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Belgium

# Invasion and metastasis

Leuven, September 4th 2009

## In 1909, Rous found that extract from sarcoma of chicken could induce tumor

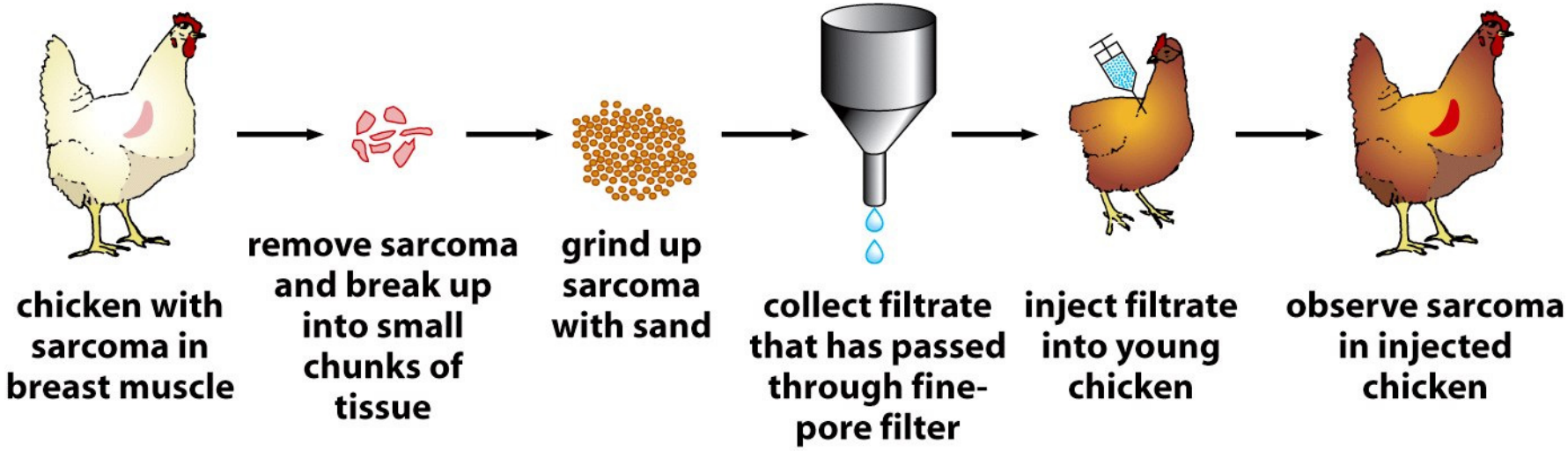
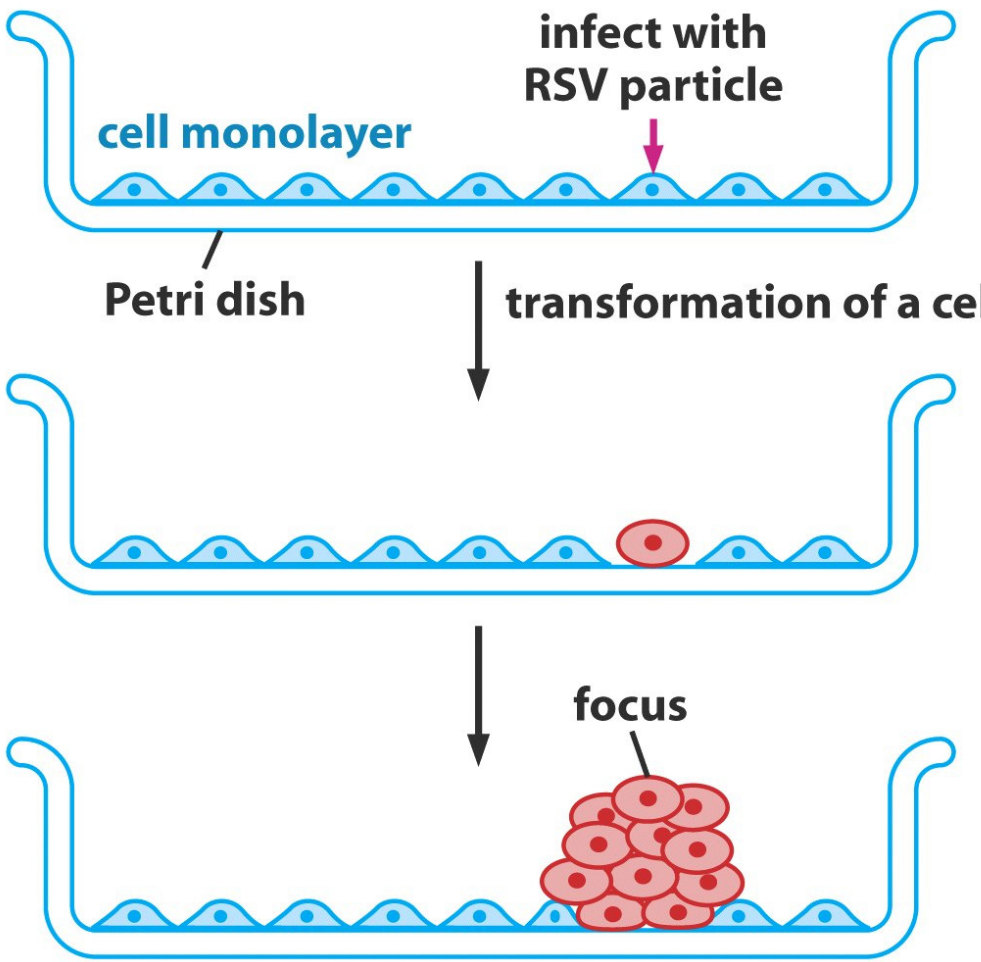
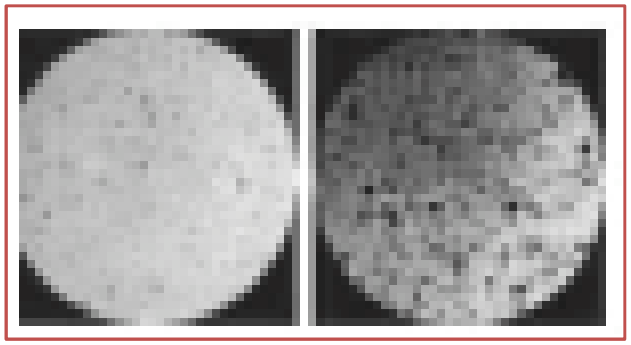



Figure 3.2 *The Biology of Cancer* (© Garland Science 2007)

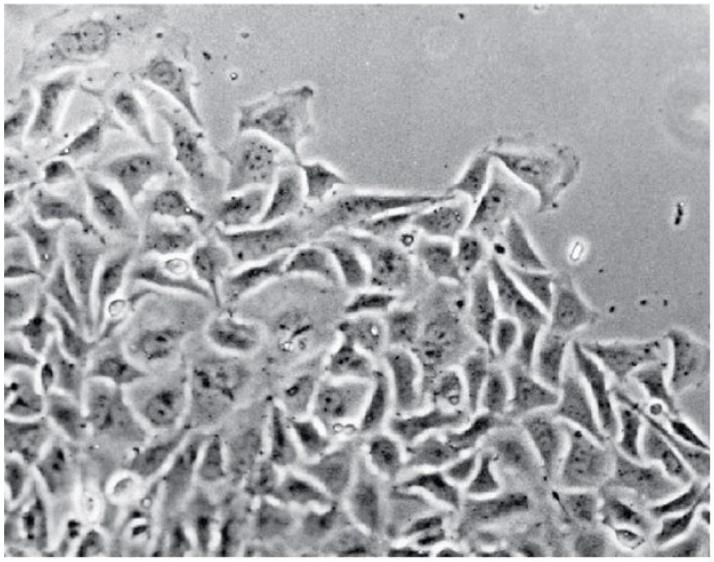
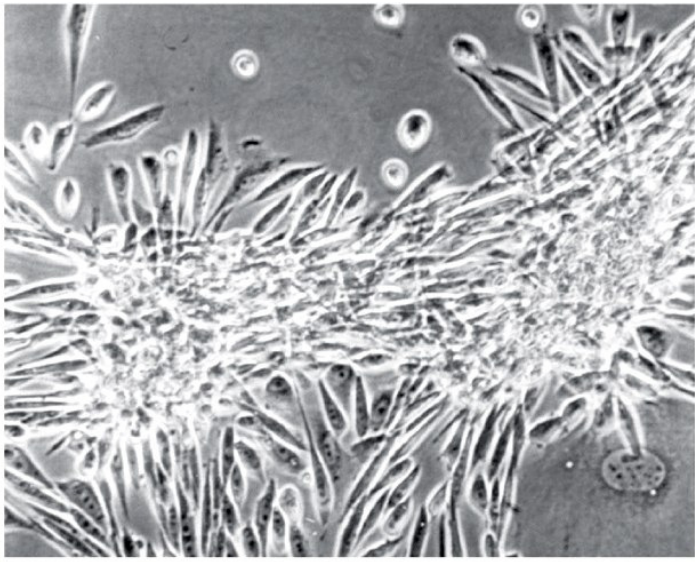
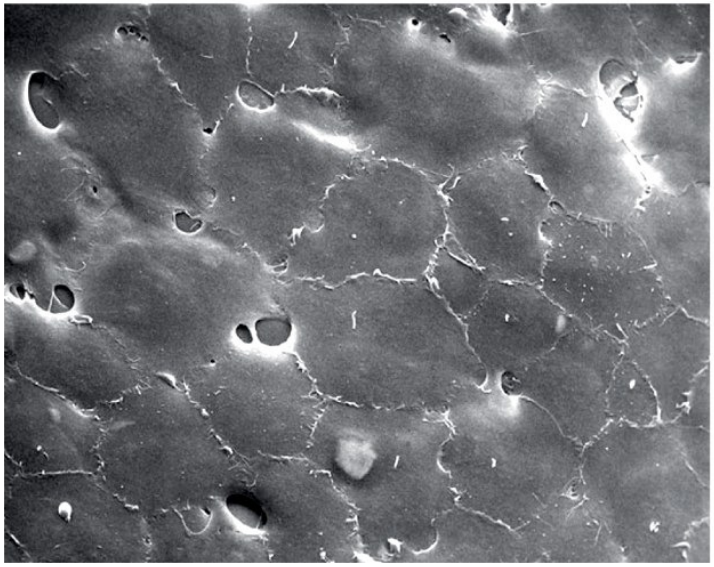
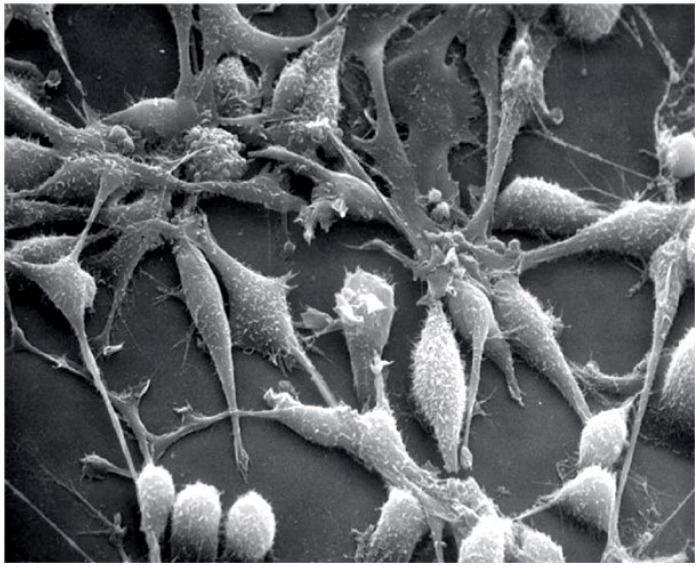
# Rous sarcoma virus is discovered to transform infected cells in culture



Proto-oncogene Activated-oncogene

Figure 3.7a *The Biology of Cancer* (© Garland Science 2007)

| | Normal cells | Transformed cells |
|---|---|---|
| LM |  |  |
| EM |  |  |
| | - Confluent<br>- Monolayer<br>- Contact inhibition, density inhibition (topoinhibition) | - Foci<br>- Elongated round morphology, abundant N/C ratio<br>- Metabolism |

# Anchorage-independent growth

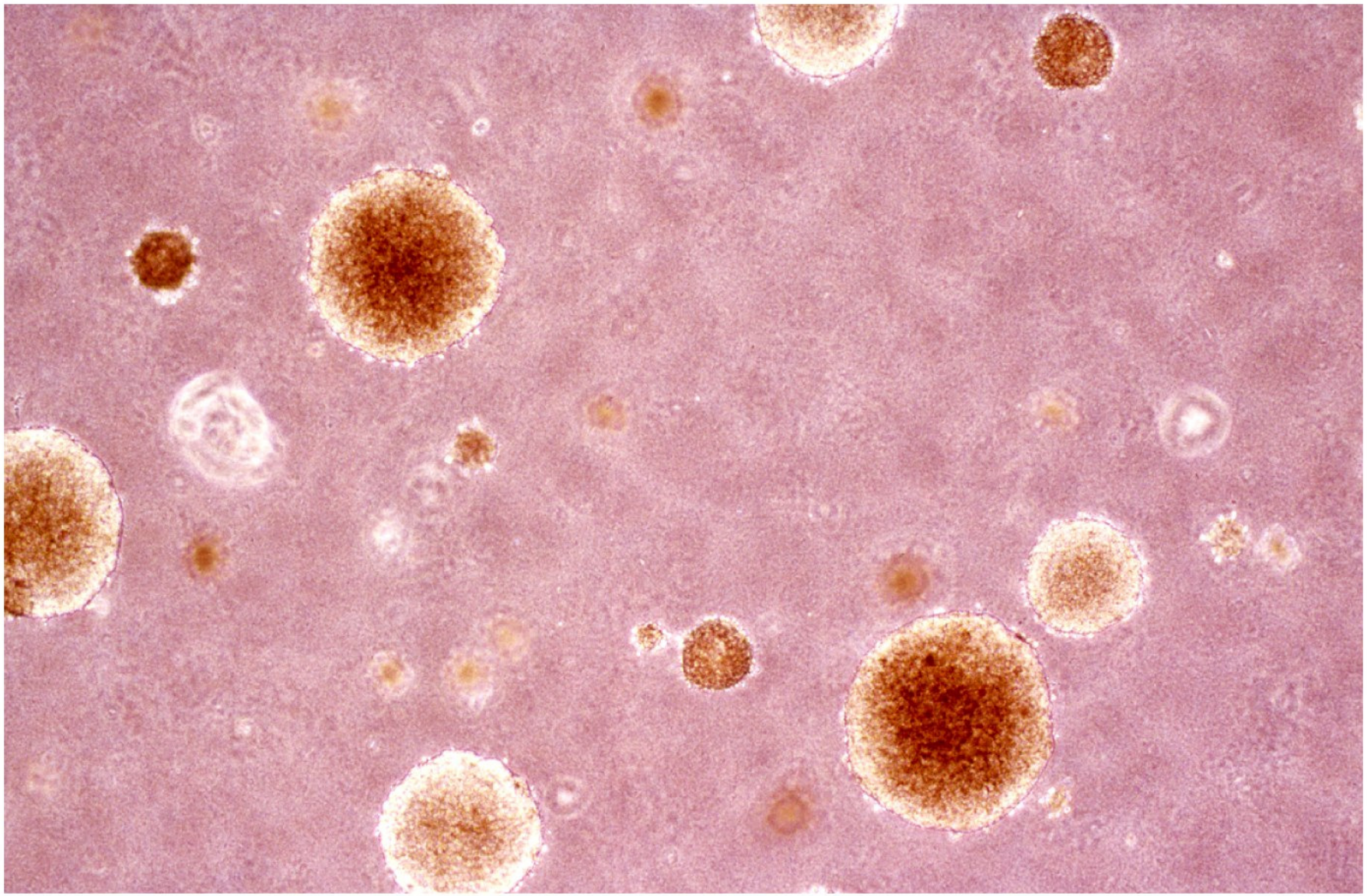


Figure 3.12 *The Biology of Cancer* (© Garland Science 2007)

# Properties of transformed cells

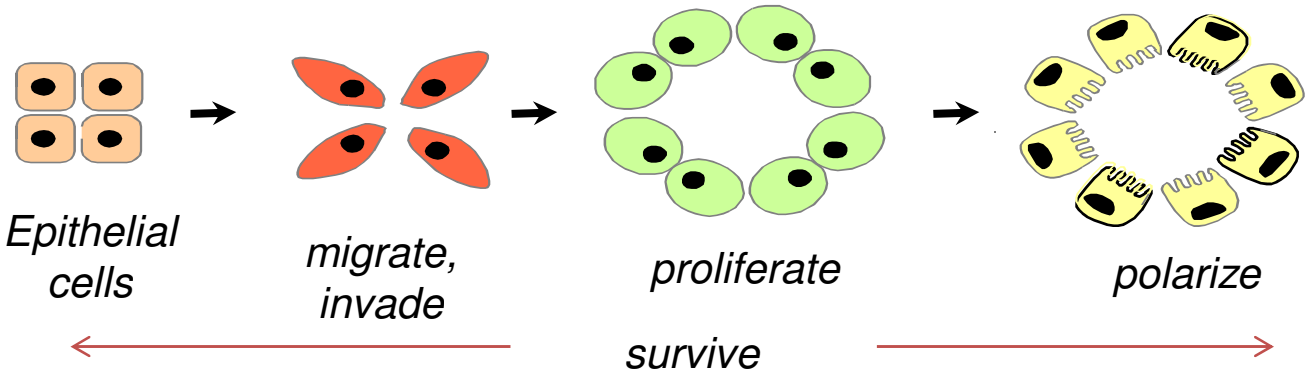
| |
|---|
| Altered morphology (rounded shape, refractile in phase-contrast microscope) |
| Loss of contact inhibition (ability to grow over one another) |
| Ability to grow without attachment to solid substrate (anchorage independence) |
| Ability to proliferate indefinitely (immortalization) |
| Reduced requirement for mitogenic growth factors |
| High saturation density (ability to accumulate large numbers of cells in culture dish) |
| Inability to halt proliferation in response to deprivation of growth factors |
| Increased transport of glucose |
| Tumorigenicity |

Adapted in part from S.J. Flint, L.W. Enquist, R.M. Krug et al., Principles of Virology. Washington, DC: ASM Press, 2000.

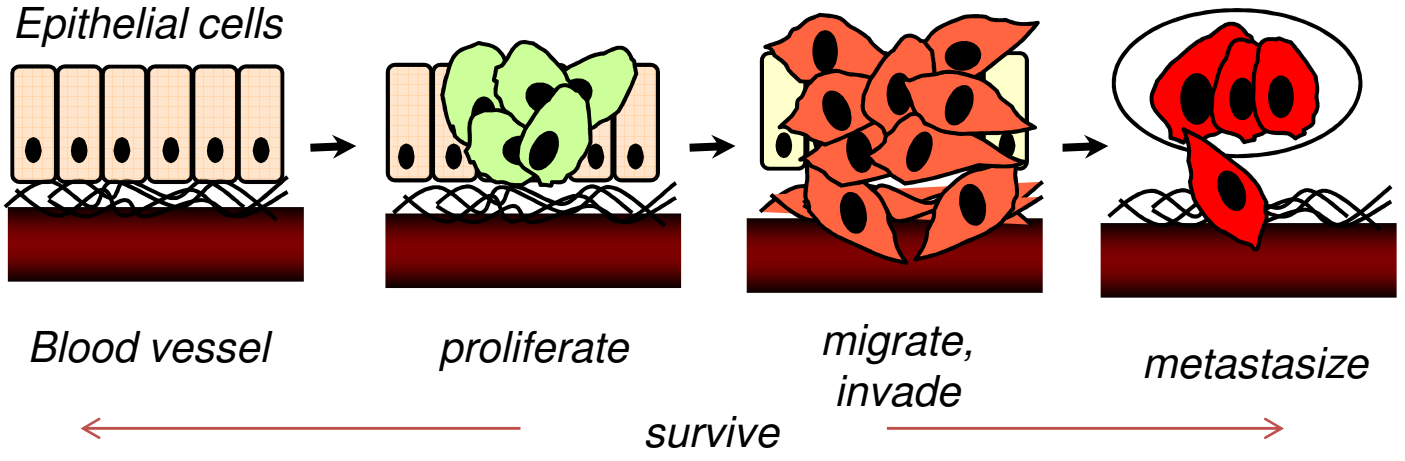
Table 3.2 *The Biology of Cancer* (© Garland Science 2007)

## Tumor progression resembles a morphogenetic process where each step is aberrantly and constitutively activated

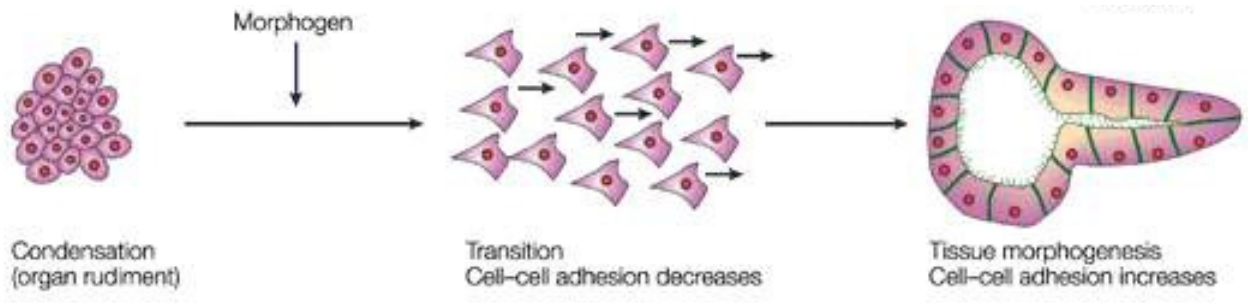
***Normal invasive growth: morphogenesis***

