Invasion and Metastasis

What is metastasis?

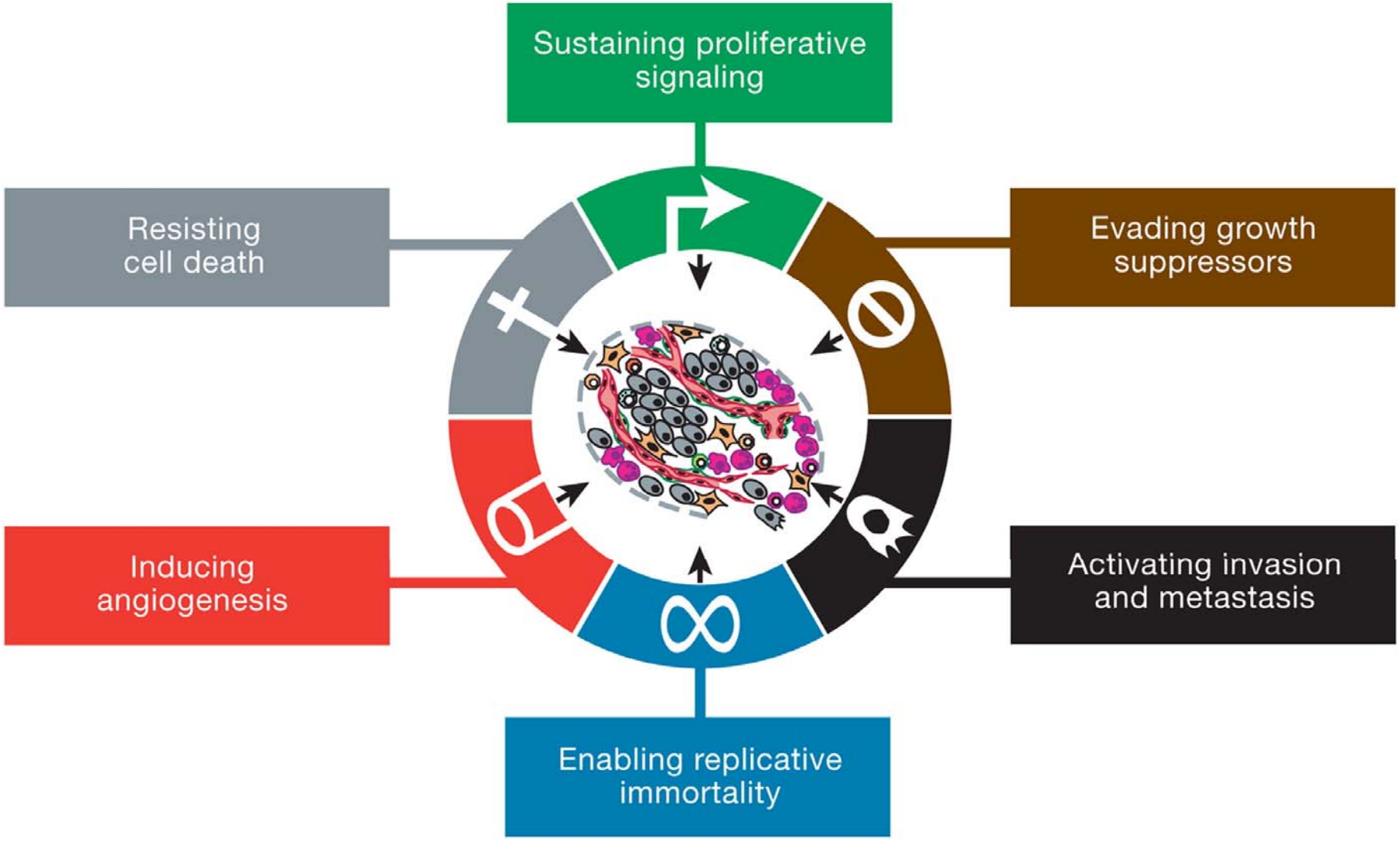
Tumors take many years before detection (>10^9 cells)- particularly if tumor growing in an extensible space.

Only when tumor begins to compromise function of the organ does it evoke symptoms. Most primary tumors can be surgically excised and account for less than 10% of cancer deaths.

For 90% of patients - primary successfully excised but patients die as a result of disease at sites distant to that of primary.

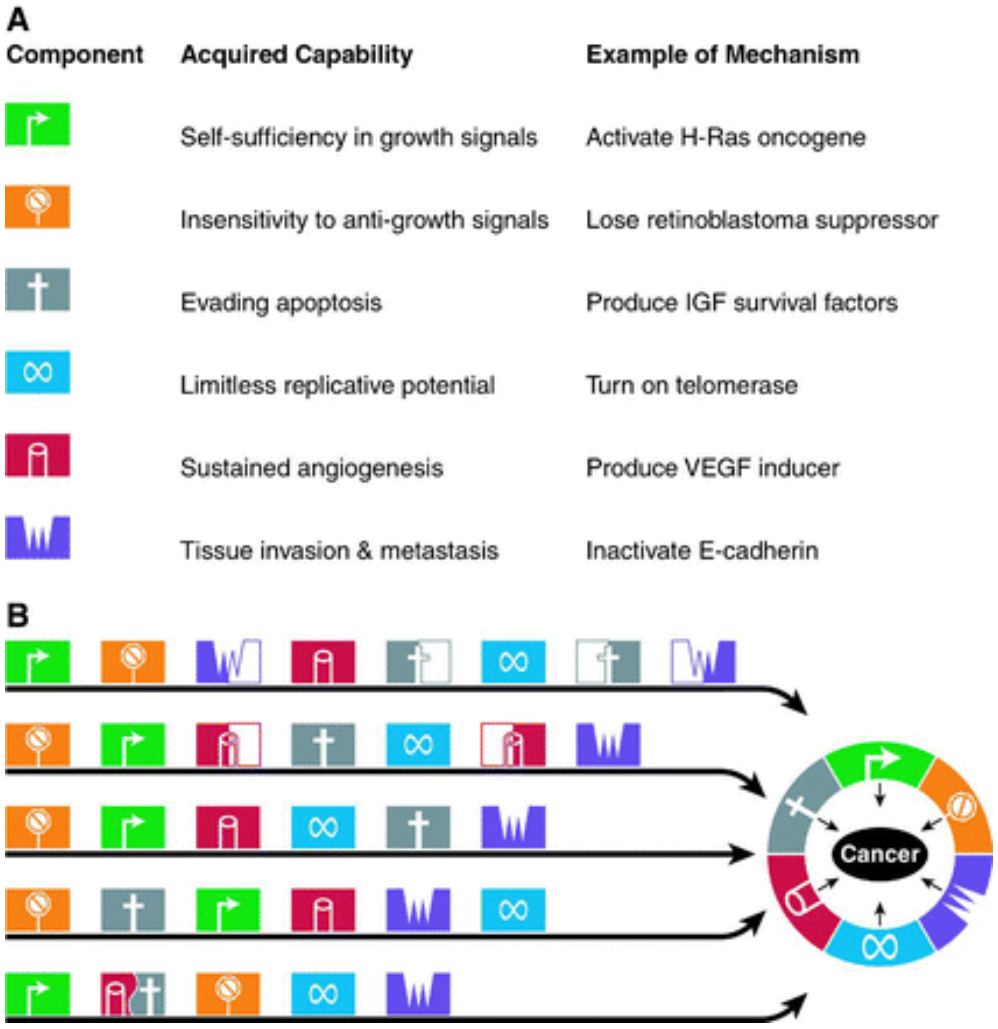
Metastasis is the truly lethal event in cancer and the process we know least about.

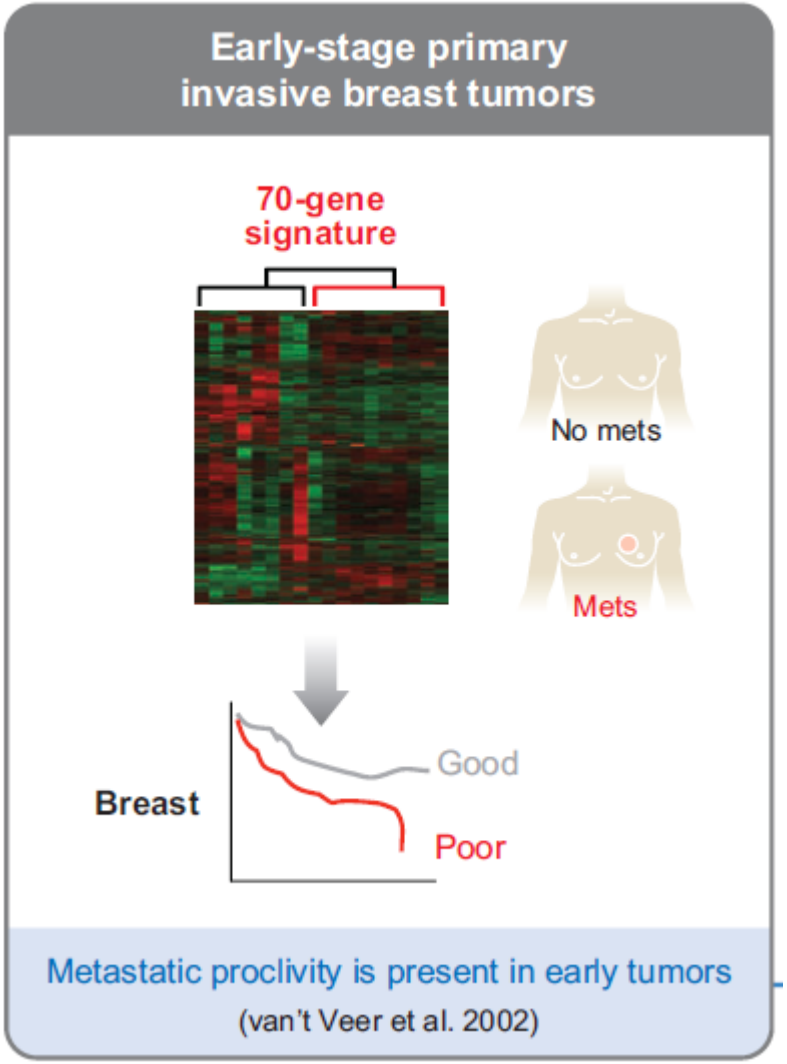
Understanding metastasis has the greatest potential to extend patient survival.



Cell 144, March 4, 2011 p 646-674 2011

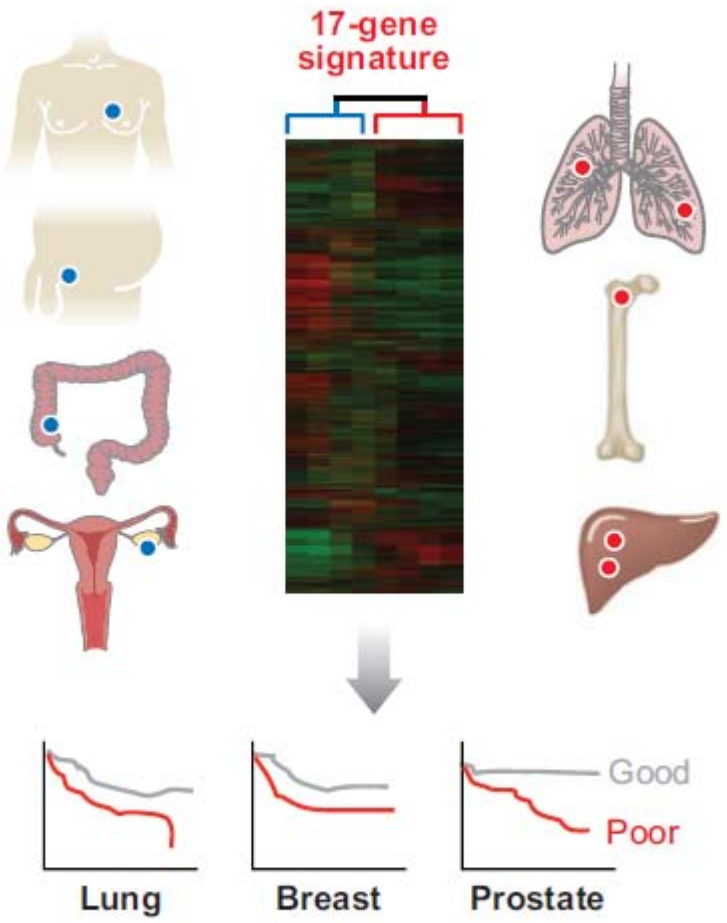
Mutations in the cancer cell





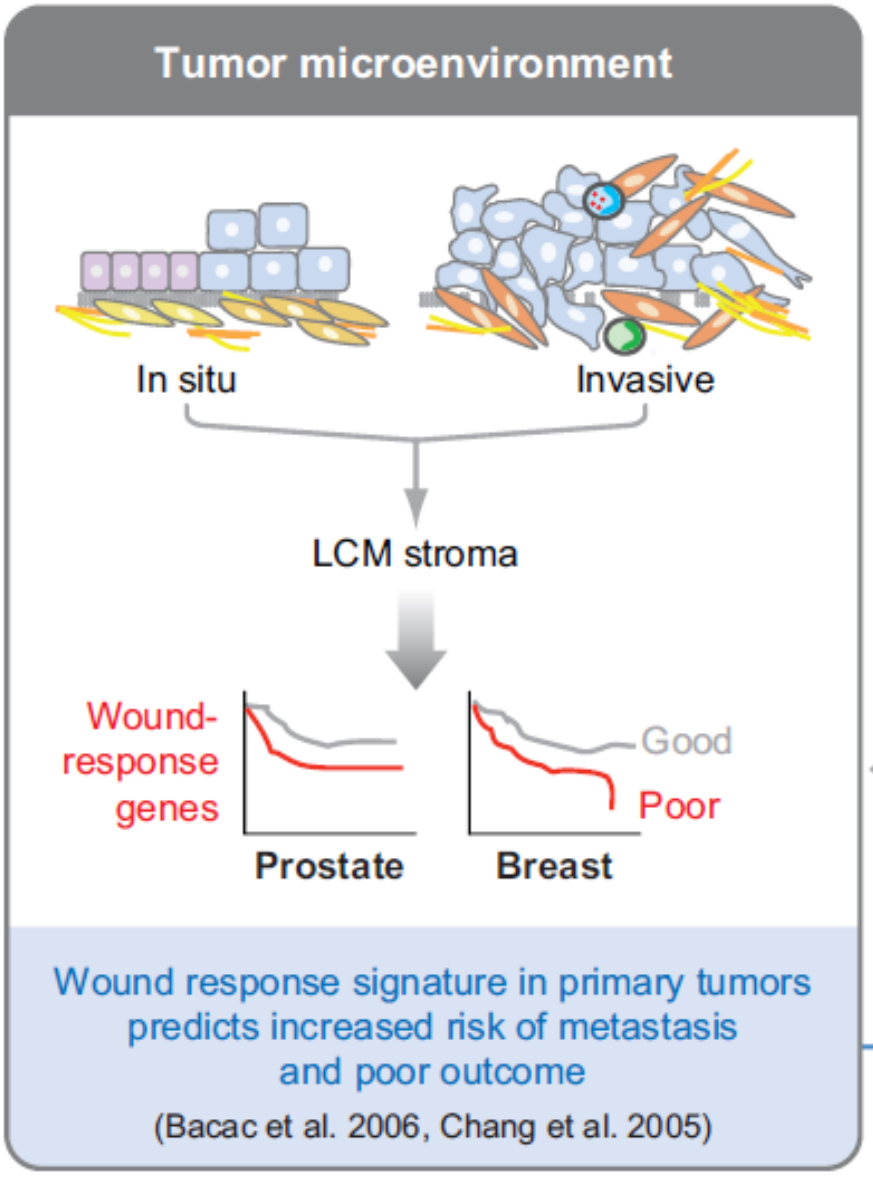
(van't Veer et al., Nature 415(6871):530–536, 2002)

Primary tumors vs. metastatic nodules

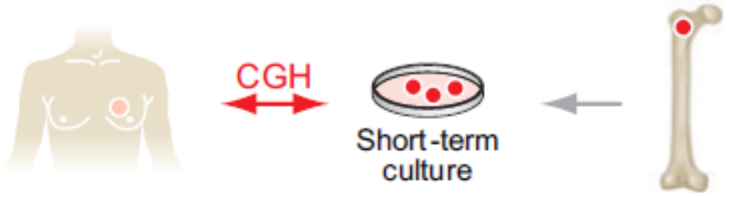


The metastatic potential is encoded in the bulk of primary tumors

(Ramaswamy et al. 2003)


Tumor microenvironment
In situ
Invasive
LCM stroma
Wound-response genes
Good
Poor
Prostate
Breast
Wound response signature in primary tumors predicts increased risk of metastasis and poor outcome
(Bacac et al. 2006, Chang et al. 2005)

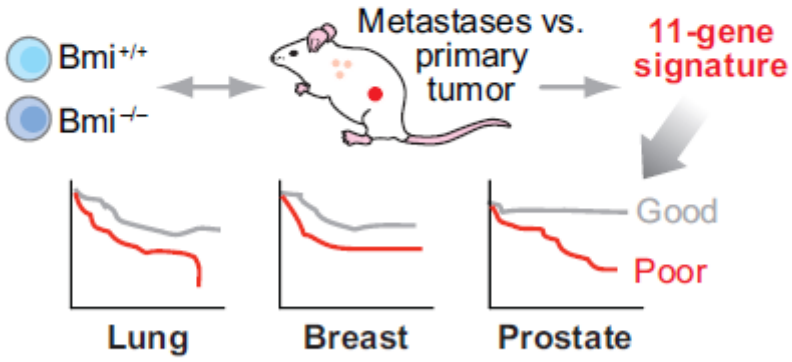
Bone metastases vs. primary tumor



Dissemination occurs early during tumorigenesis

(Gangnus et al. 2004)

Stem cell–ness and cancer



Tumors with stem cell–like signatures are likely to have poor prognosis

(Glinsky et al. 2005)

Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer

Gennadi V. Glinsky, Olga Berezovska, and Anna B. Glinskii

Sidney Kimmel Cancer Center, San Diego, California, USA.

The Journal of Clinical Investigation http://www.jci.org Volume 115 Number 6 June 2005

Metastatic disease non Hodgkins Lymphoma (NHL):

-CT scan (blue)

-PET scan (yellow) – FDG accumulates in regions of active metabolism

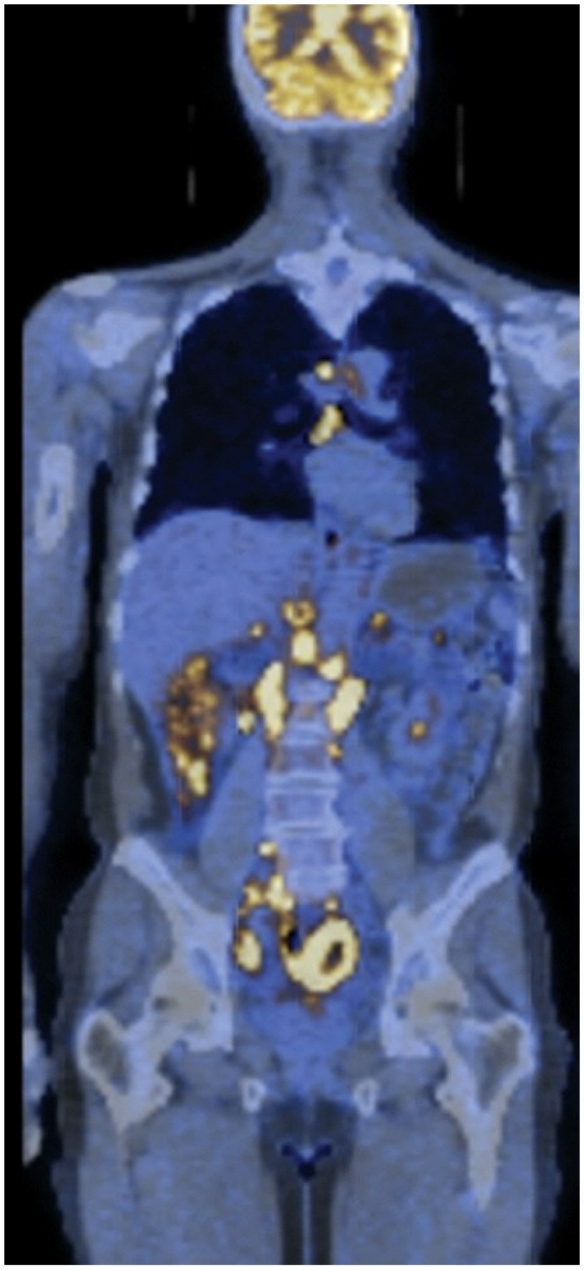


Figure 14-1 The Biology of Cancer (© Garland Science 2007)

Tumor cells metastasize through lymph and blood vessels

Pancreatic cancer cells within lymphatic vessel

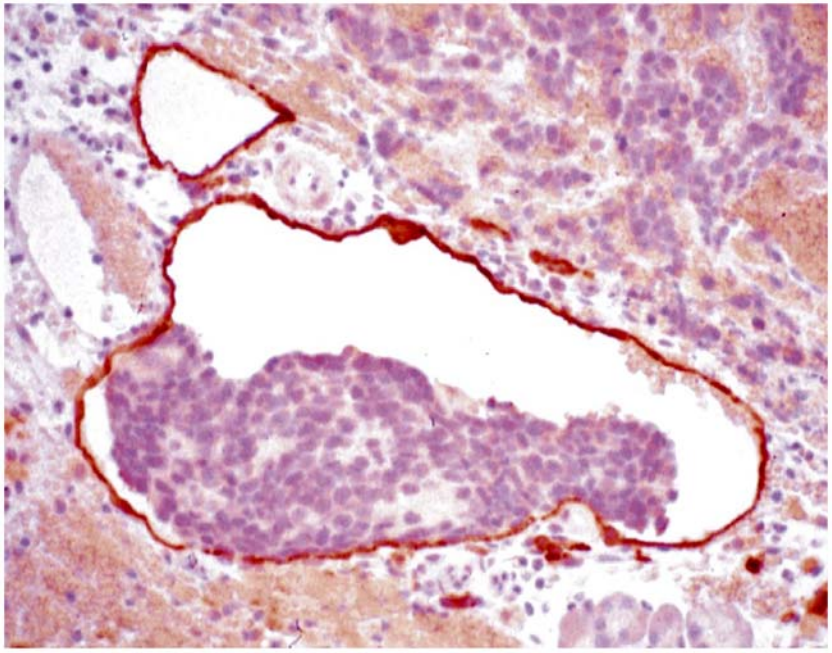


Figure 14-2a The Biology of Cancer (© Garland Science 2007)

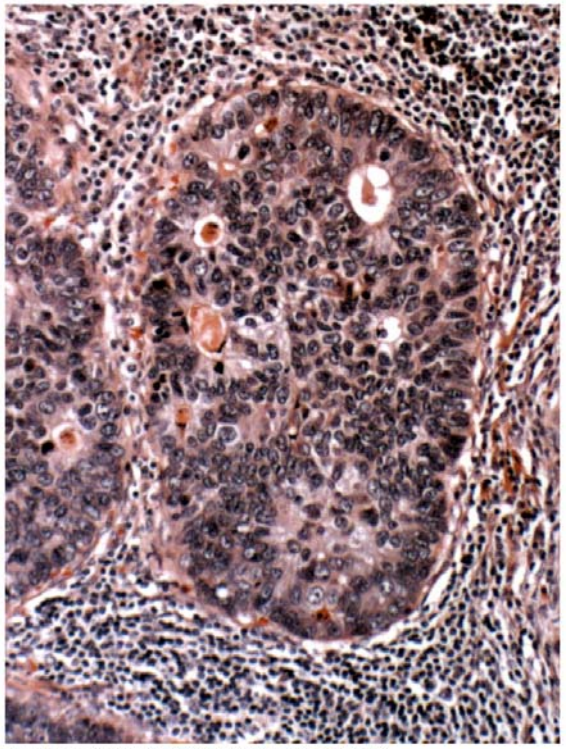


Figure 14-2b The Biology of Cancer (© Garland Science 2007)

Breast cancer in lymphatic drainage

Metastases disrupt organ functions

Breast cancer metastasizes to bone (pain and skeletal collapse); brain, lungs, liver (function).

