)

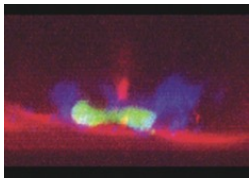
- CTCs have been detected in blood vessels and in the bone marrow of human breast and colon cancers and in mouse models
- Survival in the circulature is facilitated by adhesion to platelets:







Platelets (blue) bound to GFP+ melanoma cell:

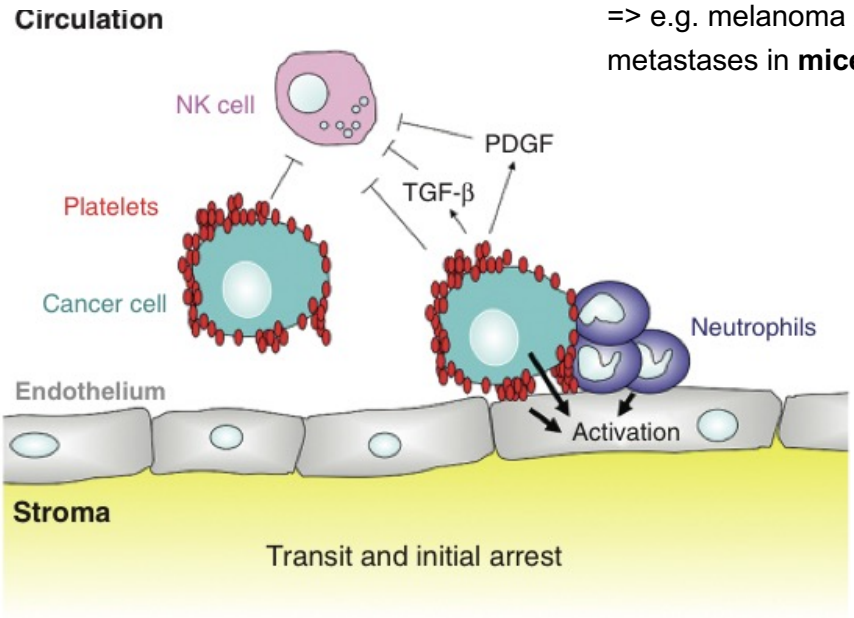


- Such **microthrombi** form within minutes after injection of tumor cells into the tail vein of mice (top right)

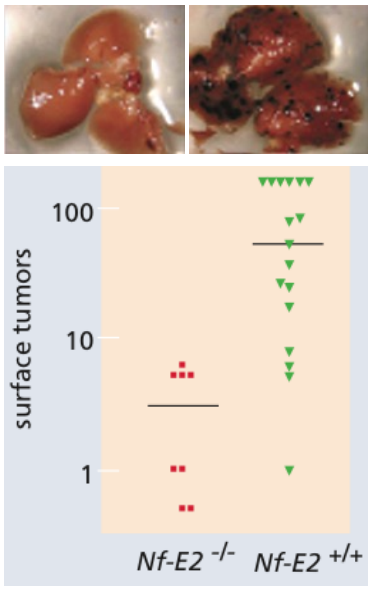
Figure 14.9 *The Biology of Cancer* (© Garland Science 2014)

③ Platelets increase cancer cell survival during transport in the circulation

Platelets protect cancer cells against Natural Killer (NK) cells:

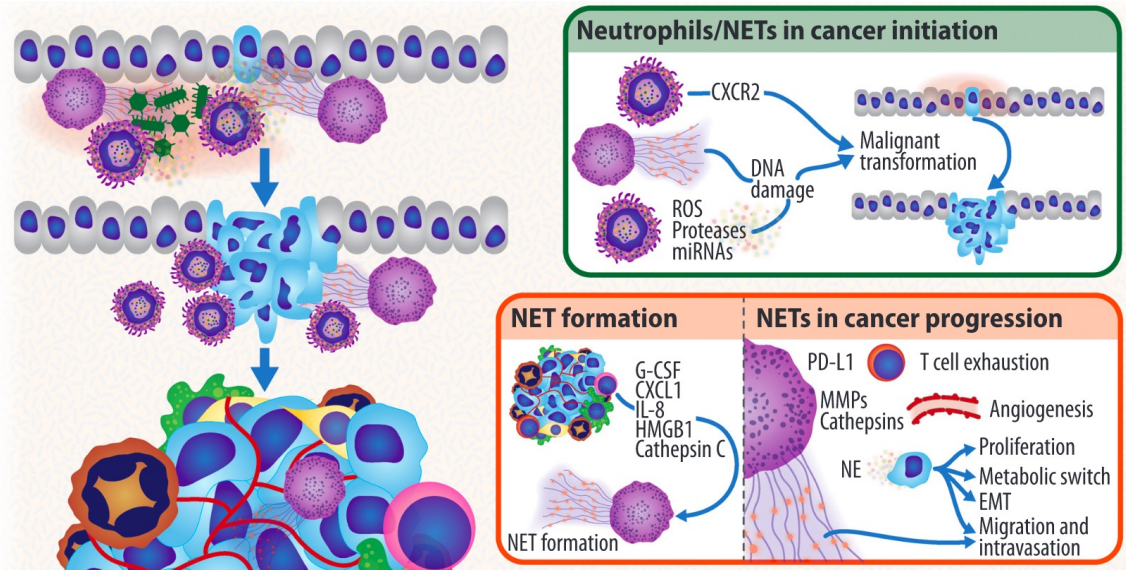


=> e.g. melanoma xenografts do not form lung metastases in **mice lacking platelets (Nf-E2$^{-/-}$)**:



sidebar 27; Labelle & Hynes 2012 Cancer Discovery 2:1091-1099

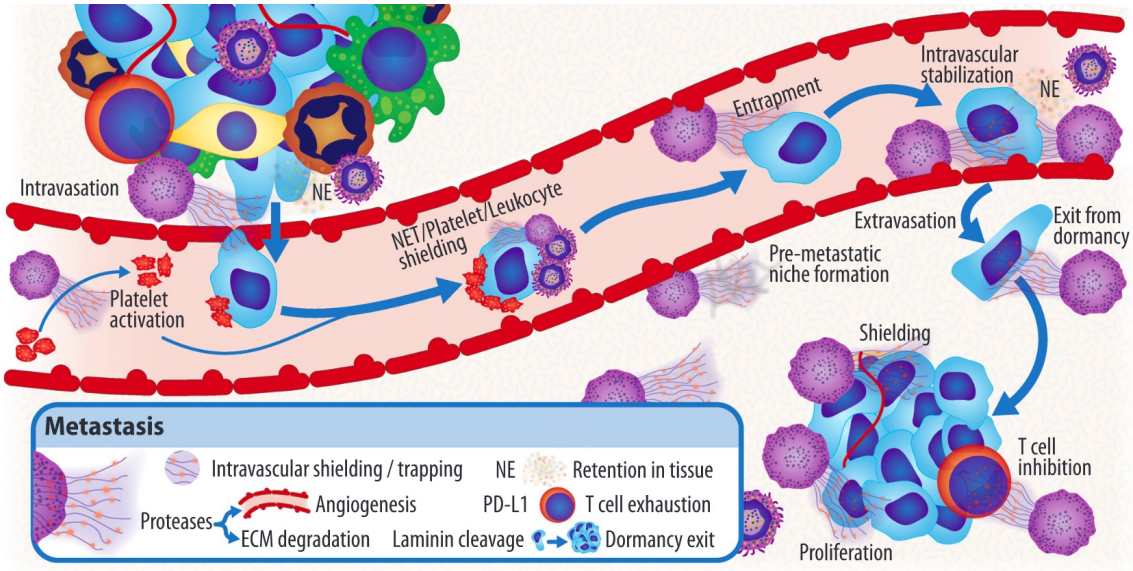
Evil roles of neutrophils in cancer



- bone marrow derived; short-lived cells; can facilitate tumorigenesis
- release their DNA as Neutrophile Extracellular Traps (NETs)

Adrover et al. 2023, Cancer Cell 41:505-526

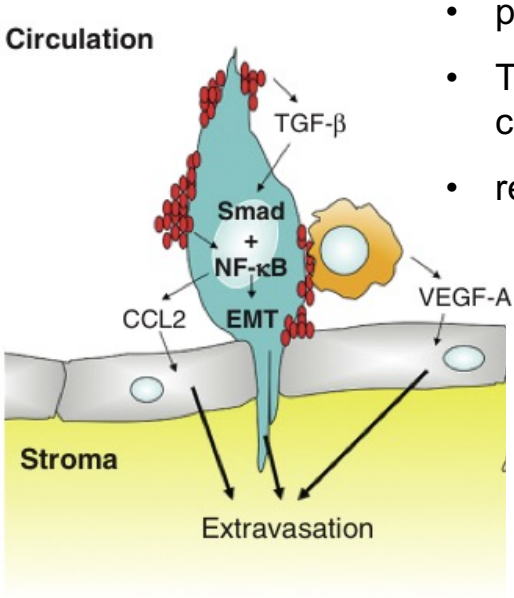
Neutrophils aid metastasis at multiple steps



- Inhibition or drug-induced degradation of NETs reduces metastasis in preclinical models

Adrover et al. 2023, Cancer Cell 41:505-526

Platelets also promote ④ *Extravasation*



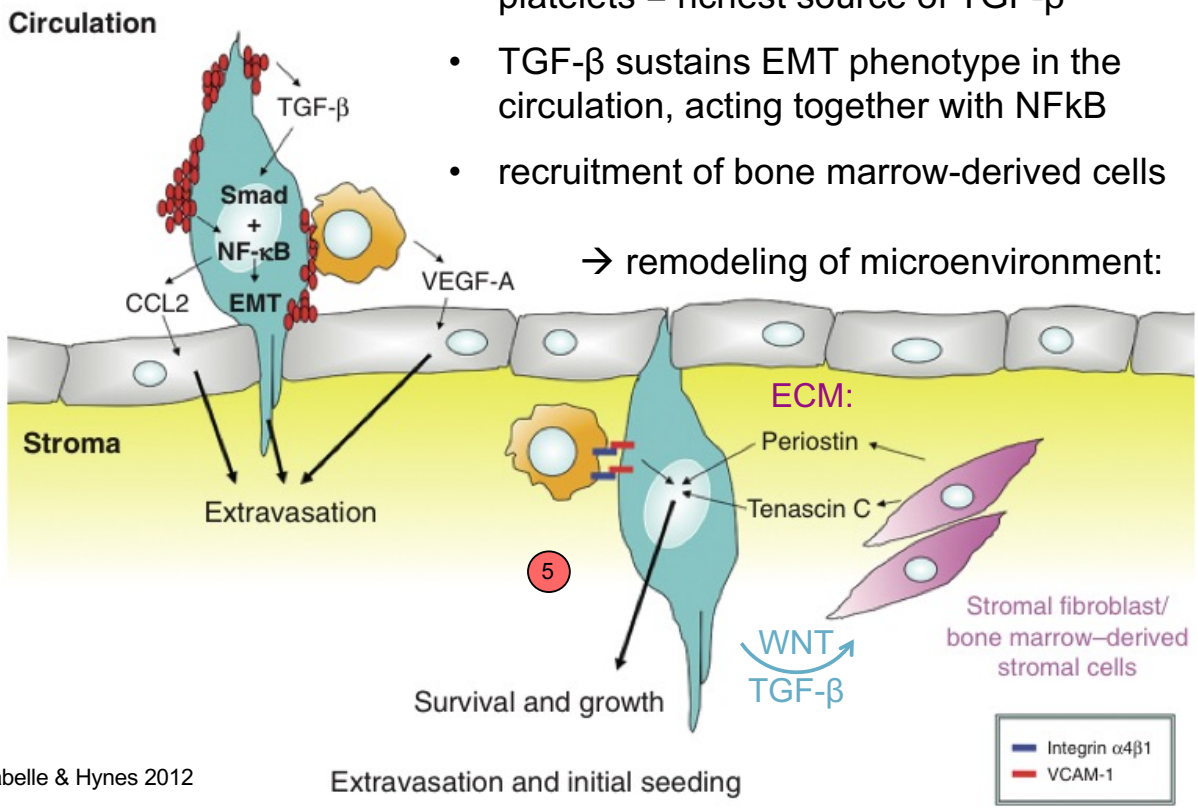
- platelets = richest source of TGF-β
- TGF-β sustains EMT phenotype in the circulation, acting together with NFkB
- recruitment of bone marrow-derived cells