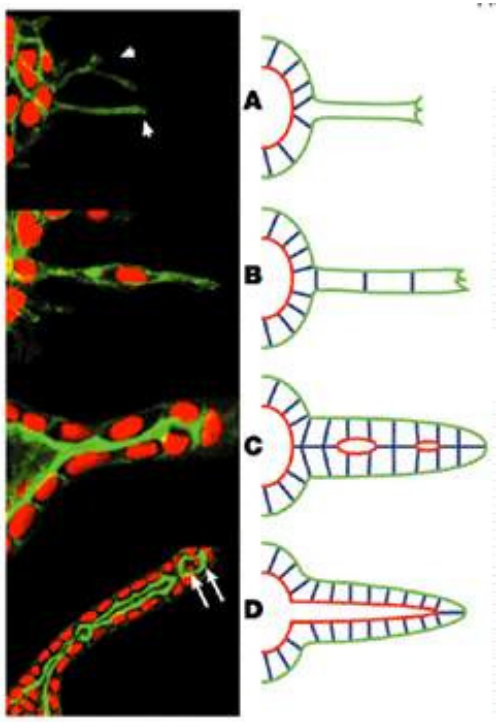



***Neoplastic invasive growth: tumor infiltration and metastasis***



# Morphogenesis and formation of hollow organs during embryogenesis

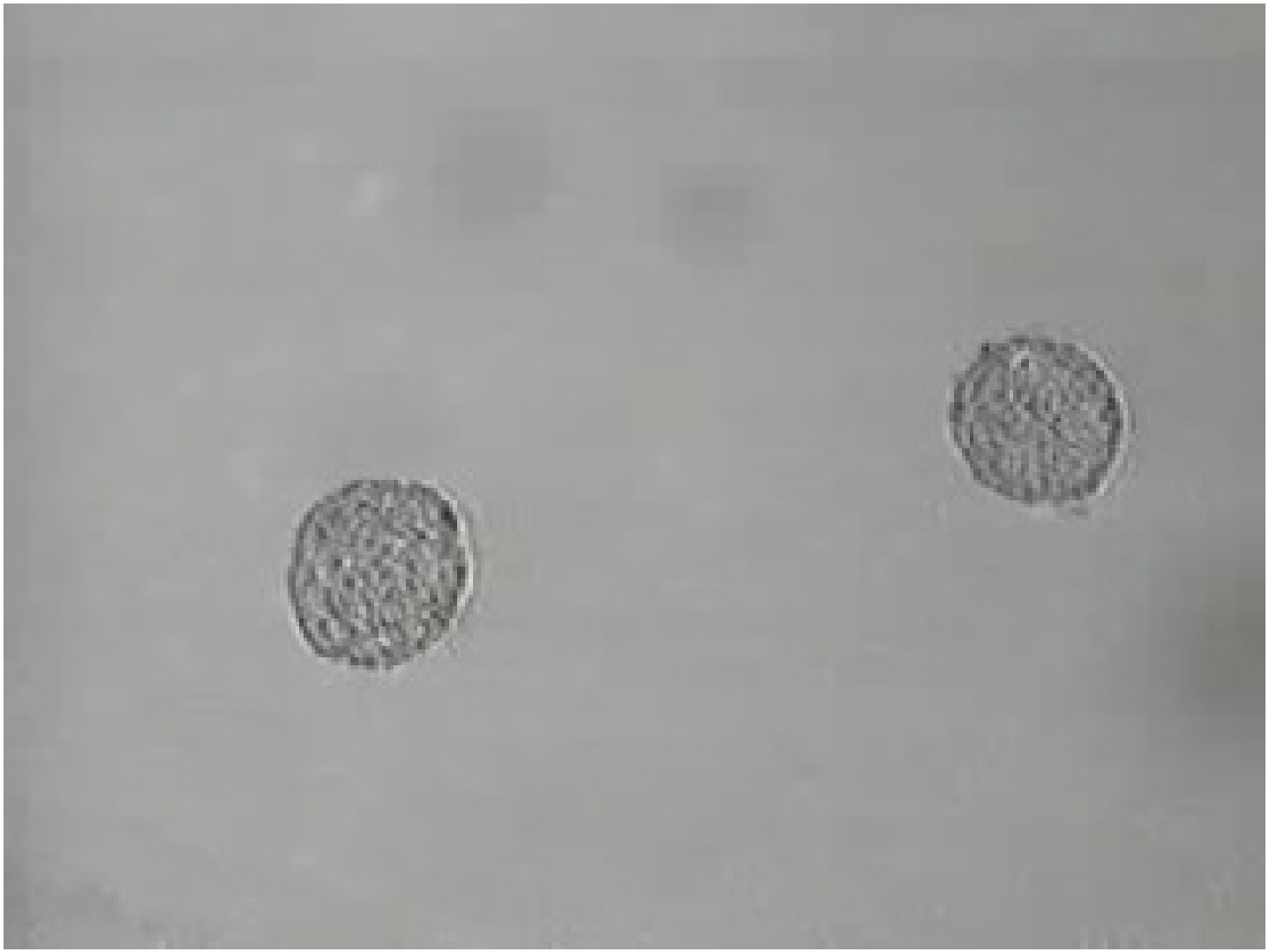


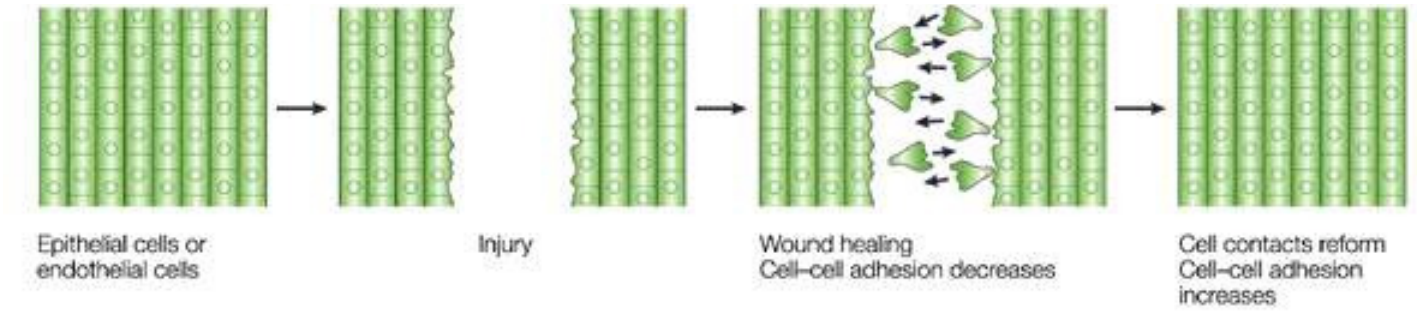

- Dedifferentiation
  1. destabilization
  2. migration
  3. proliferation

- Redifferentiation
  1. Reorganization of the tissue architecture

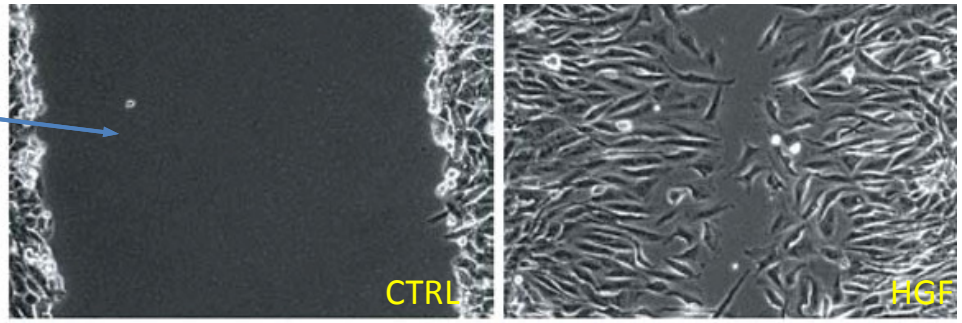
# Tubulogenesis assay *in vitro*



# Morphogenesis in adult life



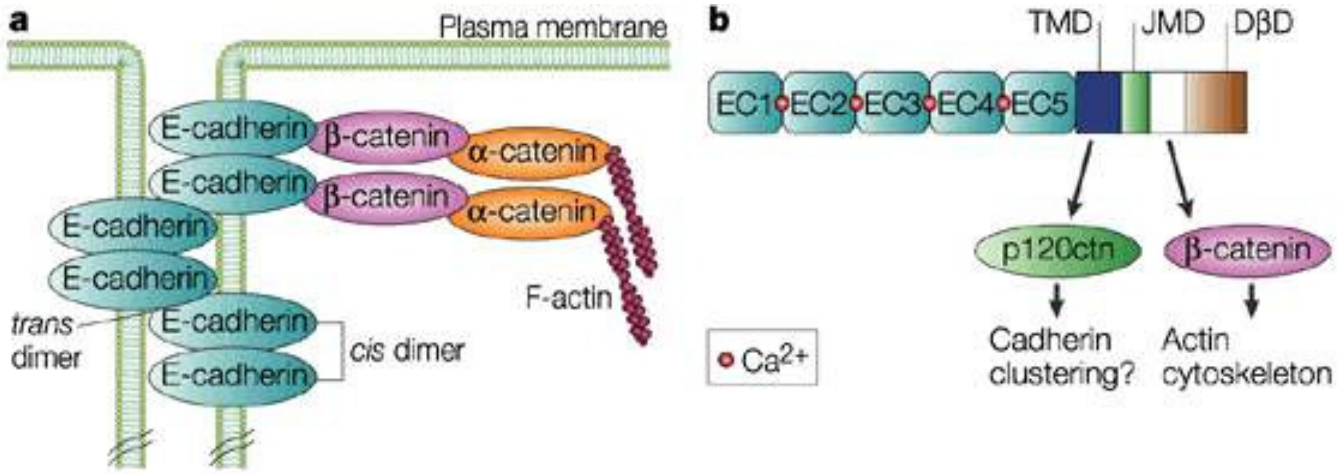

Scratch assay
(wound-healing assay)



## Tumor progression is a multistep program towards malignancy

1. Loss of cell-cell interaction
2. Acquired cell motility
3. Remodeling of the extracellular matrix
4. Aberrant activation of the dedifferentiation program

# The first step towards malignancy: loss of cell-cell interaction (epithelial-mesenchymal transition, EMT)




1. Transcriptional repression of *E-cadherin*: Snail, Slug e Twist

2. *Nonsense* or *frameshift* mutations in *E-cadherin*: breast lobular carcinoma, gastric carcinoma

3. Methylation of the *E-cadherin* promoter

4. Phosphorylation and degradation of E-cadherin: Hakai

# EMT (epithelial-mesenchymal transition): cellular changes

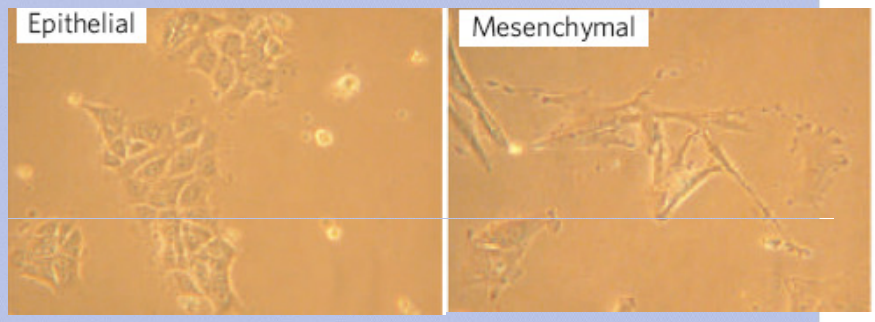
**Table 14.2** Cellular changes associated with the epithelial–mesenchymal transition

**Loss of**

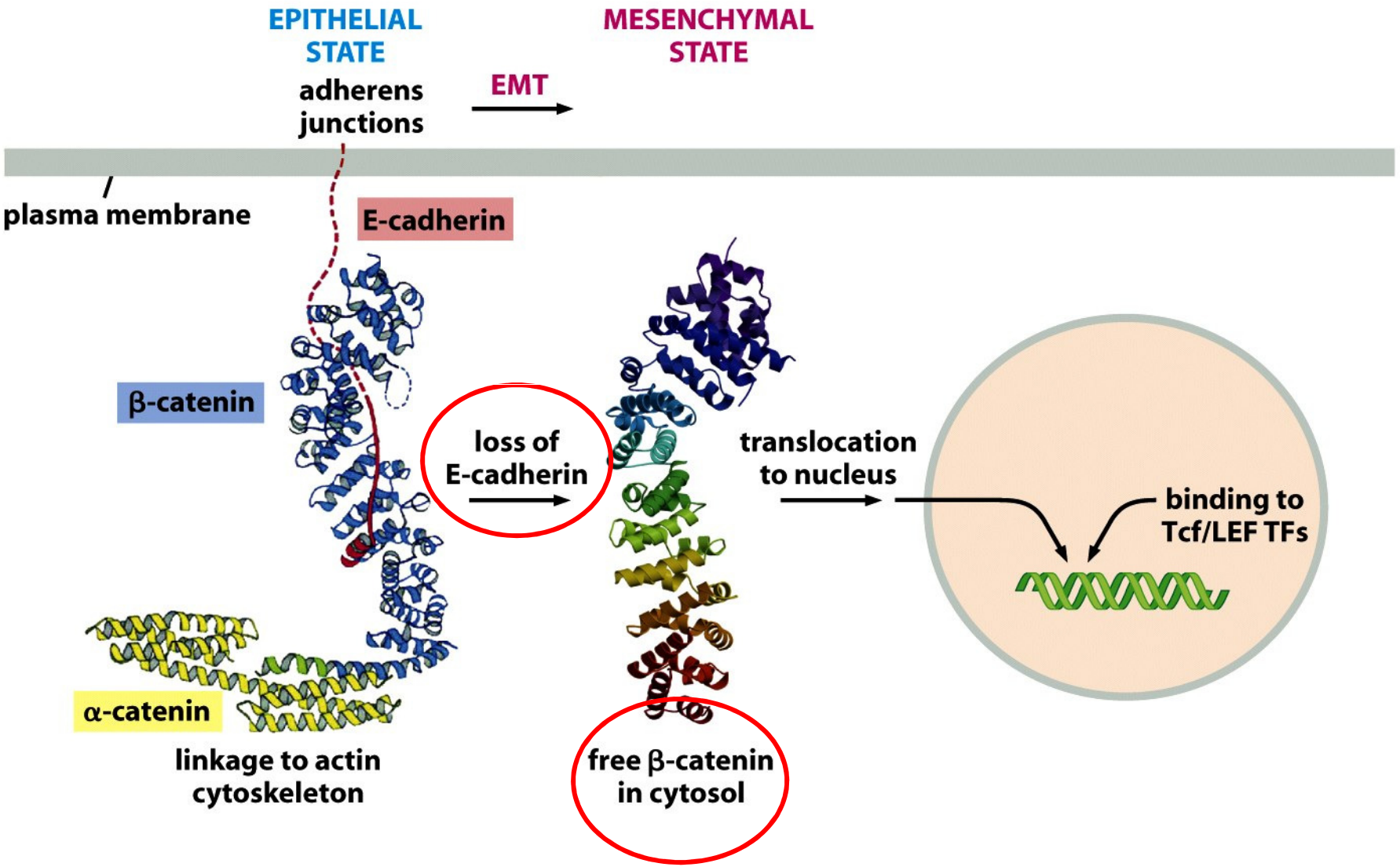
- Cytokeratin (intermediate filament) expression
- Epithelial adherens junction protein (E-cadherin)
- Epithelial cell polarity

**Acquisition of**

- Fibroblast-like shape
- Motility
- Invasiveness
- Mesenchymal gene expression program
- Mesenchymal adherens junction protein (N-cadherin)
- Protease secretion (MMP-2, MMP-9)
- Vimentin (intermediate filament) expression
- Fibronectin secretion
- PDGF receptor expression
- αvβ6 integrin expression

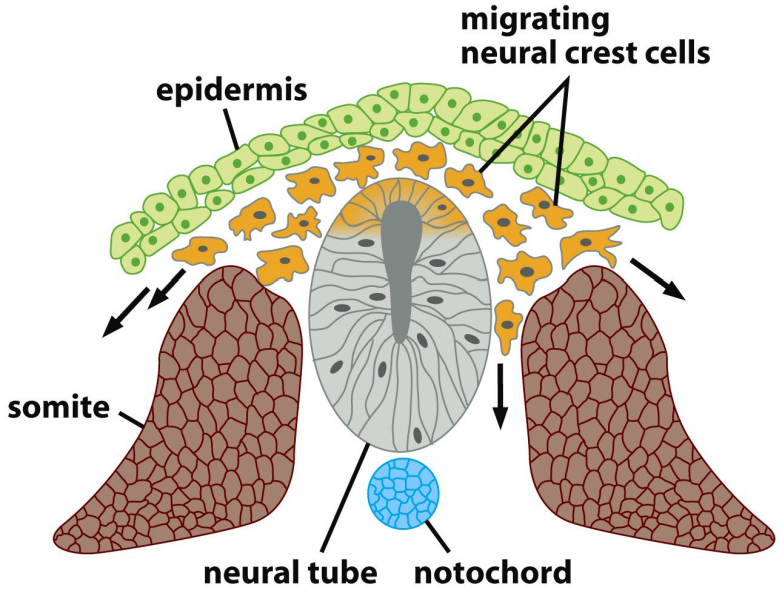


# EMT: molecular changes



# Epithelial-mesenchymal transition in physiology

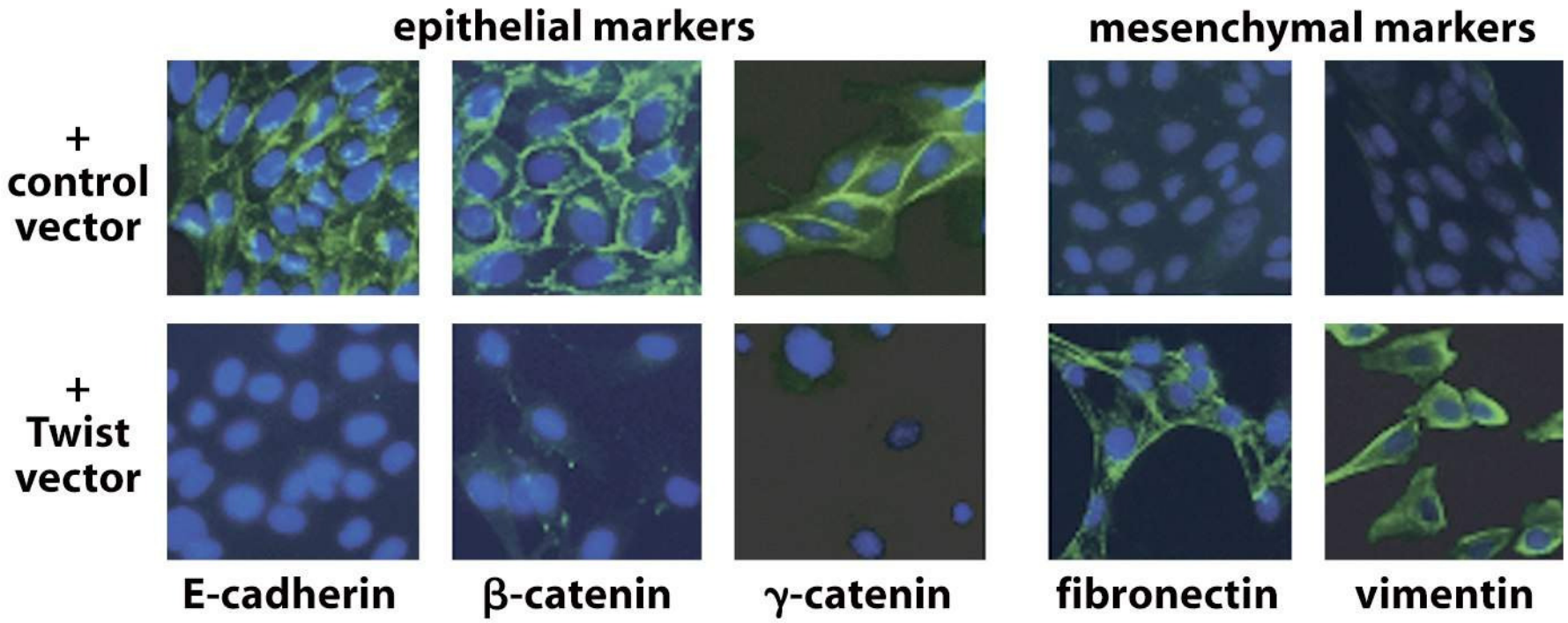
gastrulation




neural crest formation

## Loss and acquisition of markers during the epithelial-mesenchymal transition:

## Tumor cells *in vitro*




Yang et al., Cell, 2004

# Loss and acquisition of markers during the epithelial-mesenchymal transition:

## Tumors *in vivo*

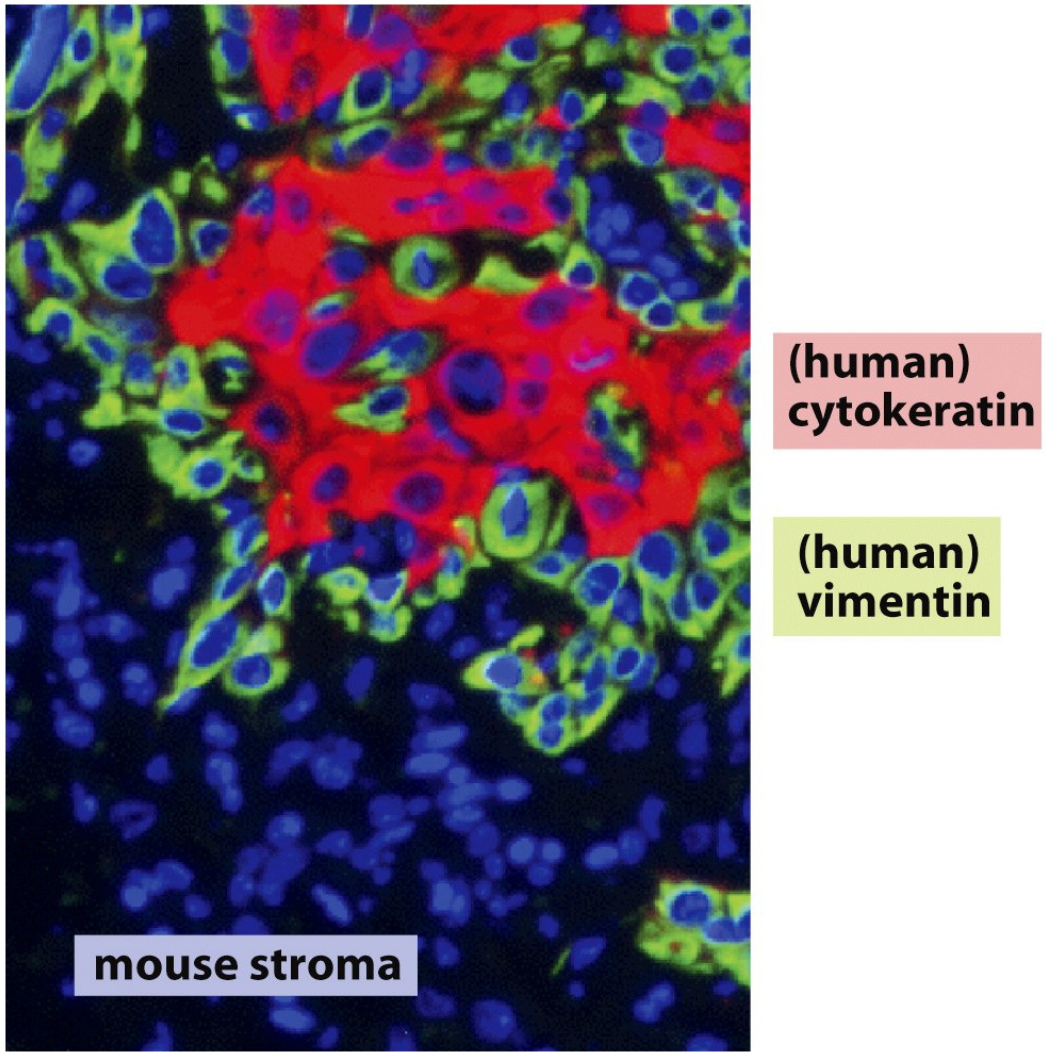
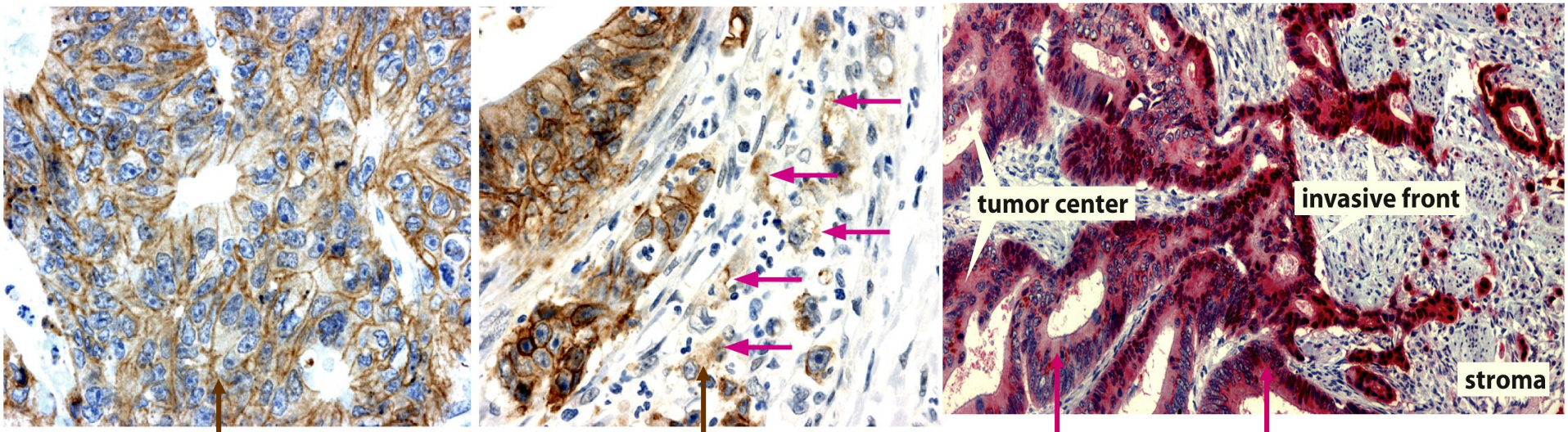



Figure 14.19c *The Biology of Cancer* (© Garland Science 2007)

# EMT: colon carcinoma cells at the invasive edge

Primary tumor

Invasive edge



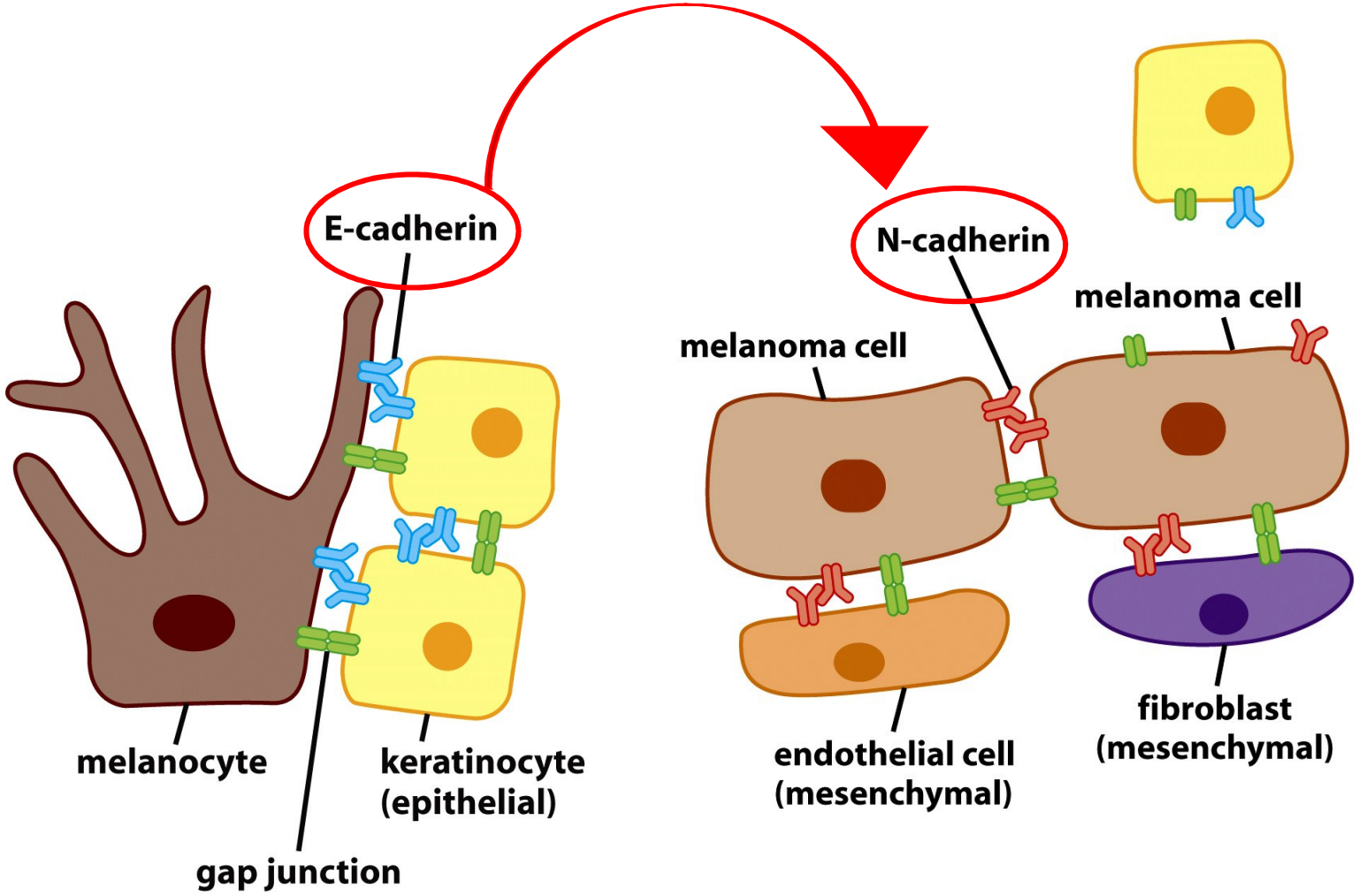

E-cadherin on the plasma membrane: adherent junctions

Low E-cadherin: loss localization at the plasma membrane

β–catenin: cytoplasm localization

β–catenin: nuclei localization

# Cadherin shift: an example from the melanoma cell invasiveness




**E-cadherin**: homodimeric bridges. Strong interaction.
**N-cadherin**: homophilic interactions, binds to other molecules of the same type displayed by nearby cells, increased affinity for **stromal cells** (as fibroblast). Weak interaction.