Other cancers spread to different organs.

Some cancers have very high rates of metastasis-while others hardly metastasize at all.

Understanding the basis for these differences could lead to control over tumor spread.

Size of primary correlates with risk of breast cancer metastasis

1589 tumors followed for up to 46 years.

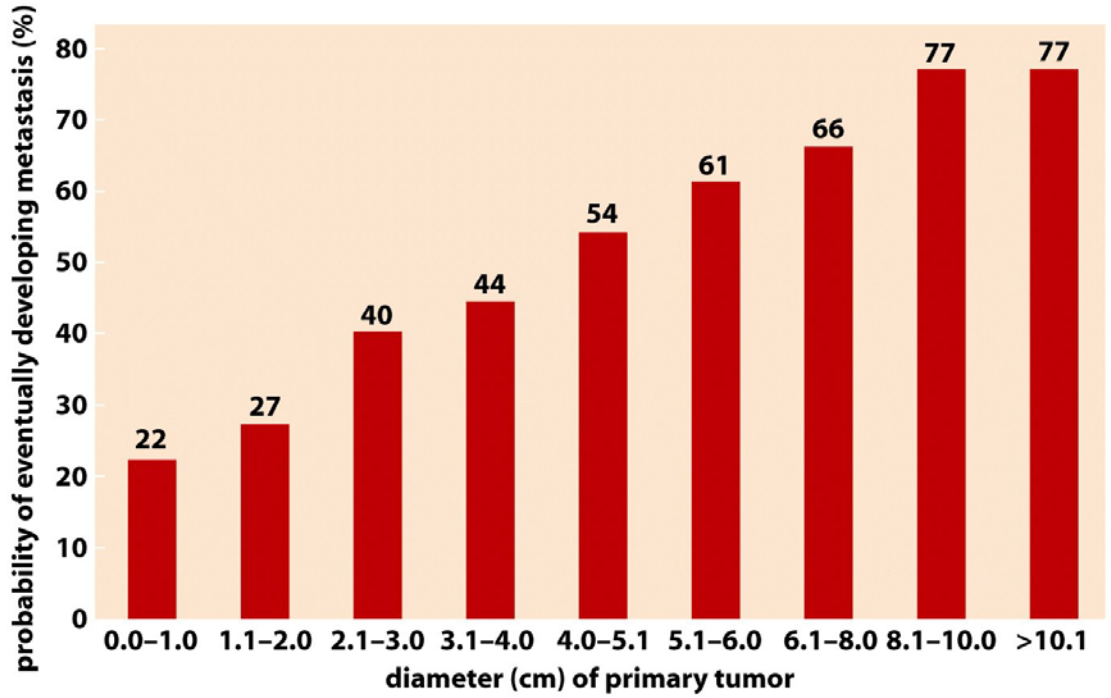


Figure 14-3 The Biology of Cancer (© Garland Science 2007)

If larger tumors associated with increased risk of metastasis then is metastasis a late event (mutations in metastasis genes)?

Simplistic deduction:

Since 4% breast cancers under 1 cm ~ mutant p53 alleles.

And 42% breast cancers over 3 cm ~ mutant p53 cancers acquire further mutations as they grow.

Mutations that favor metastasis may therefore arise as late events.

Just as likely:

a) Cells in large and small tumors equally capable of metastasizing but large tumors seed more cells

b) Large tumors contain more proliferative cells and mutations that influence proliferation influence metastasis.

Difficult to predict when metastasis first occurs.

If the metastatic phenotype arises early then most tumors will have already metastasized by the time of detection

Consensus: most tumors do metastasize early and give rise to micrometastases that are too small to detect.

Aggressive therapy given soon after detection of primary may therefore reduce the incidence of metastatic disease.

How do tumors spread?

The concept of the invasion-metastasis cascade: 6 distinct steps

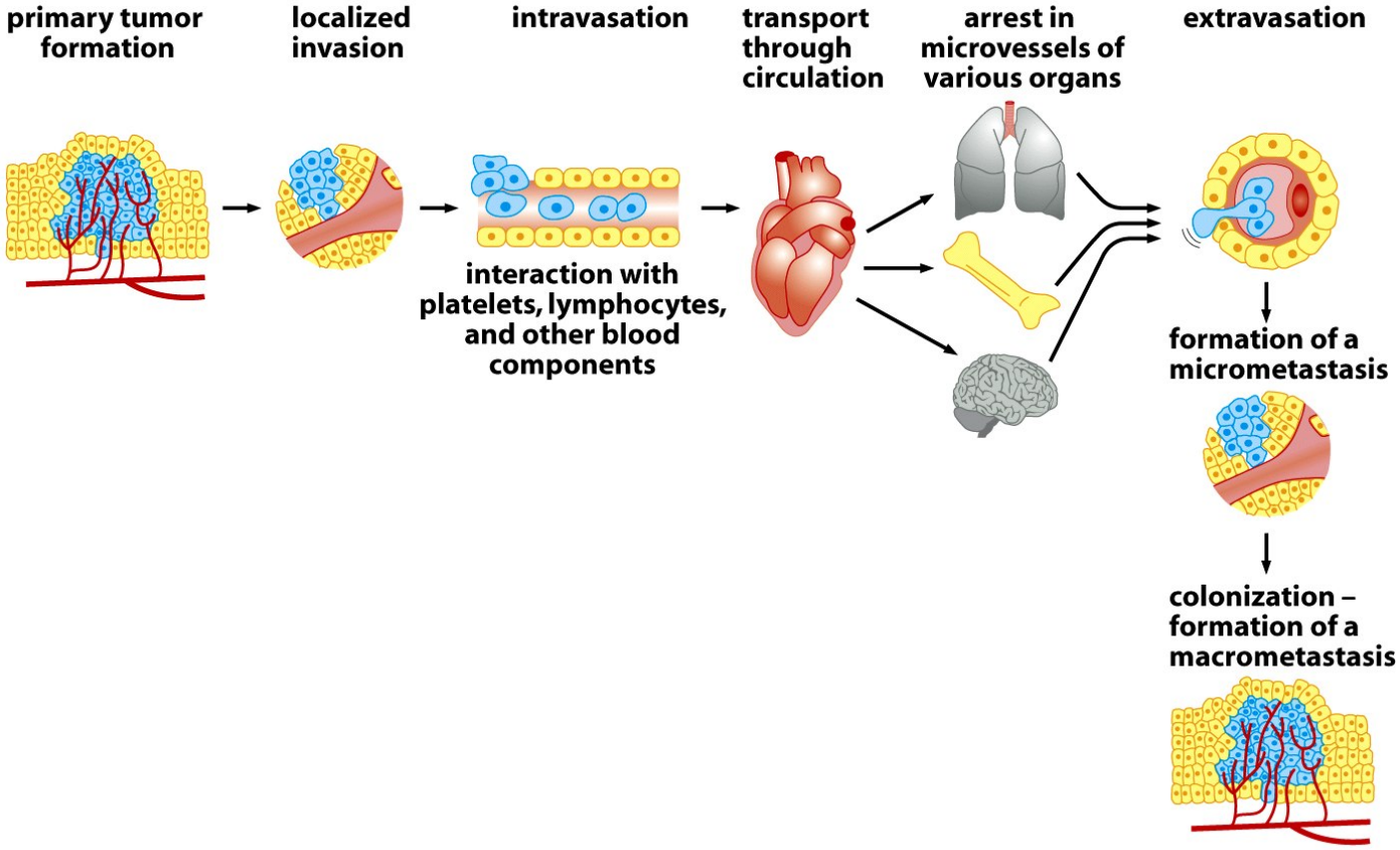


Figure 14-4 The Biology of Cancer (© Garland Science 2007)

Probability of an individual cancer cell completing cascade is very small

Step 1: Invasion

Invasive lobular mammary carcinoma progressing in single file from left to right through channels they have carved in stroma

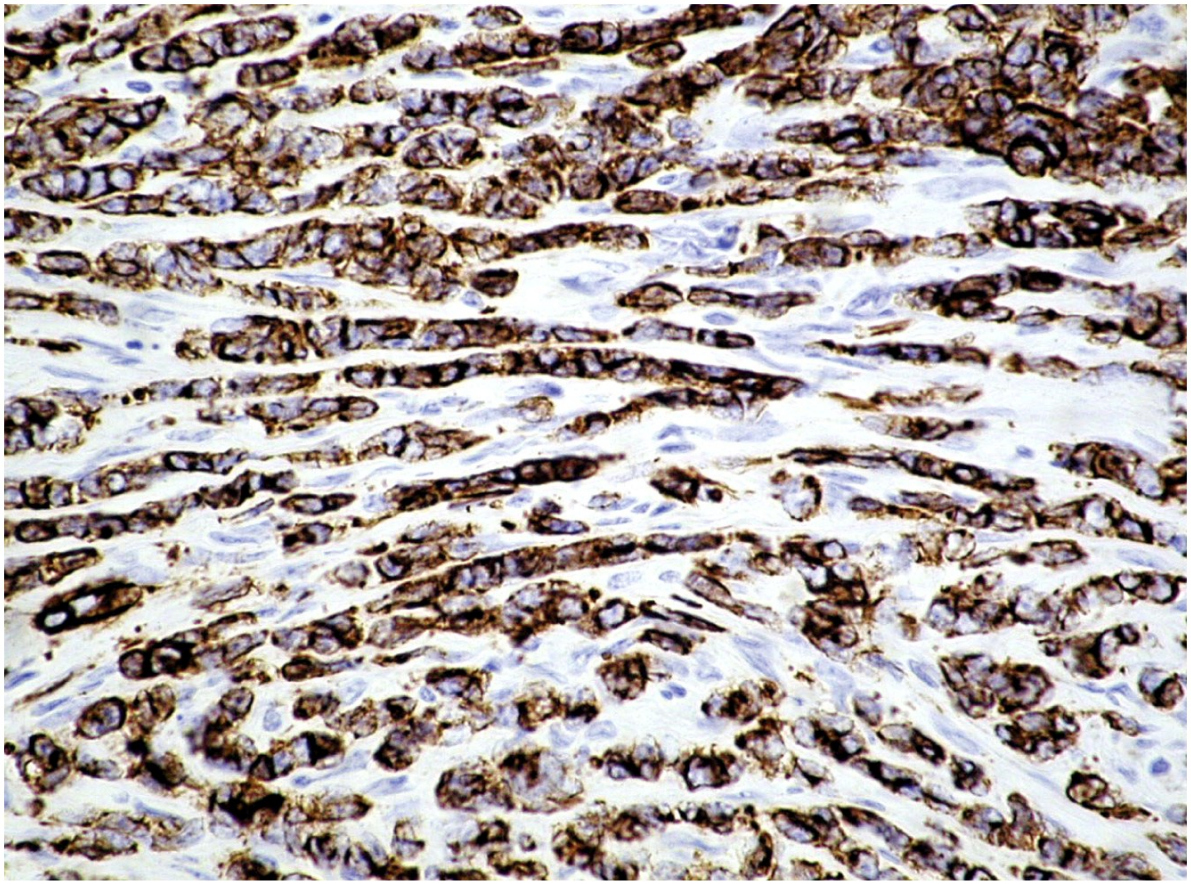


Figure 14-5a The Biology of Cancer (© Garland Science 2007)

More typically cells invade as a posse

Melanoma cells moving as a cohort through collagen matrix. Adherens junctions red (E-cadherin).

β1 integrins

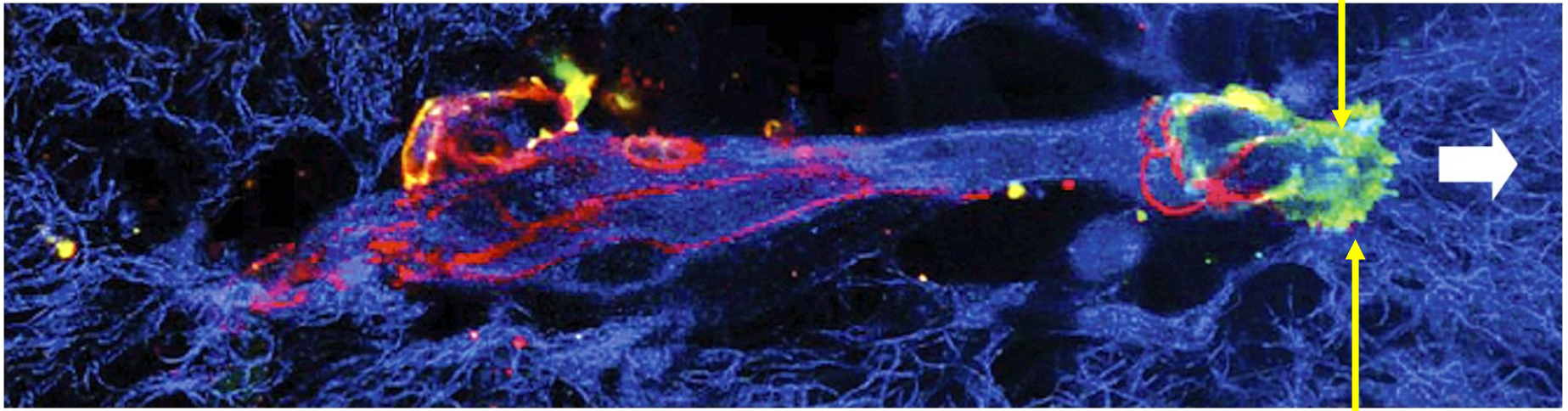


Figure 14-5b The Biology of Cancer (© Garland Science 2007)

metalloproteinases

Breach of basement membrane

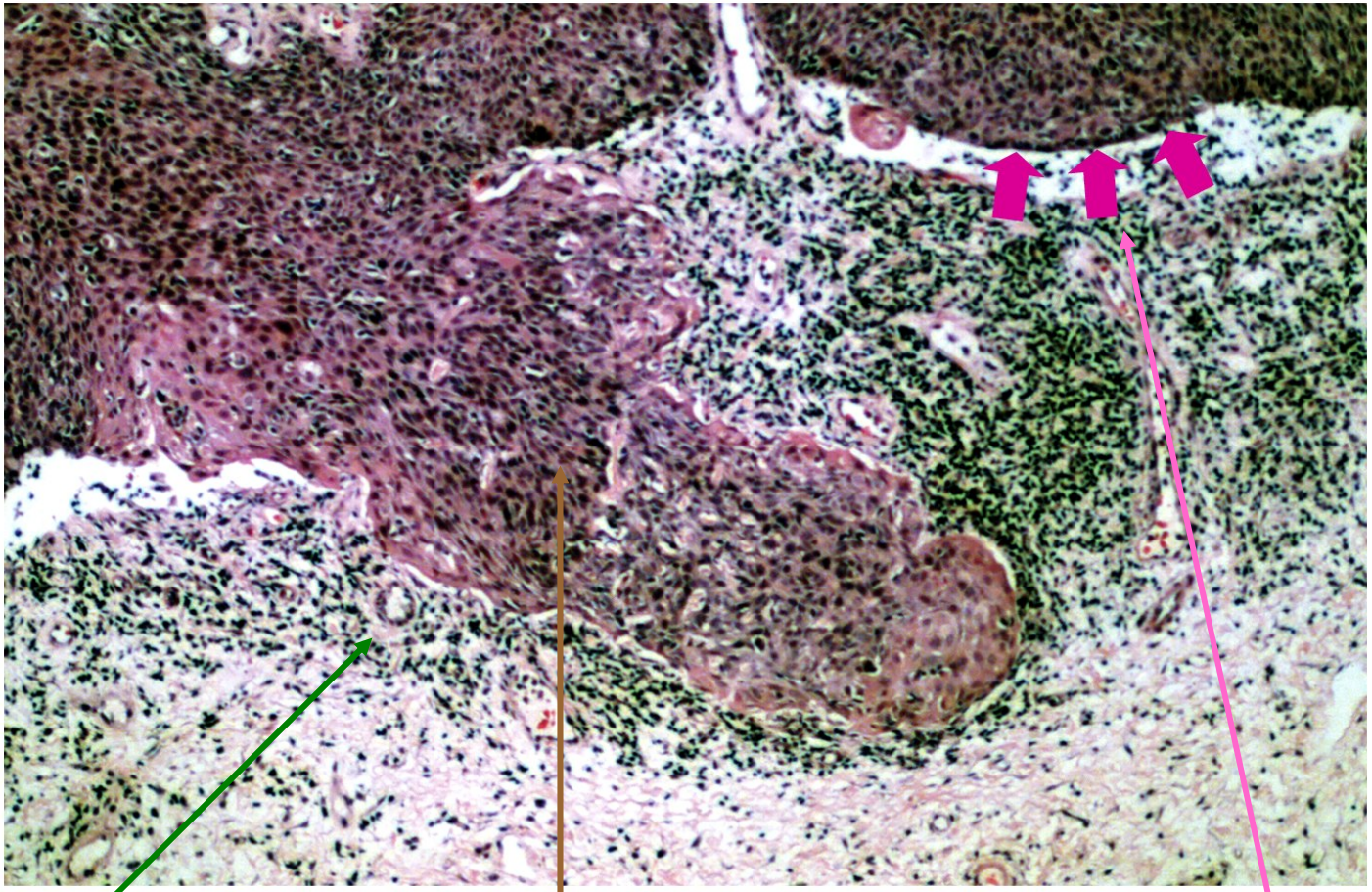


Figure 14-5c The Biology of Cancer (© Garland Science 2007)

Inflammatory cells

Squamous carcinoma

Basement membrane

Step 2 Intravasation: transport thro vasculature

Hazards:

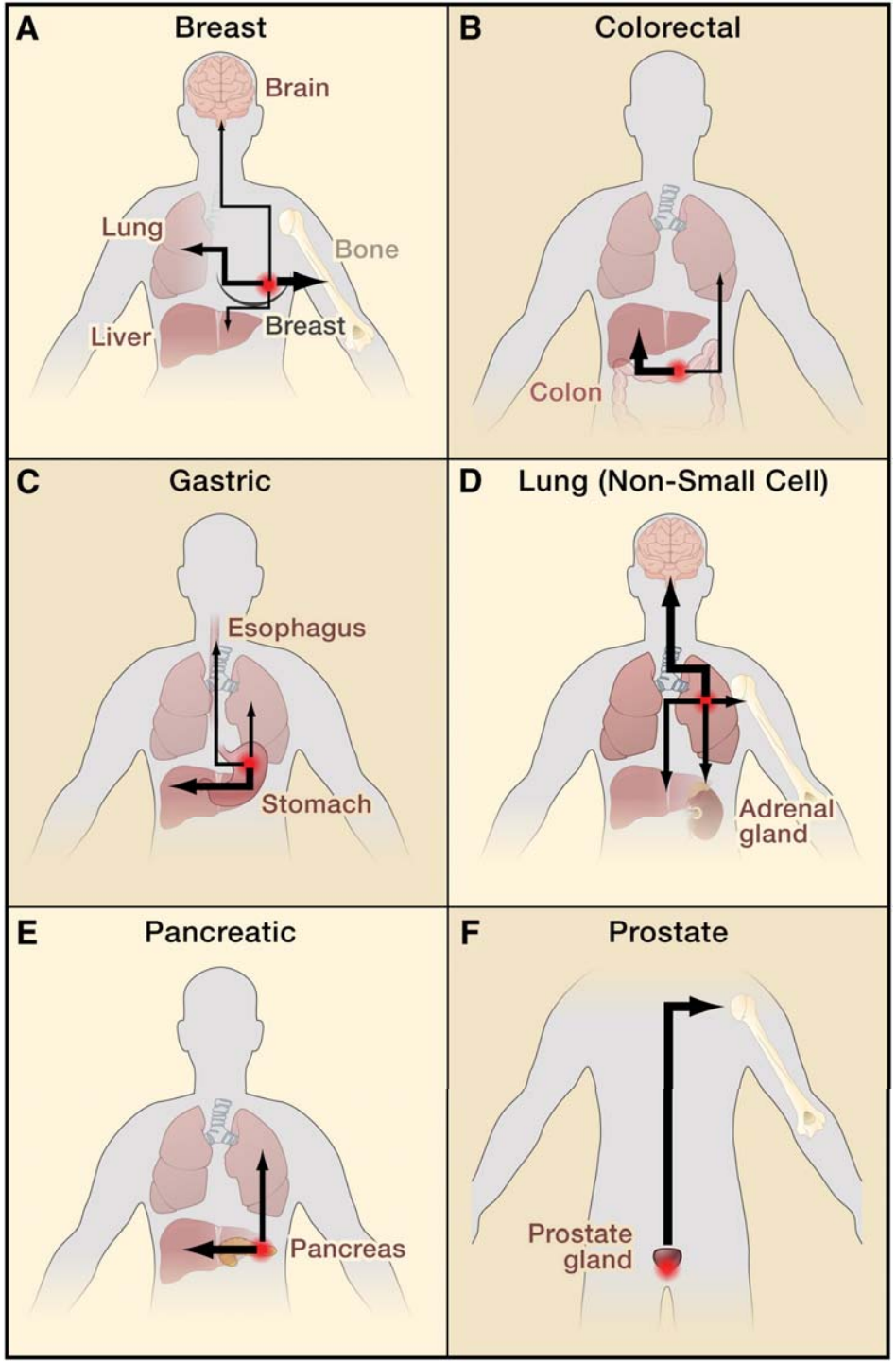
Anoikis (homelessness)- loss of substrate contact-triggers apoptosis.

Shear forces.

Lack of stromal support (survival factors).

Exposure to cells of the immune system.

Exposure to platelets (beneficial).