Labelle & Hynes 2012

Platelets also promote ④ *Extravasation and* ⑤ *Seeding*

- platelets = richest source of TGF-β
- TGF-β sustains EMT phenotype in the circulation, acting together with NFkB
- recruitment of bone marrow-derived cells

→ remodeling of microenvironment:



Labelle & Hynes 2012

Extravasation and initial seeding

Outline

1. **Localized invasion**
   - **Breach of basal lamina:** Invadopodia and the proteolytic cascase
   - **Cell motility and survival:** integrin-mediated adhesion to ECM
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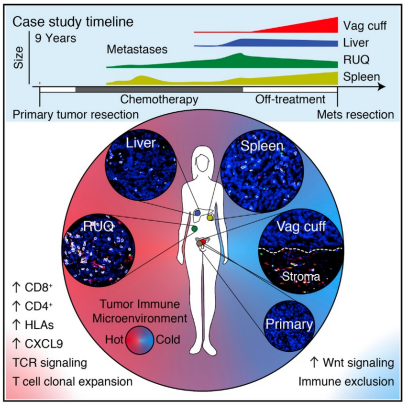
6. **Colonization:** Mesenchymal-Epithelial Transition (MET) and its regulation by Id1

Article

Cell (2017)

Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient

Graphical Abstract



Authors
Alejandro Jiménez-Sánchez, Danish Memon, Stephane Pourpe, ..., Taha Merghoub, Alexandra Snyder, Martin L. Miller

Correspondence
snyderca@mskcc.org (A.S.), martin.miller@cruk.cam.ac.uk (M.L.M.)

In Brief
Distinct tumor immune microenvironments co-exist within a single individual and may help to explain the heterogeneous fates of metastatic lesions often observed post-therapy.

- The load of passenger mutations continues to increase in metastases
- As adaptive immunity co-evolves, it becomes *spatially non-uniform* among metastases:

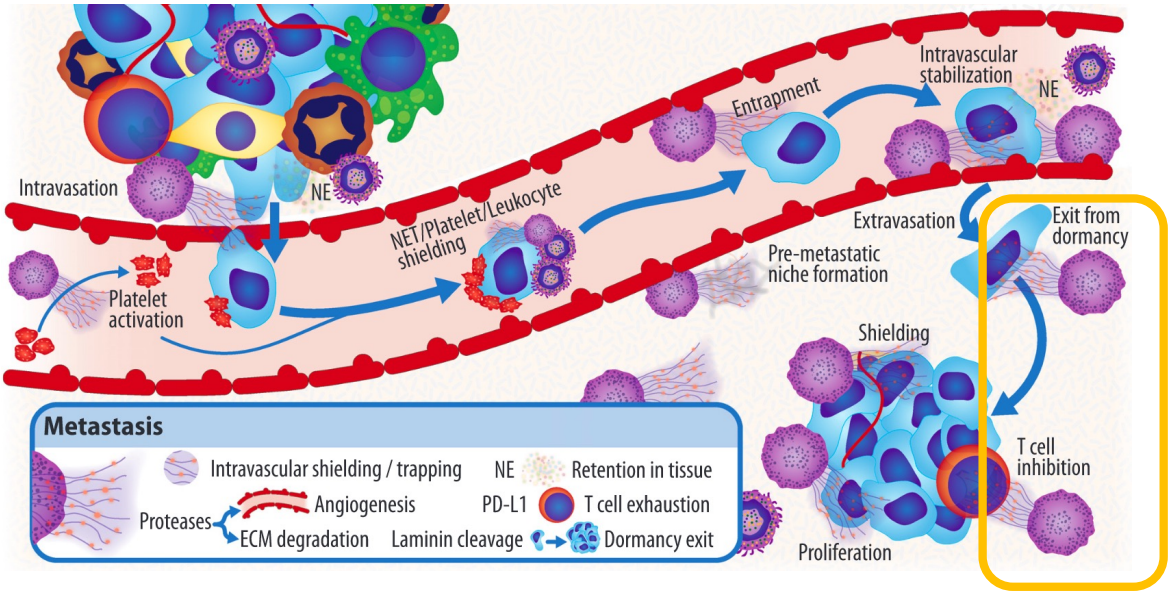
→ Is this heterogeneity *responsible* for selecting a few metastatic outgrowths?

The Genomic and Immune Landscapes of Lethal Metastatic Breast Cancer PDF

Leticia De Mattos-Arruda, Stephen John Sammut et al.