# The epithelial-mesenchymal transition is a *temporary* state

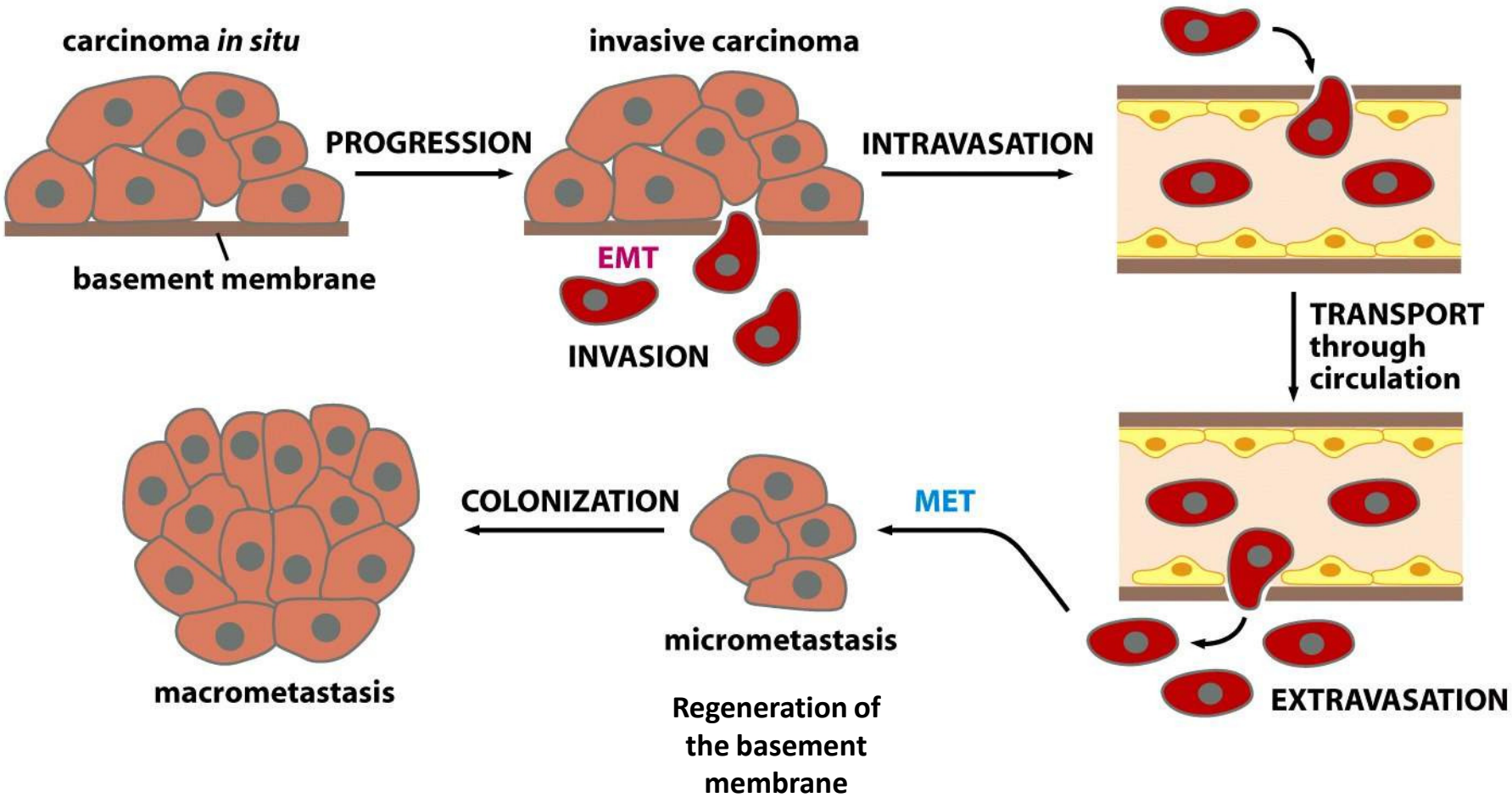
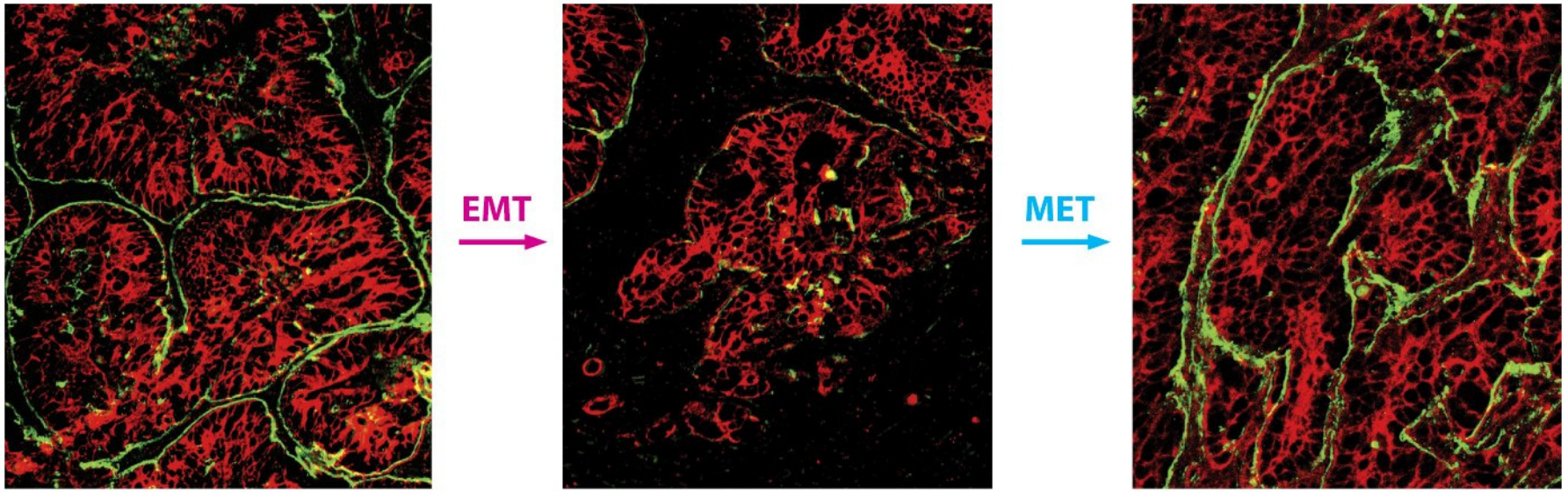



Figure 14.17b *The Biology of Cancer* (© Garland Science 2007)

# The EMT is reversible!
# Mesenchimal-epithelial transition: MET

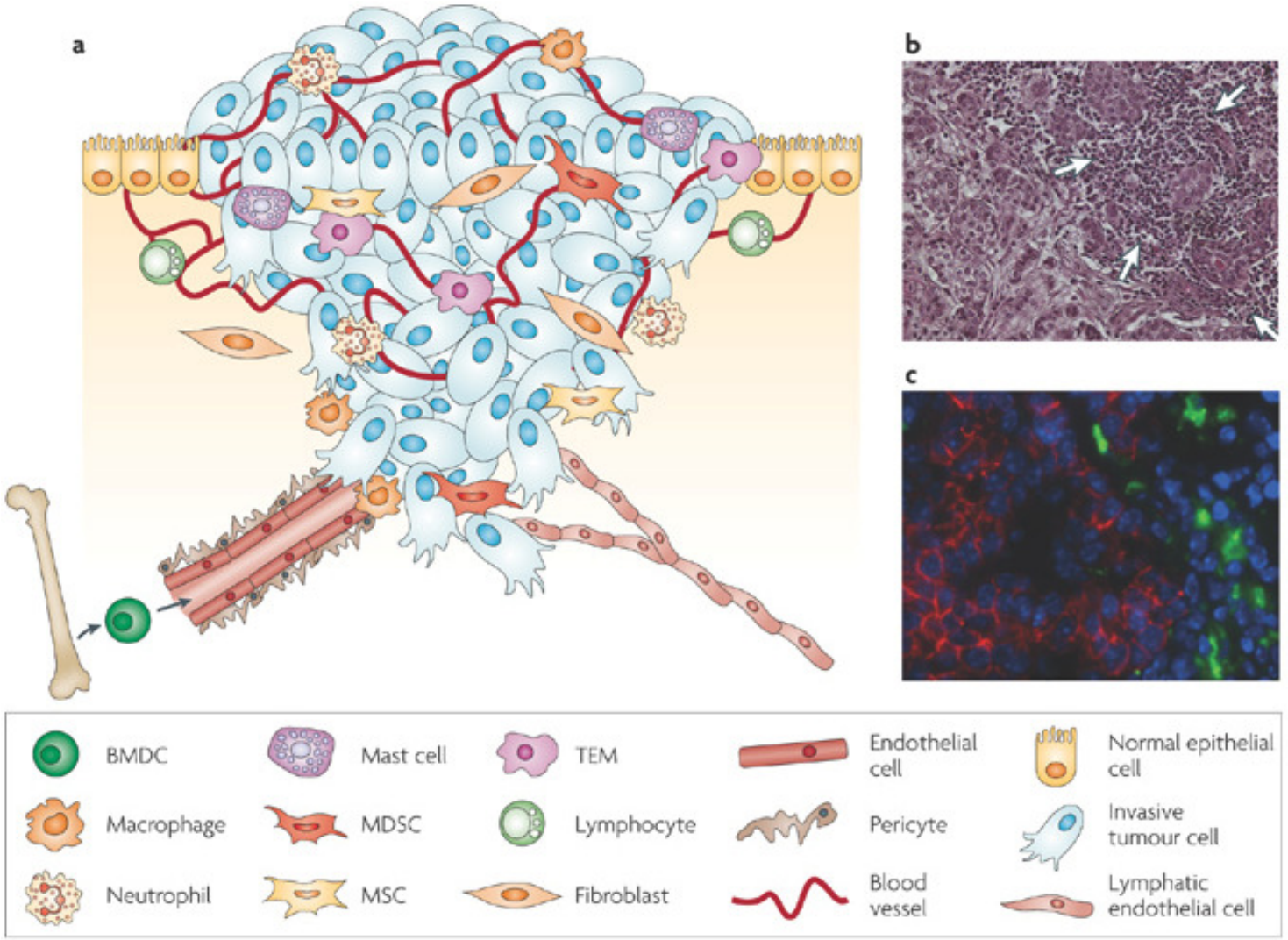



(Colorectal carcinoma)

Cytokeratin 18 (epithelial marker)

Basement membrane

# Cancer is not only a disease of neoplastic cells

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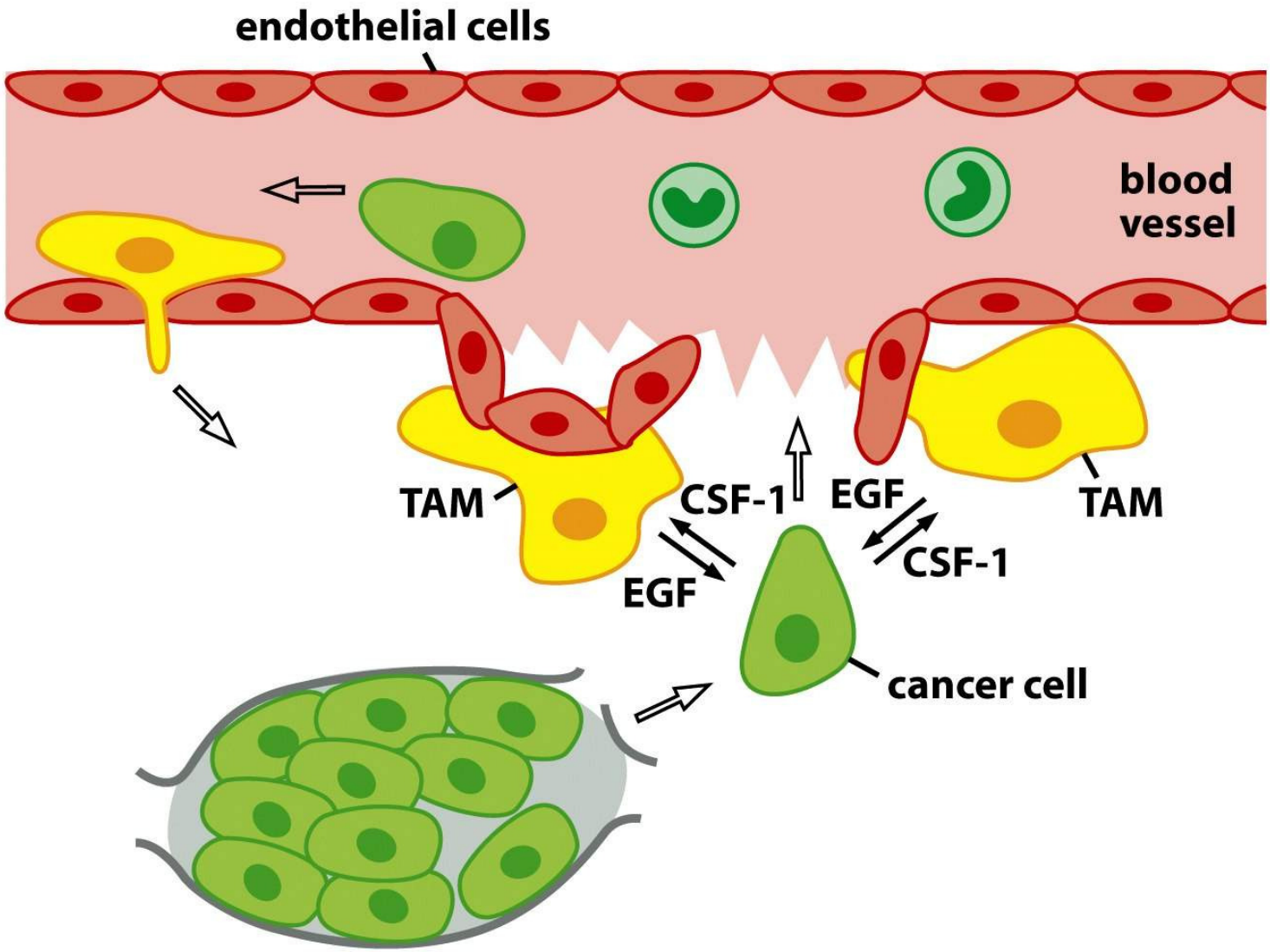
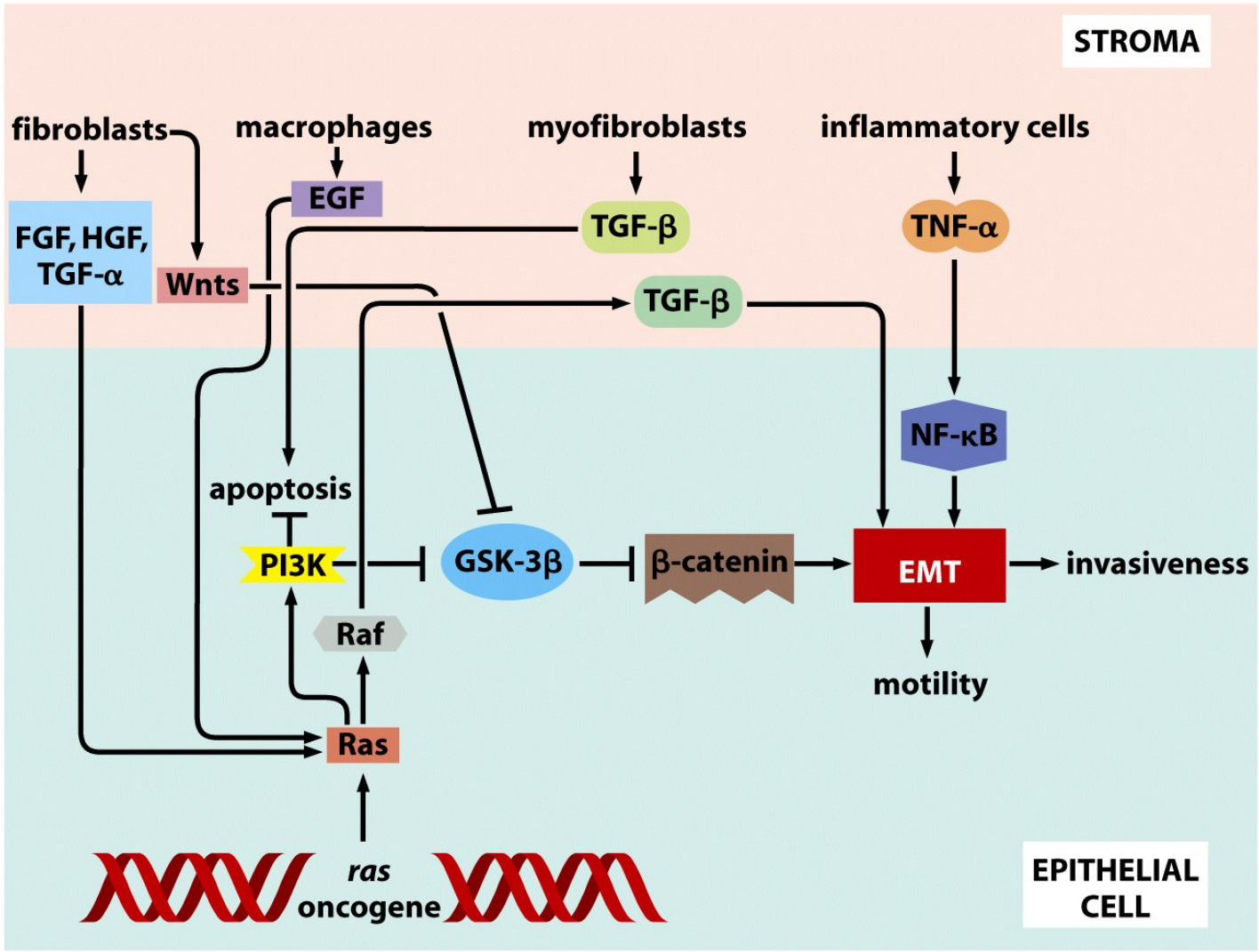
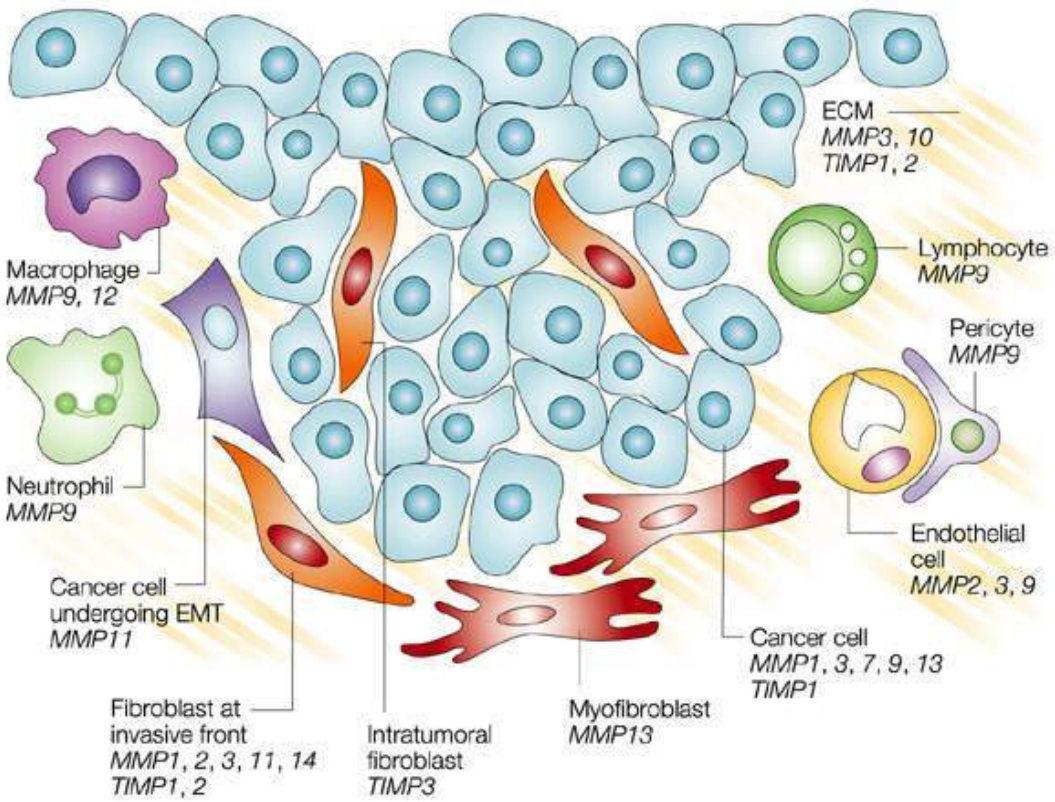
# EMT: the dialogue substitutes the monologue



# Signal to EMT: stroma influence



# Metalloproteinases: matrix degrading enzymes produced by tumor and stromal cells




Matrix metalloproteinases (MMPs):
187 known MMPs, 28 secreted.

- Activation growth factors / inactivation pro-apoptotic factors
- Release of growth factors bound to the extracellular matrix
- Digestion of the extracellular matrix
- Cleavage of laminin-5 and exposure of the cryptic pro-migratory binding site
- Cleavage of E-cadherin
- Angiogenesis

## *In vitro* models do not take in account tumor-stroma interactions

Tumor cell lines behave more naturally when implanted in (immune-deficient) rodents, and develop stromal interactions important to their growth and therapeutic sensitivity.