Cell 147: October 14 2011
p275-292

Routes of circulation

Tumors usually lodge in first capillary bed they encounter

Tumors that attract platelets ~ may lodge in wider vessels such as arteriole

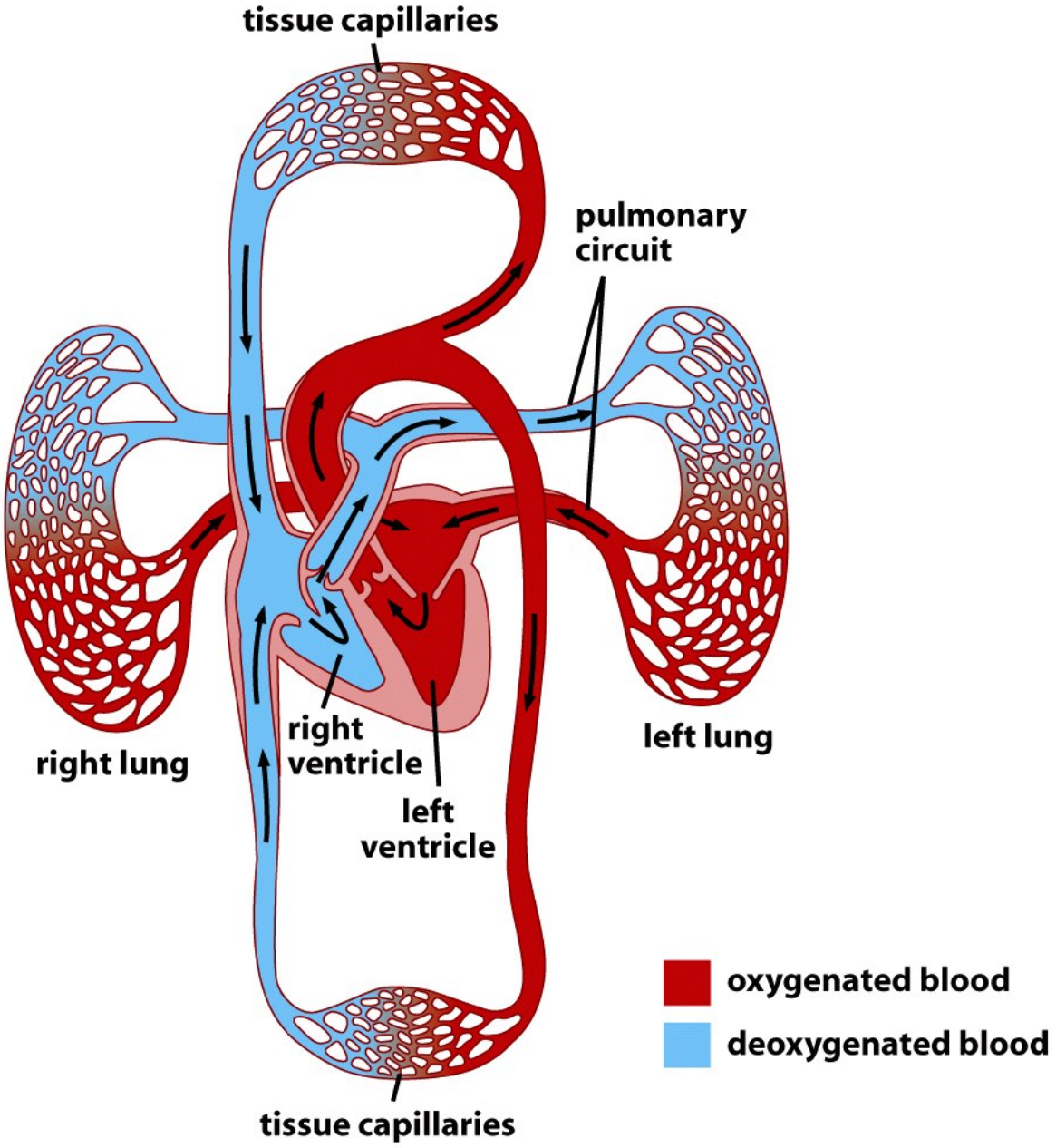
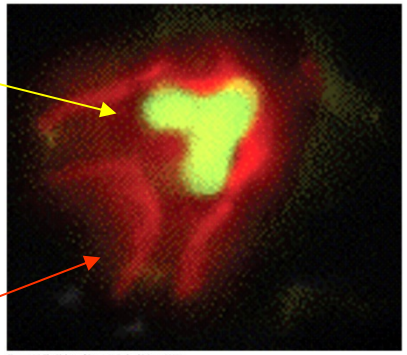


Figure 14-6 The Biology of Cancer (© Garland Science 2007)

Step 3: extravasation

tumor clump



vessel

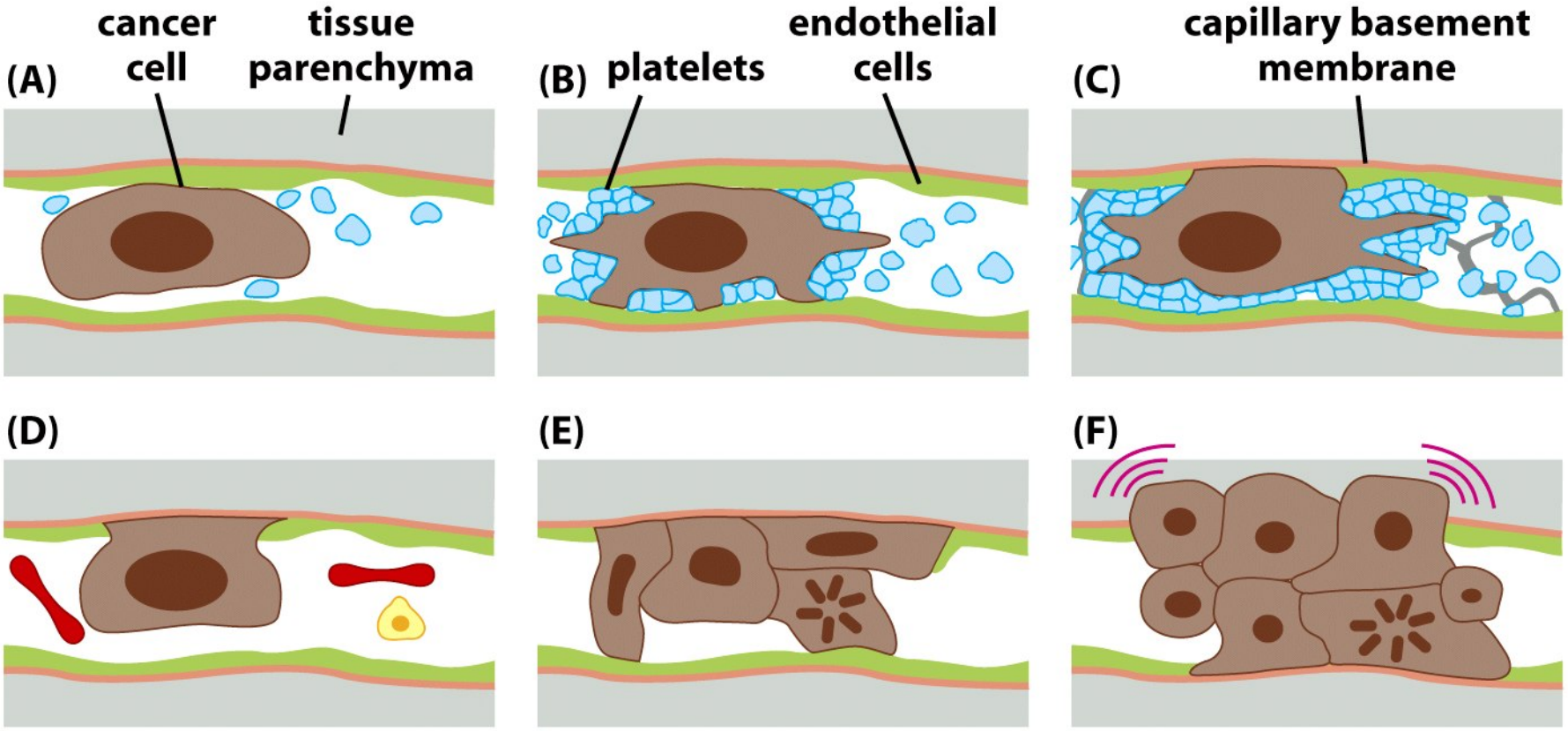


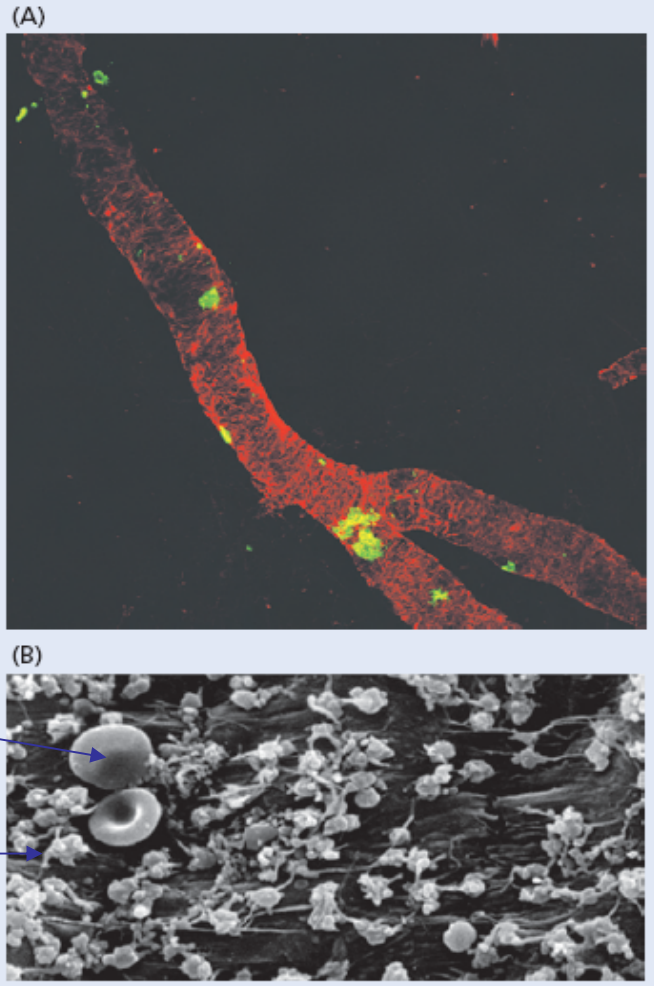
Figure 14-9 The Biology of Cancer (© Garland Science 2007)

Tumors attach to vessel walls thro microthrombi

Localized exposure of basement membrane provides attachment points for potential microthrombi attachment

red blood cells

platelets



Tumors stimulate microthrombi

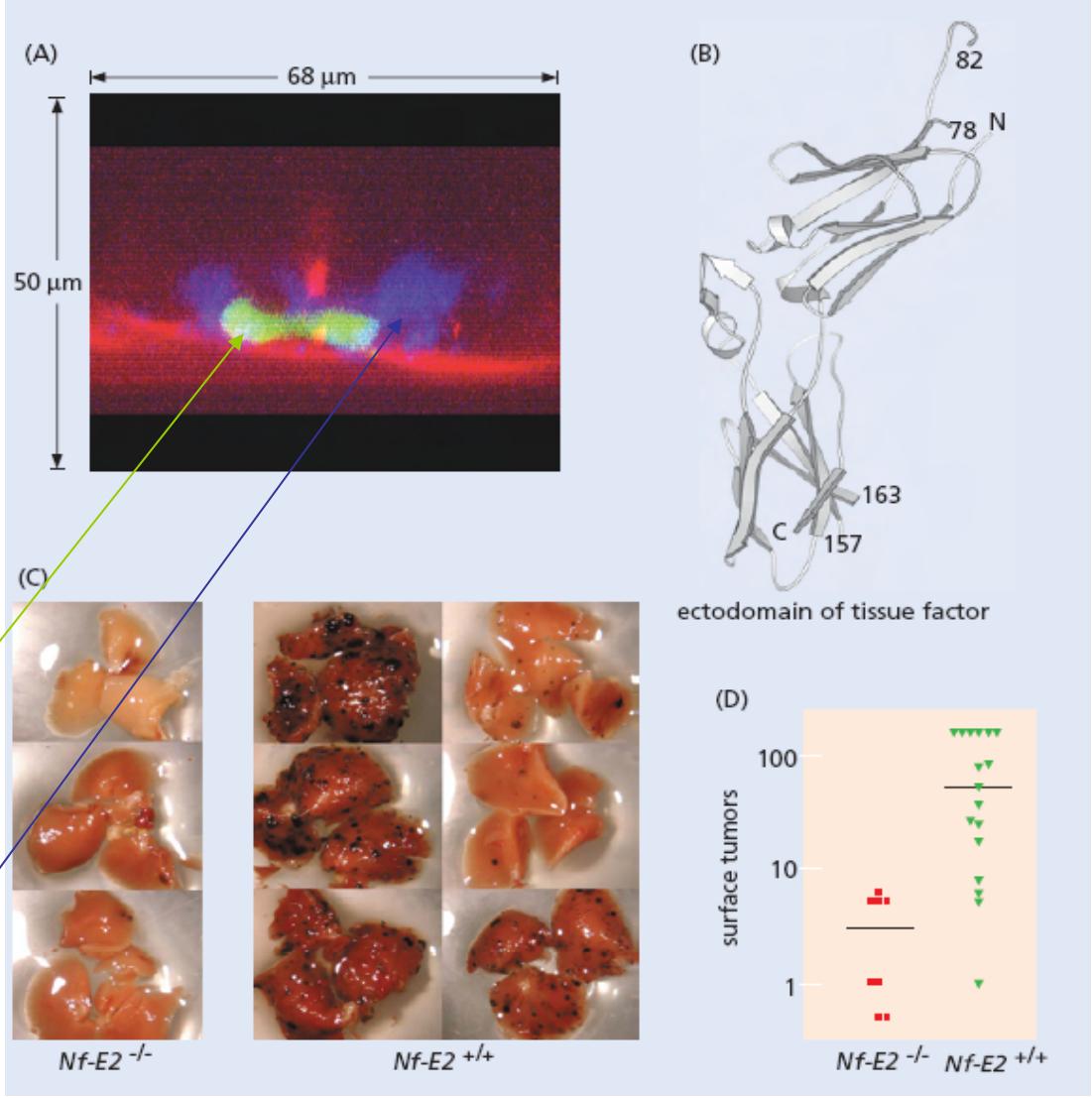
Within seconds of entering blood stream tumors stimulate thrombus formation (platelets and red blood cells).

Tissue factor activates thrombin and converts fibrinogen to fibrin.

Mice lacking platelets (Nf-E2-) do not support metastasis of malignant melanoma C and D.

Tumor cell

platelet



How efficient is metastasis?

Mice carrying primary tumors of 1 gm (10^9 cells)

10^6 cells enter circulation per day.

However less than 5 tumors result.

Metastatic inefficiency.

Step 4: colonization

Colonization - the rate-limiting step.

Unlike the primary tumor tissues lack stroma that provide support. Consequently most micrometastases remain dormant for long periods or die before they can give rise to 2ndary tumors.

Evolution of metastatic ability can occur outside the primary tumor

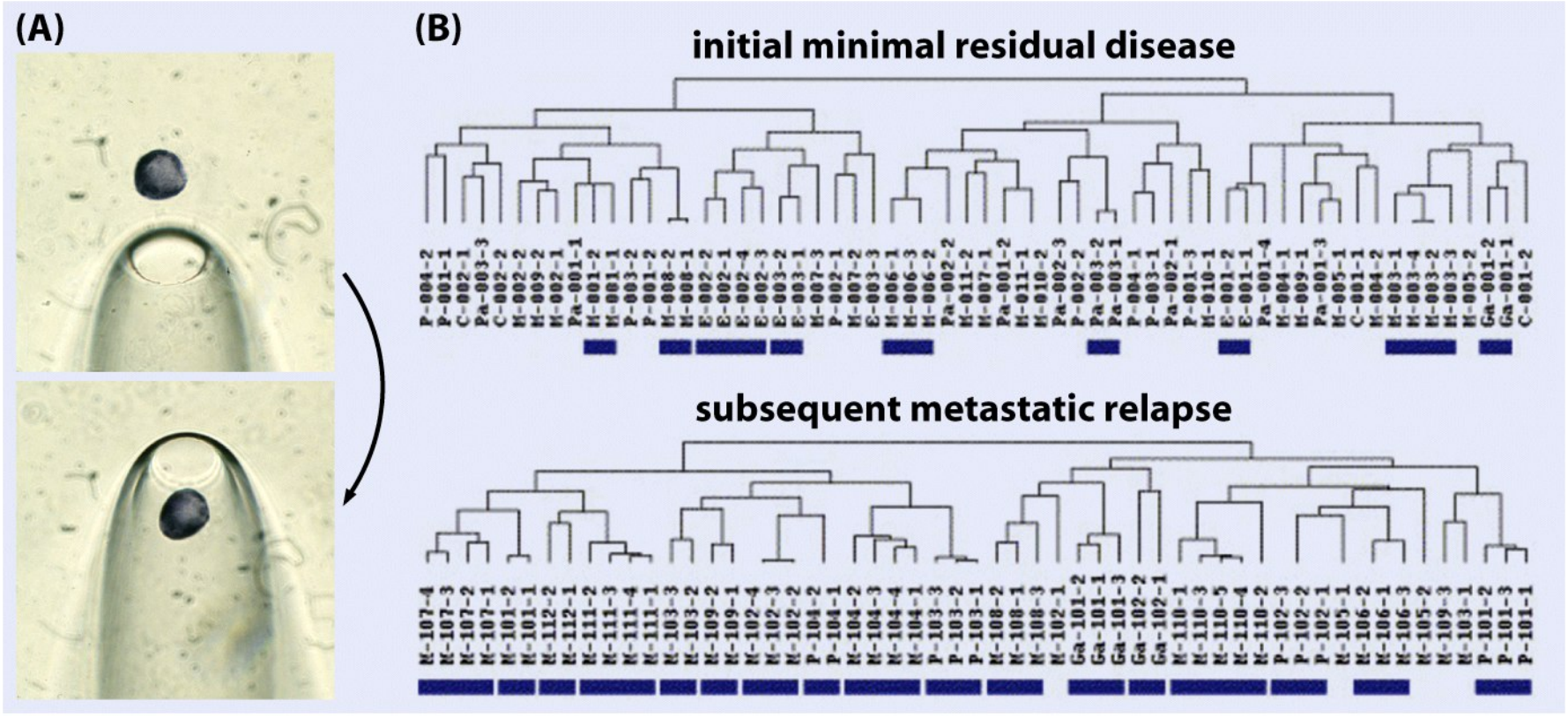


Figure 14-11ab The Biology of Cancer (© Garland Science 2007)

The evolution of colonizing ability

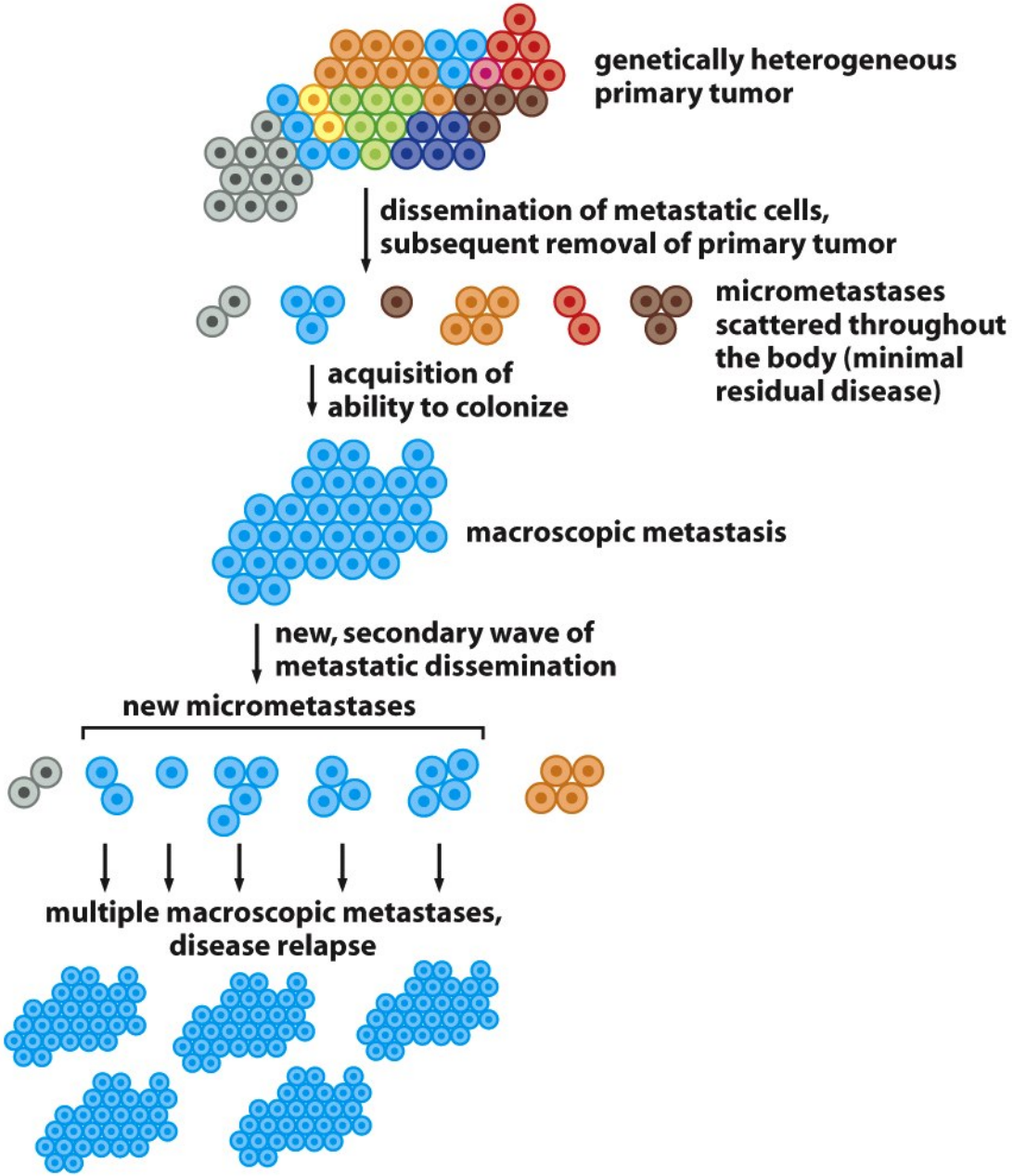


Figure 14-11c The Biology of Cancer (© Garland Science 2007)

Micrometastases (MM) detectable in bone marrow

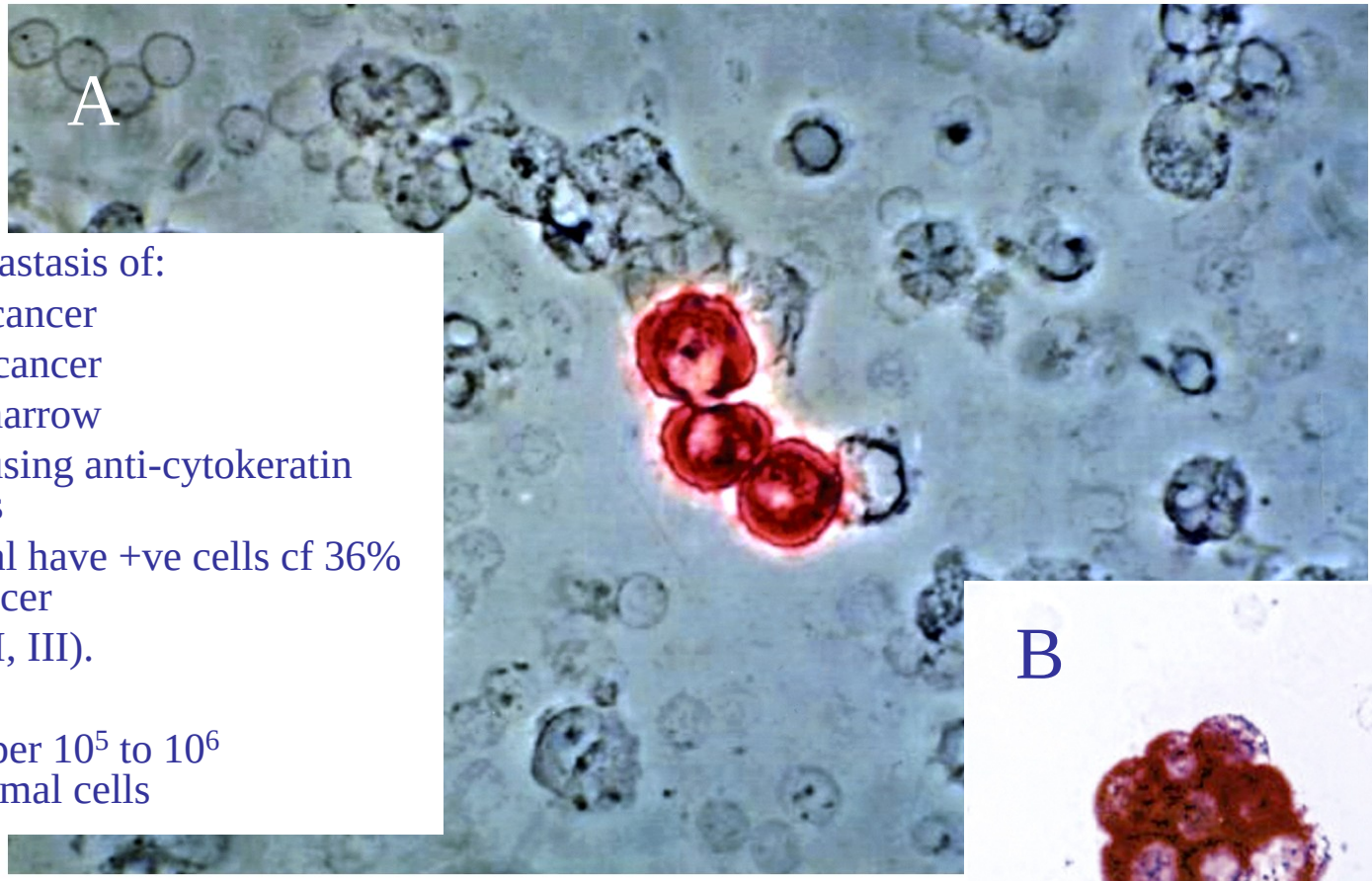


Figure 14-10a The Biology of Cancer (© Garland Science 2007)

Micrometastasis of:

A. colon cancer

B. breast cancer

in bone-marrow

detected using anti-cytokeratin antibodies

1% normal have +ve cells cf 36% breast cancer

(stage I, II, III).