Cell Reports, 27, 9, 5 2019

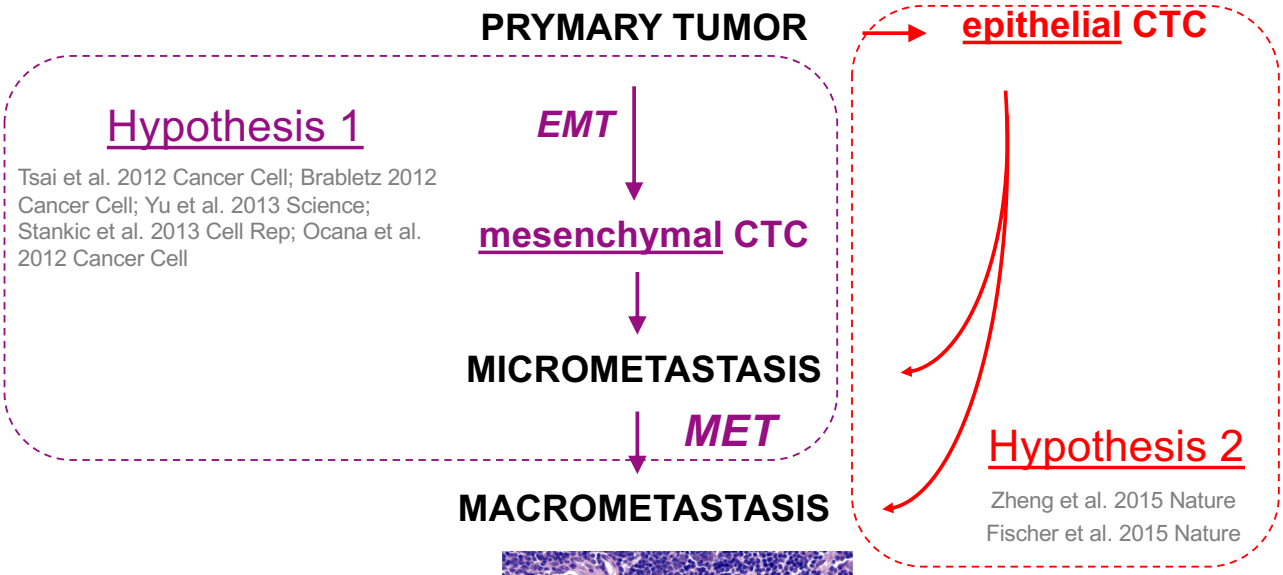
Neutrophils aid metastasis at multiple steps



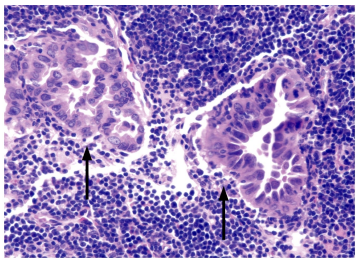
- Inhibition or drug-induced degradation of NETs reduces metastasis in preclinical models

Adrover et al. 2023, Cancer Cell 41:505-526

Is MET rate-limiting for metastatic colonization?

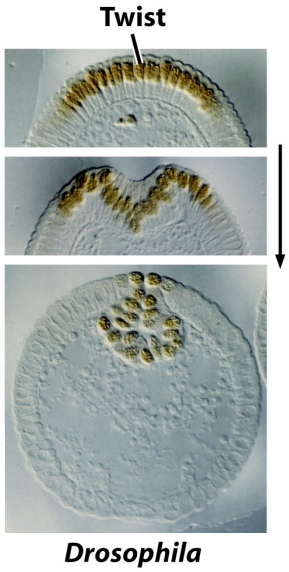


Observation:
Macrometastases often share the epithelial morphology of primary tumors

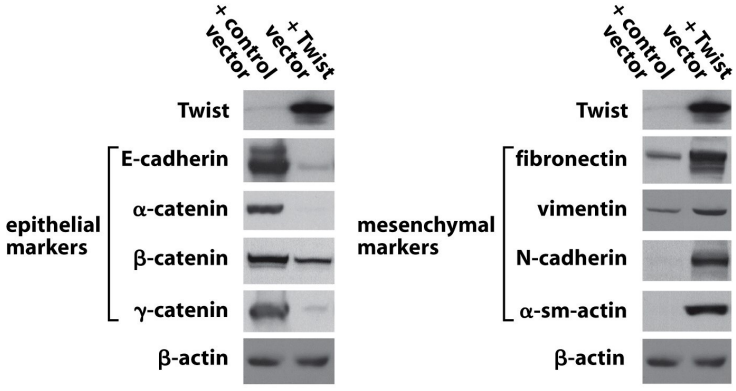


Lymph node containing lung adenocarcinoma micrometastases (one with duct-like structure)

Testing if transient EMT promotes metastasis: But how?



Twist is required for EMT *in vivo* (left) can induce EMT in cultured epithelial MDCK cells *in vitro* (below):



What will happen if this potent EMT-inducing transcription factor is expressed by tumor cells *in vivo* as an inducible transgene?