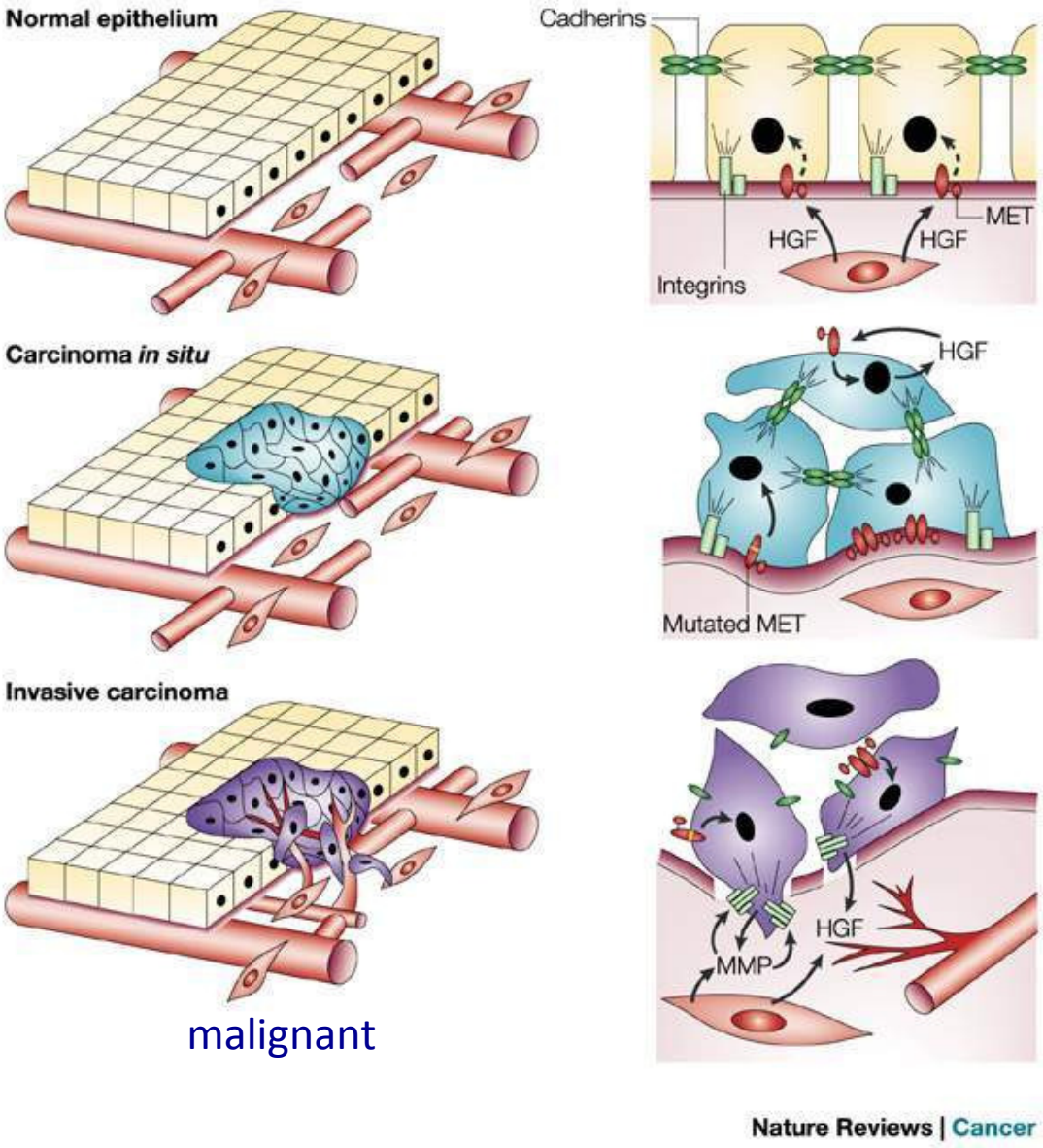
**Orthotopic** tumor

**Subcutaneous** tumor


**Syngeneic** tumors


**Xenograft**

# The tumor alters progressively its interaction with the environment




- Growth factors
- Basal lamina adhesion
- Cell-cell interaction

- Cell-autonomous growth
- Protection against apoptosis
- Anchorage-independent growth

- Basement membrane disruption
- Matrix remodeling
- Angiogenesis

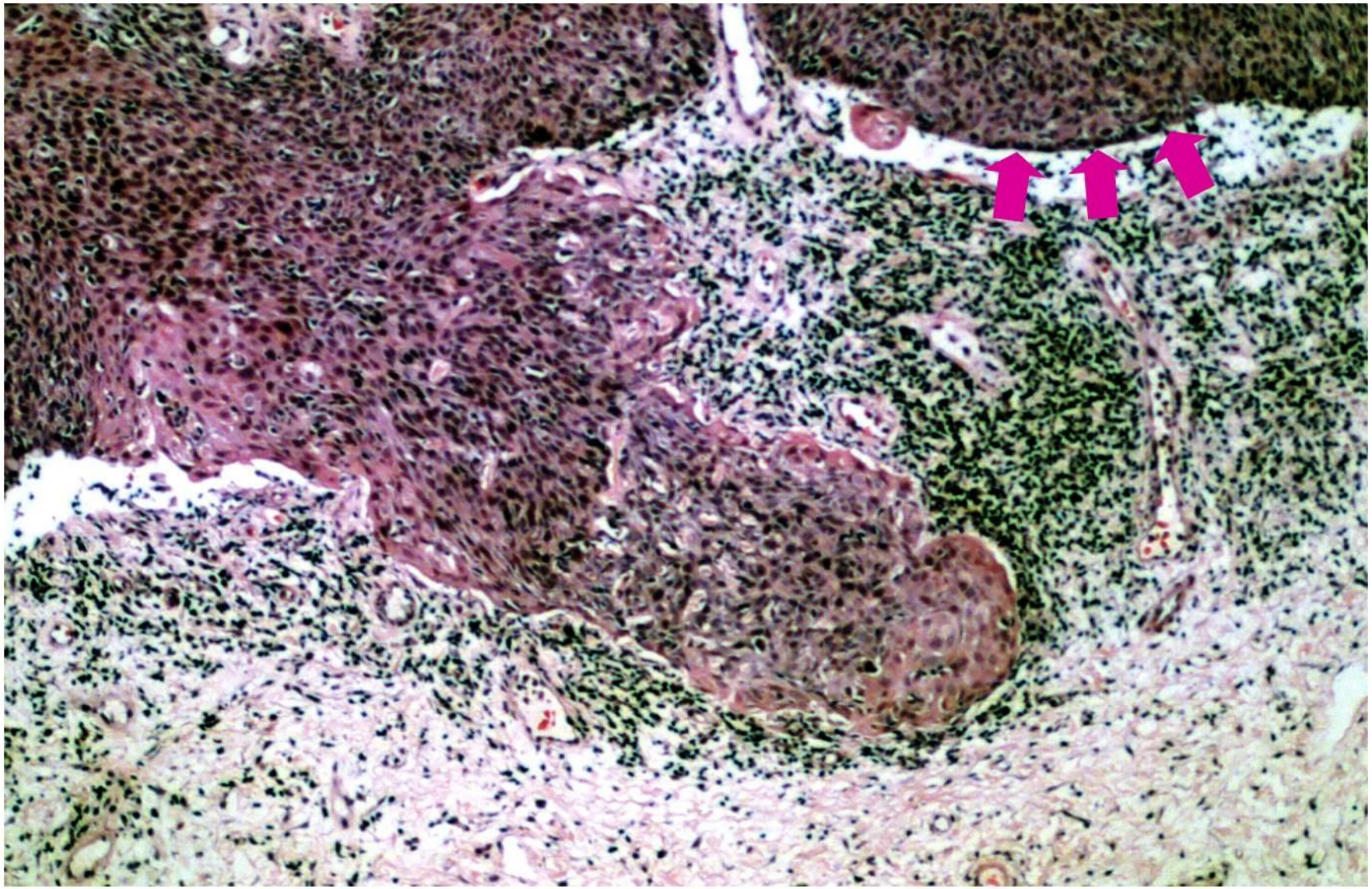
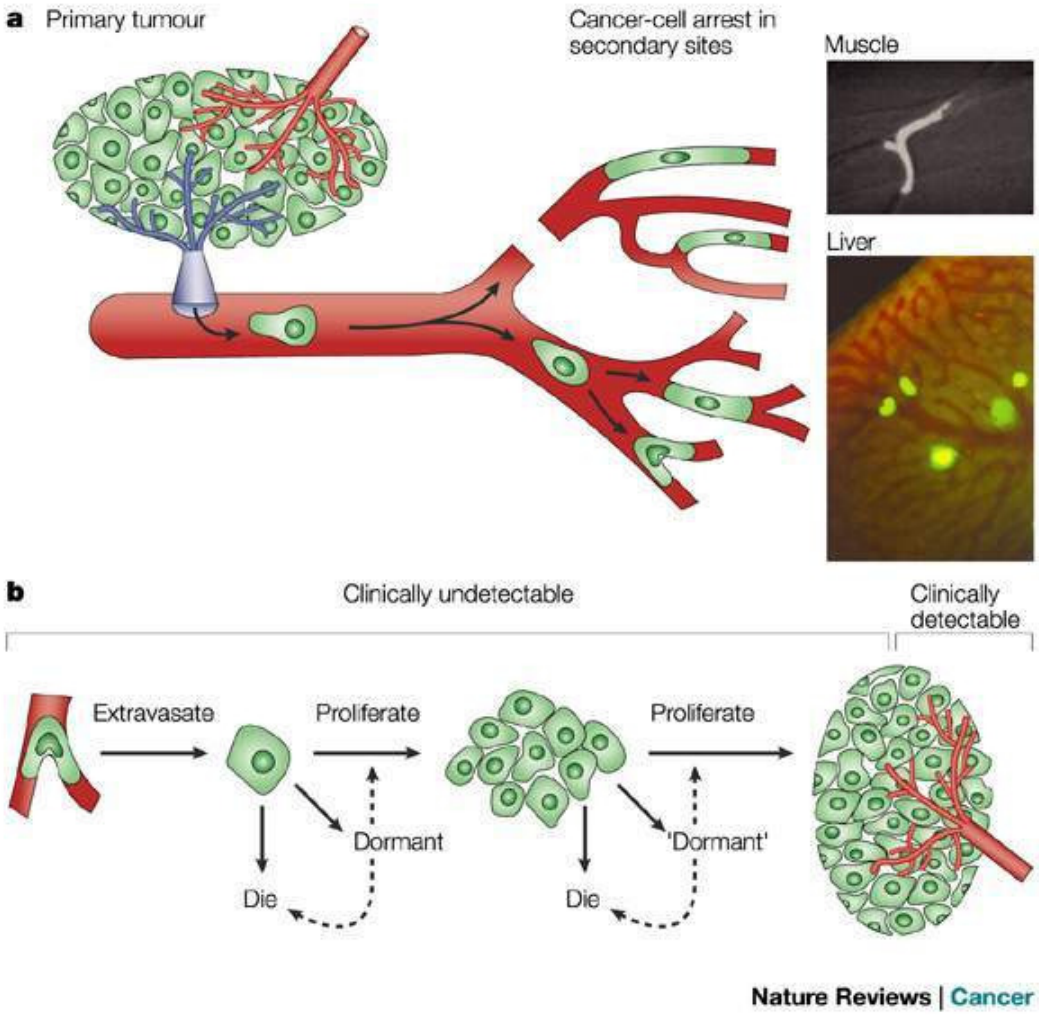
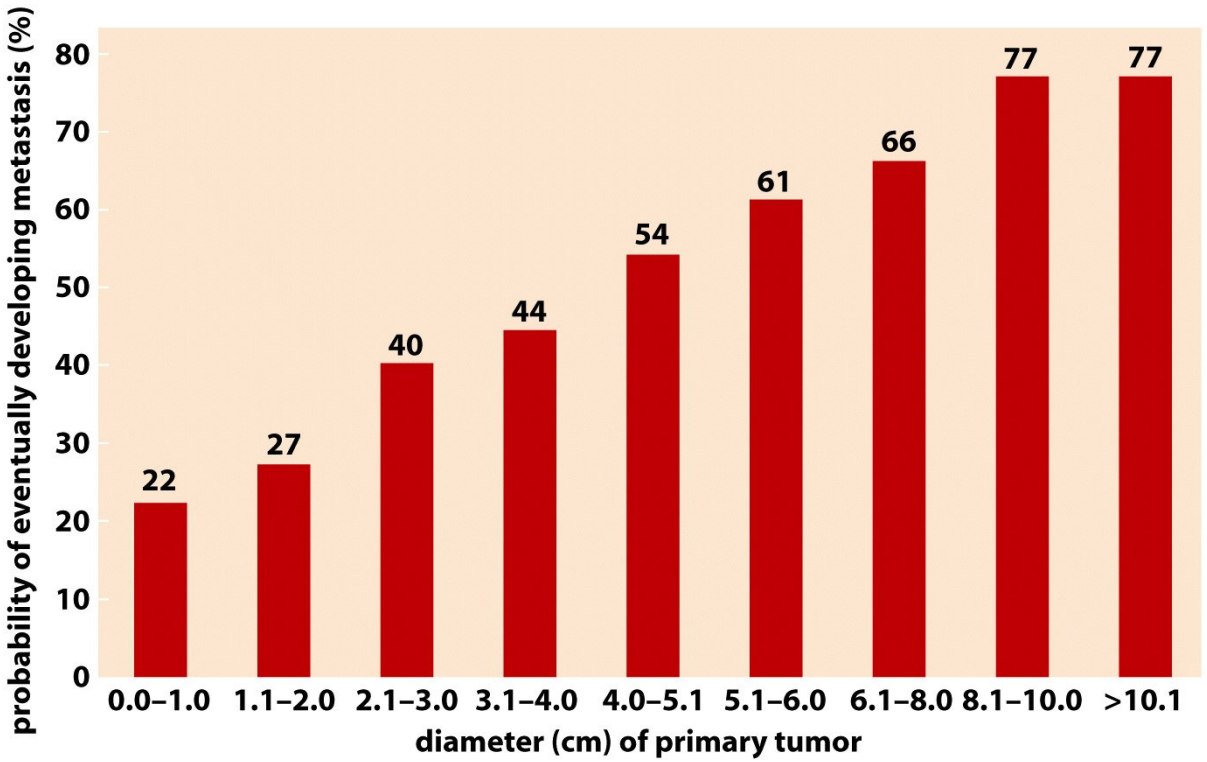


Figure 14.5c *The Biology of Cancer* (© Garland Science 2007)

# To metastasize, cancer cells have to intravasate



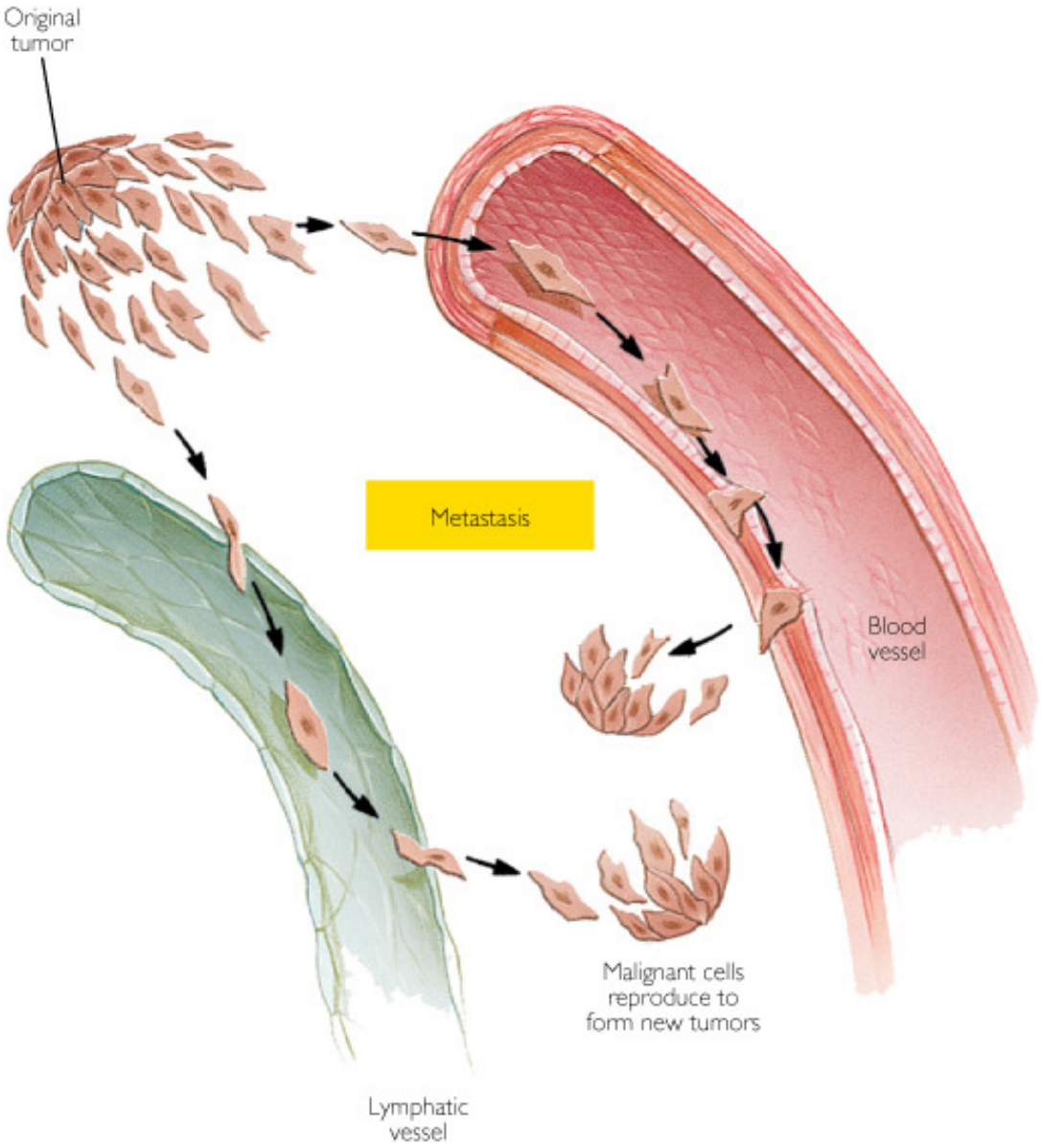
# Primary tumor size and risk of metastases




1. Metastasis trait is acquired relatively late in the growth of the primary tumor

2. Larger tumors may have greater number of metastasizing cells, although small and large tumors could be equally capable of metastasizing

# Routes of metastasis: lymphatic and blood vessels



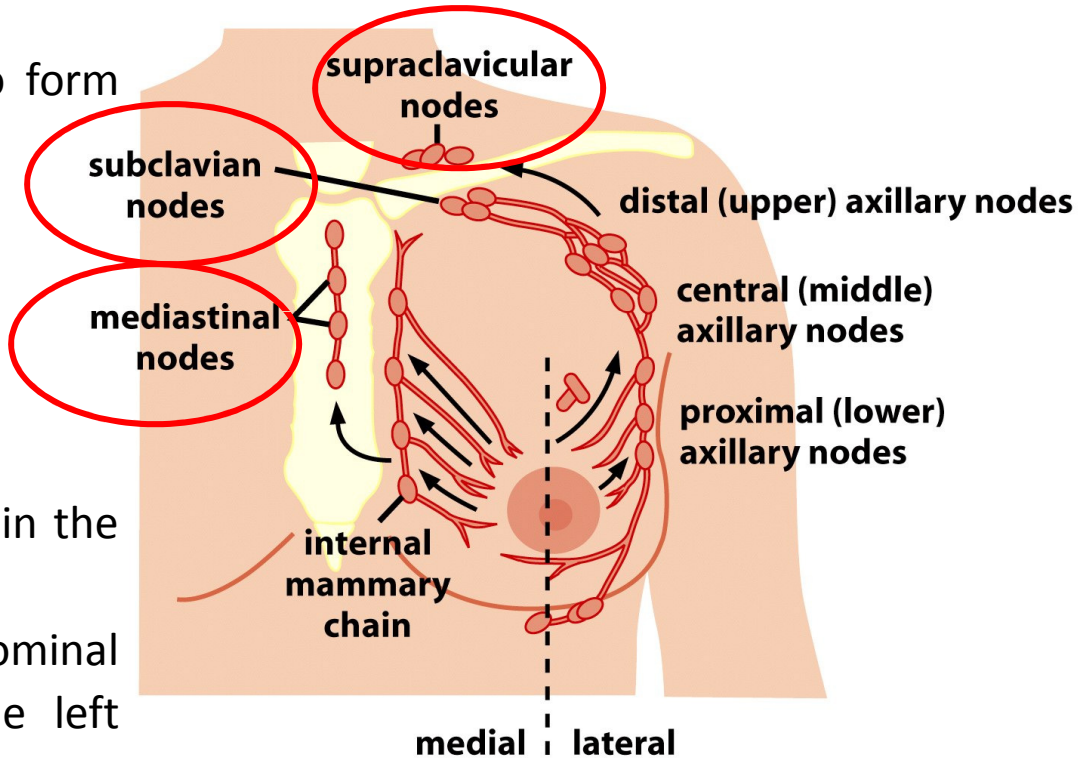
# Routes of metastasis: lymphatic and blood vessels

**Angiogenesis:**

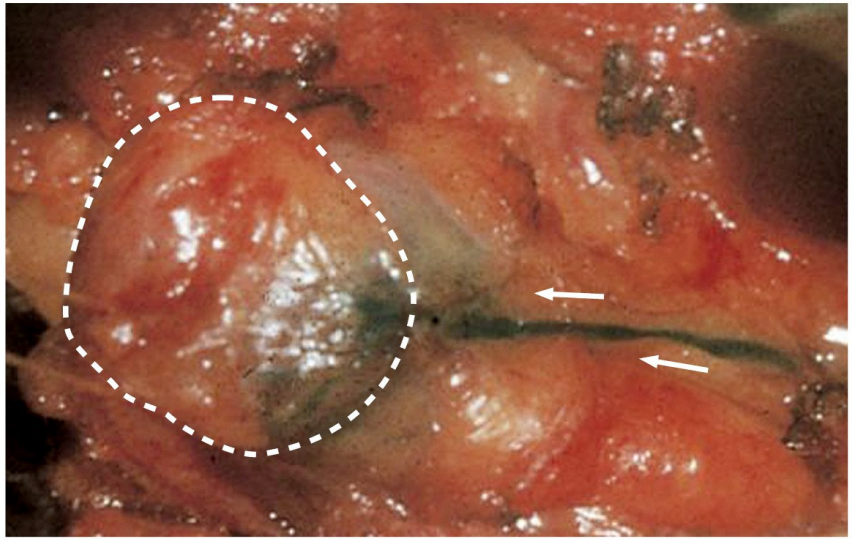
- Support the metabolic activity of cancer cells, so they can survive and proliferate
- Provides the access to distant sites to form metastases

**Lymphatic vessel contribution:**

Drain the interstitial fluids accumulated in the spaces between cells.
The vessels converge to major abdominal vessel that empties its lymph into the left subclavian vein near the heart.
Connession with blood circulation: cancer cell dissemination

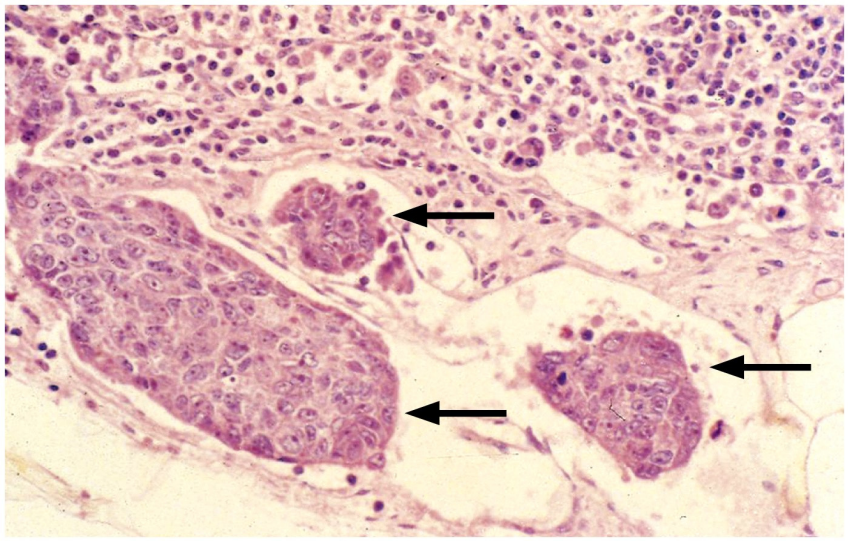


# Lymph node as a sentinel node

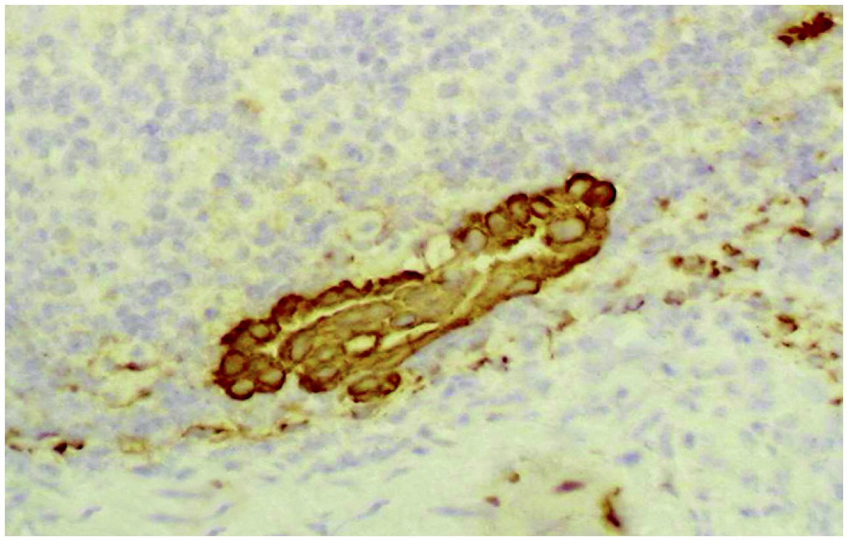


(tumor)

- Dye injection in the tumor mass
- Follow the trail of the dye via the lymphatic duct