1 colony per 10^5 to 10^6 mesenchymal cells

Figure 14-10b The Biology of Cancer (© Garland Science 2007)

Does marrow micrometastasis predict relapse?

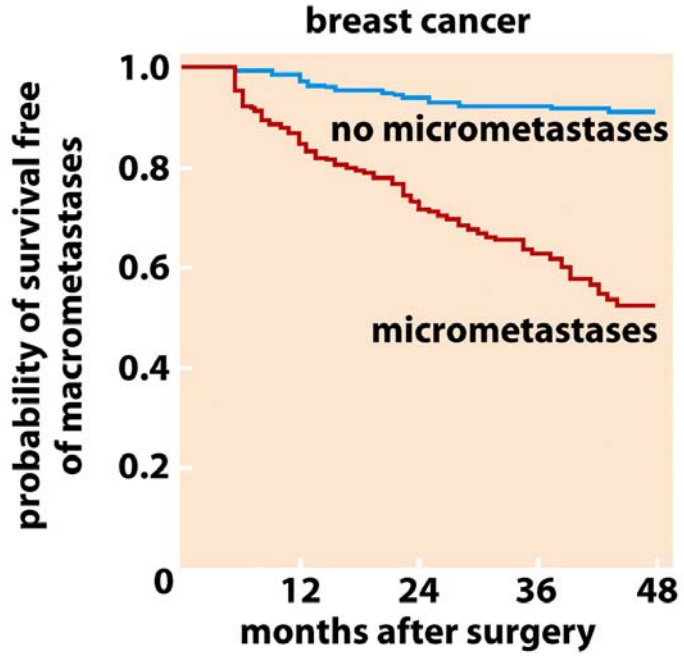


Figure 14-50a The Biology of Cancer (© Garland Science 2007)

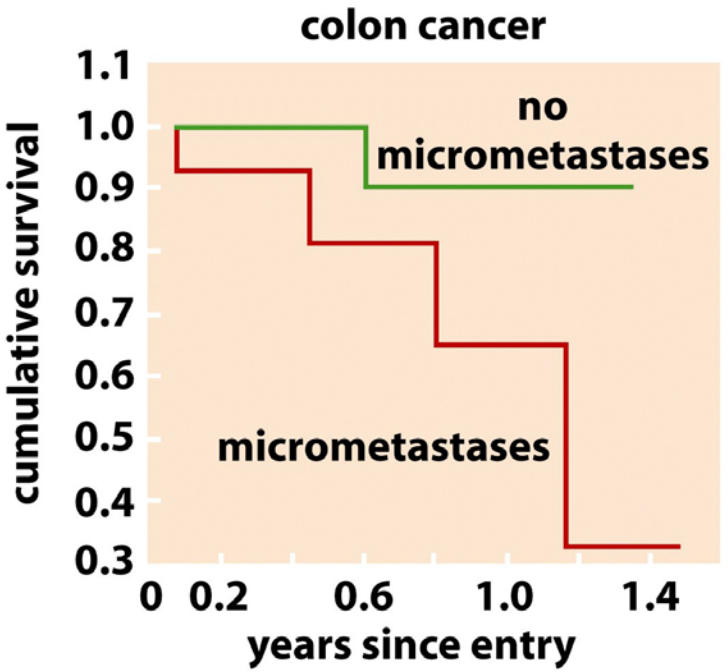


Figure 14-50b The Biology of Cancer (© Garland Science 2007)

Patients with MM at time of surgery have higher relapse rates.

Breast cancer MM increase risk of developing metastatic disease 4 to 10 fold.

Colon cancer patients 90% patients who lack MM alive at 15 months compared to 30% of those with marrow MM

Cancer esophagus – 79-88% pts have MM in rib marrow at resection

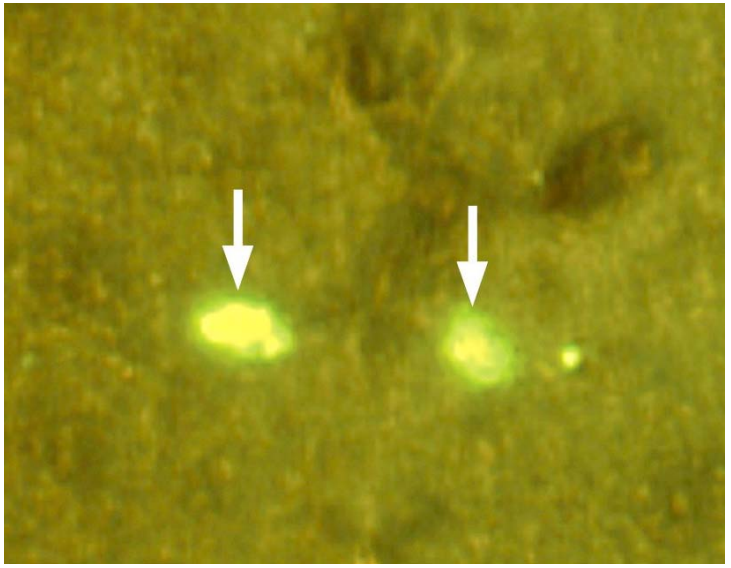
Can MM remain dormant?

Are MM truly dormant?

Fluorescently-labeled cells lose fluorescence with each division. Breast cancer cells injected into portal vein lodge in liver were viable and had not lost fluorescence 11 weeks later.

Cells could be excised, cultured and retained tumorigenicity when introduced subcutaneously.

Cells were resistant to chemotherapy that killed growing cells.



What we have learned so far

Invasion-metastasis cascade has multiple steps.

As many changes in phenotype involved as precede inititial tumor formation.

Cells readily enter circulation and seed distant sites. However, only a small fraction grow.

Nevertheless vast numbers of cells seeded by growing tumors ensure that some of these will eventually grow.

Clinically these pose a major threat to survival of patient.

Important question:

To what extent do the steps represent changes in gene expression. Are there metastasis specific genes and their suppressors?

Epithelial-mesenchymal transition (a wolf in sheep's clothing)

Migration and motility require epithelial cells to lose their differentiated adhesive nature and become mesenchyme-like.

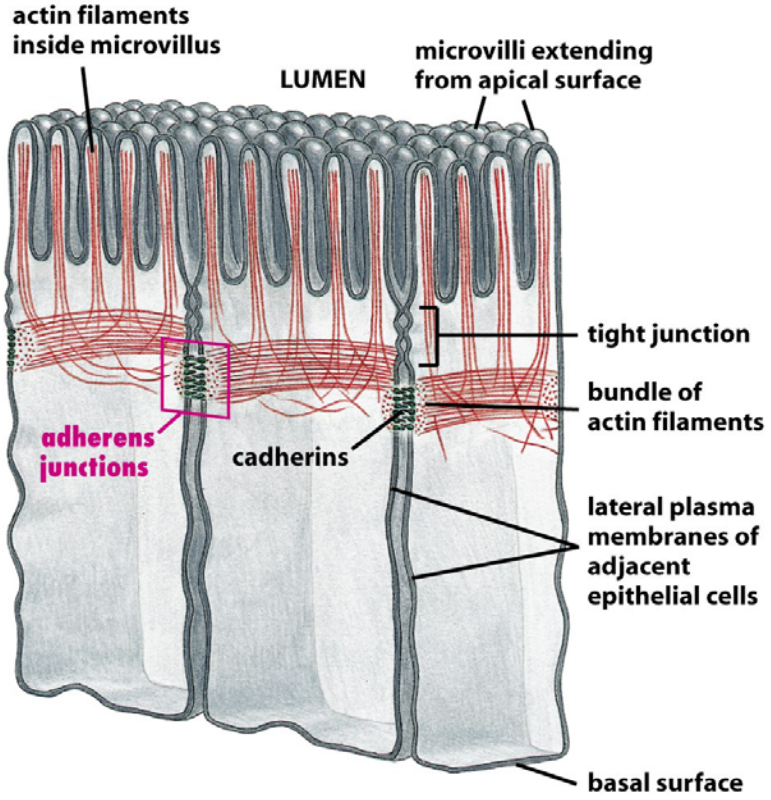


Figure 13-12a The Biology of Cancer (© Garland Science 2007)

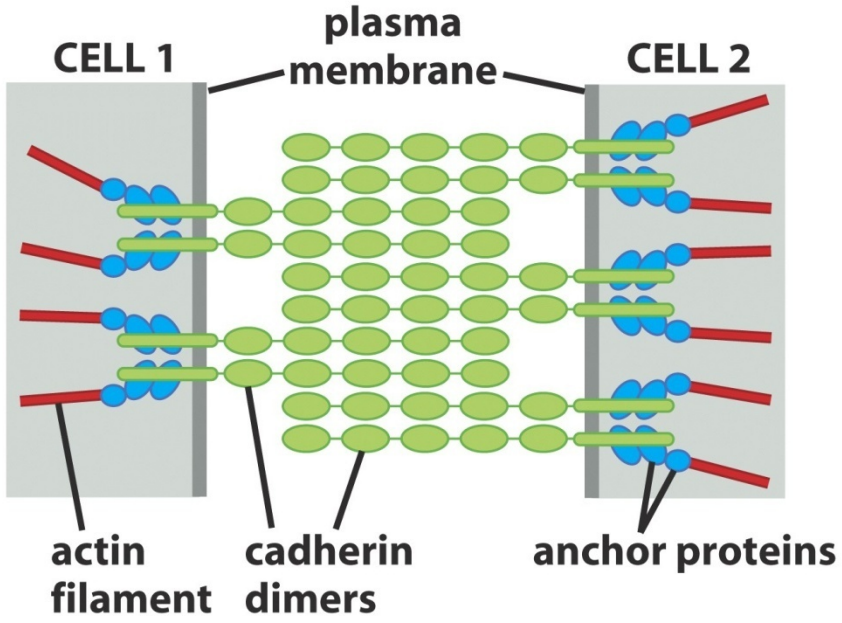


Figure 13-12b The Biology of Cancer (© Garland Science 2007)

EMT

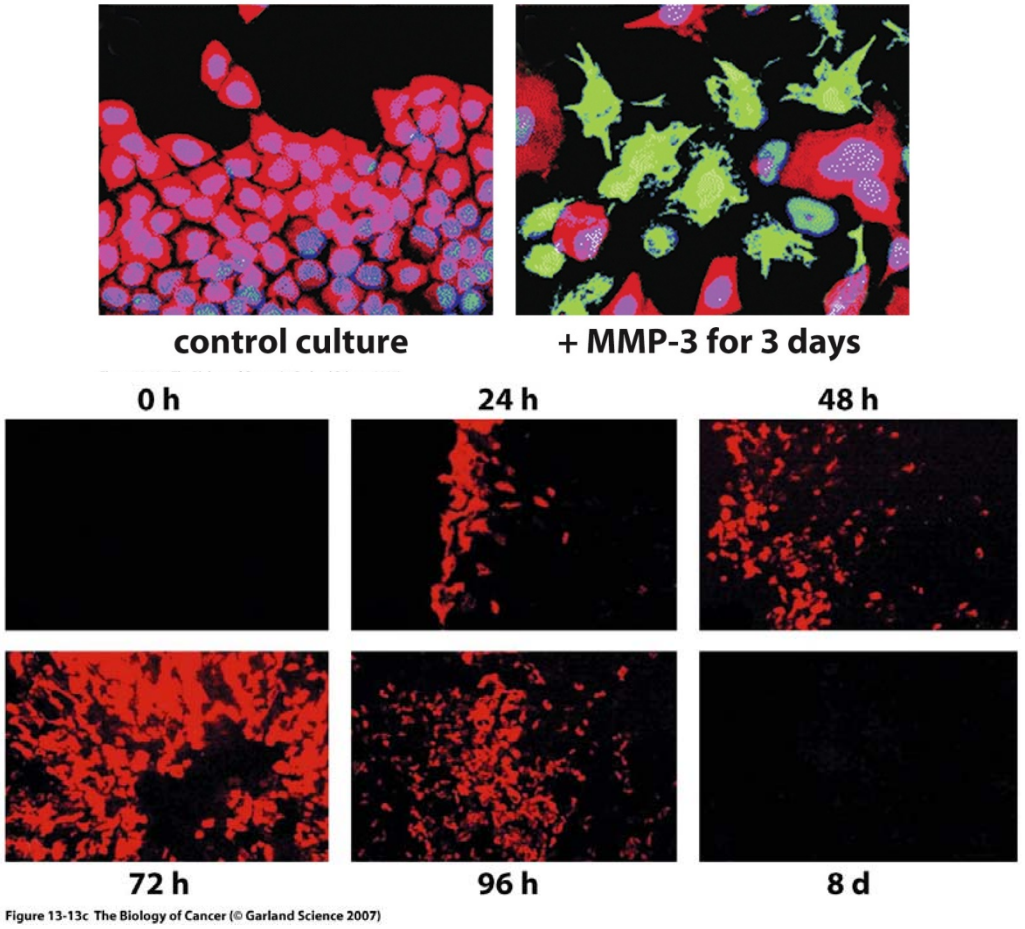


Figure 13-13c The Biology of Cancer (© Garland Science 2007)

Upper panel: Epithelial cells in monolayer culture exposed to metalloproteinase MMP-3.

Cells lose cytokeratins (red) and E-cadherin and acquire vimentin (green) fibronectin and N-cadherin become motile and invasive.

Become **indistinguishable** from mesenchyme.

Lower panel: Wounding MCF10A (pre-malignant) monolayer –induction of vimentin (red) at wound site. Vimentin lost once cells revert to epithelial.

Embryogenesis and EMT

Ectodermal-mesodermal transition at **gastrulation**

Delamination neuroepithelial cells from neural tube to form melanocytes, peripheral NS

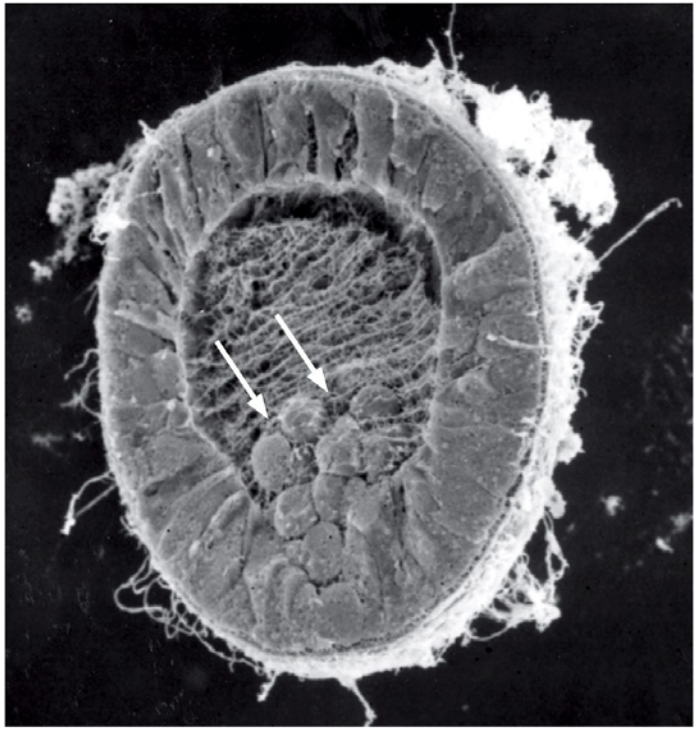


Figure 14-13a The Biology of Cancer (© Garland Science 2007)

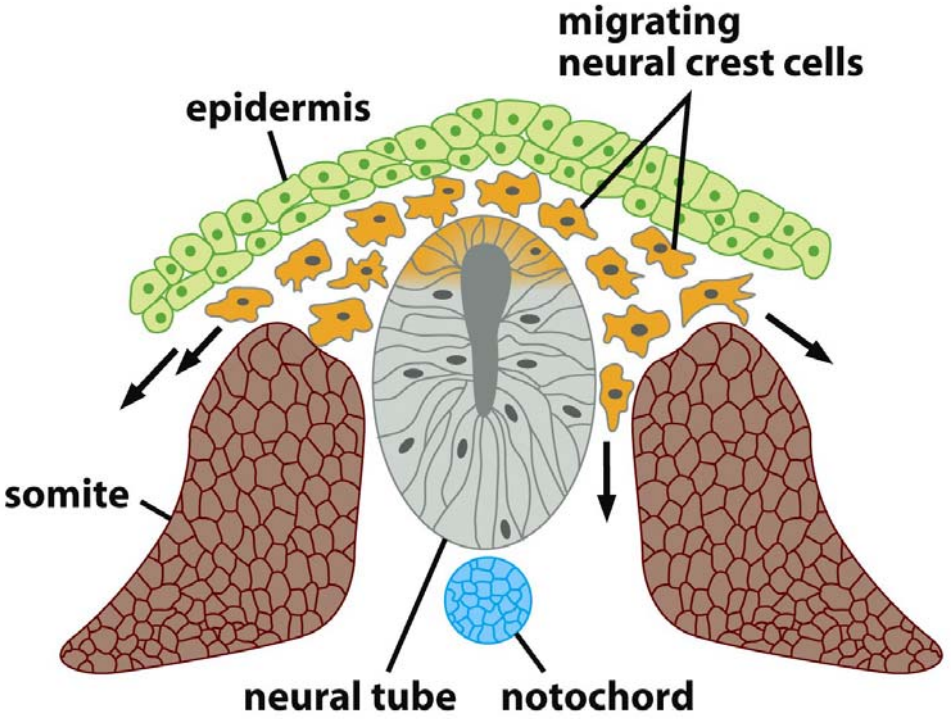


Figure 14-13b The Biology of Cancer (© Garland Science 2007)

Can we see EMT in tumors?