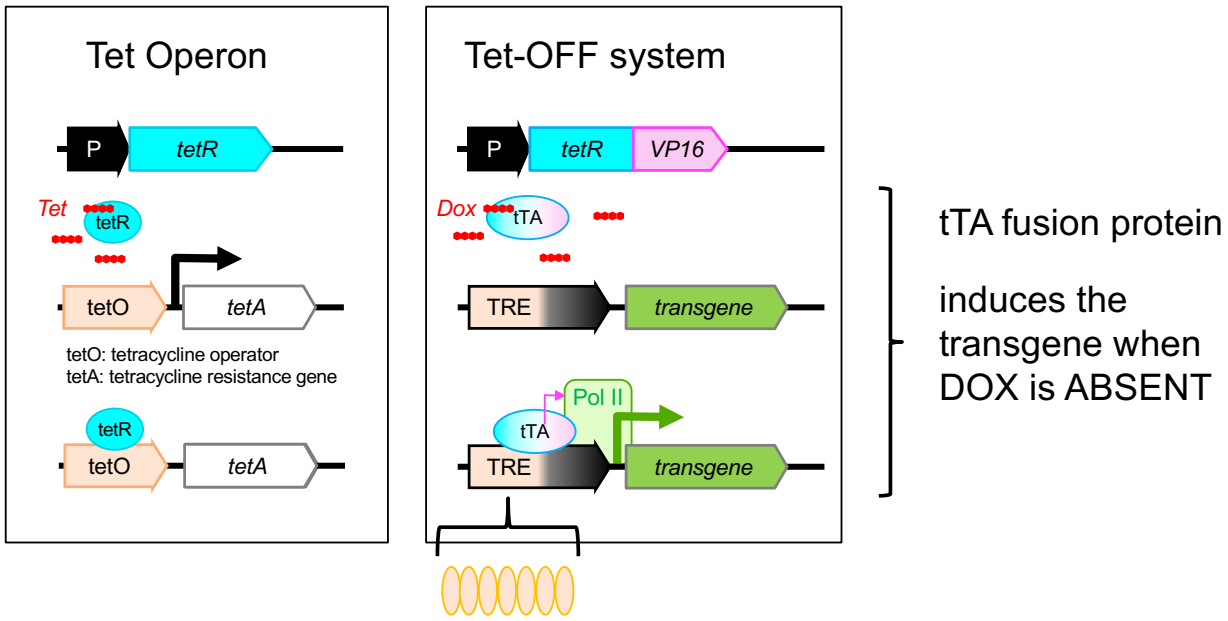
→ see next 3 slides

Figure 14.15b *The Biology of Cancer* (© Garland Science 2007)

Induction of transgenes by tTA upon *removal* of Doxycyclin (Tet-OFF system)

tetR: tetracycline repressor (*E.coli*)

tTA: tet Trans-Activator (tetR DBD + VP16 AD)



tTA fusion protein

induces the transgene when DOX is ABSENT

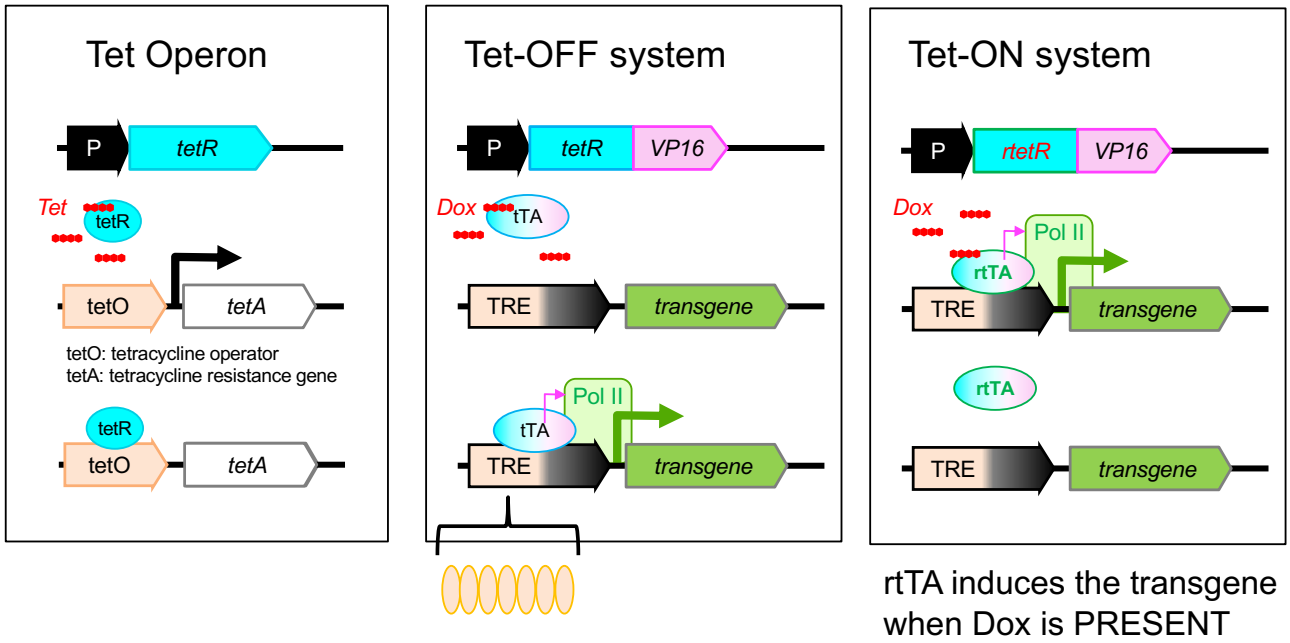
7x tetO fused to a minimal promoter
= Tet-responsive element (TRE)

Induction of transgenes by reverse tTA (rtTA) upon *addition* of Doxycyclin (Tet-ON system)

tetR: tetracycline repressor (*E.coli*)