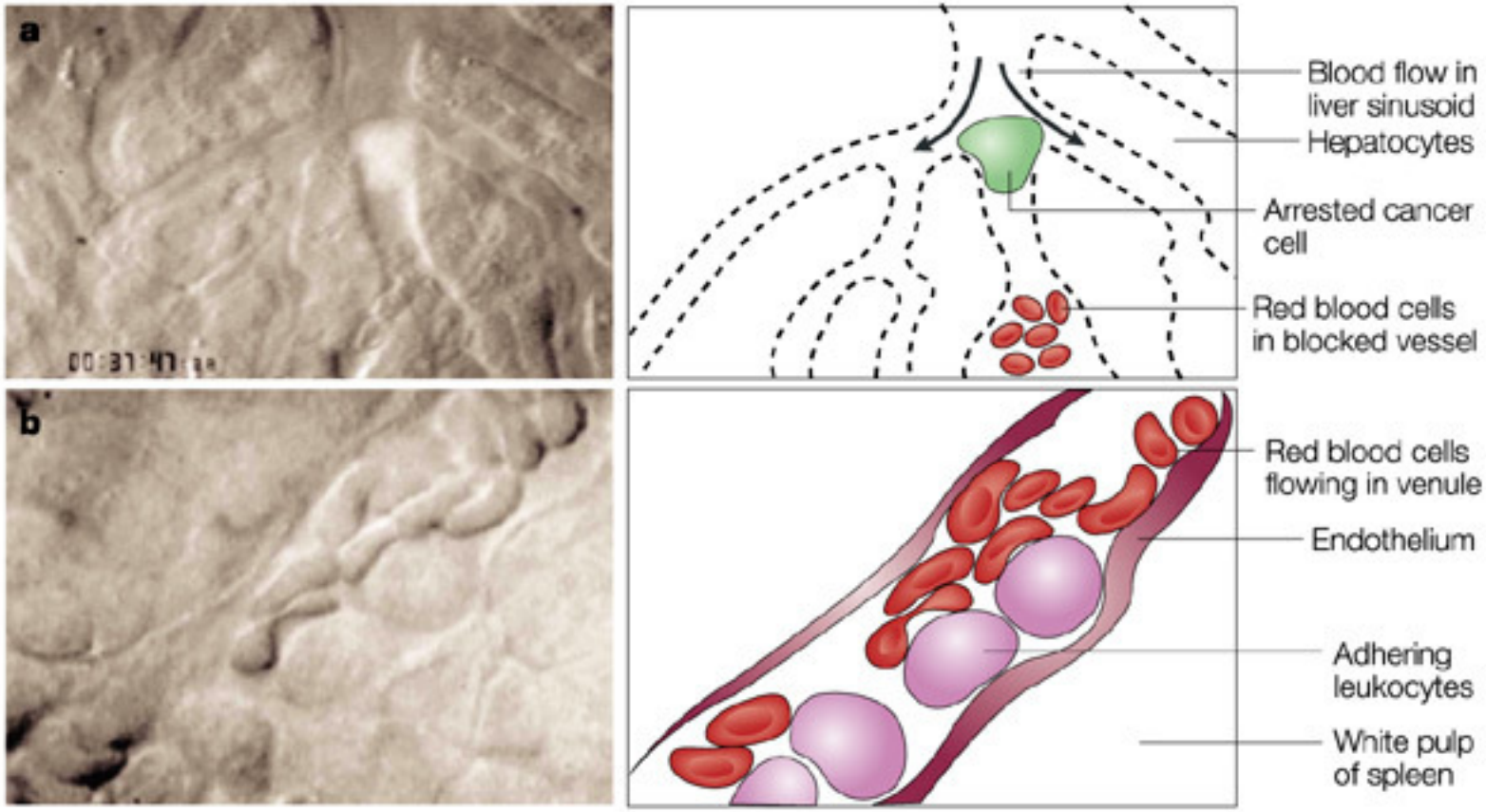


**Axillary limph node: 3 micrometastases**



**Sentinel lymph node: micrometastases. Staining Ab anti-cytokeratin**

# Cancer cell arrest in microvessel: lacking flexibility

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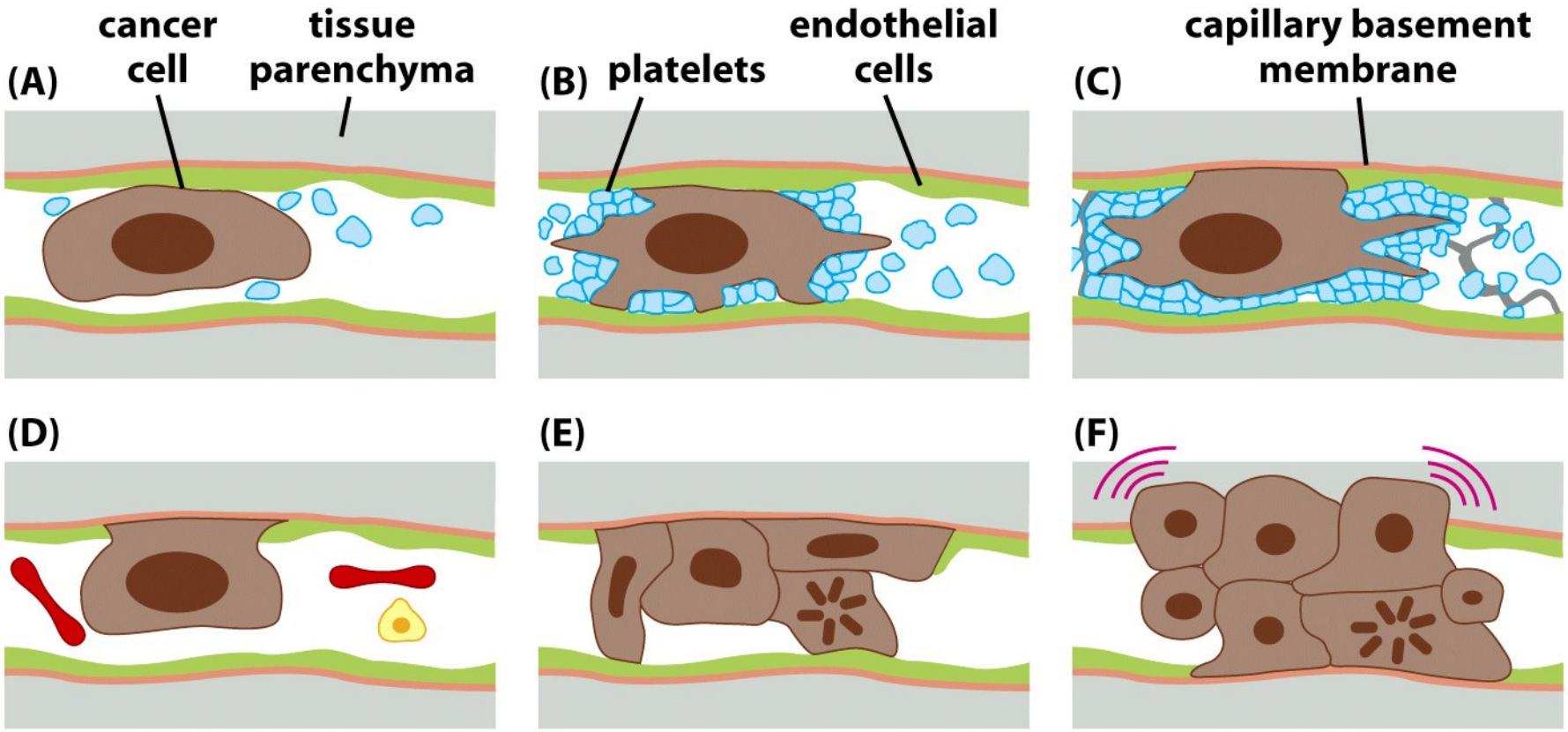
# Extravasation: between passive and active processes

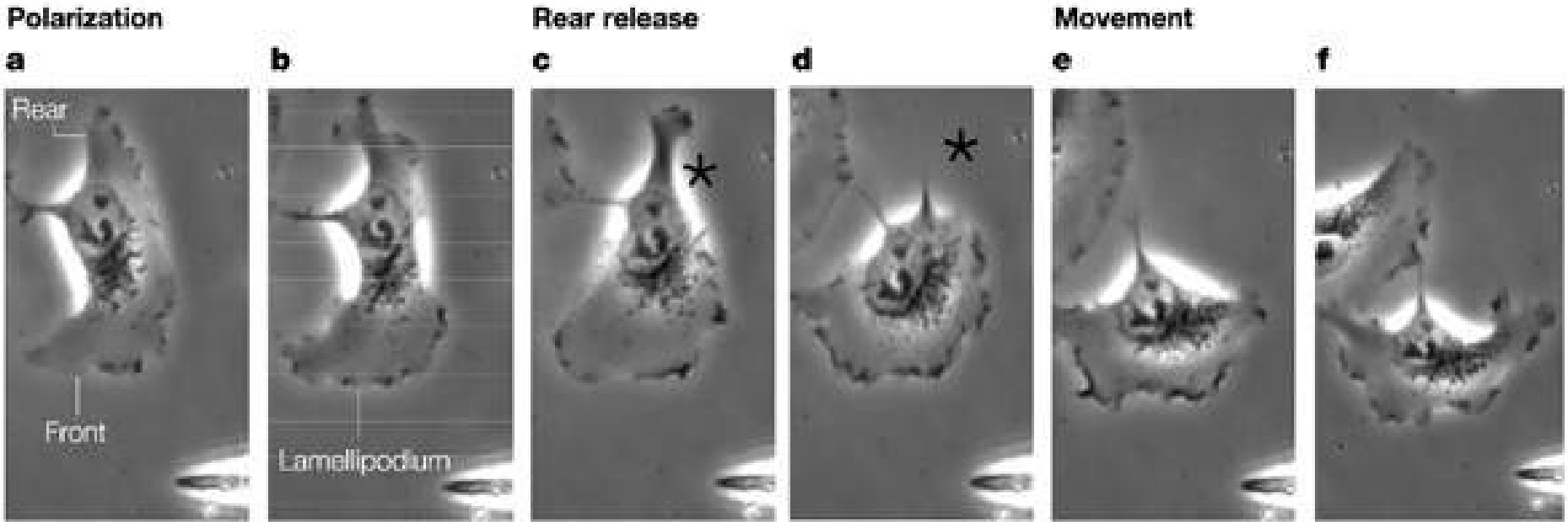
1min




1 day

Several days

# Different modes of migration: single cell migration

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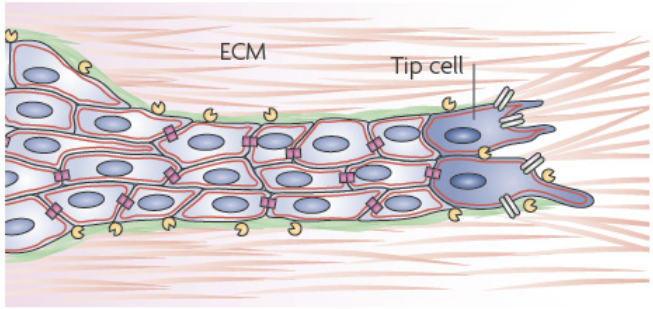
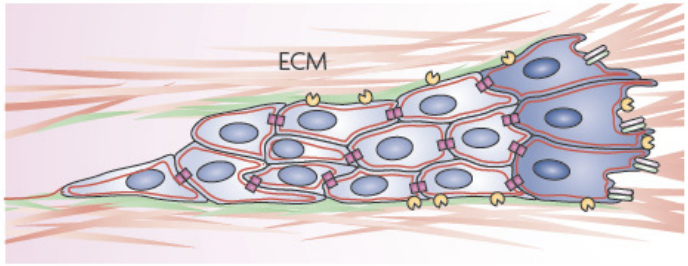
# Different modes of migration: collective cell migration

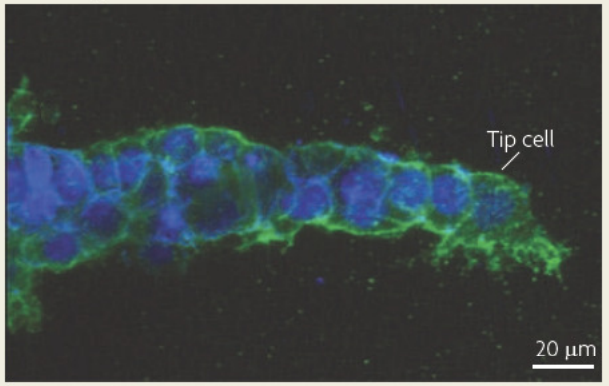
**d** Multicellular 3D invasion strands



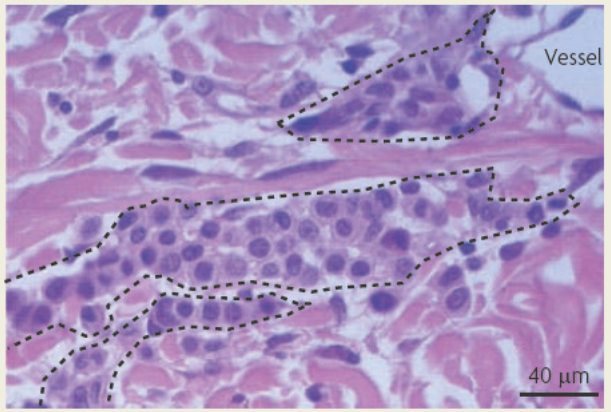

**f** Detached cluster



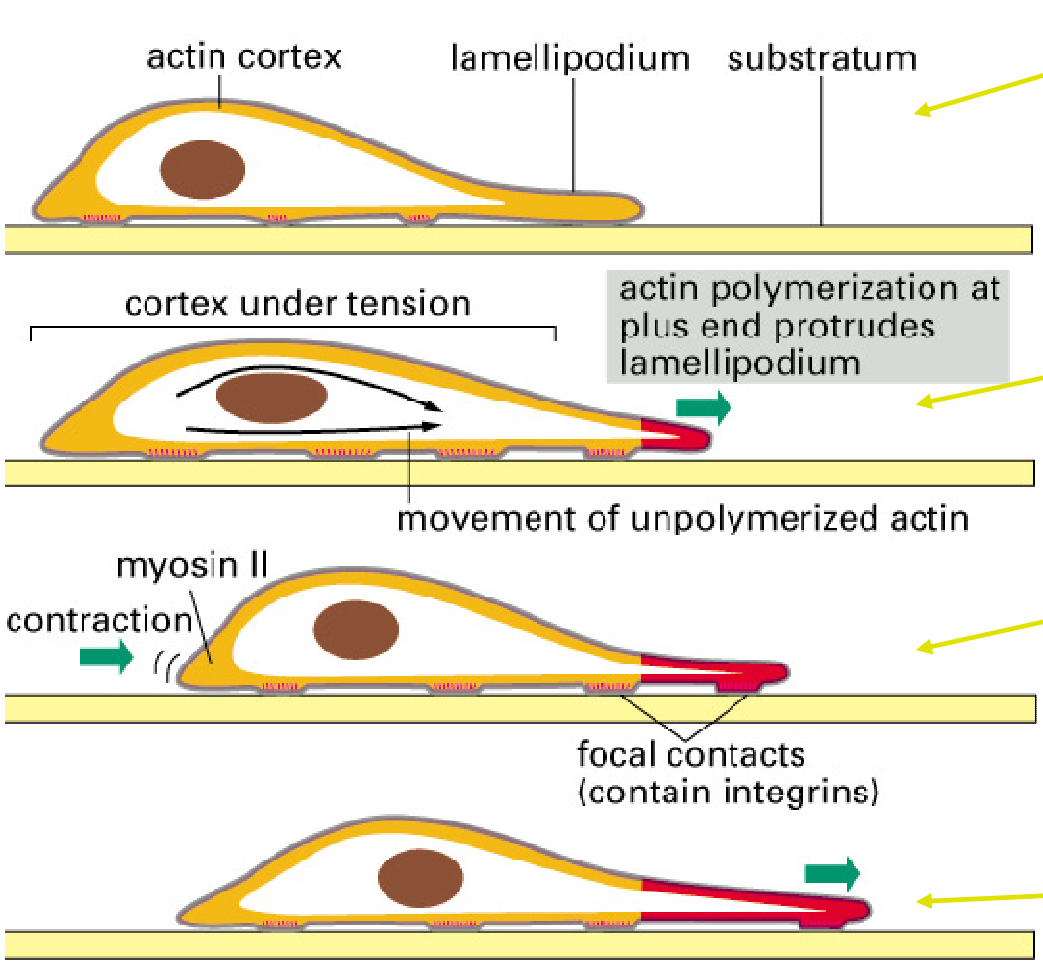

Mammary carcinoma




Human melanoma in deep dermis



# Cell migration relies on coordinated activation of several cytoskeletal functions




Polarization

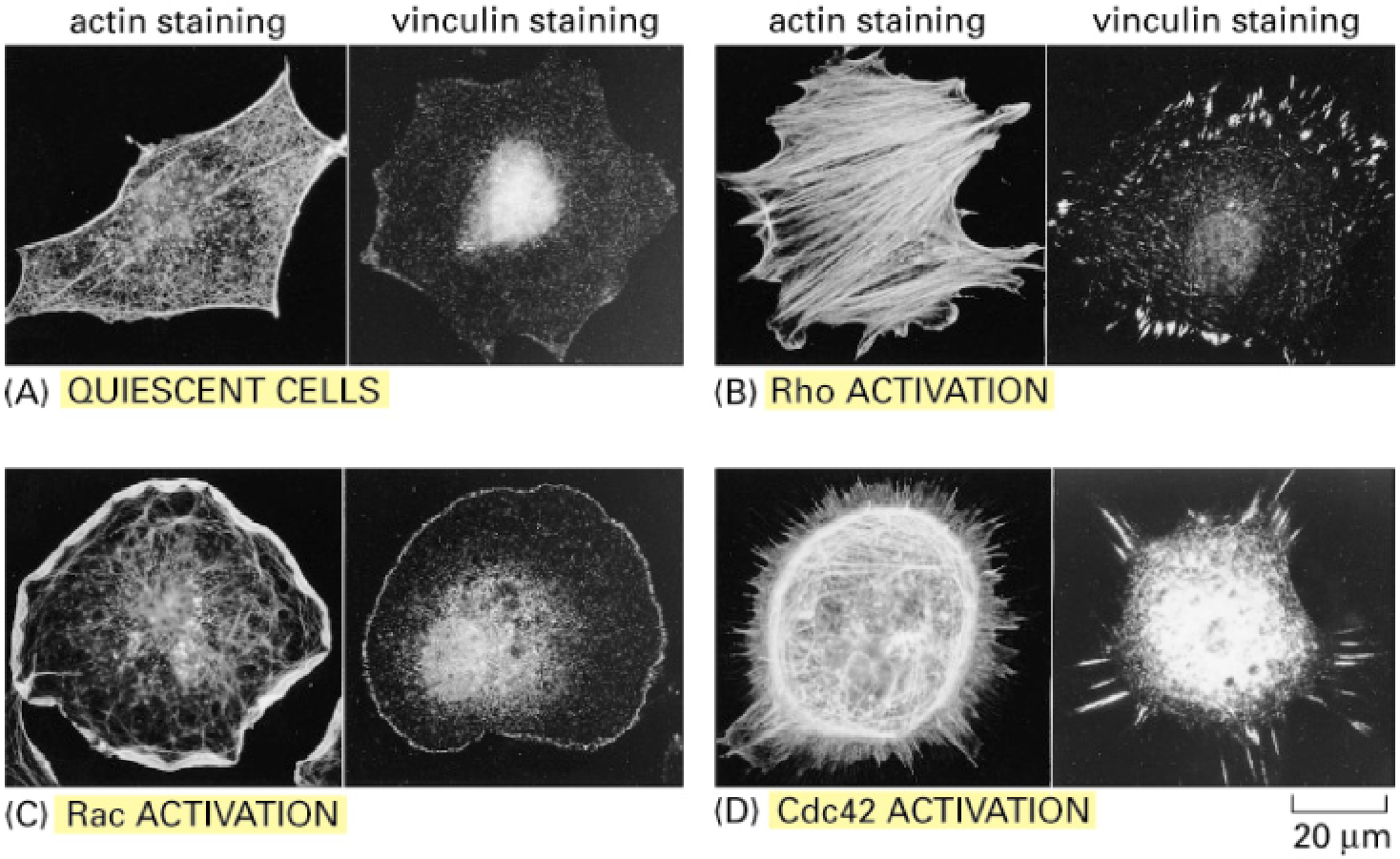
Protrusion of the leading edge

Adhesion and contraction

(deadhesion at the trailing edge)

New protrusion

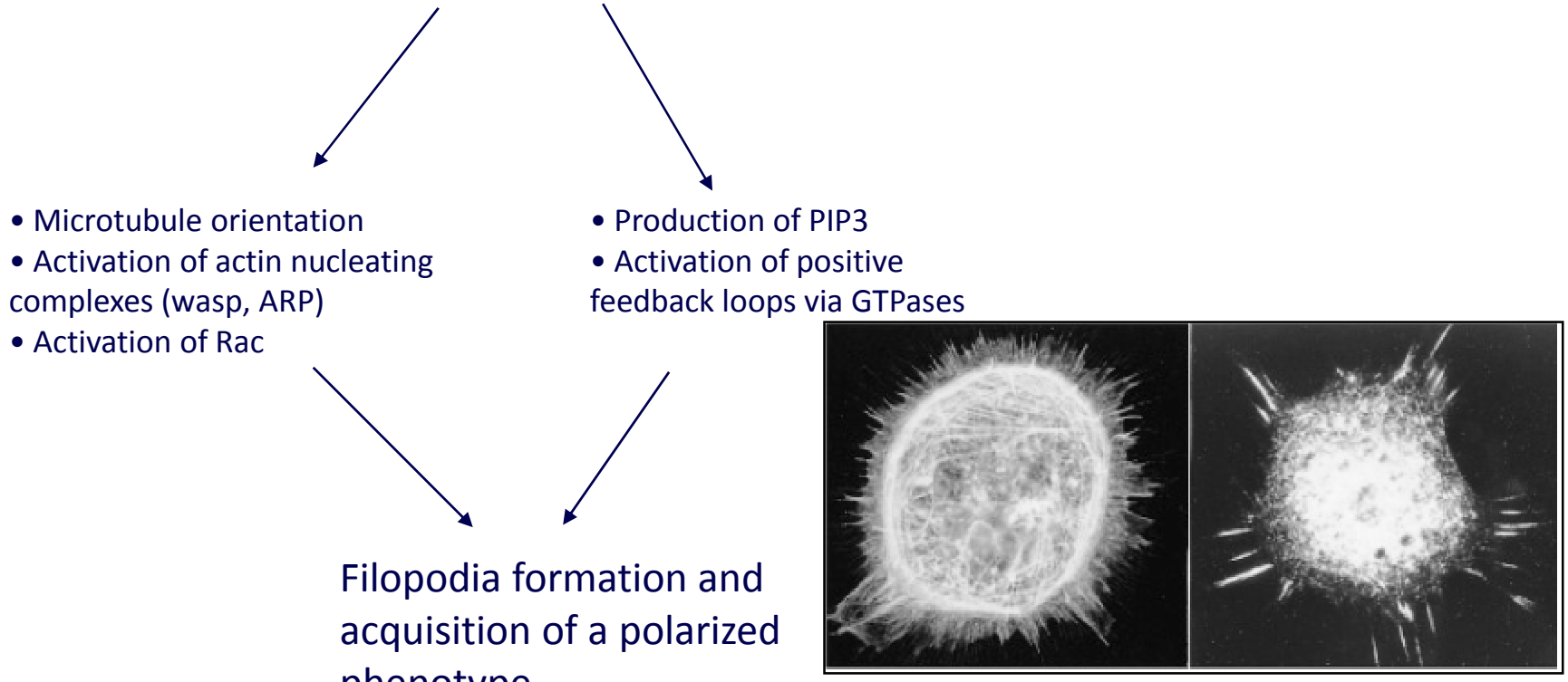
# Small GTPases belonging to the Rho family control cytoskeletal rearrangement during migration



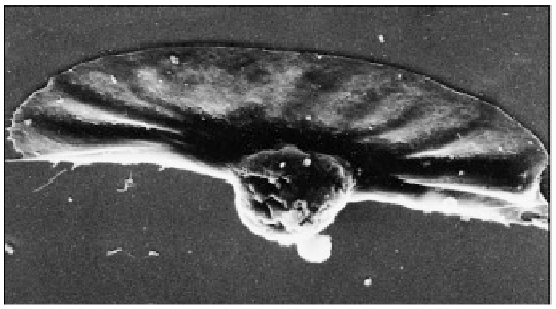
# Phase I: Polarization

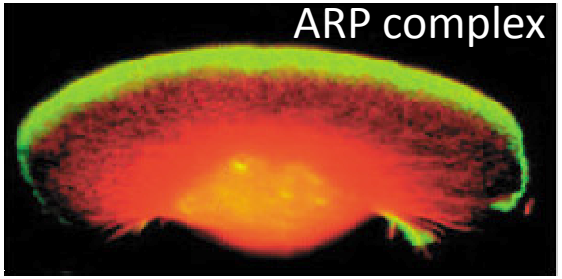
• Chemotactic stimulus (gradient of growth factors, or bacterial proteins in case of neutrophils, etc)

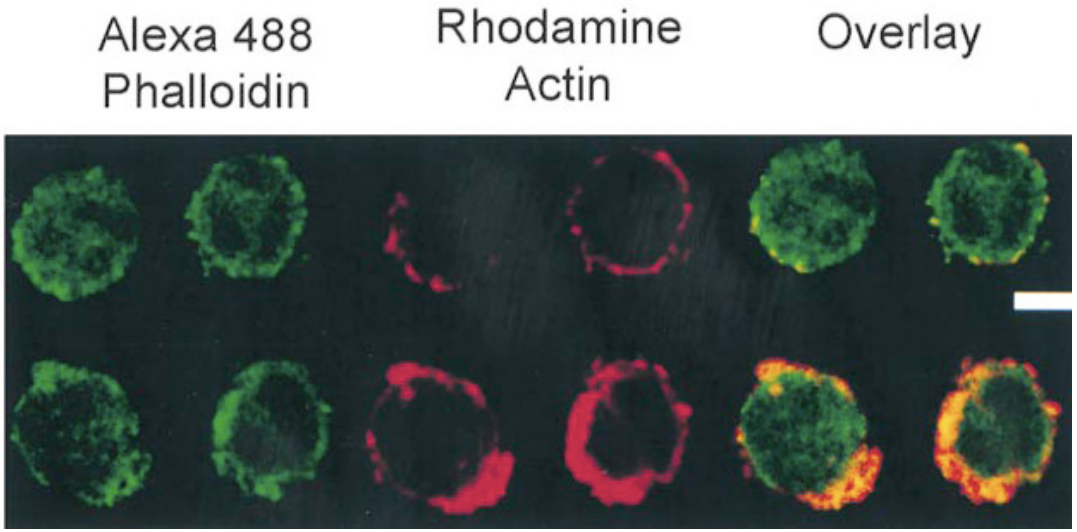
• Polarized activation of Cdc42 and PI3K

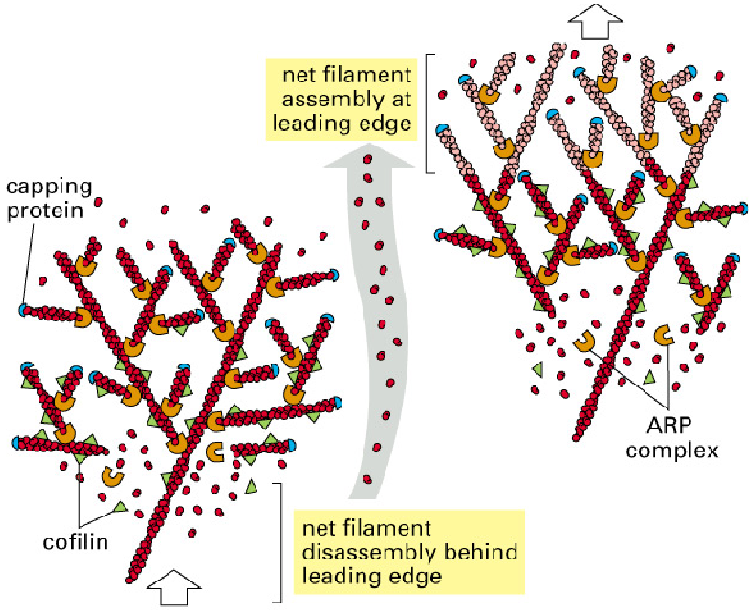


# Phase II: Protrusion



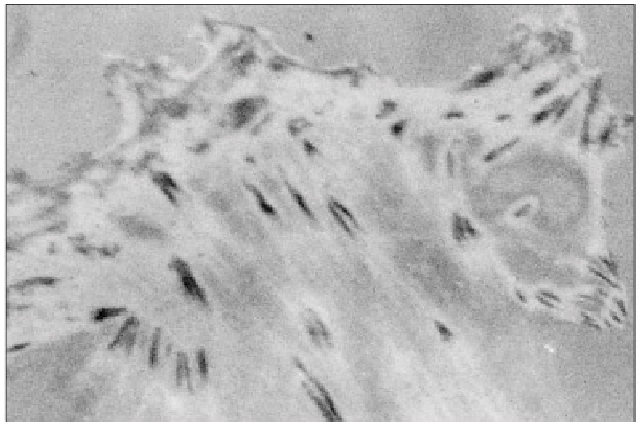


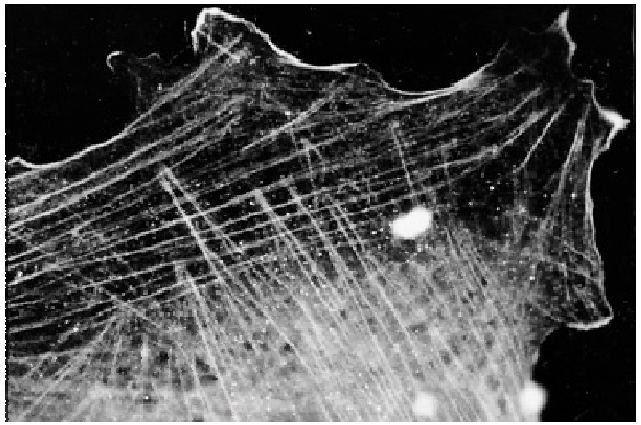




- Activation of Rac
- Productions of PIPs
- Decreased capping and increased polymerization