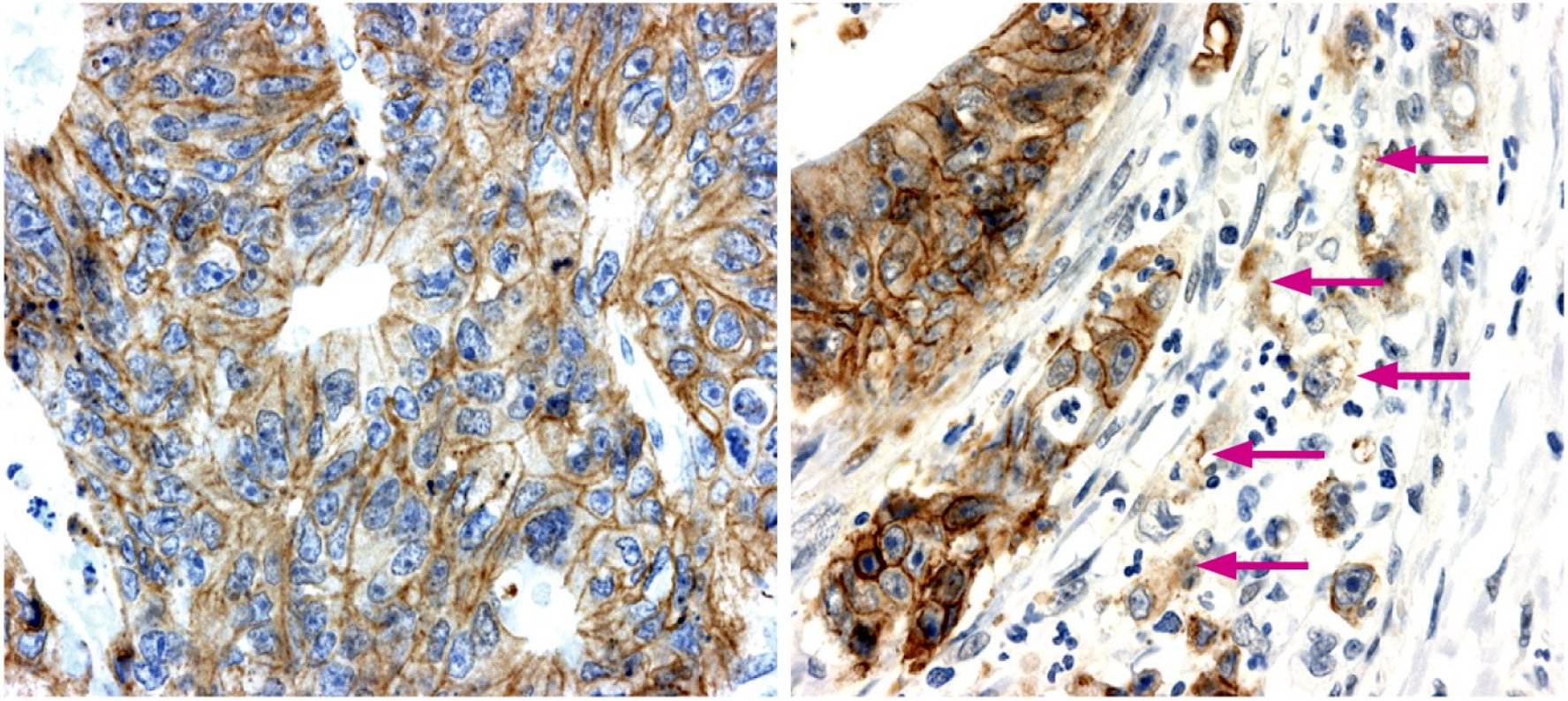


Figure 14-14a The Biology of Cancer (© Garland Science 2007)

Loss of E-cadherin (brown) from membrane of cells at invasive edge of colon carcinoma (arrows)

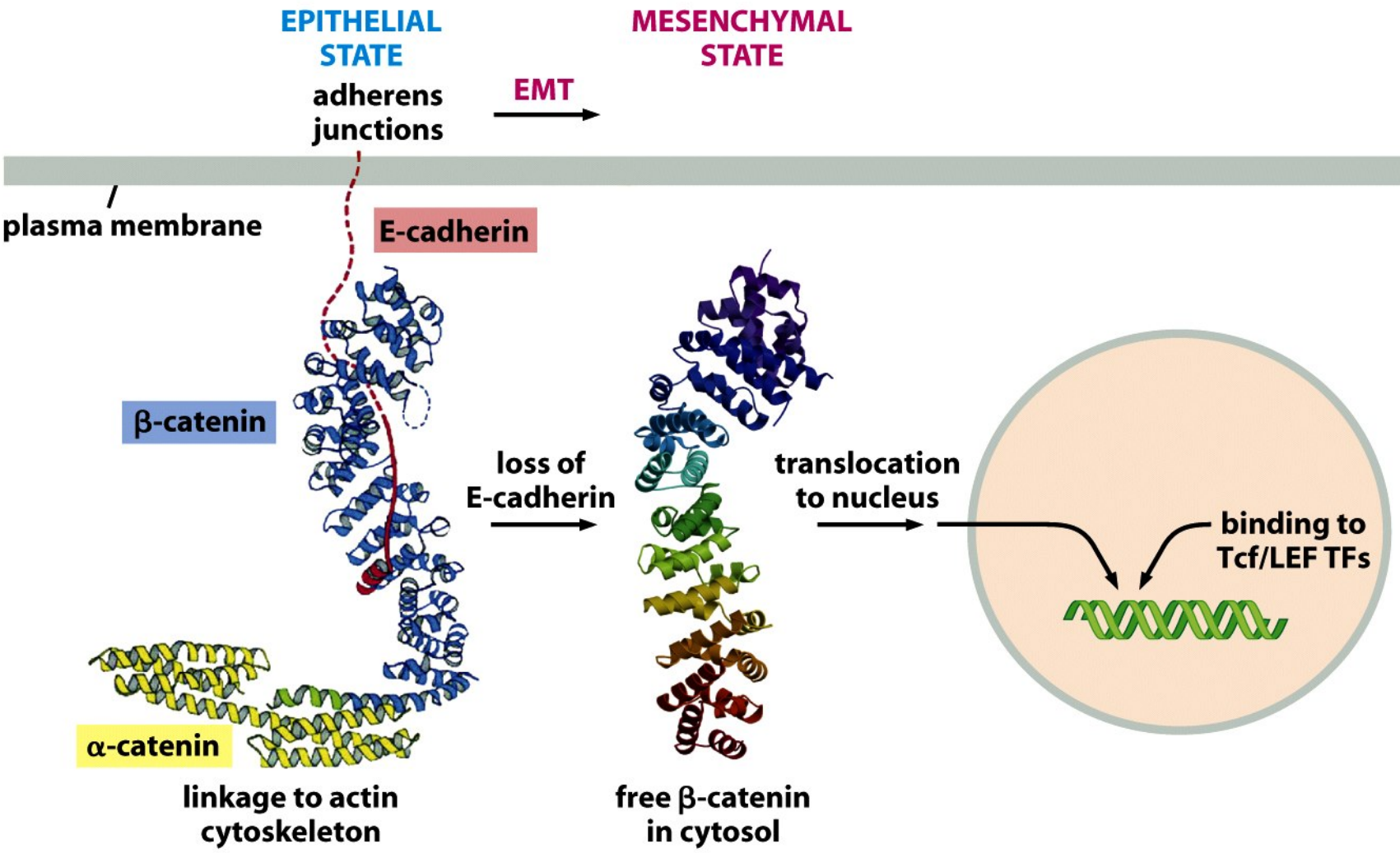


Figure 14-14c The Biology of Cancer (© Garland Science 2007)

β-catenin translocates to nuclei

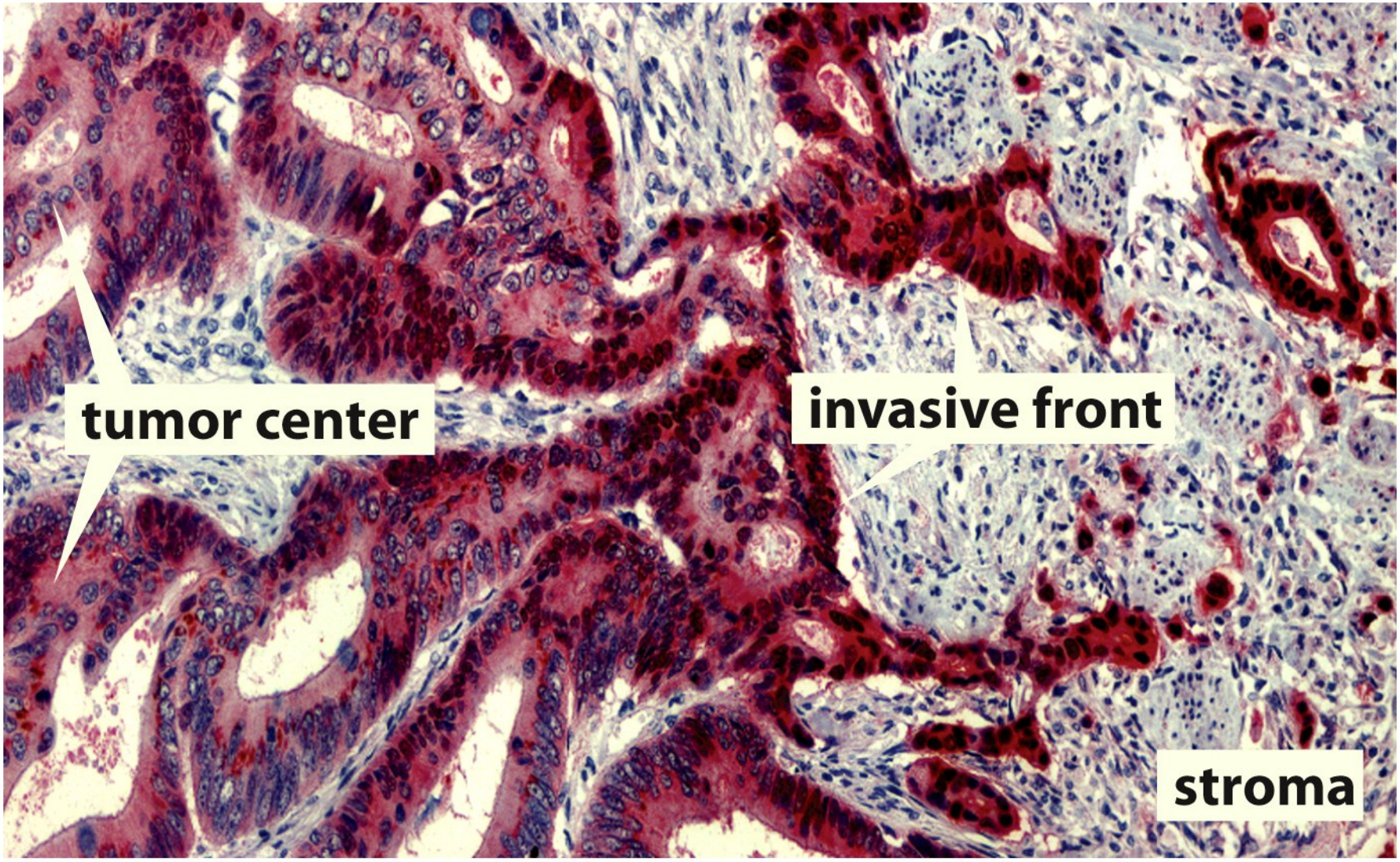


Figure 14-14b The Biology of Cancer (© Garland Science 2007)

Biochemical changes accompanying EMT

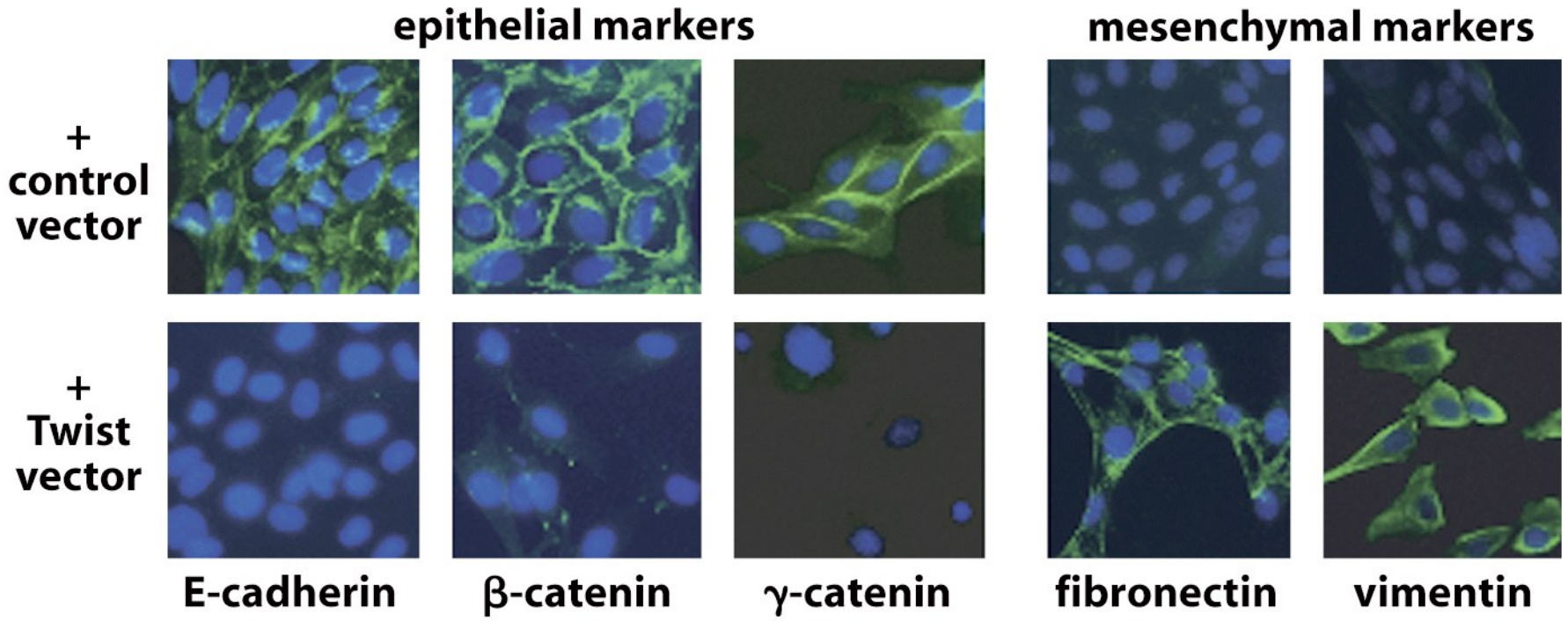


Figure 14-15a The Biology of Cancer (© Garland Science 2007)

Expressing Twist transcription factor in MDCK cells induces fibronectin and vimentin

Confirmation by immunoblot

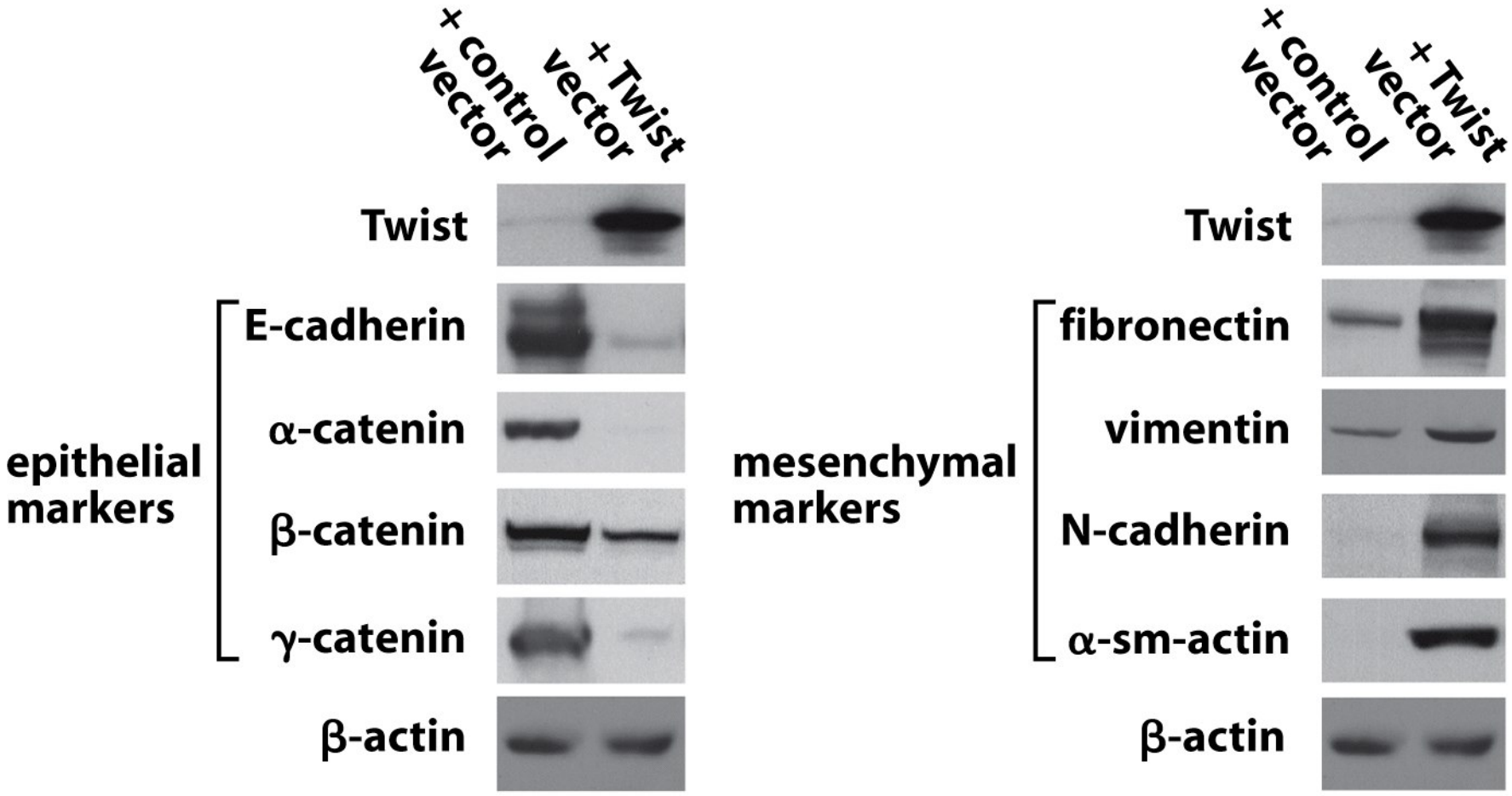


Figure 14-15b The Biology of Cancer (© Garland Science 2007)

E cadherin N-cadherin switch plays a pivotal role in EMT

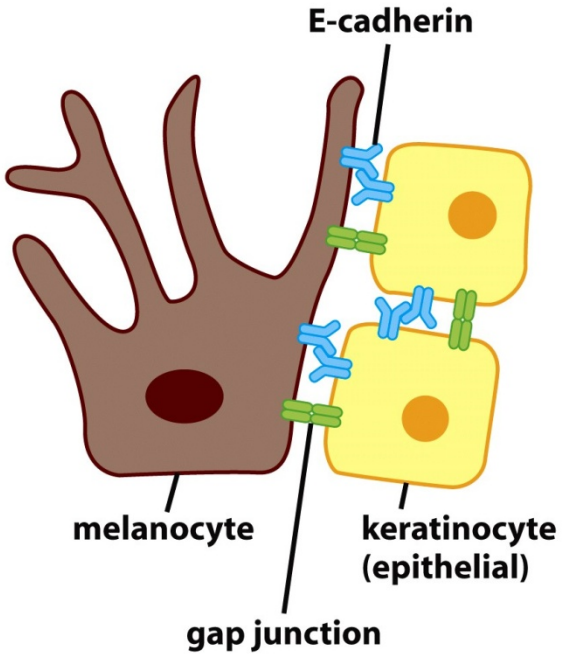


Figure 14-16a The Biology of Cancer (© Garland Science 2007)

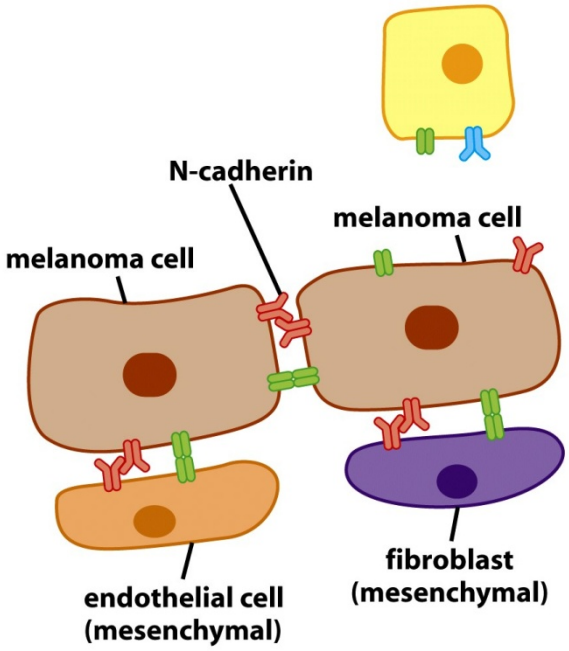


Figure 14-16b The Biology of Cancer (© Garland Science 2007)

E-cadherin to N-cadherin switch permits new associations with mesenchymal cells. Similarly gastrulation as well as HGF embryonic ectodermal layer—emigration and dermal precursors from primitive dermomyotome. Homotypic interactions permit invasion into and establishment of epithelial cells in mesenchyme. N-cadherin bonds weaker than E-cadherin.

HGF (stromal factor) induces scattering in MDCK cells

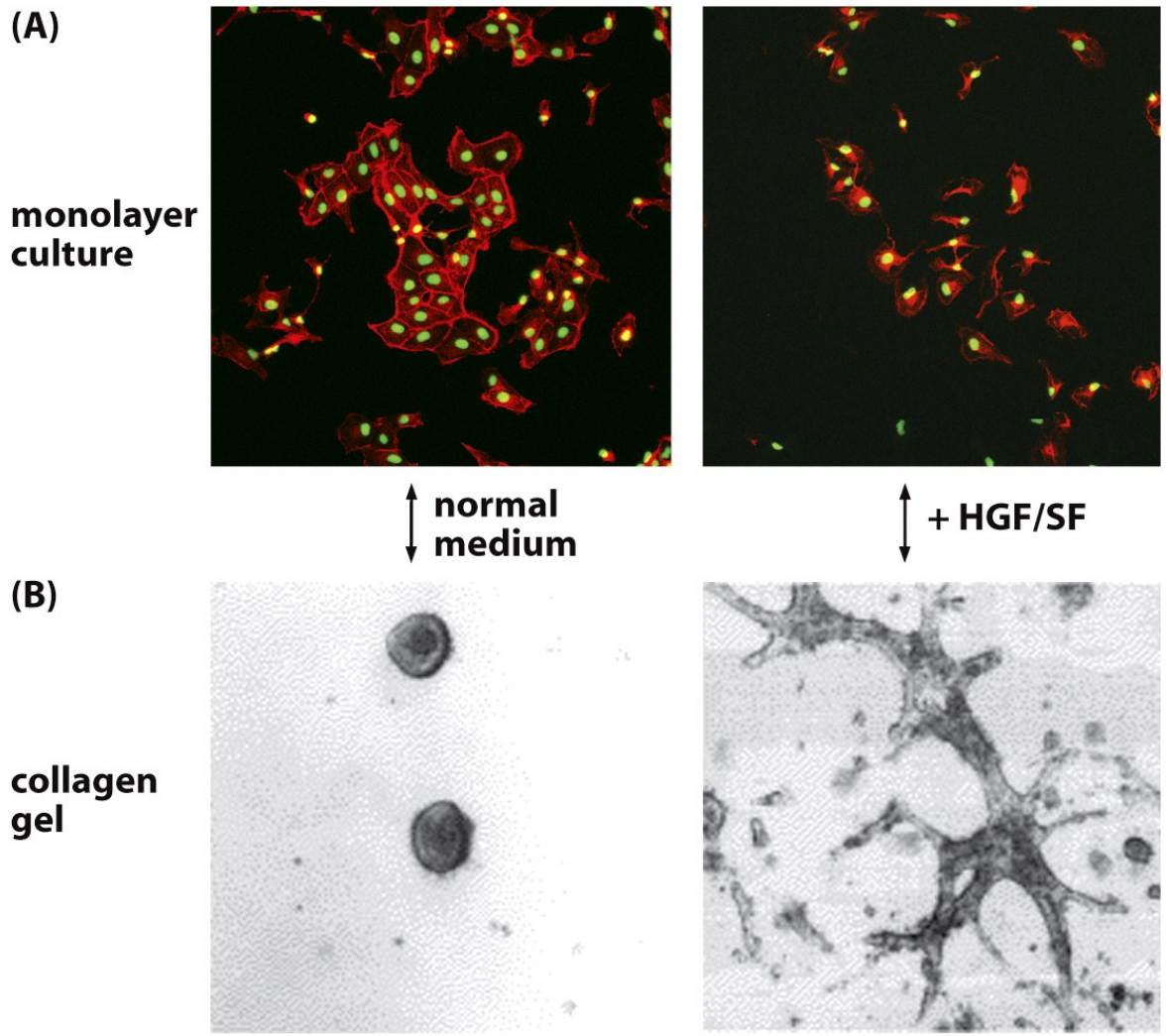


Figure 14-24 The Biology of Cancer (© Garland Science 2007)

EMT induced by stromal signals and therefore reversible

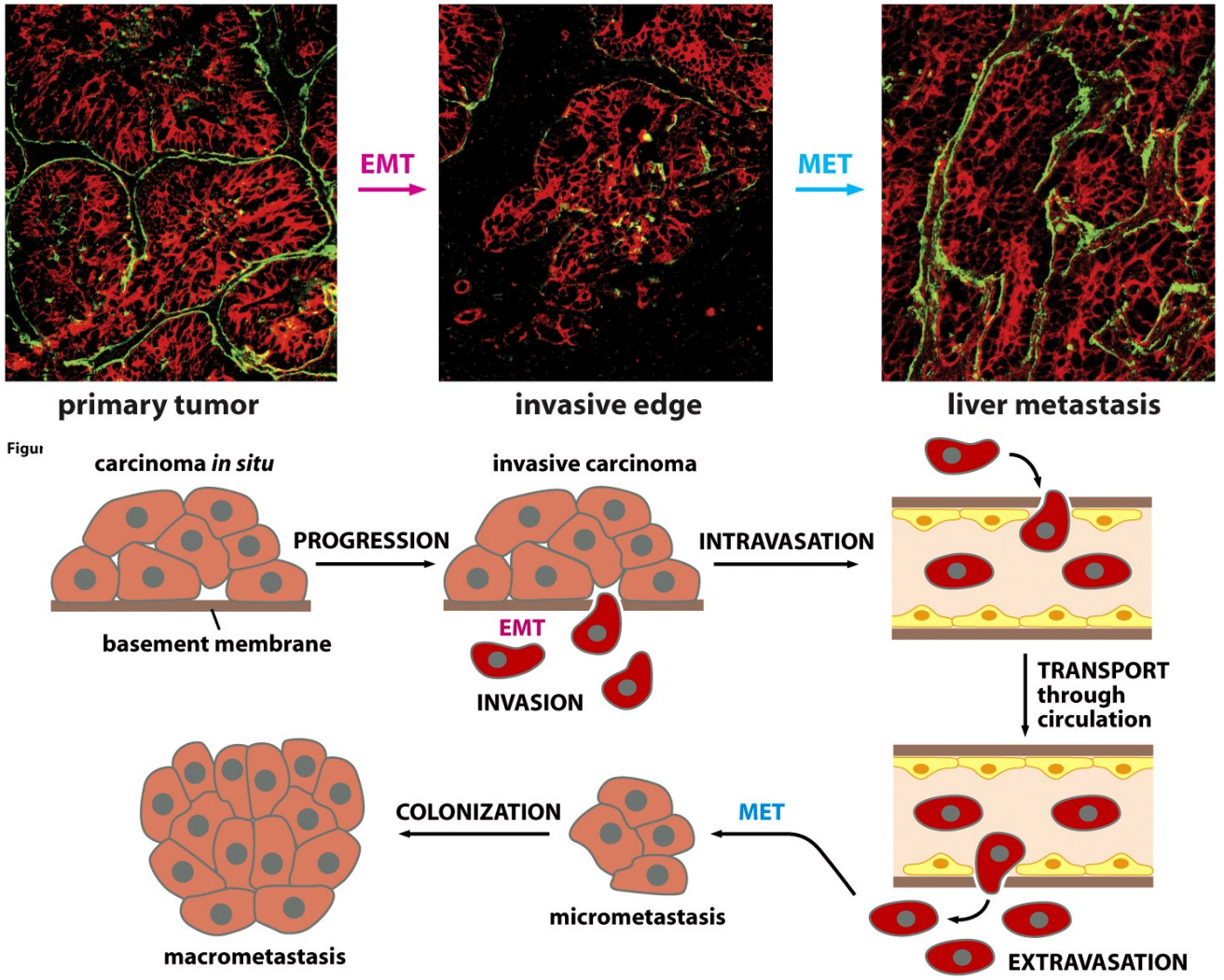


Figure 14-17b The Biology of Cancer (© Garland Science 2007)

Why do 2ndary tumors resemble primary tumors and not mesenchyme? Reversibility may be the key

Histopathology of primary and secondary tumors often strikingly similar.