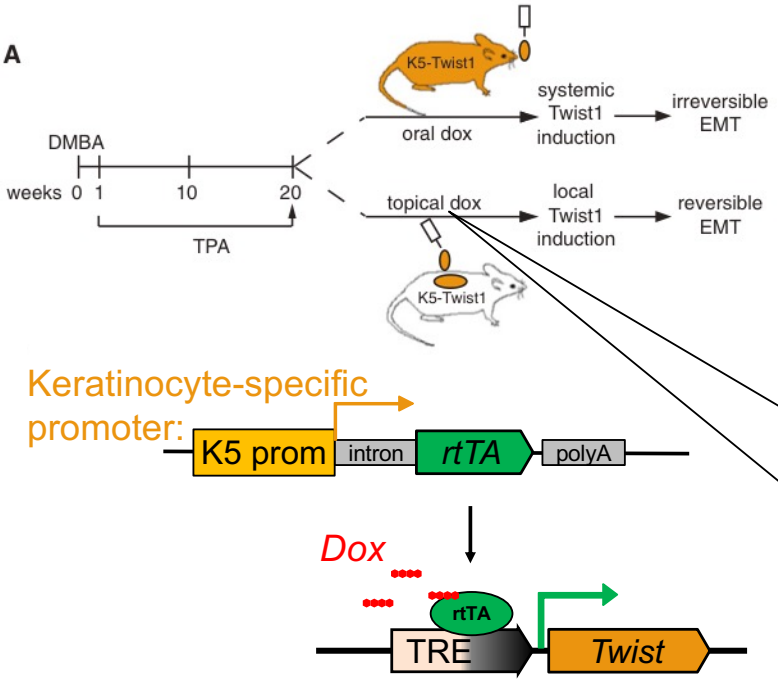
tTA: tet Trans-Activator (tetR DBD + VP16 AD)

rtTA: *reverse* tTA (a mutated version)



rtTA induces the transgene when Dox is PRESENT

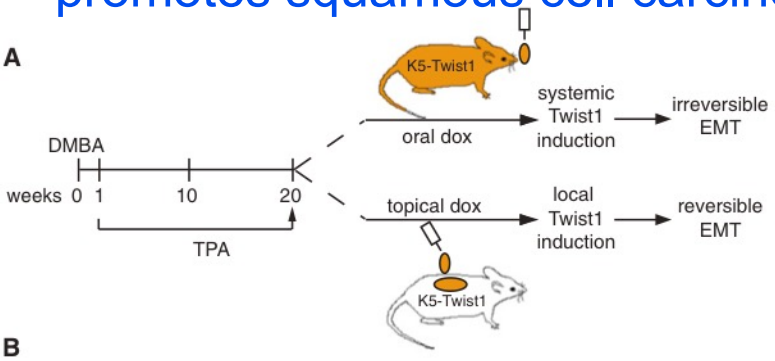
Dox-induced Twist expression in skin keratinocytes

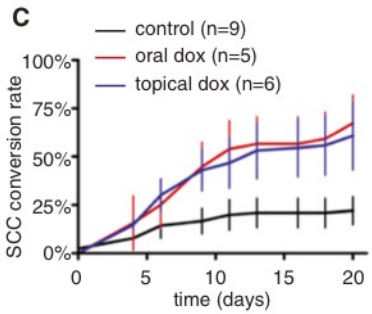


In cells migrating away from the dox-treated area, rtTA is still synthesized but will cease to maintain their *Twist* expression

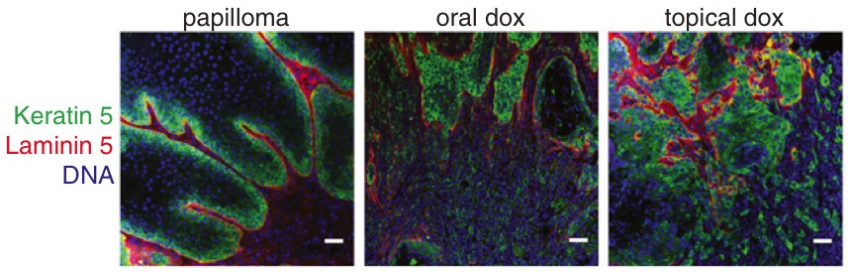
Tsai et al. 2012 Cancer Cell 22:725-736

1. Transgenic Twist expression in skin keratinocytes promotes squamous cell carcinoma (SCC) invasion





B

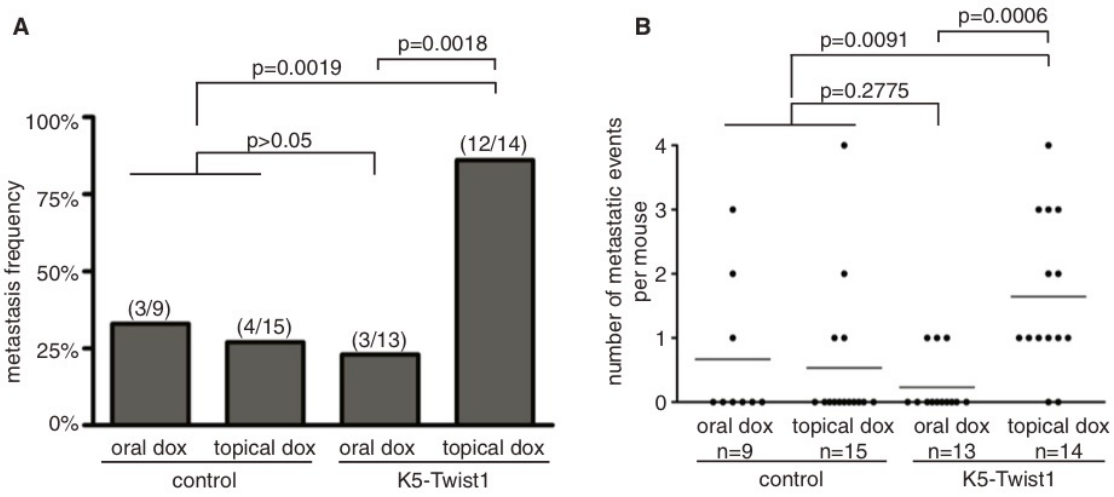


- A Dox-inducible Twist transgene converts benign skin papillomas into squamous cell carcinoma (SCC)

- *Transient* as well as *sustained* Twist expression equally stimulated tumor <u>invasiveness</u>