# Phase III: Contraction

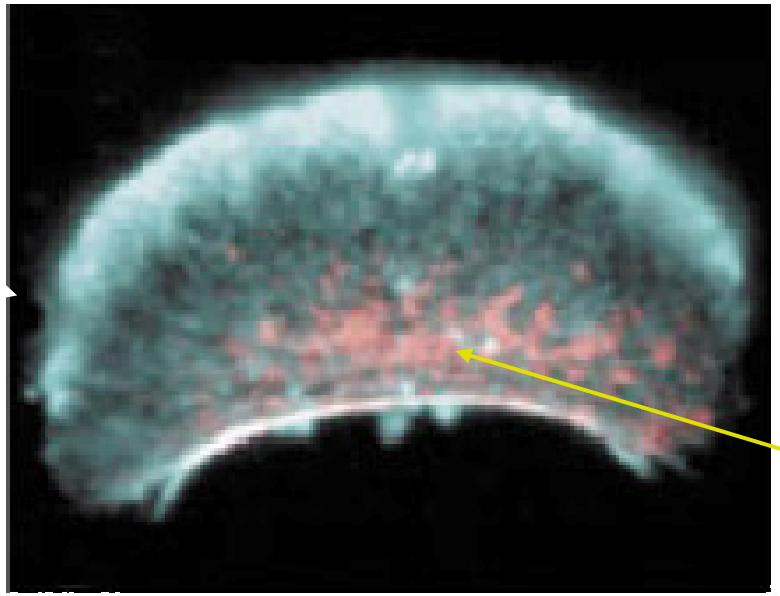


Activation of Rho



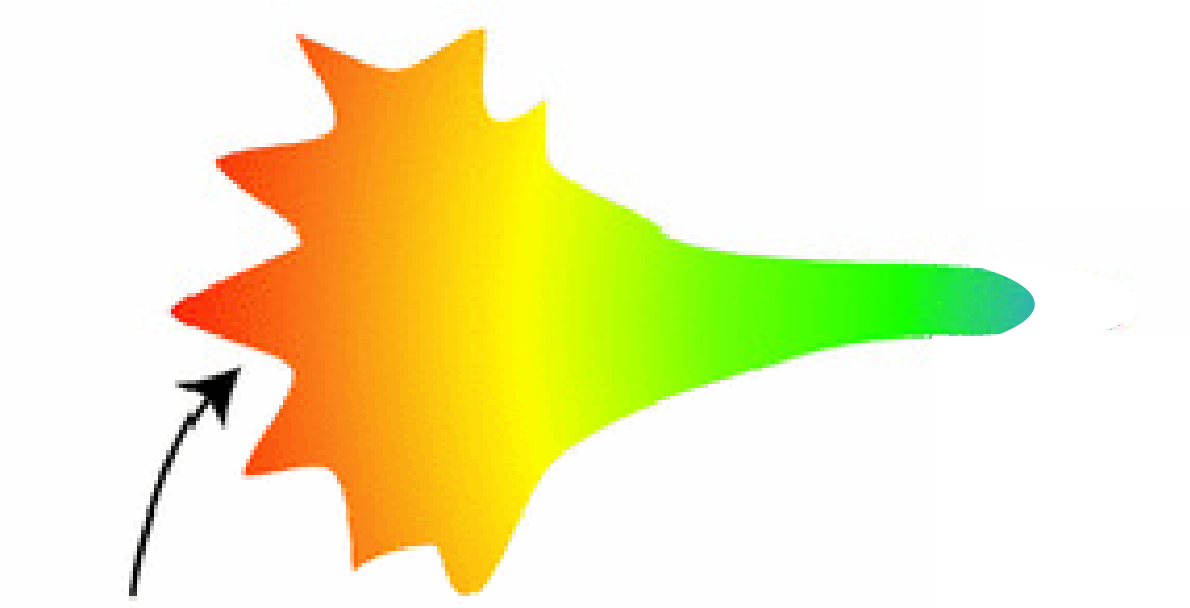
Formation of focal contacts and stress fibers, in part via formins

Contraction acto-myosin



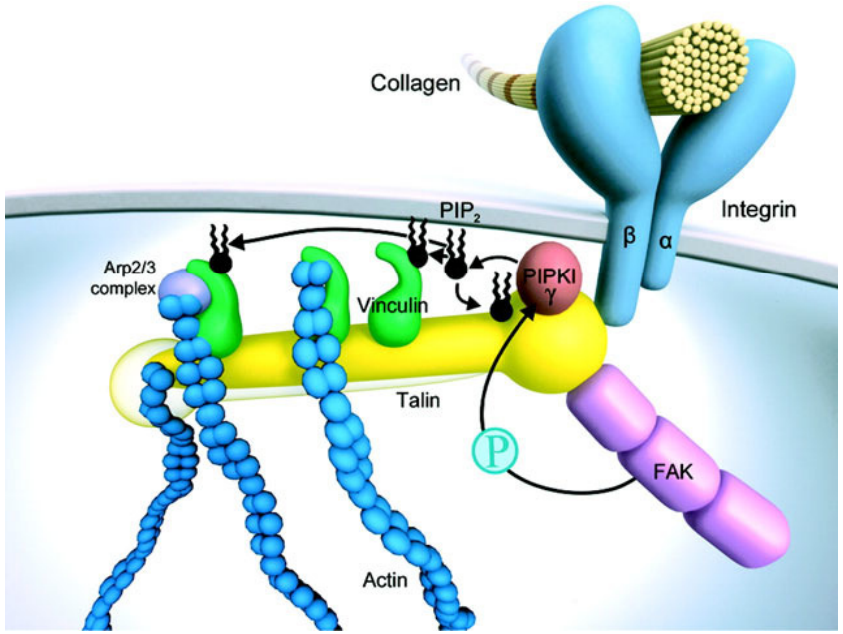
Myosin

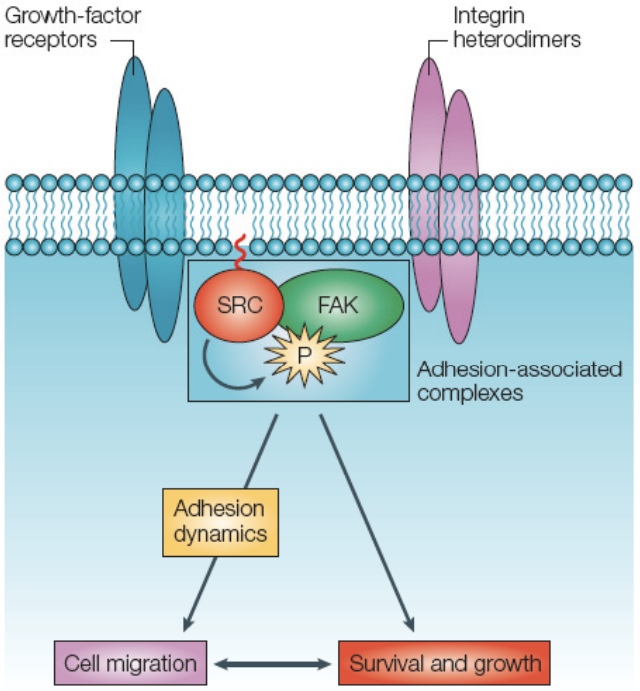
# Small GTPases activity is compartmentalized within the migrating cell



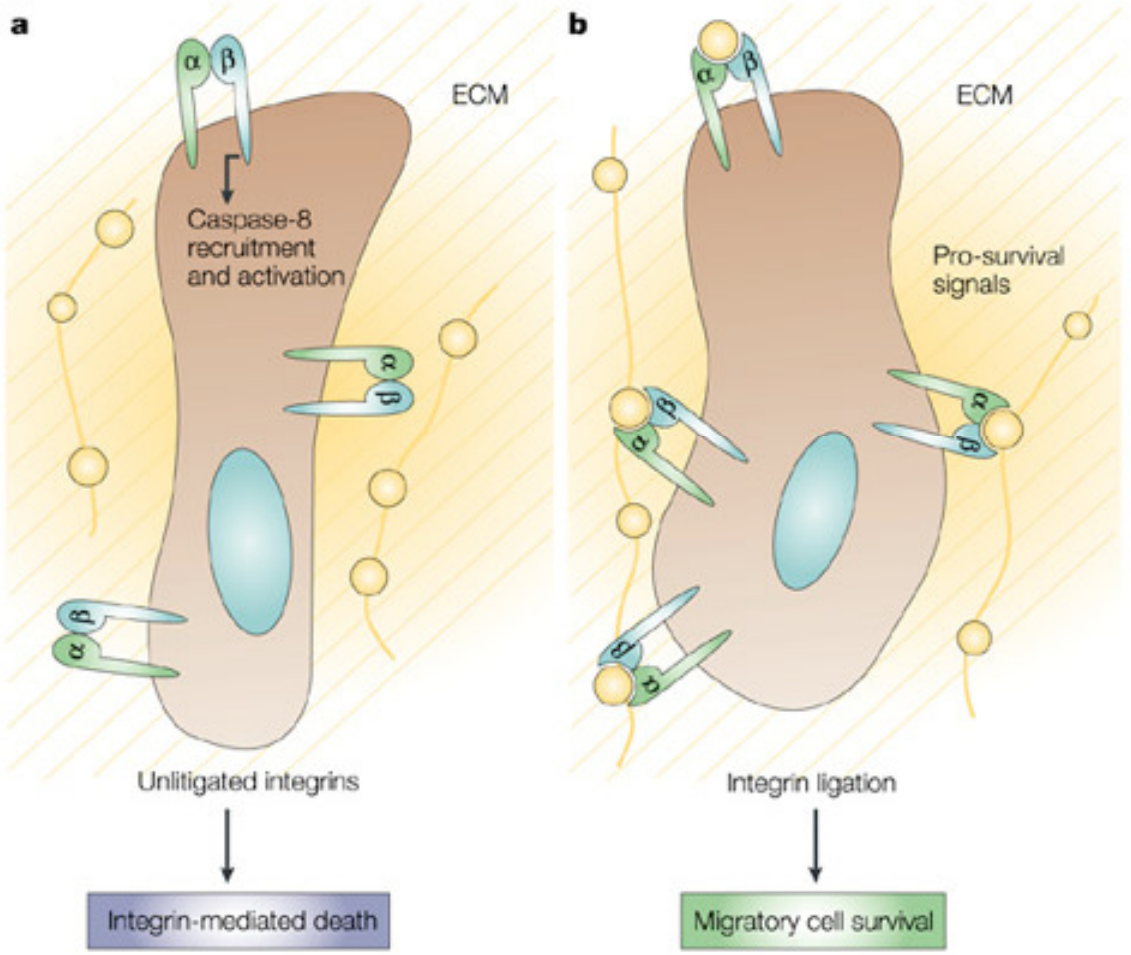
lamellipodium

# Stress fibers are connected to focal adhesions



# Integrin binding to extracellular matrix ligands prevents apoptosis

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# Integrins and cell invasion

The recognition of the substrate (matrix) by integrins leads to:

1. Mechanical adhesion to allow the contraction

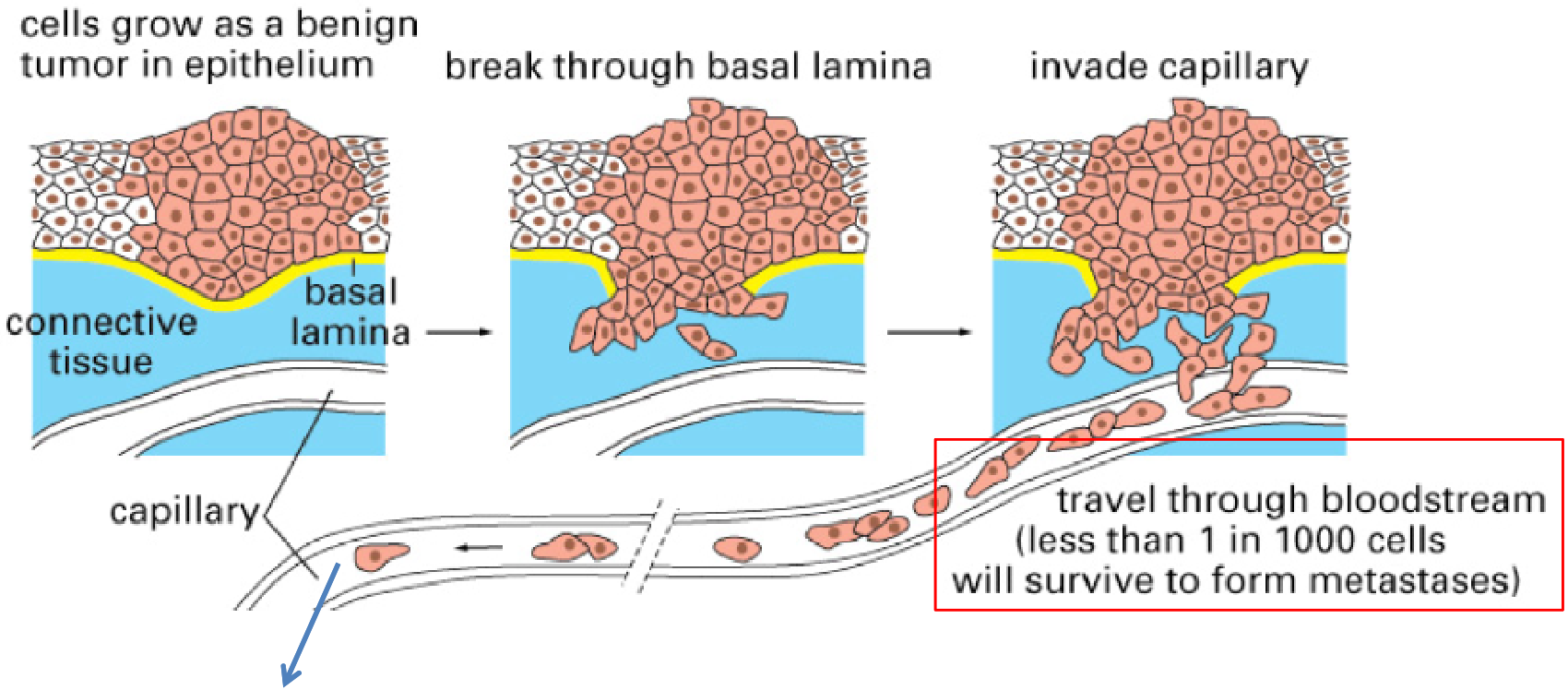
2. Protection against apoptosis

During cell invasion, the tumor cells need to survive and migrate in a different environment:

1. Expression of new integrins *ad hoc* (ie: $\alpha v\beta 3$ in melanomas)

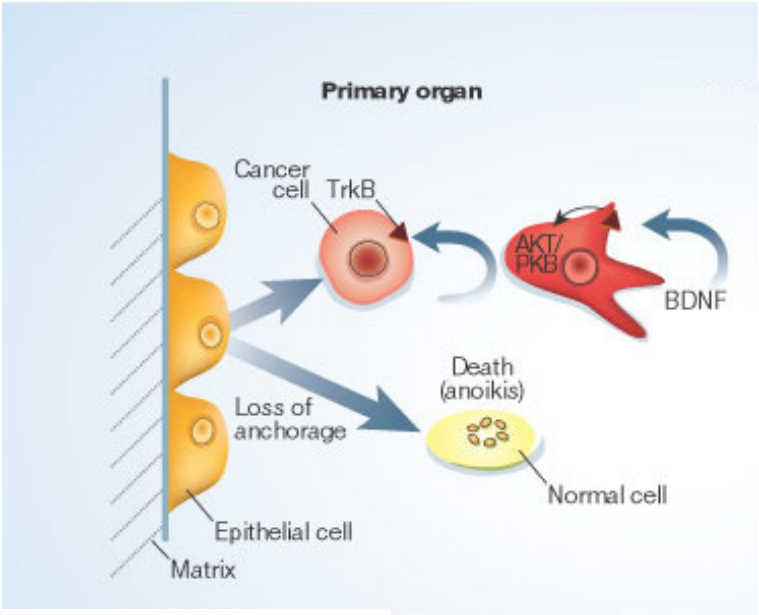
2. Increased affinity and avidity of pre-existing integrins

## Anoikis (homelessness):
## programmed cell death in absence of “home ground”

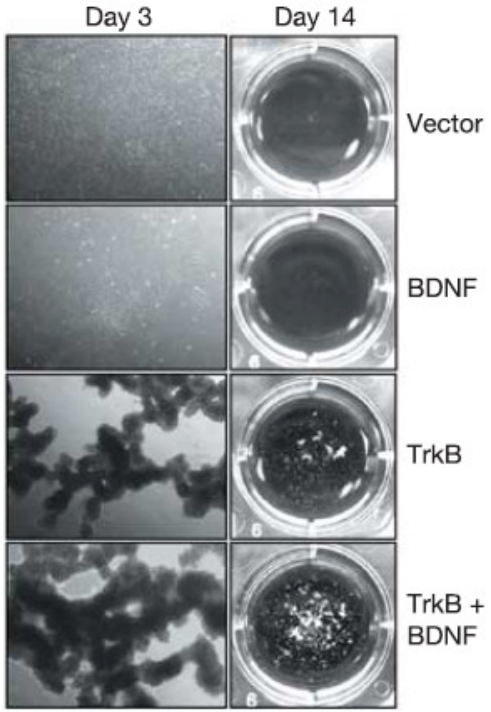



**Metastasis** to distant organ

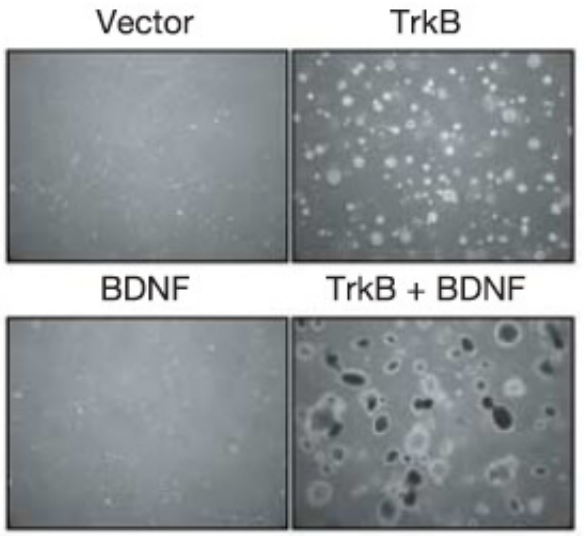
# Tricks to survive far from home




Poly-HEMA-coated plates (ULC plates)