In fact used by pathologists to

a) Identify tumors of unknown origin

and

b) Dismiss the concept of EMT!

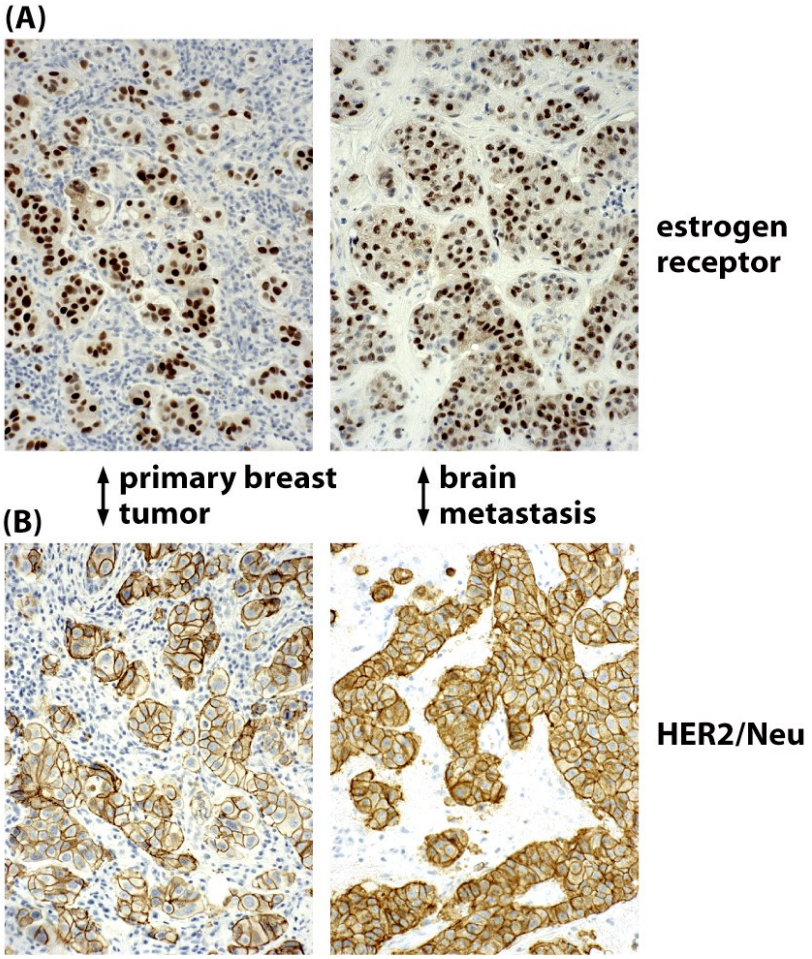


Figure 14-18 The Biology of Cancer (© Garland Science 2007)

Exposing the wolf

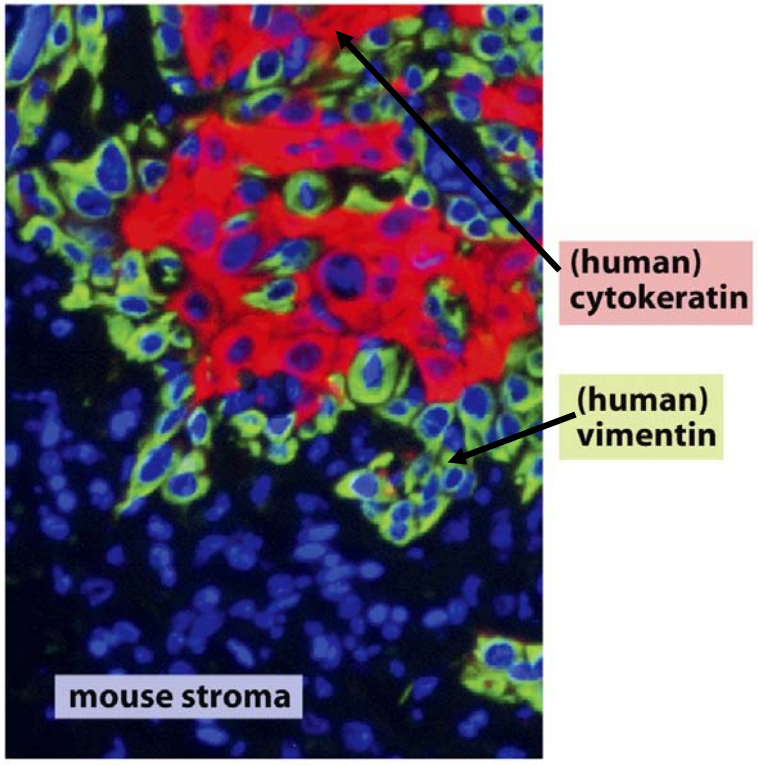


Figure 14-19c The Biology of Cancer (© Garland Science 2007)

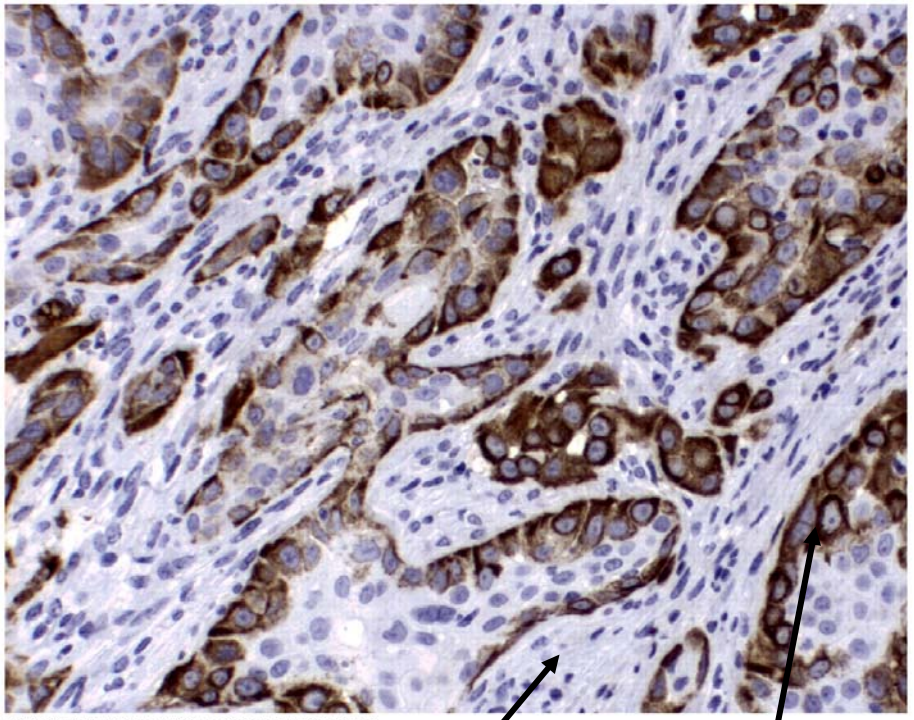


Figure 14-19d The Biology of Cancer (© Garland Science 2007)

EMT at invasive edge of **human** mammary epithelial cells in **mouse** host.

Mouse stroma

Human vimentin

How do stromal cells control EMT?
Role of TGF-β

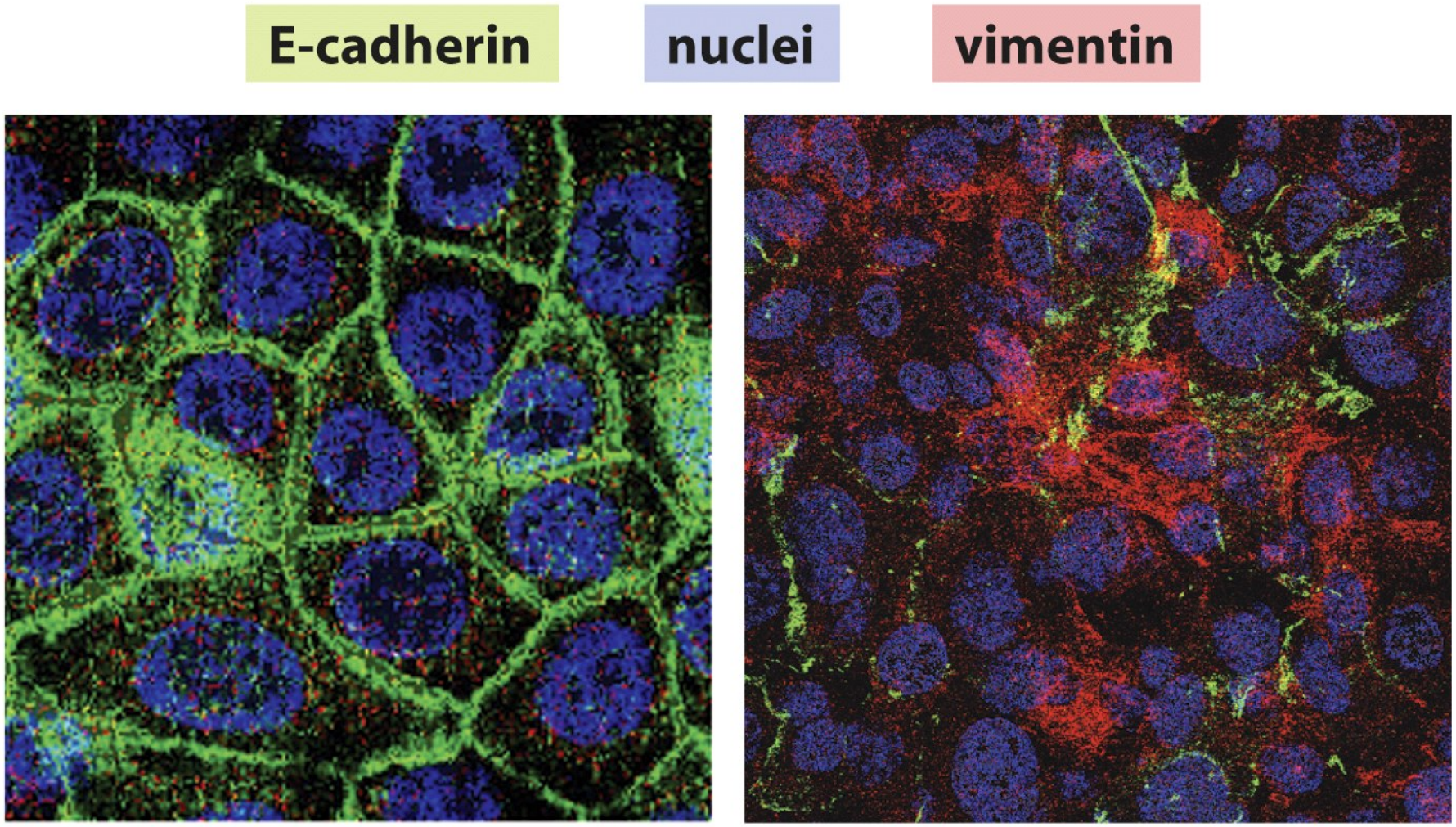


Figure 14-20a The Biology of Cancer (© Garland Science 2007)

Macrophages: role in invasion and metastasis

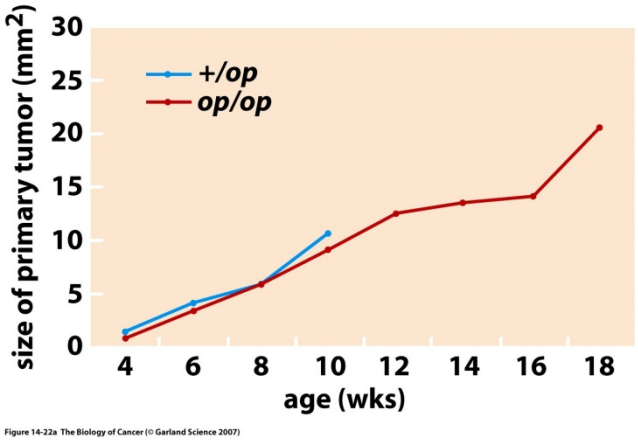


Figure 14-22a The Biology of Cancer (© Garland Science 2007)

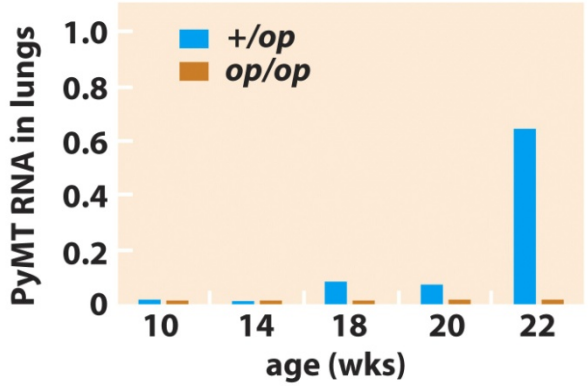


Figure 14-22c The Biology of Cancer (© Garland Science 2007)

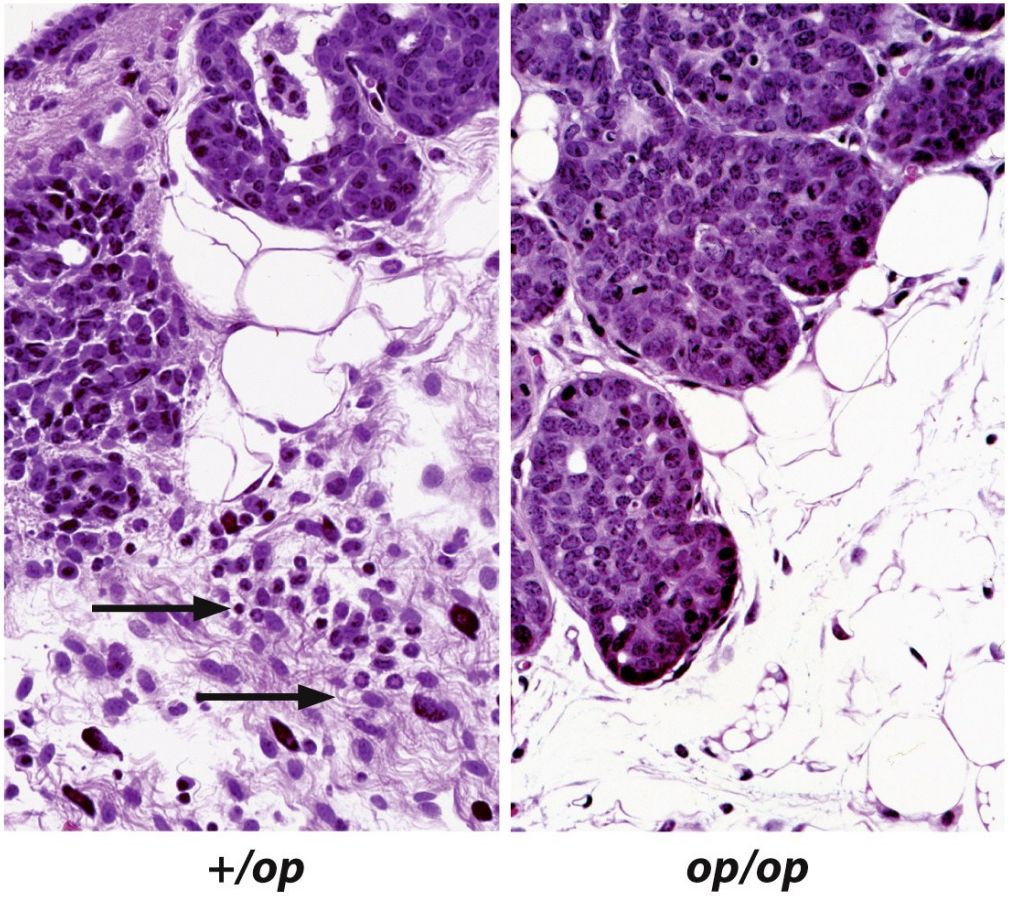


Figure 14-22b The Biology of Cancer (© Garland Science 2007)

+/op mice make CSF1 and recruit macrophages to tumors-mets form in lungs
op/op mice lack CSF1 and cannot recruit macrophages- mets do not form in lungs

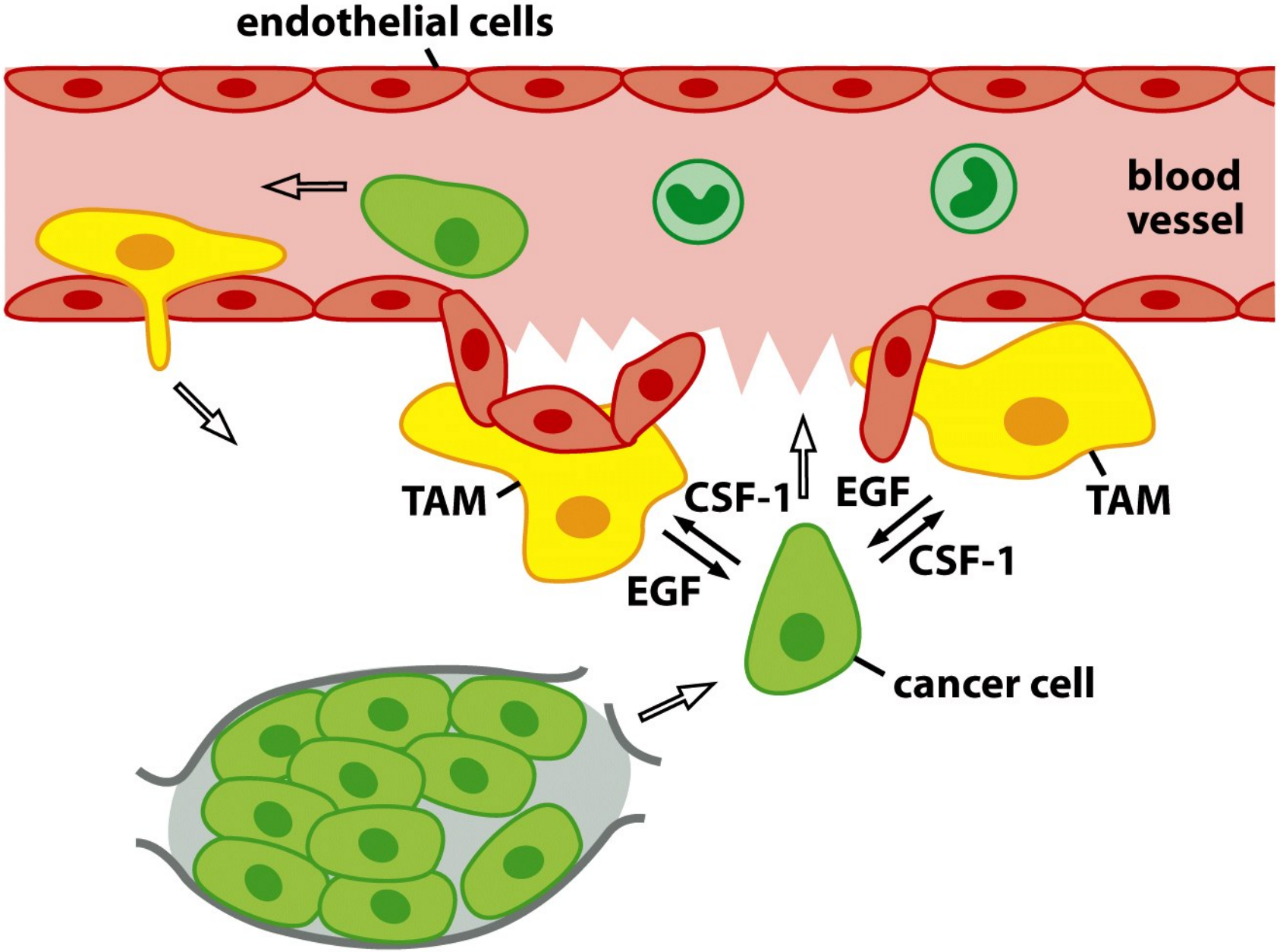


Figure 14-23c The Biology of Cancer (© Garland Science 2007)