Tsai et al. 2012 Cancer Cell 22:725-736

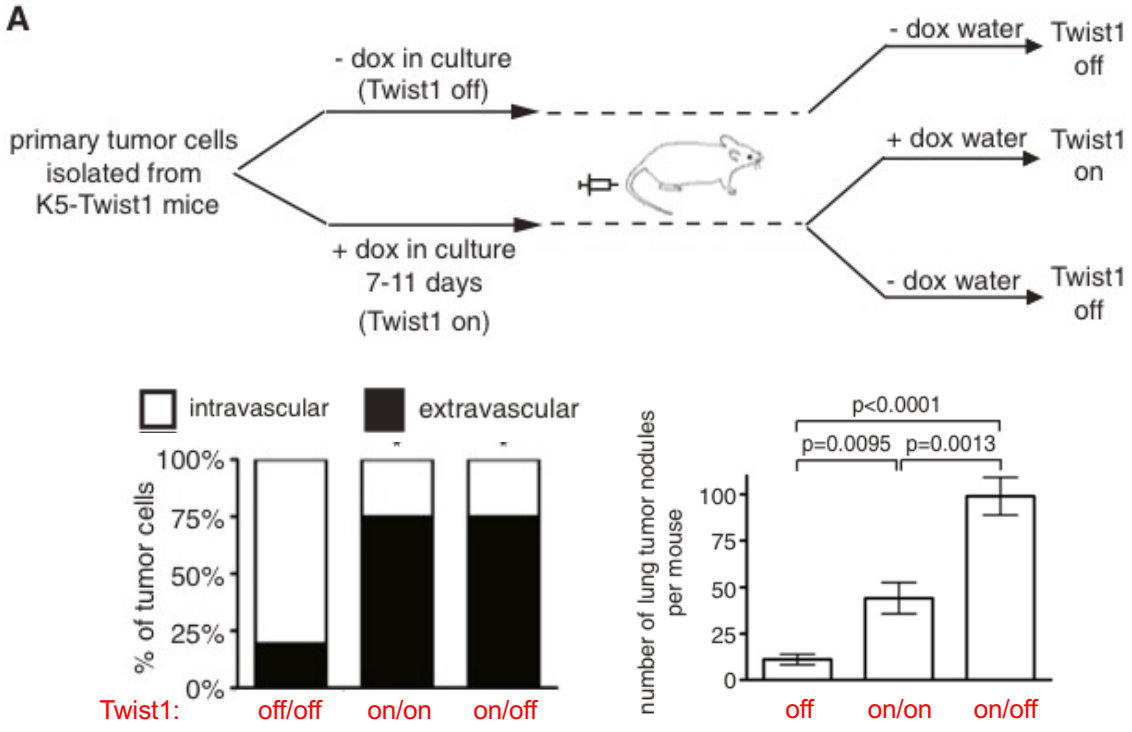
2. Twist also promotes skin SCC *metastasis* - *but only if it is expressed transiently*



- *transient* induction of tetO-Twist1 by K5-rtTA transgene (topical dox administration) increases frequency of metastases
- *sustained* Twist1 induction by orally administred dox does not

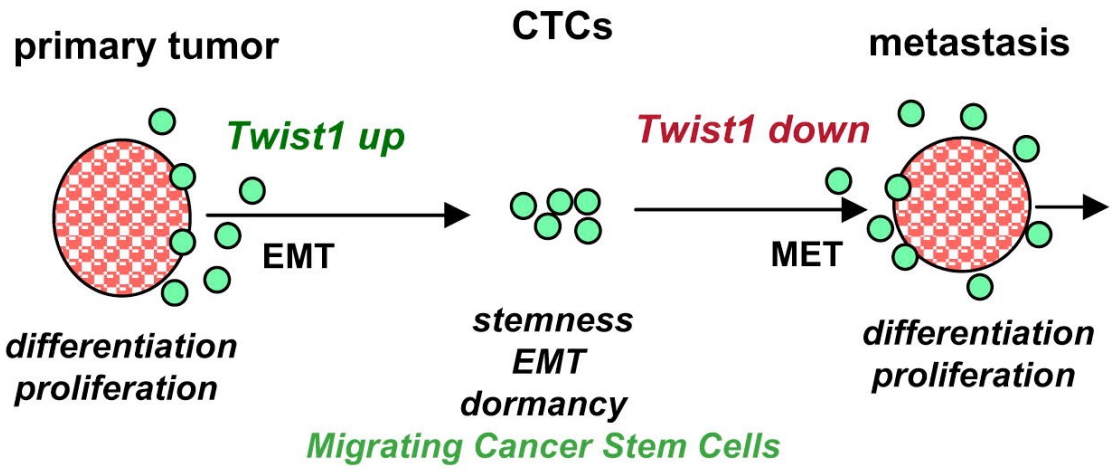
Tsai et al. 2012 Cancer Cell 22:725-736

3. Twist stimulates extravasation, but then must be switched off to efficiently promote colonization



Tsai et al. 2012 Cancer Cell 22:725-736

Model



- A “stem cell” phenotype may depend on tumor-host interactions that maintain EMT at the invasive front and in CTCs (e.g. TGFβ)

Brabletz 2012 Cancer Cell 22:699 - 701

Summary - Invasion-metastasis, steps 3-6

- **Circulating tumor cells (CTCs)** interact with **neutrophils** and with **platelets**, a rich source of factors that aid survival and extravasation, incl. TGF-β
- Downstream of **TGF-β and additional factors** such as NFkB, EMT TFs **can maintain CTCs in a state of "partial EMT"** (Vimentin expression, loss of E-cadherin at the surface)
- In melanoma and triple-negative breast cancer, **partial EMT of CTCs correlates with poor prognosis** and increased **drug resistance**
- The **rate-limiting** step of metastasis called **colonization** is **facilitated by MET** and by **remodeling of the metastatic niche**, probably combined with **immune escape**.