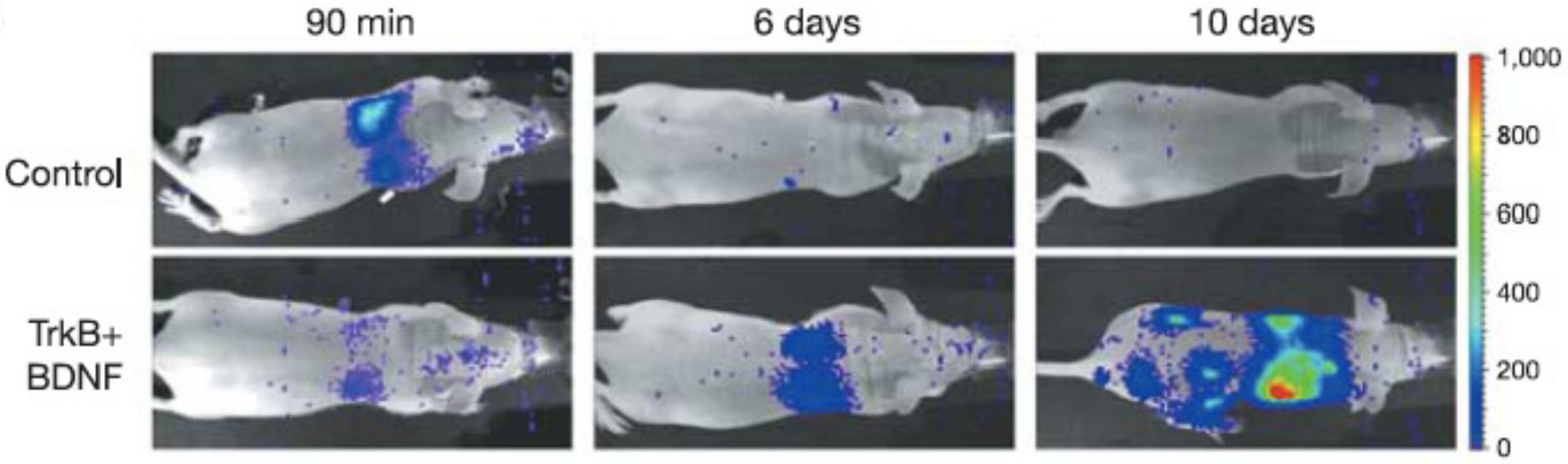



Soft agar assay



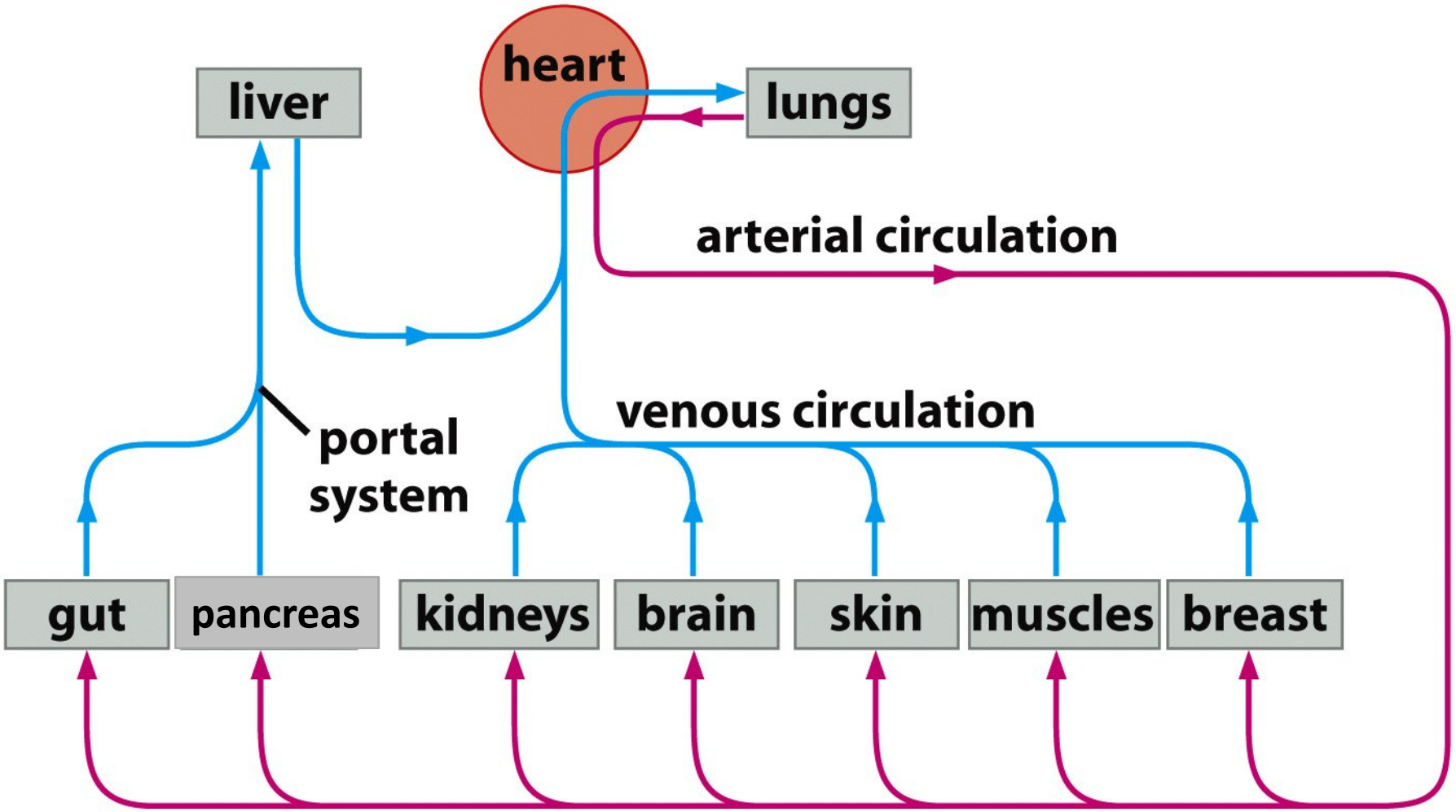

Douma et al, *Nature*, 2004

# TrkB activation prevents anoikis of circulating tumor cells and therefore promotes their lodging in distant organs

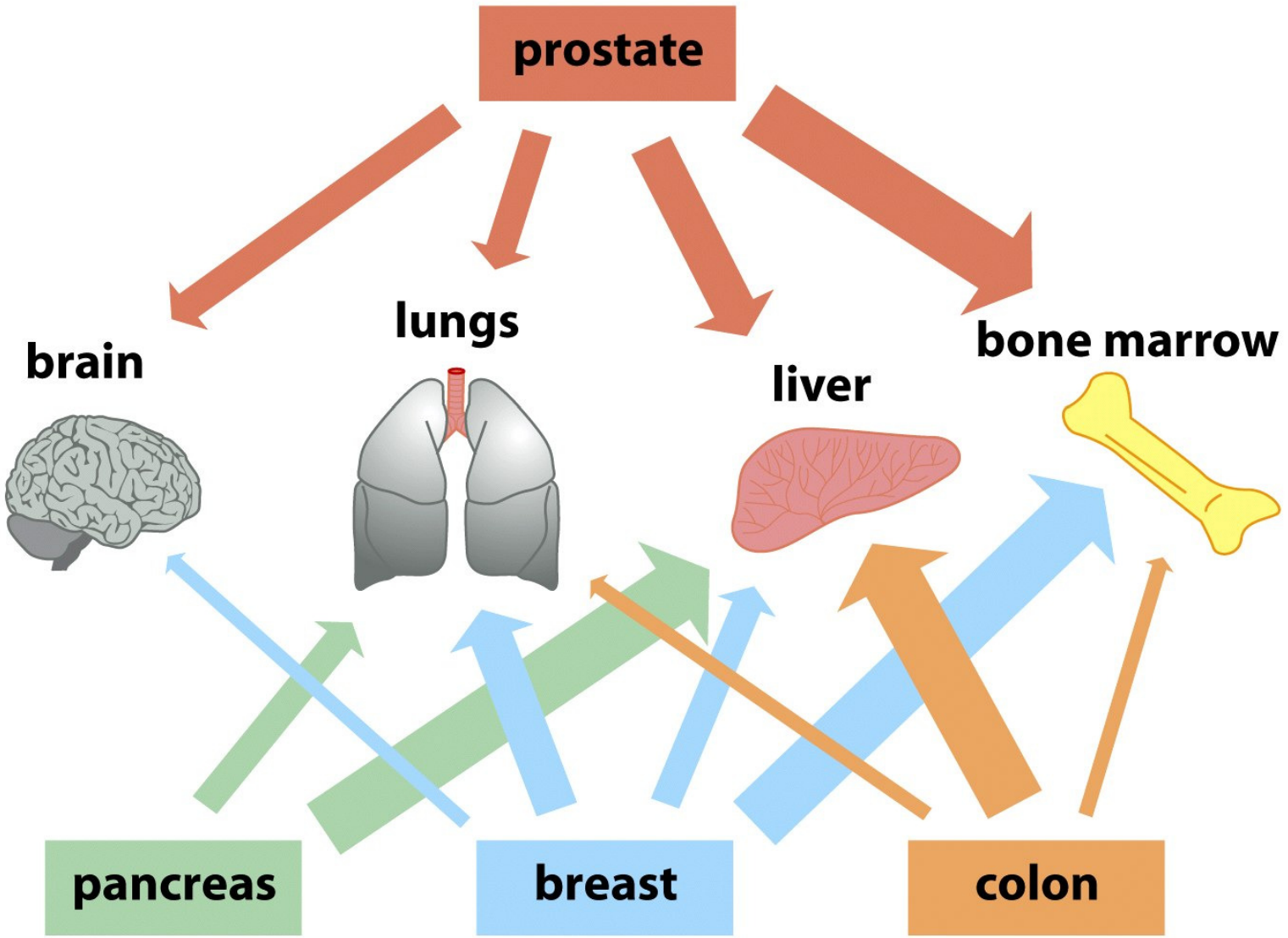



Douma et al, *Nature*, 2004

# Portal circulation and liver metastasis



# Primary tumors and their metastatic tropism



# “Seed and soil” hypothesis (1889)

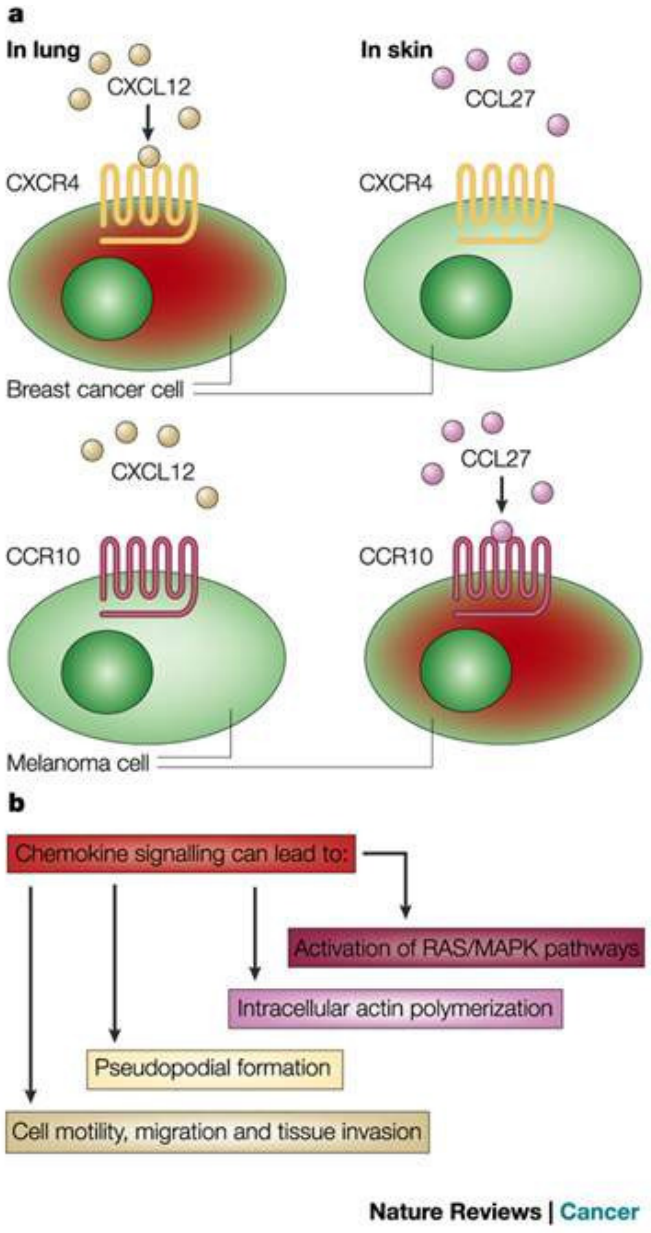
***Stephen Paget***

By analysing 735 case histories of fatal breast cancer, he found that the patterns of the metastasis formation could not be explained by random scattering or by patterns.

The sites of secondary growths are not a matter of chance, some organs provide a more fertile environment than others for the growth of certain metastases.

***“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 soil."***

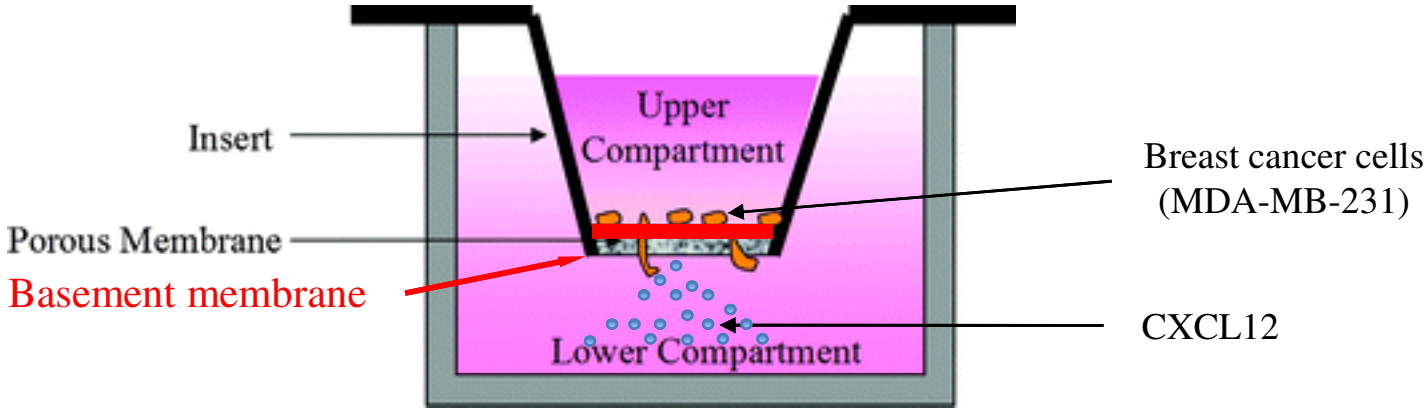
# Chemokines can influence organ-specific metastatic growth of cancer cells

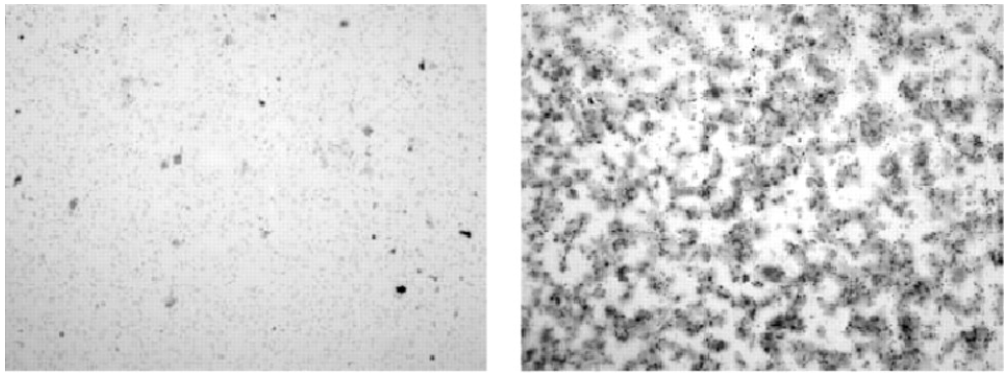


# CXCL12 induces cell migration and invasion of breast cancer cells *in vitro*

Migration

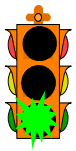
Invasion



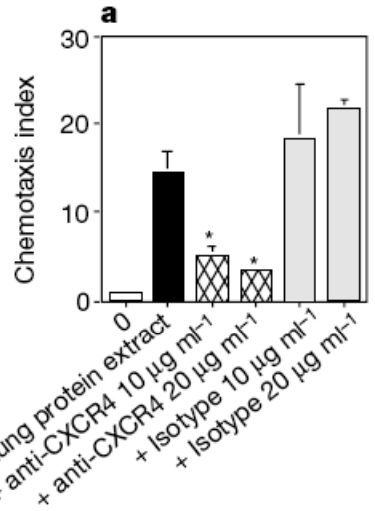
Control CXCL12

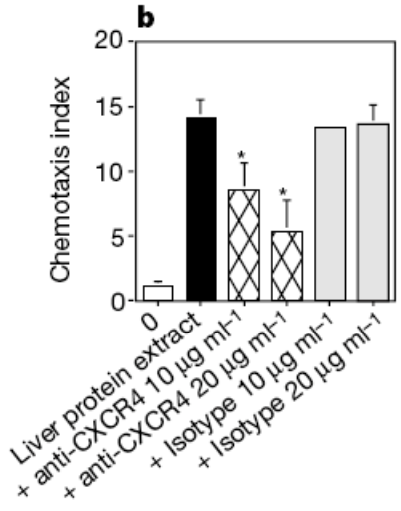
# CXCR4-neutralization inhibits breast cancer cell chemotaxis *in vitro*

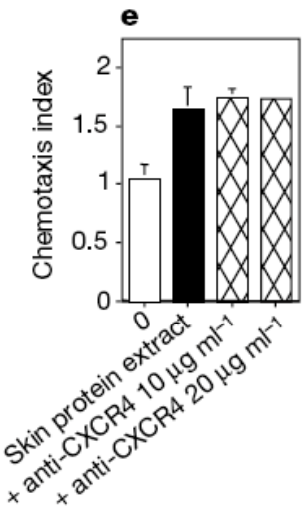


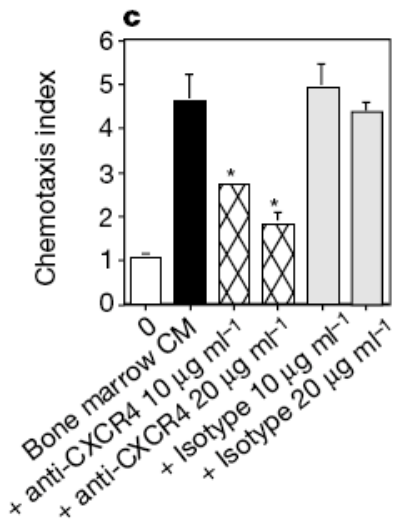
Lung, liver, bone marrow, lymph nodes

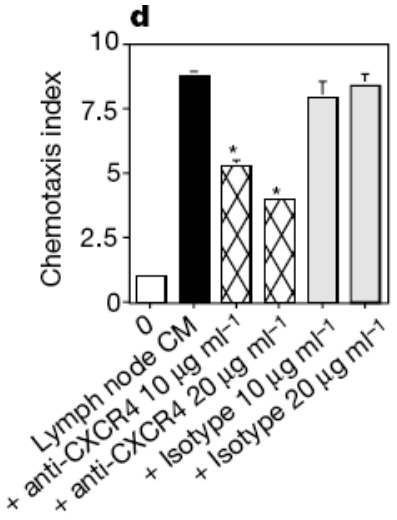
Skin, muscle

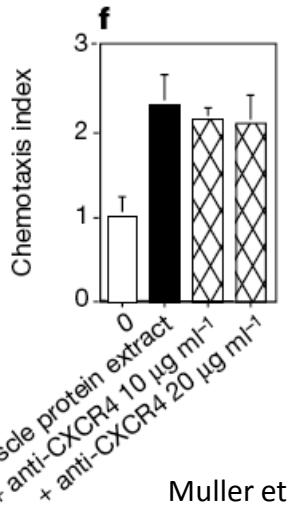


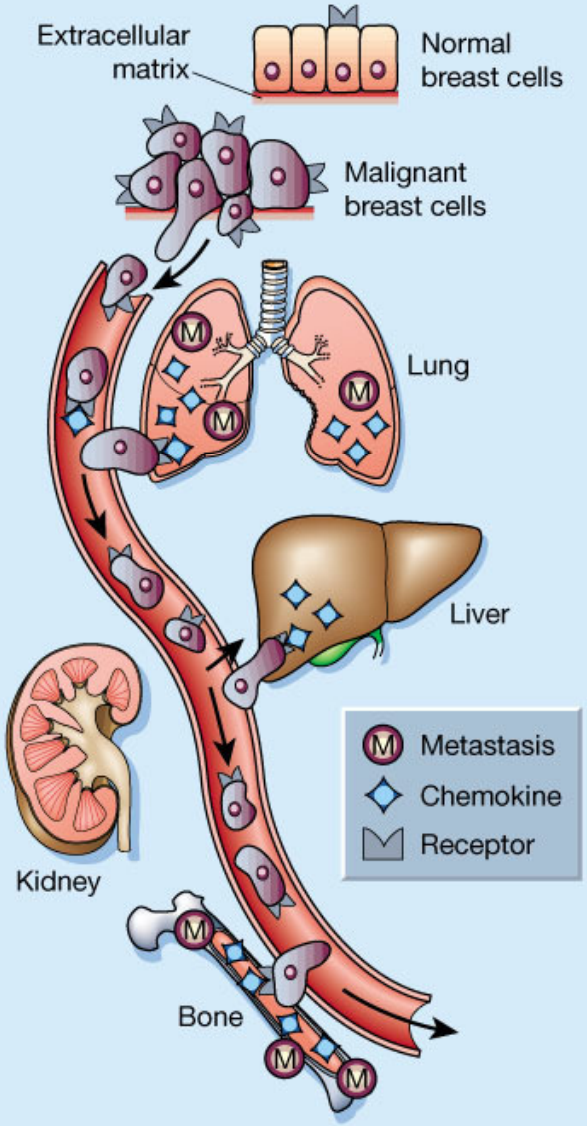



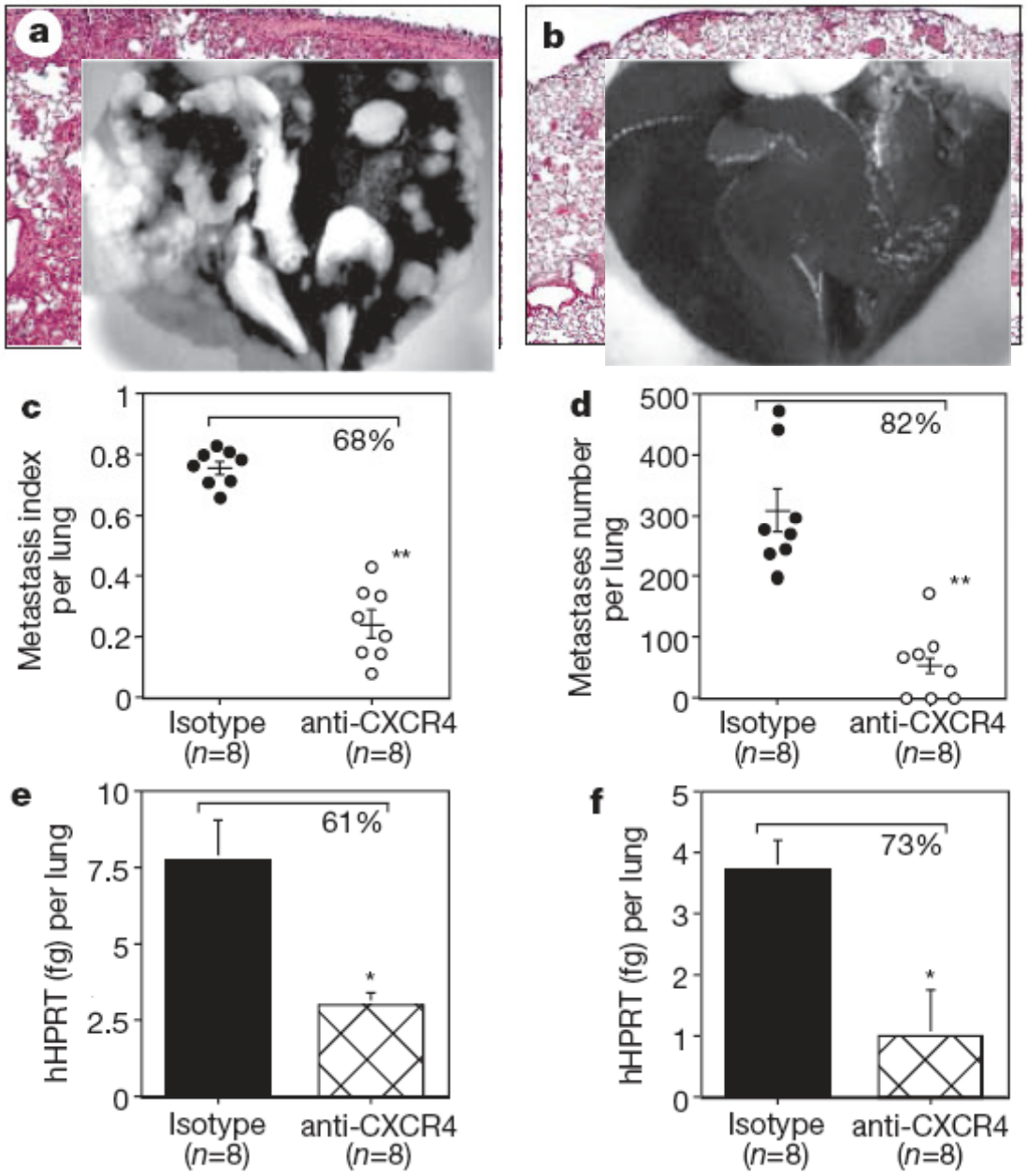




Muller et al., *Nature*, 2001

# CXCR4-neutralization inhibits breast cancer metastasis *in vivo*

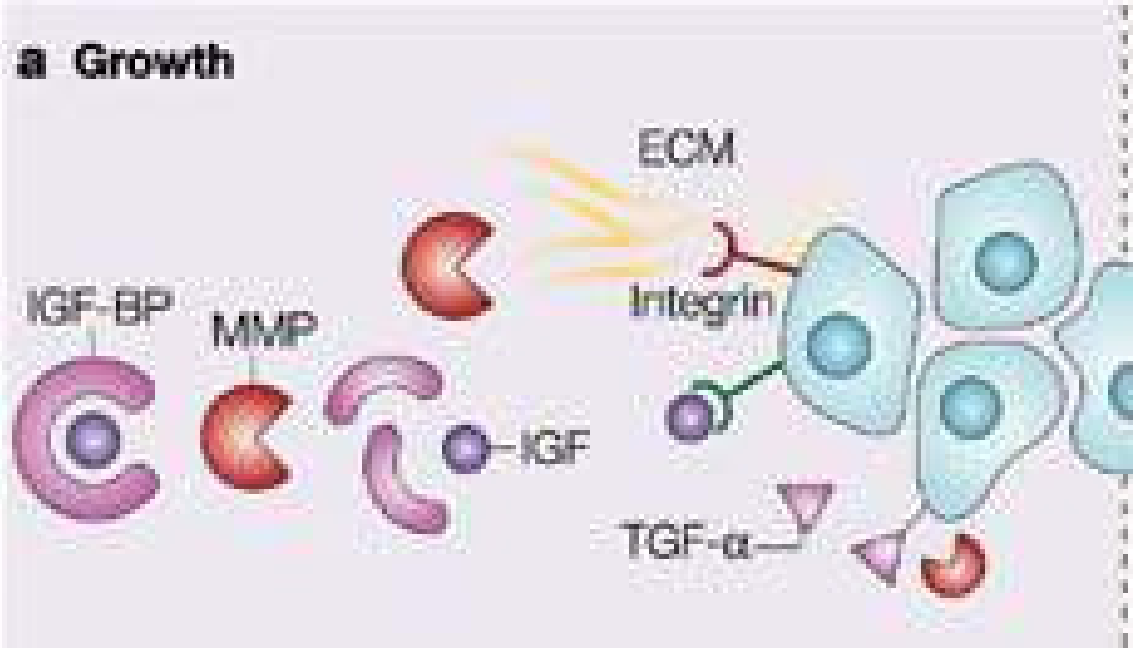



Muller et al., *Nature*, 2001



*Thank you!*

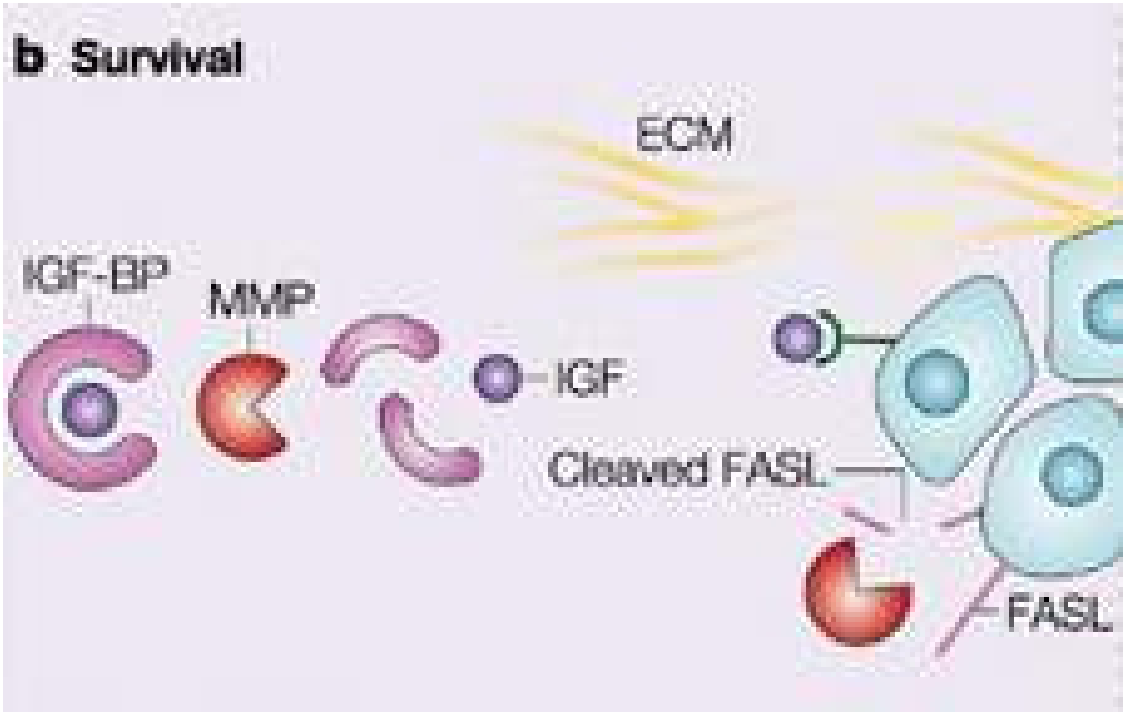
# Metalloproteases and growth factor release




1. Proteolytic cleavage of (inactive) transmembrane precursors of growth factors (i.e. TGFα, EGF, TNFα, etc)

2. Release of growth factors bound to the extracellular matrix (i.e. *Insulin-like Growth Factor* (IGF) bound to IGF-BP, VEGF, etc)