Signals that trigger EMT

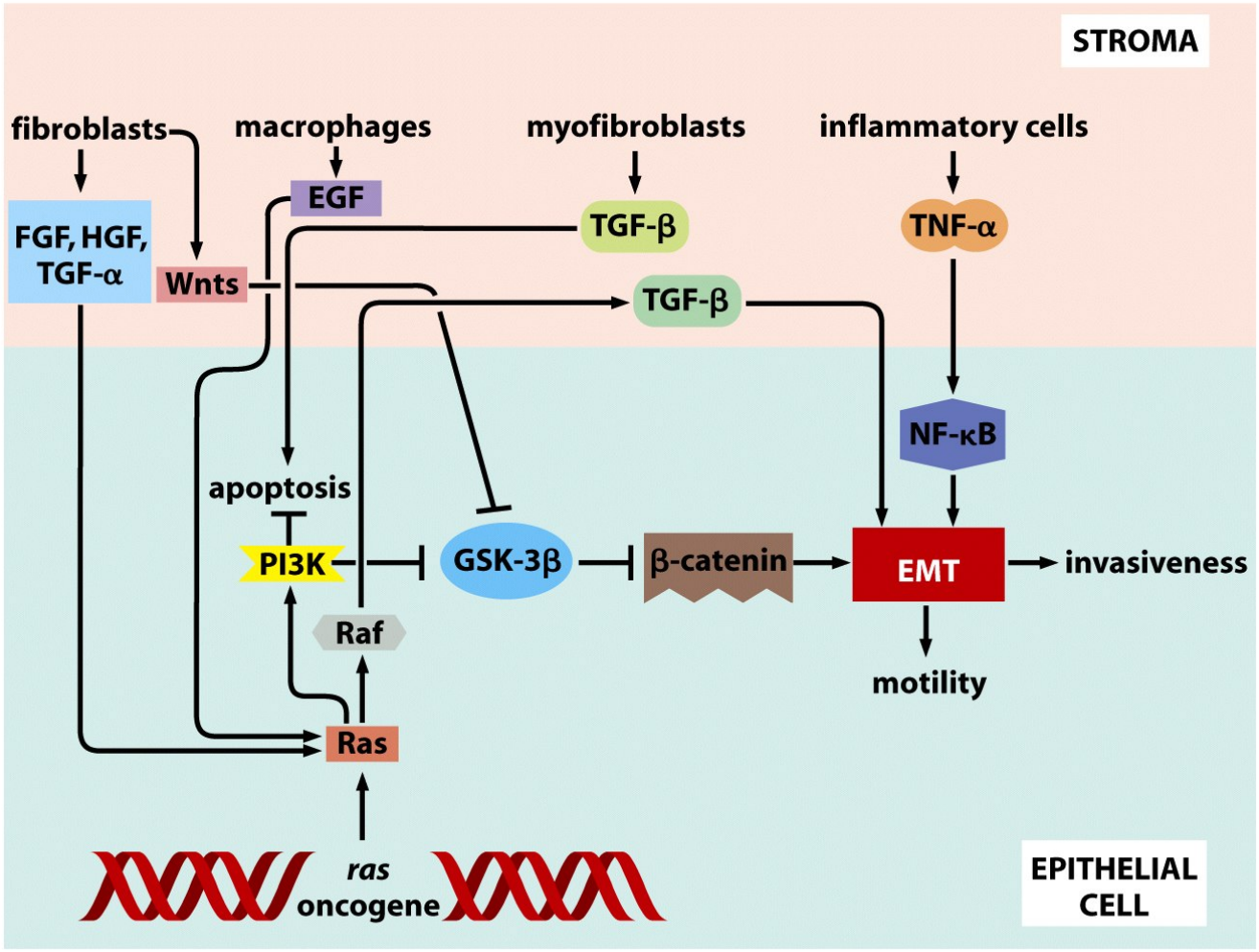


Figure 14-25 The Biology of Cancer (© Garland Science 2007)

Embryonic transcription factors programming EMT

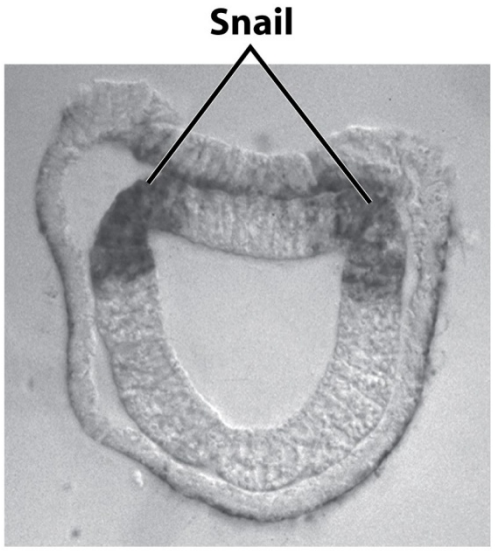


Figure 14-26a The Biology of Cancer (© Garland Science 2007)

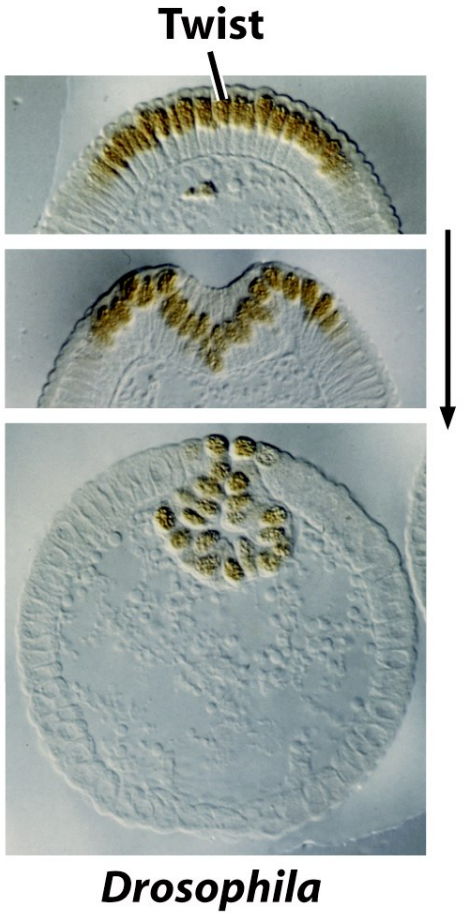


Figure 14-26b The Biology of Cancer (© Garland Science 2007)

Figure 14-26c The Biology of Cancer (© Garland Science 2007)

Transient expression Slug in wound healing

Monolayer of keratinocytes scraped to induce wound

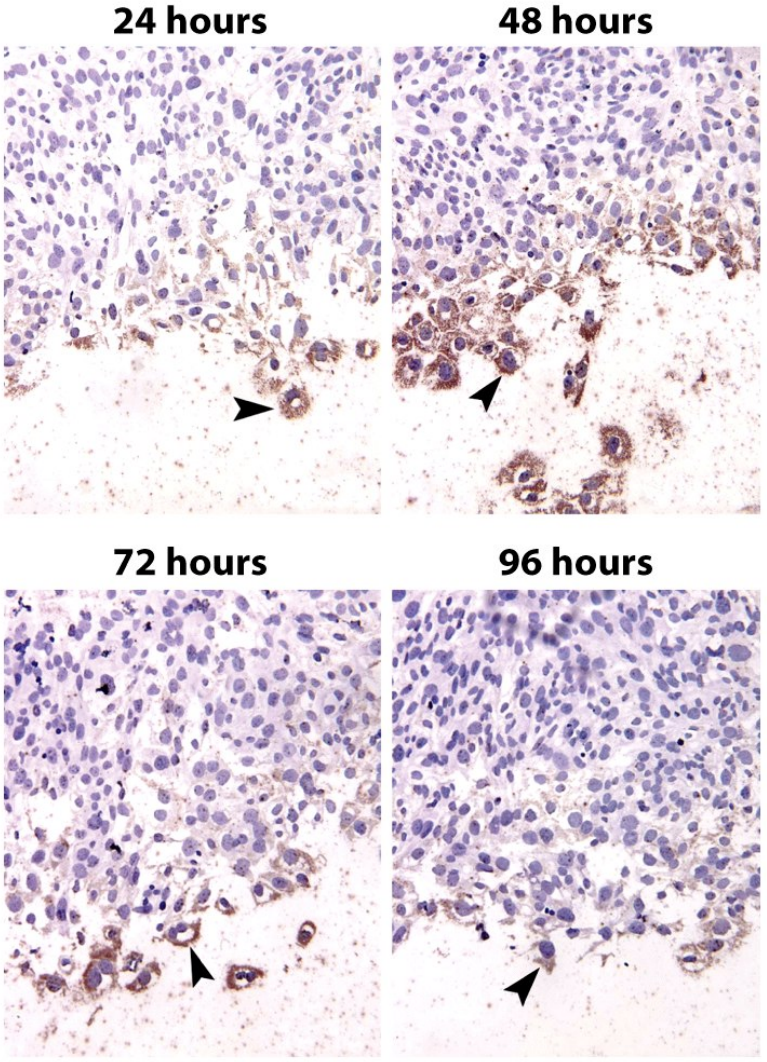


Figure 14-27 The Biology of Cancer (© Garland Science 2007)

Expression EMT-inducing transcription factors in tumors

Slug binds to
E-box sequence
in E-cad promoter

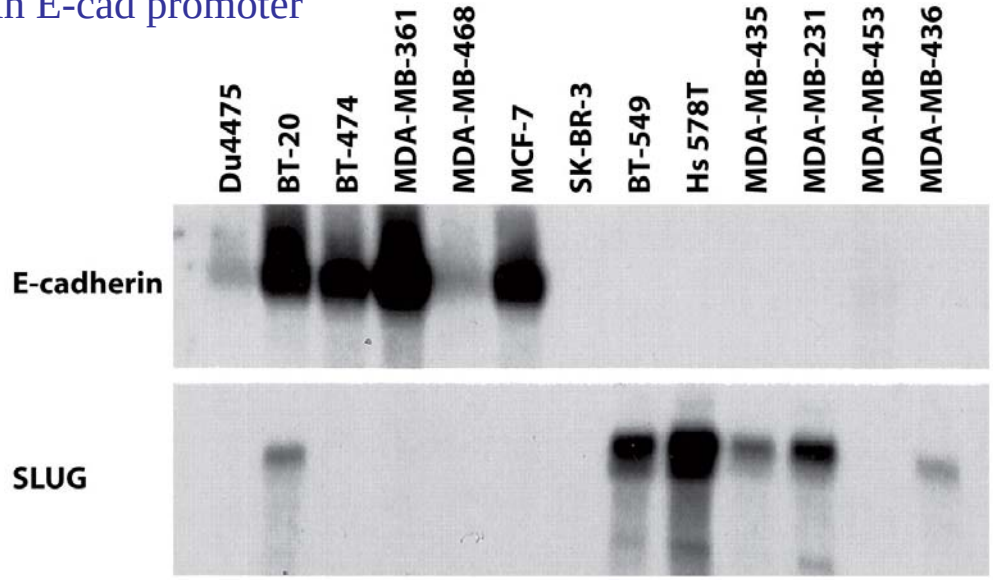


Figure 14-28a The Biology of Cancer (© Garland Science 2007)

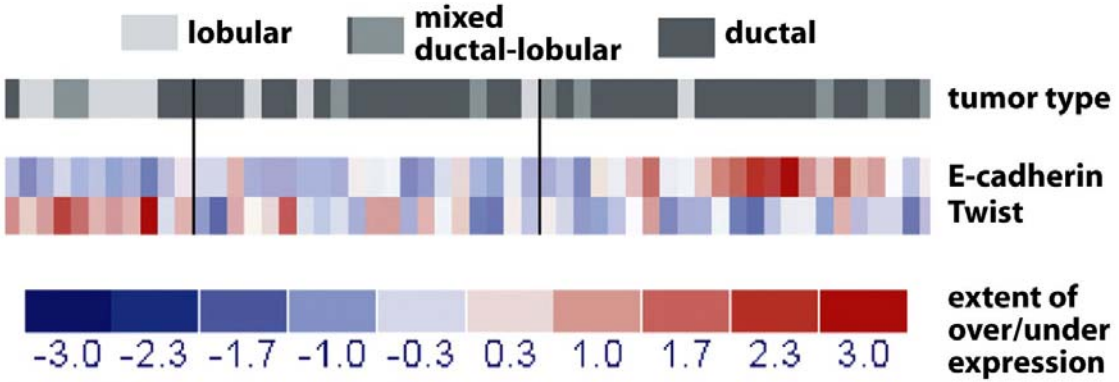


Figure 14-28b The Biology of Cancer (© Garland Science 2007)

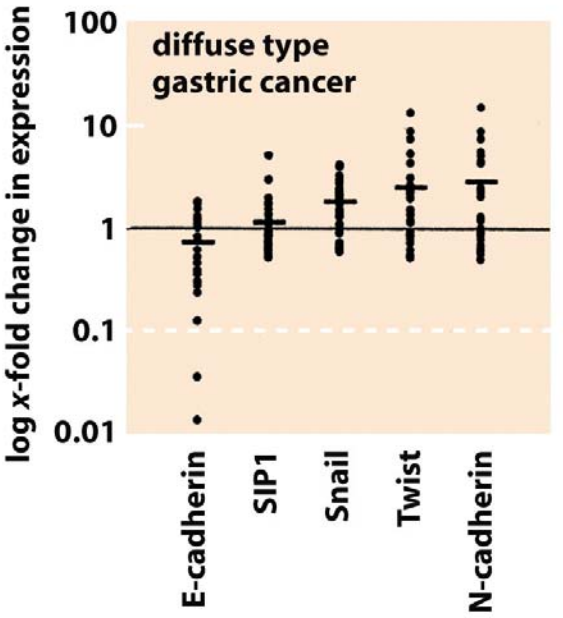
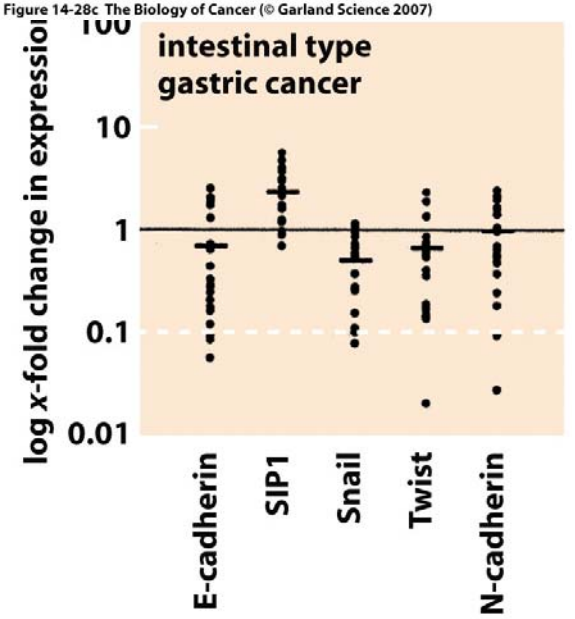


Figure 14-28c The Biology of Cancer (© Garland Science 2007)



Similarities between EMT embryogenesis and tumor progression

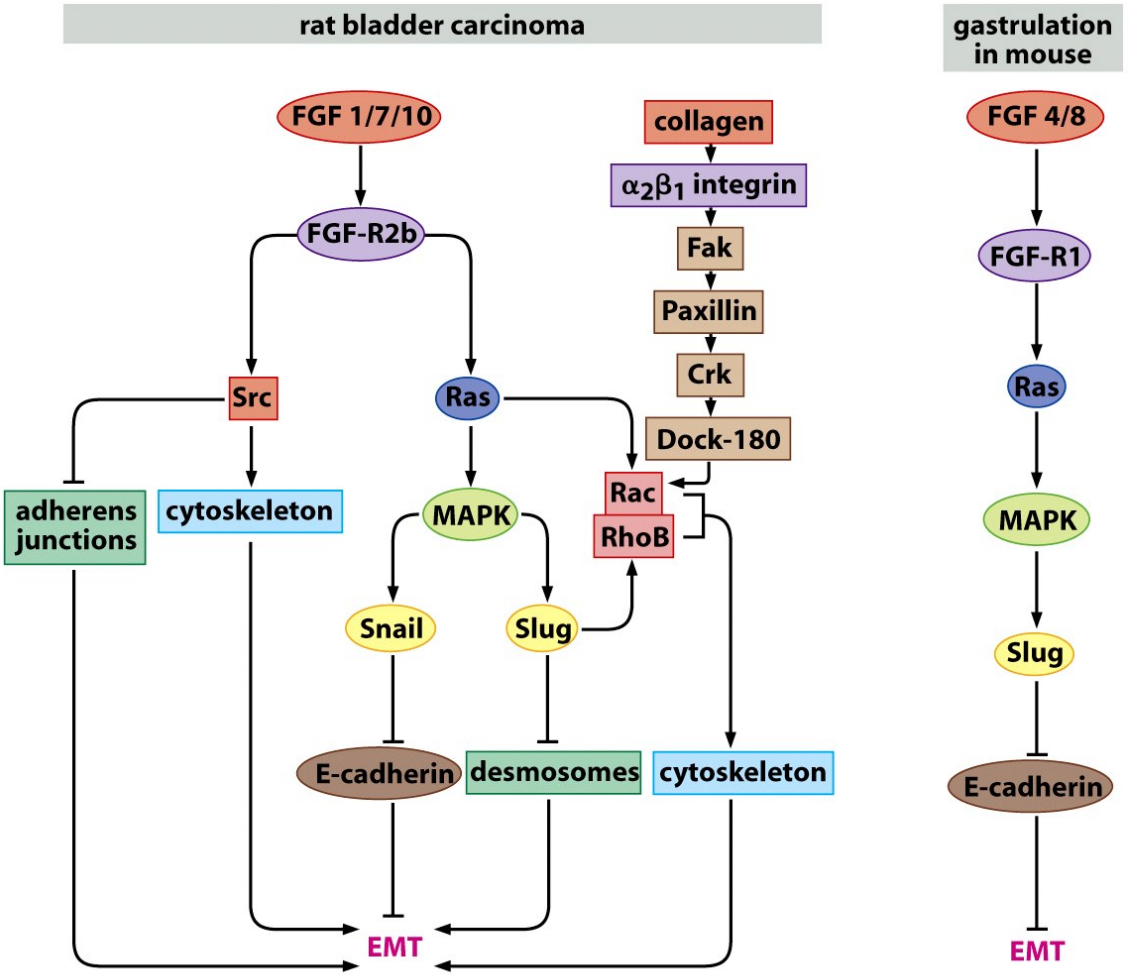


Figure 14-30 The Biology of Cancer (© Garland Science 2007)

Extracellular proteases and invasiveness

Mammary carcinoma in MMTV-polyoma middle T mouse

proteolysis

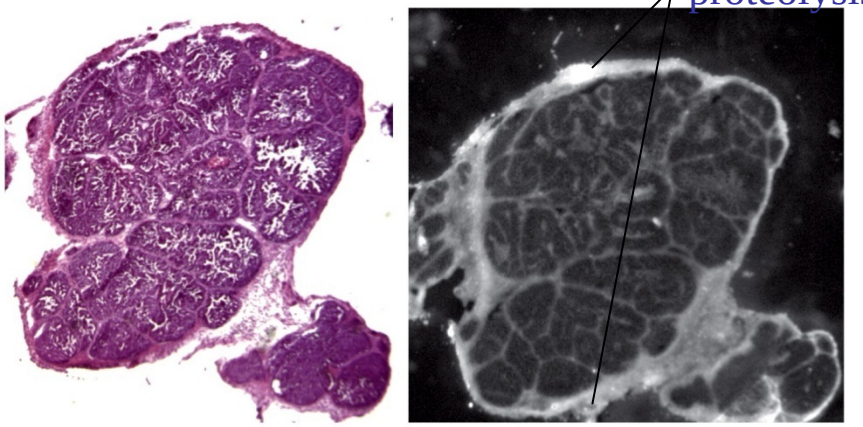


Figure 14-31a The Biology of Cancer (© Garland Science 2007)

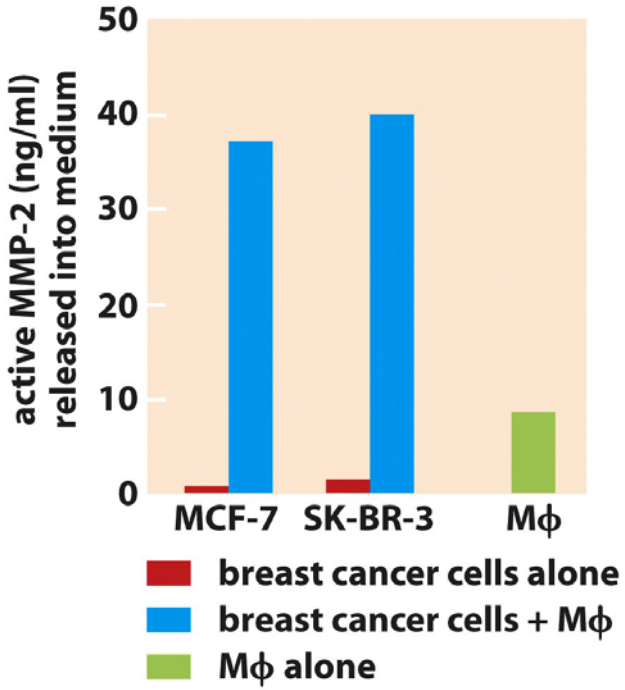


Figure 14-31c The Biology of Cancer (© Garland Science 2007)

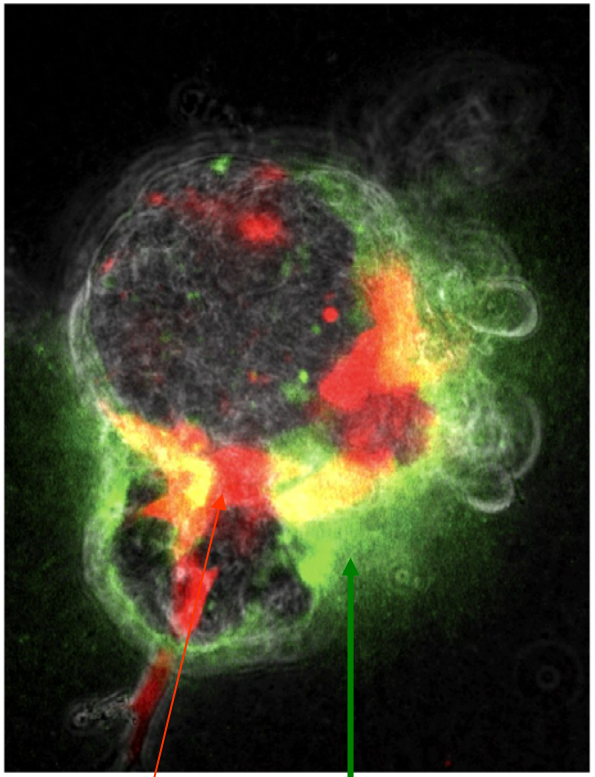


Figure 14-31b The Biology of Cancer (© Garland Science 2007)

fibroblasts

collagen IV cleavage (inhibited by MMP inhibitors)

Podosomes and focal degradation of ECM (MT1-MMP)

Actin (red)

FITC - fibronectin (green)

Degradation fibronectin (black)

Colocalization podosome and degradation (arrow)