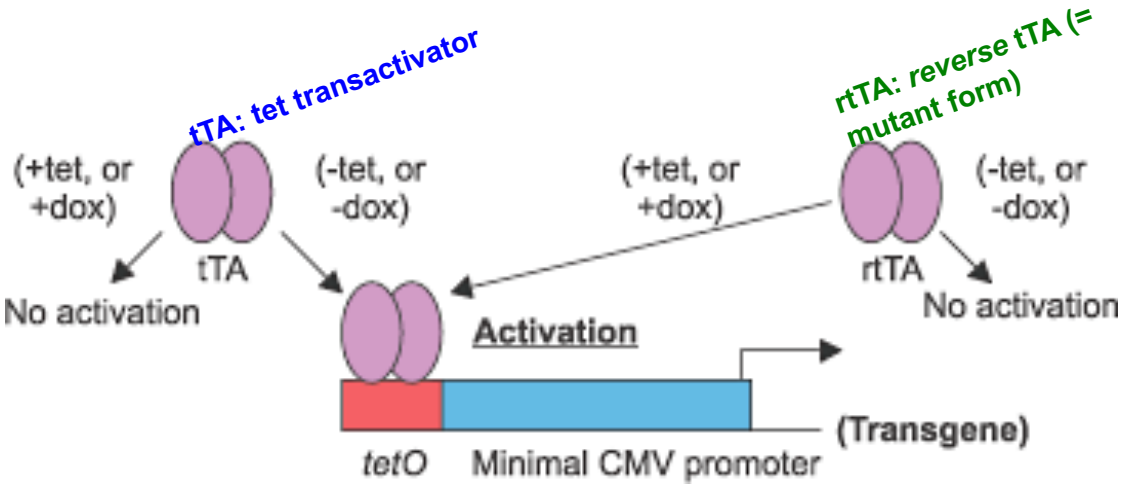
Exercise

For today's exercise (reminder)

Doxycycline-regulated transgenes (once again):

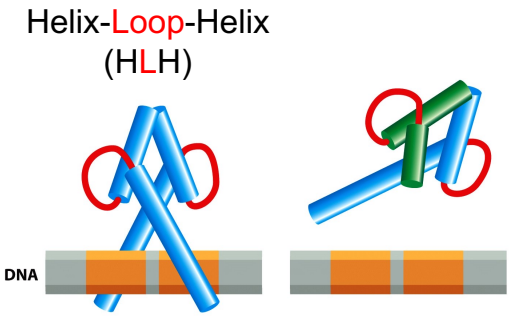
Tet-OFF system

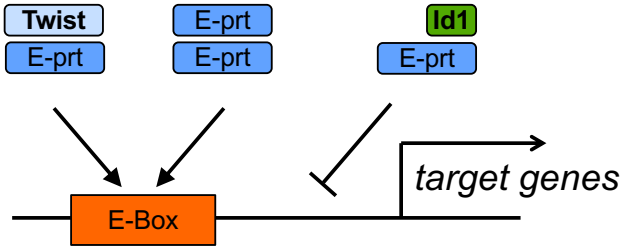
Tet-ON system



For today's exercise:
Reversal of Twist-induced EMT by Id1

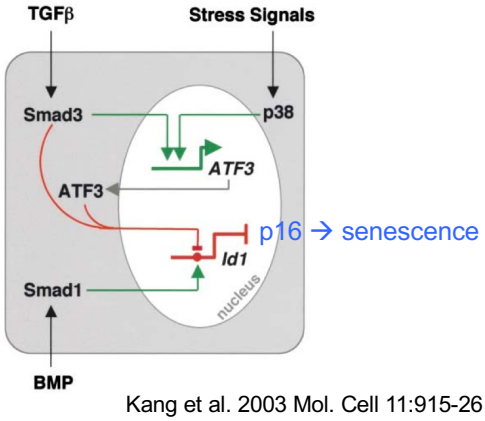
- Twist belongs to the family of bHLH transcription factors
- To bind DNA, Twist must dimerize with E-protein
- Alternatively, E-proteins bind with high affinity to Id
- Since Id1,2,3,4 lack a DNA binding domain, they function like natural 'dominant negative' inhibitors of E-proteins:





(Replicative Immortality lecture):

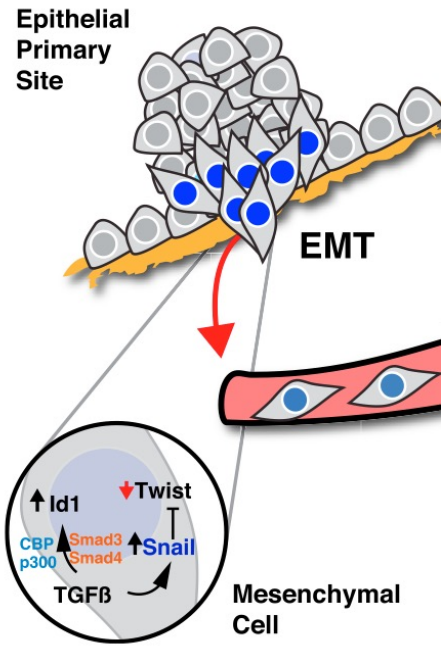
Tumor suppressive TGFβ signaling can *repress* ID1 in premalignant epithelial cells and induce senescence:



Kang et al. 2003 Mol. Cell 11:915-26

TODAY (exercise):

Oncogenic TGFβ signaling *induces* ID1 (basal-type breast cancer):



Stankic et al. 2013 Cell Reports *5*, 1228–1242