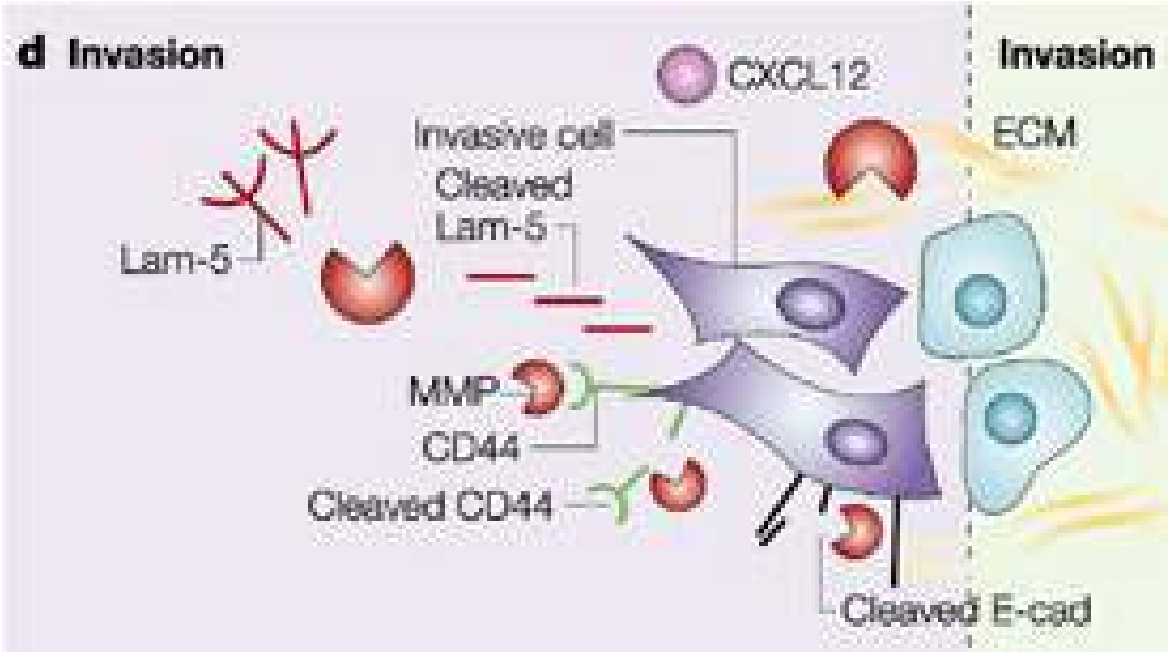
# Metalloproteases and cell survival




1. Proteolytic inactivation of Fas ligand

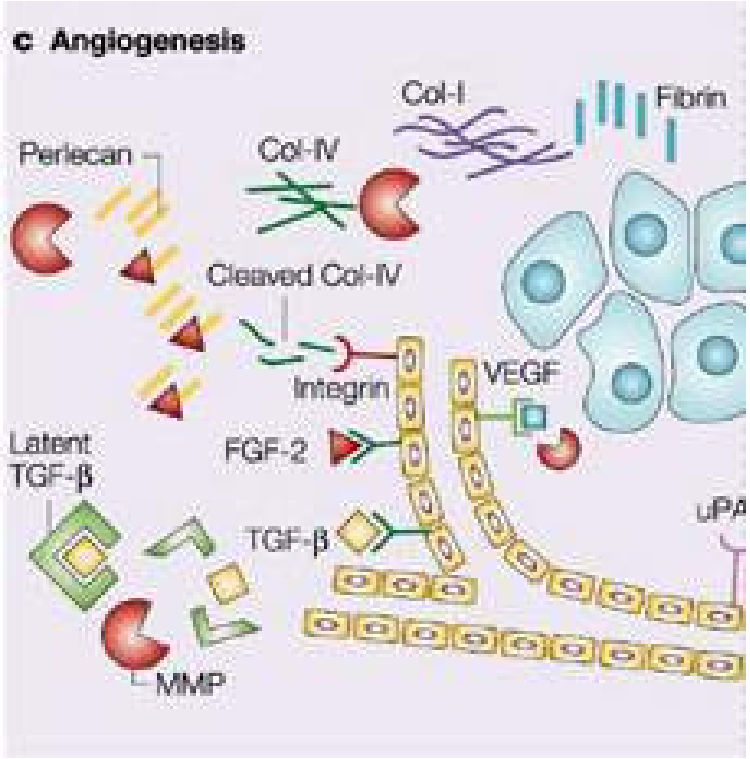
2. Release of growth factors bound to the extracellular matrix (i.e. *Insulin-like Growth Factor* (IGF) bound to IGF-BP, VEGF, etc)

# Metalloproteases and cell invasion, intravasation and extravasation




1. Cleavage of laminin-5 displaying the cryptic pro-migratory binding site
2. Cleavage of E-cadherin
3. Cleavage of the hyaluronic acid receptor CD44
4. Digestion of the extracellular matrix

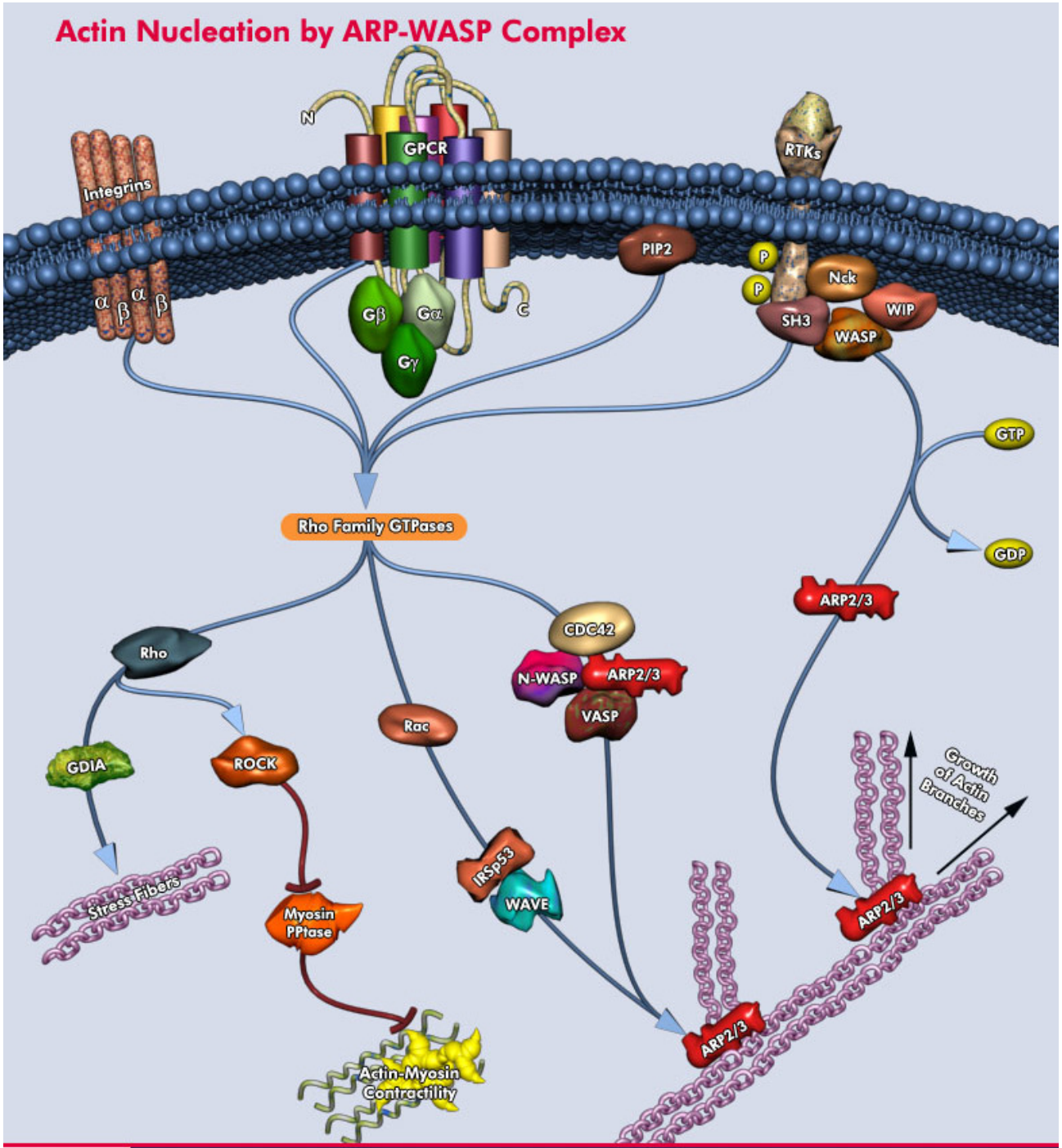
# Metalloproteases and angiogenesis



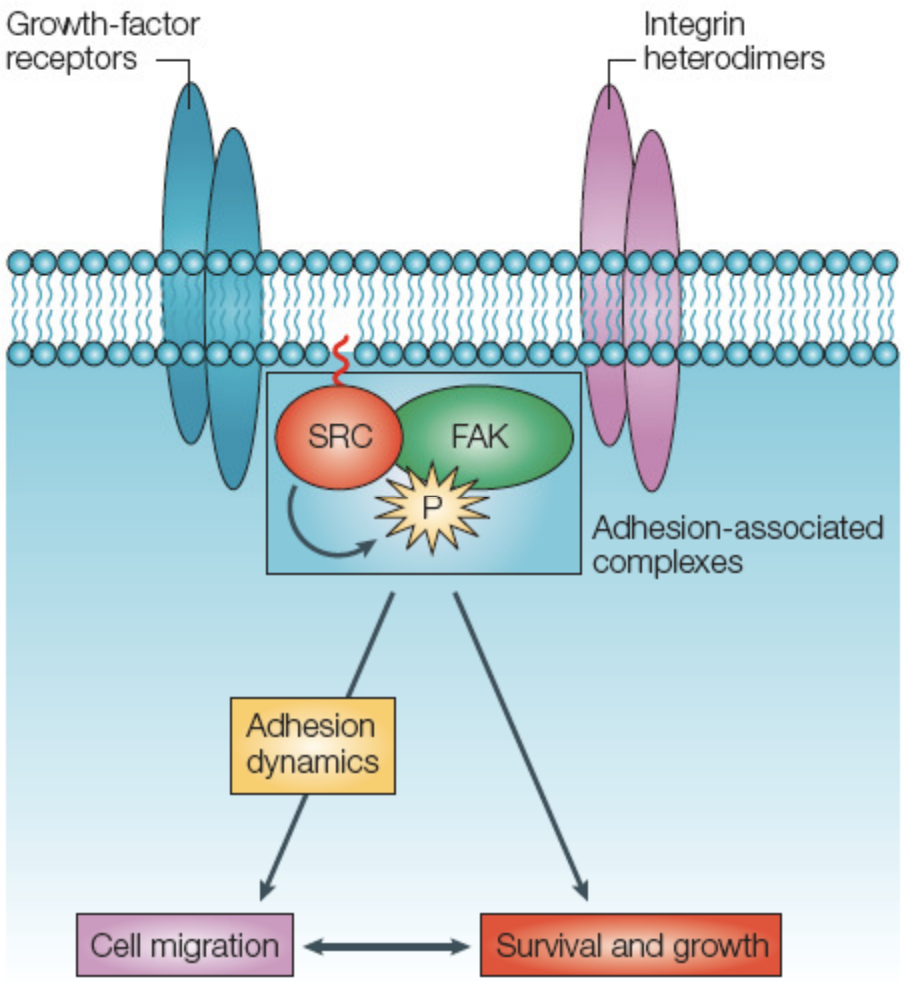

1. Digestion of the extracellular matrix (collagen-I, fibrin)

2. Proteolytic cleavage of collagen-IV, displaying the cryptic binding site for integrin $\alpha v\beta 3$

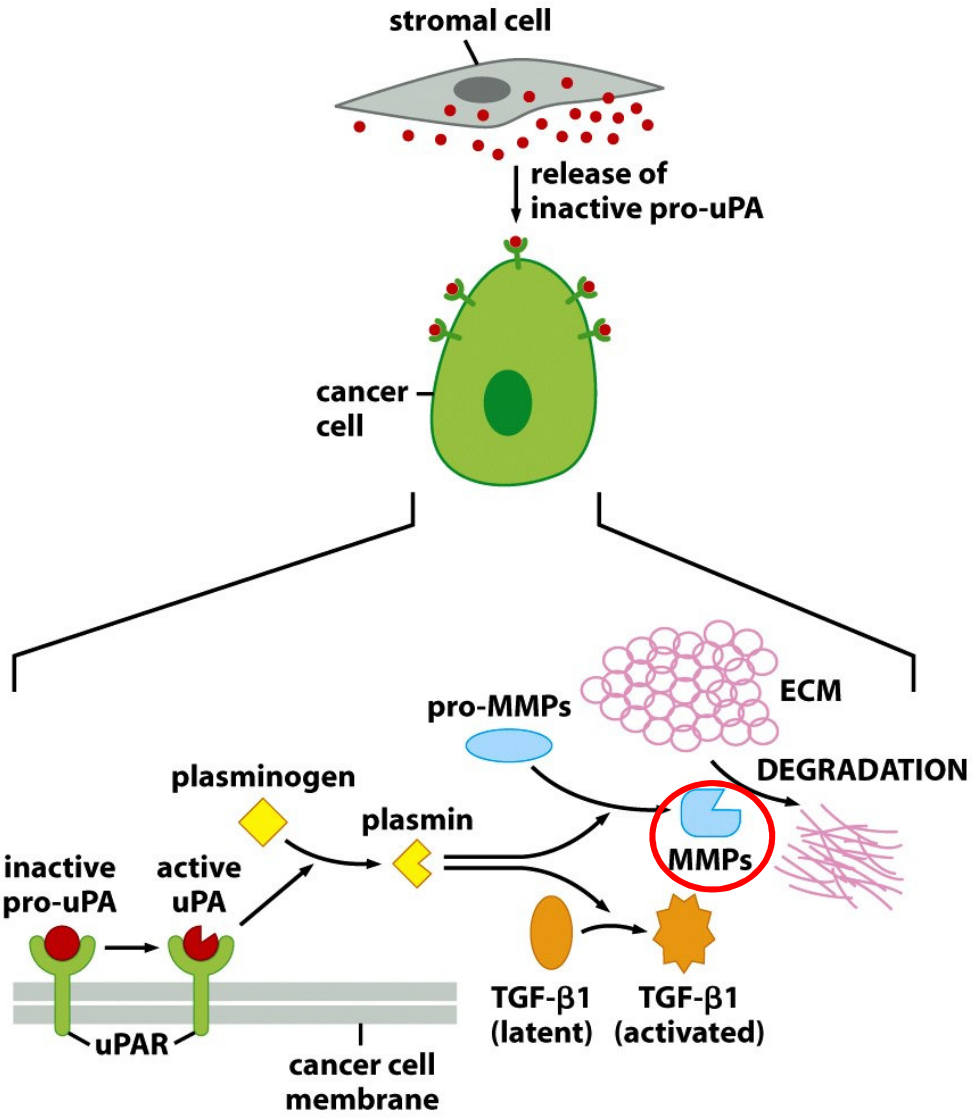
3. Release of angiogenic growth factors bound to the extracellular matrix: *Fibroblast Growth Factor* (FGF), *Vascular Endothelial Growth Factor* (VEGF), *Transforming Growth Factor-$\beta$* (TGF-$\beta$)


Actin Nucleation by ARP-WASP Complex
N
GPCR
Integrins
RTKs
PIP2
P
P
Nck
SH3
WIP
WASP
Gβ
Gα
Gγ
C
GTP
GDP
Rho Family GTPases
ARP2/3
Rho
CDC42
N-WASP
ARP2/3
VASP
Rac
GDIA
ROCK
Growth of Actin Branches
IRSp53
WAVE
Stress Fibers
Myosin PPtase
ARP2/3
ARP2/3
Actin-Myosin Contractility

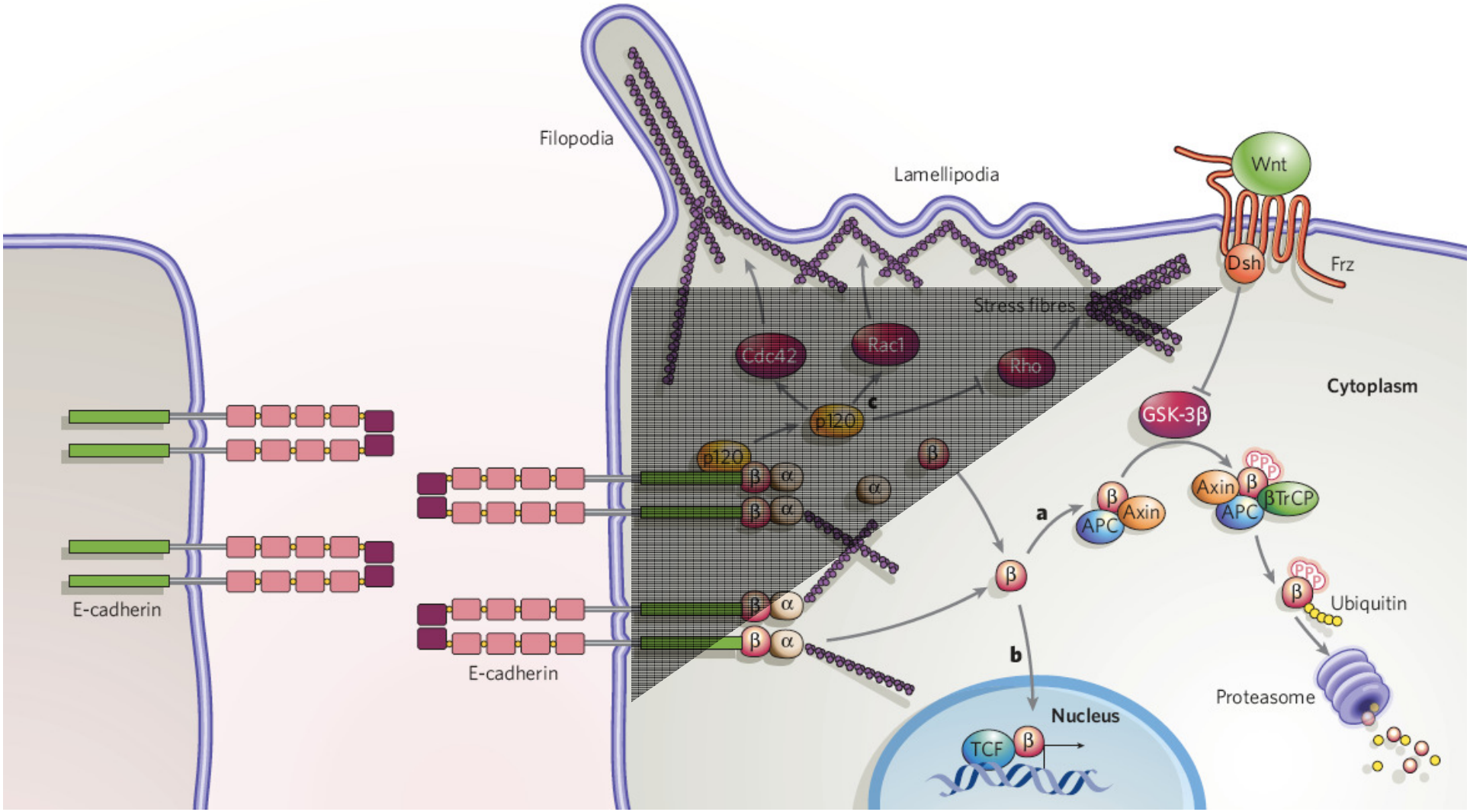
# Focal adhesions contribute to cancer progression



# Metalloproteases: key effectors to alter the interactions with the environment

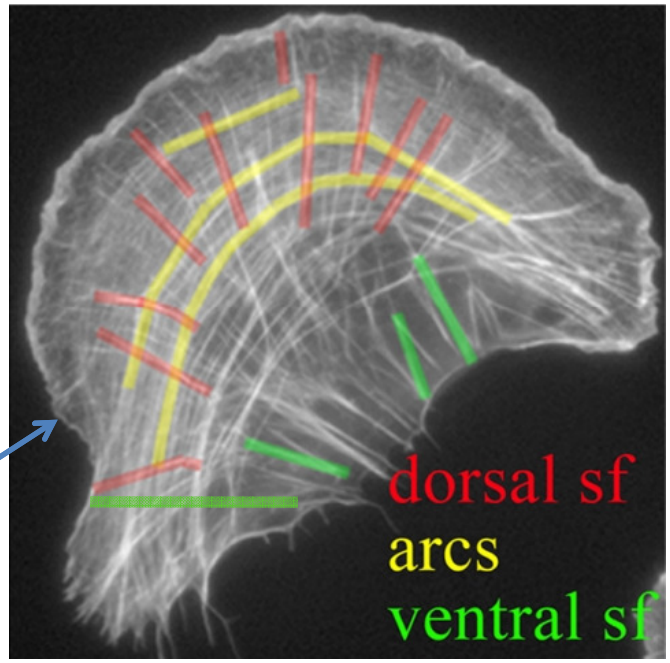


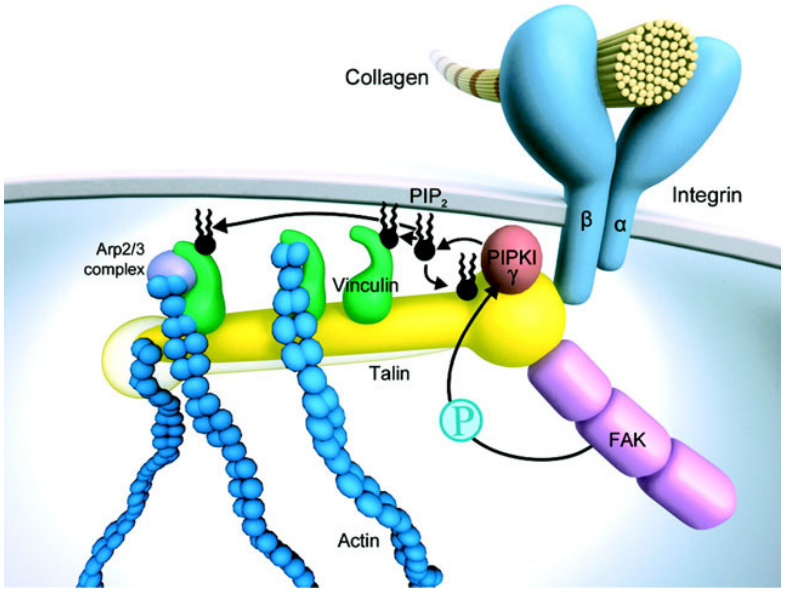
# Small GTPases activity: signalling pathways downstream of the loss of E-cadherin function



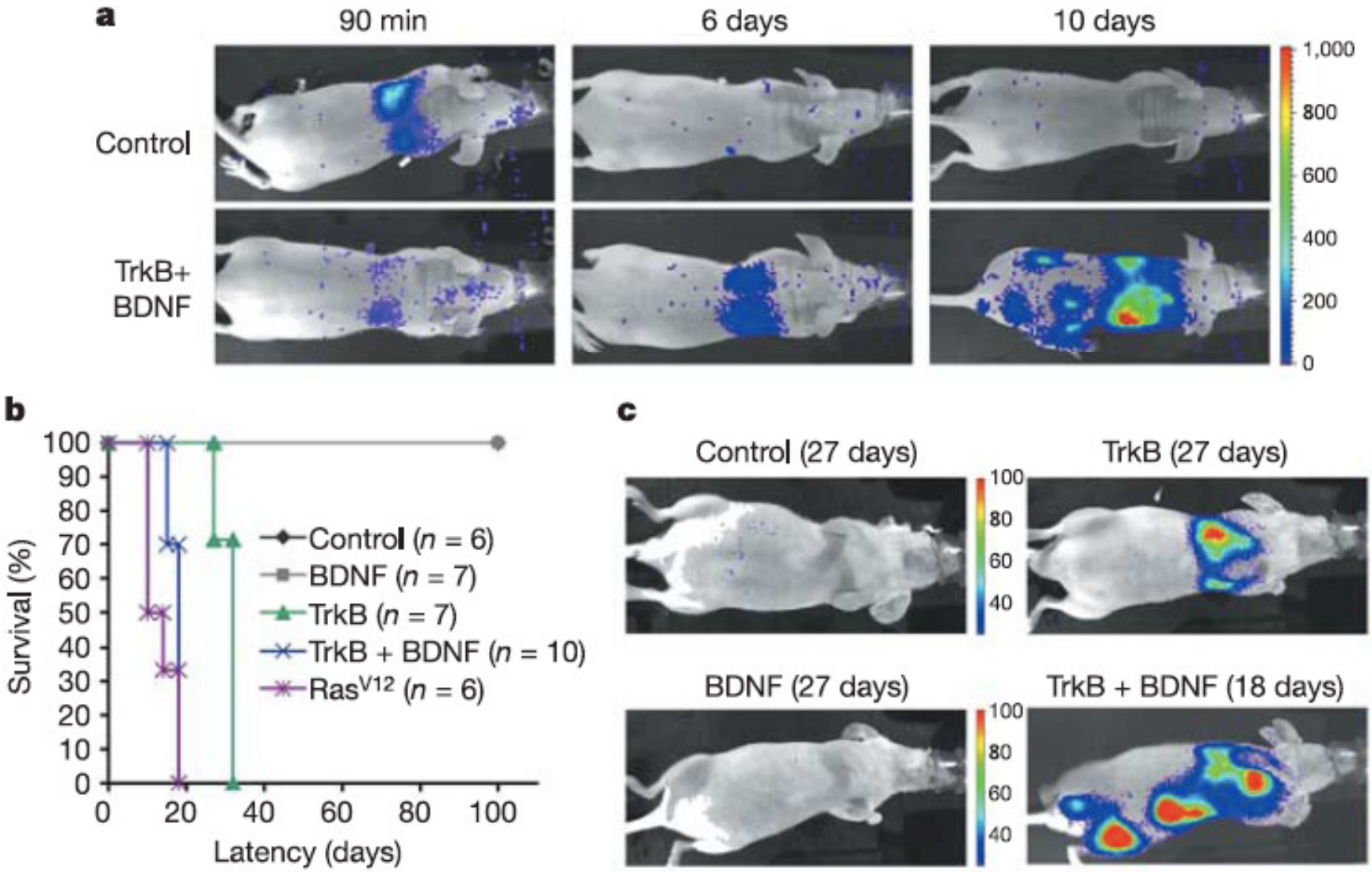
# Stress fibers form as dorsal, ventral fibers and arcs

Stress fibers



# TrkB activation prevents anoikis of circulating tumor cells and therefore promotes their lodging in distant organs




Douma et al, *Nature*, 2004