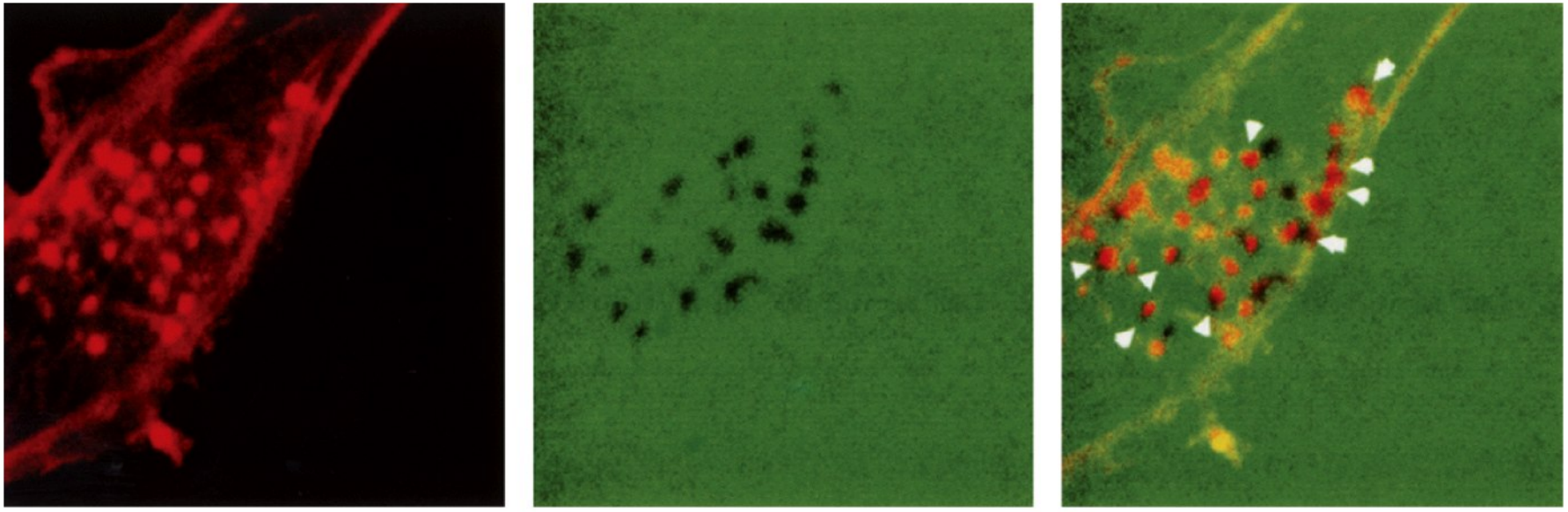


Figure 14-32 The Biology of Cancer (© Garland Science 2007)

Protease cascade induced by stroma

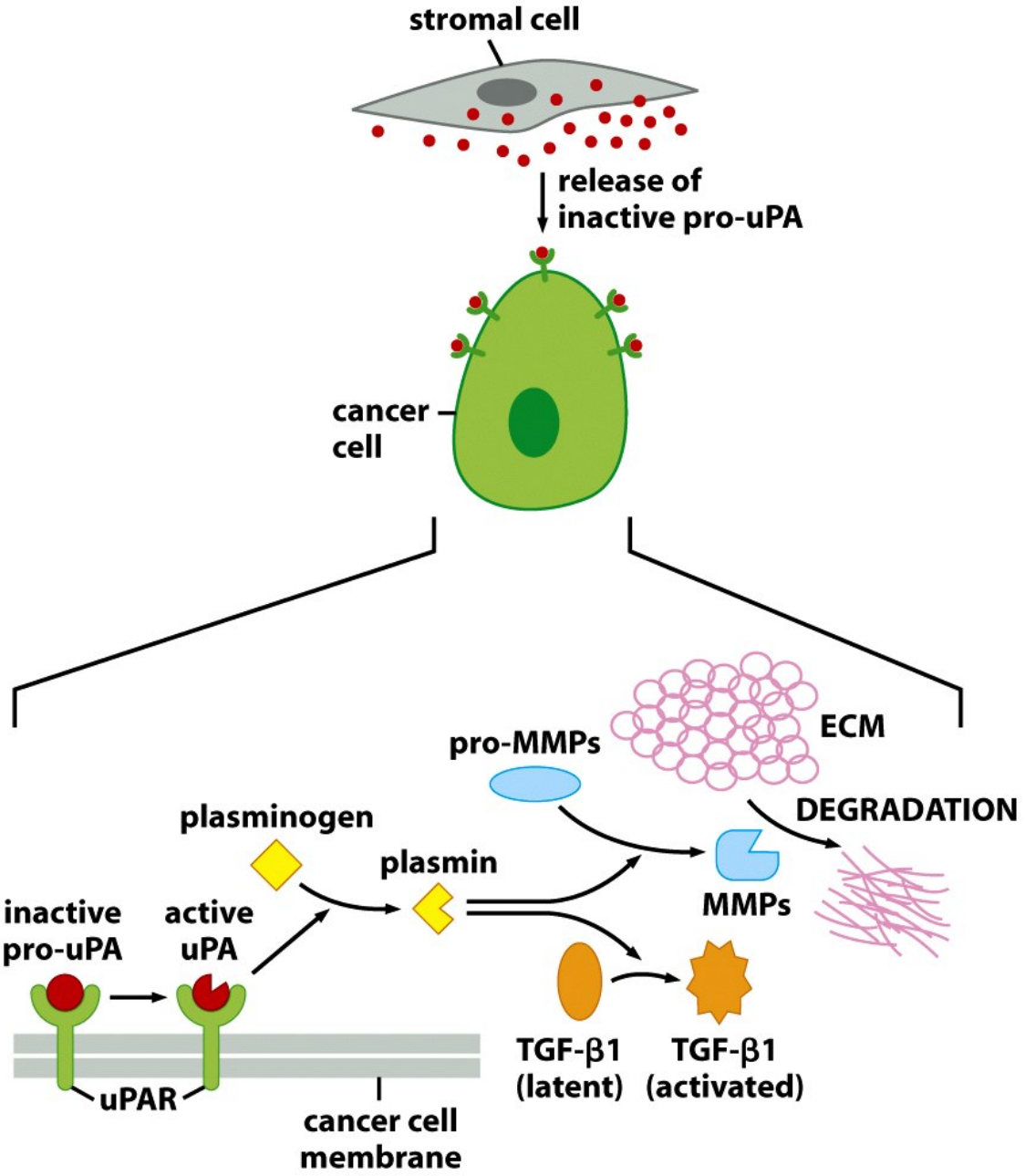


Figure 14-34 The Biology of Cancer (© Garland Science 2007)

Rho-C and metastasis

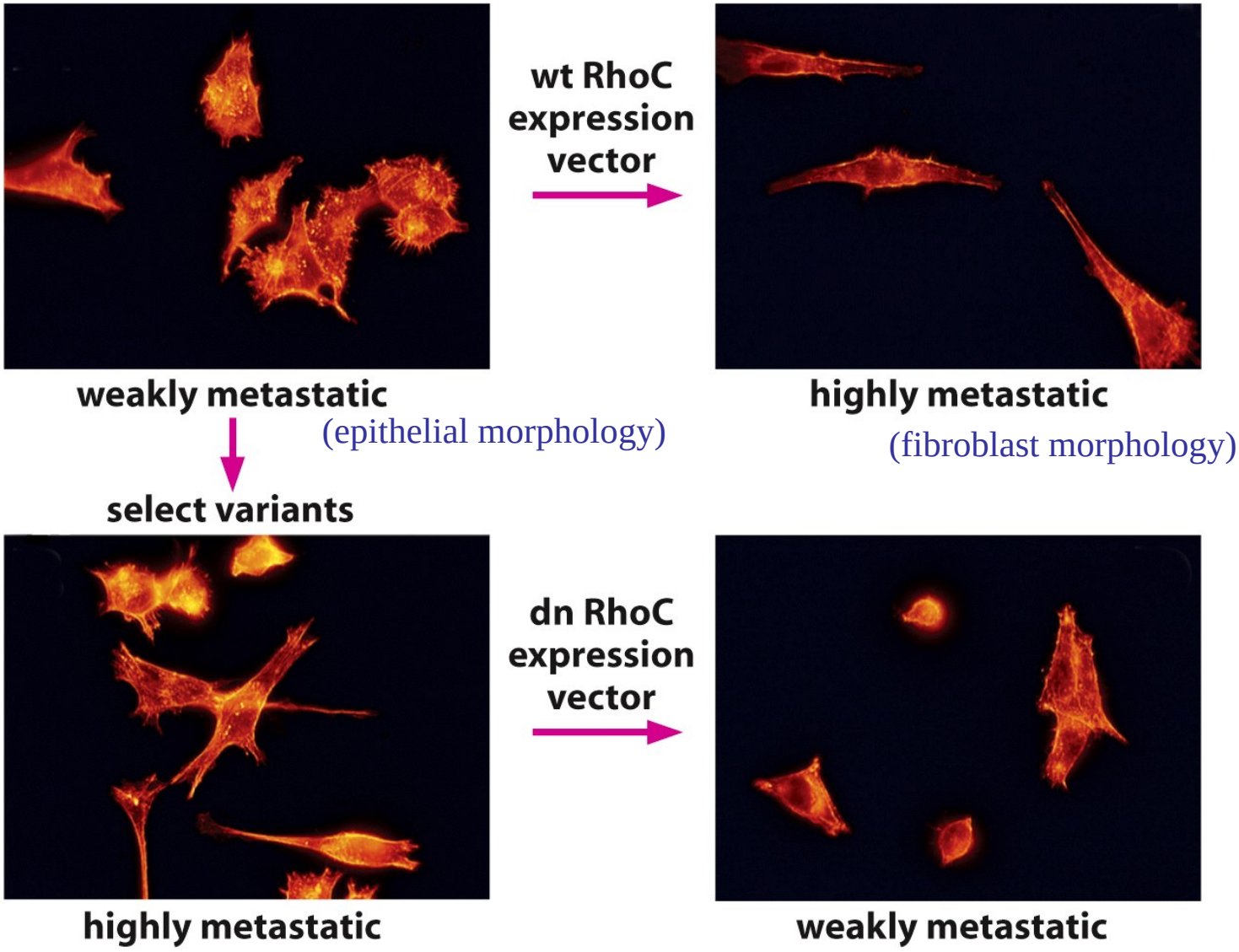


Figure 14-40 The Biology of Cancer (© Garland Science 2007)

Metastatic dissemination via lymphatics

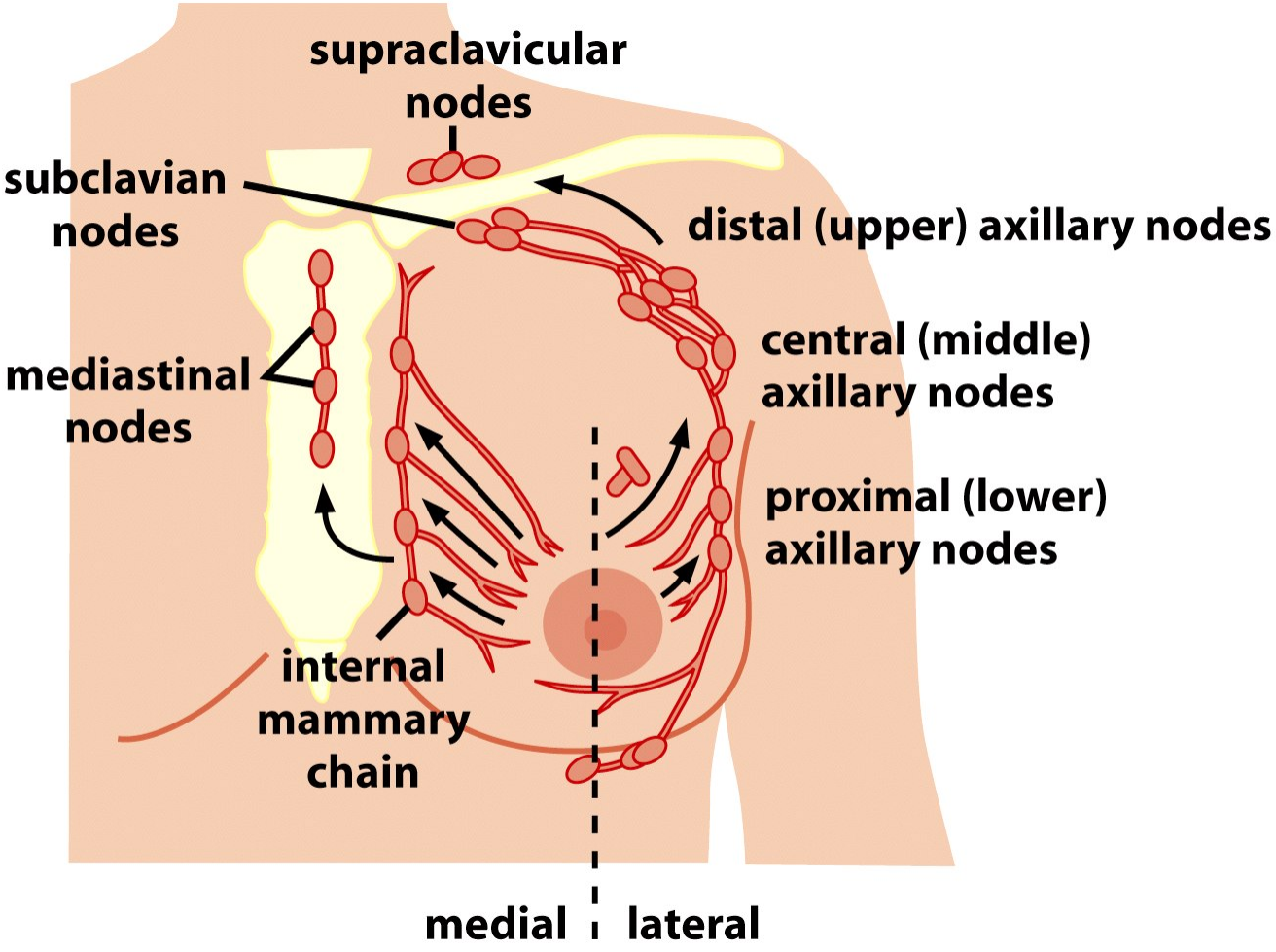


Figure 14-41a The Biology of Cancer (© Garland Science 2007)

Metastatic tropisms-seed and soil or hydrodynamics?

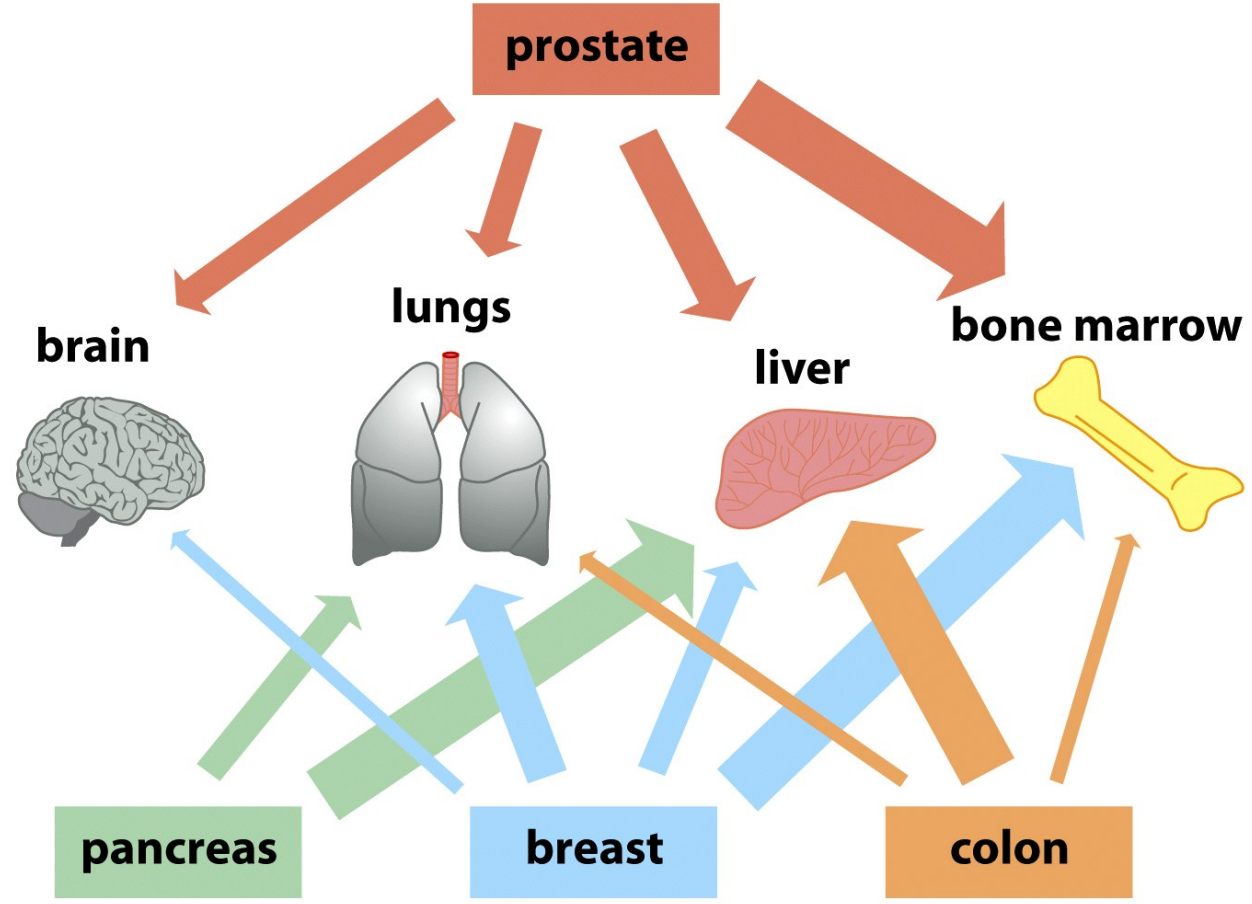


Figure 14-42 The Biology of Cancer (© Garland Science 2007)

Colorectal cell metastasis: a matter of drainage

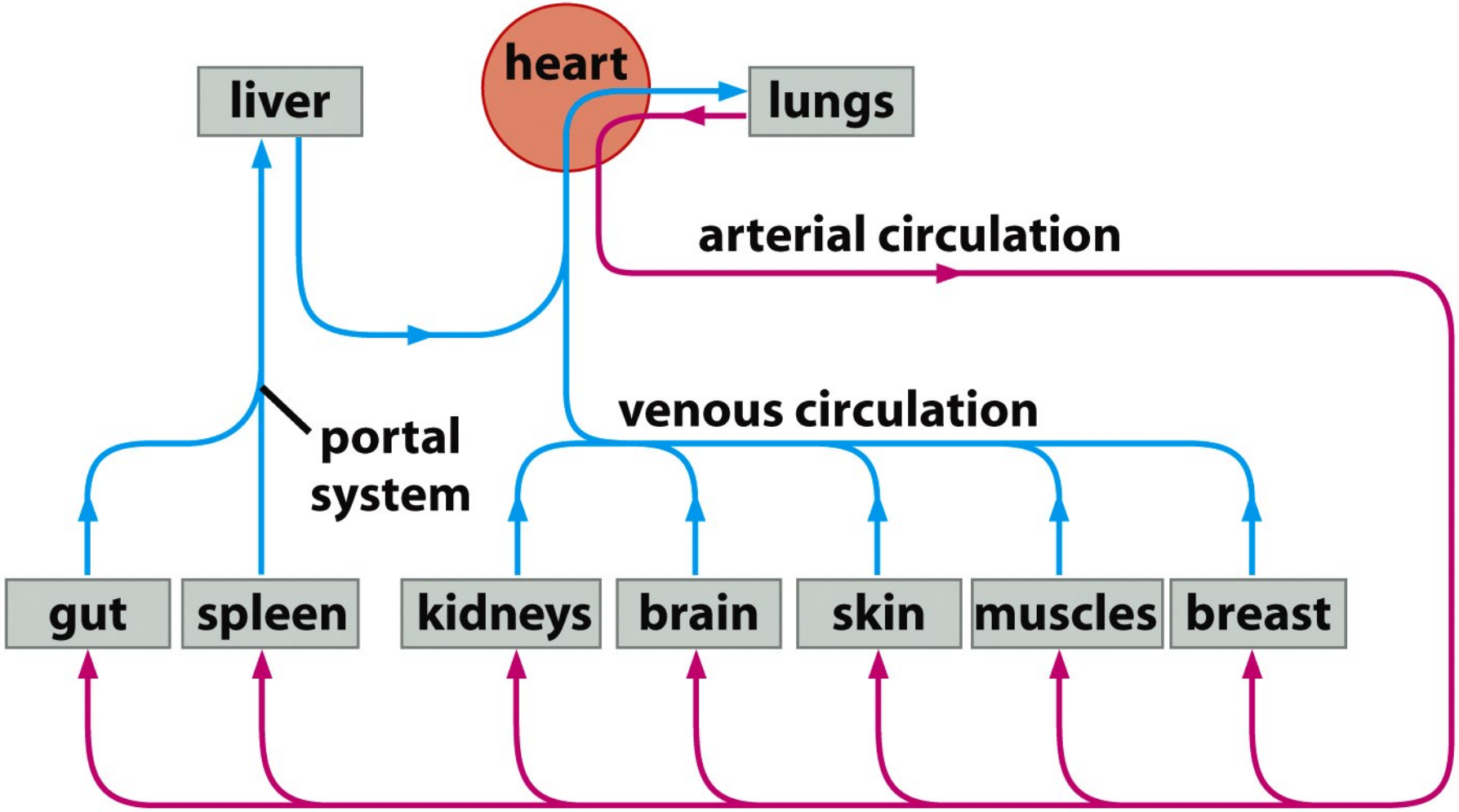


Figure 14-44 The Biology of Cancer (© Garland Science 2007)

Metastasis to bone (osteotropic metastasis) requires the subversion of osteoblasts and osteoclasts

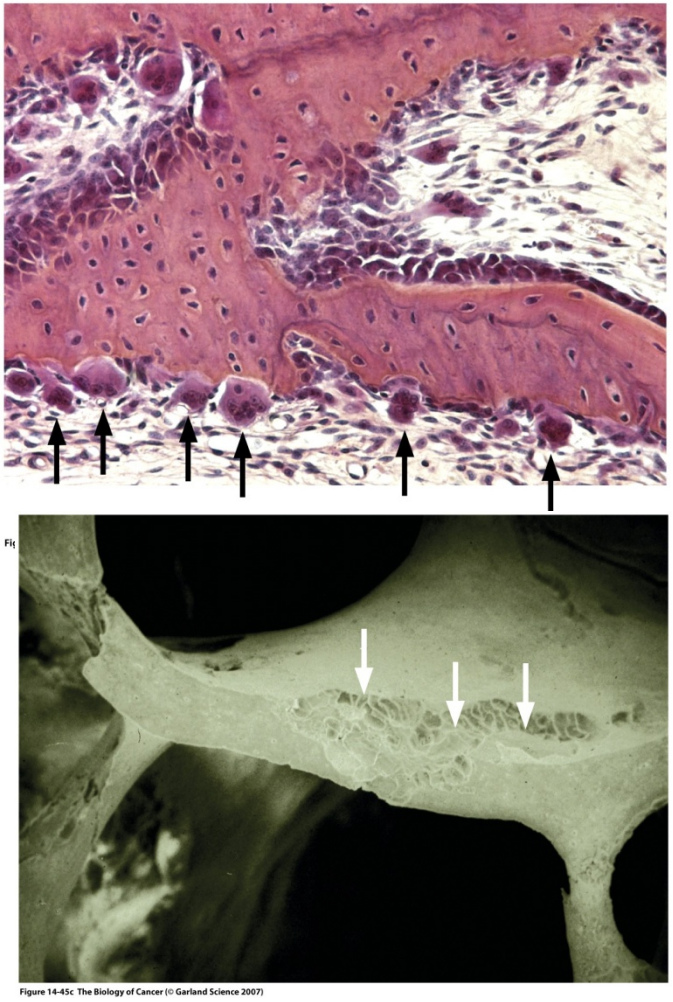


Figure 14-45c The Biology of Cancer (© Garland Science 2007)

Osteoclasts (arrowed) excavating jaw bone

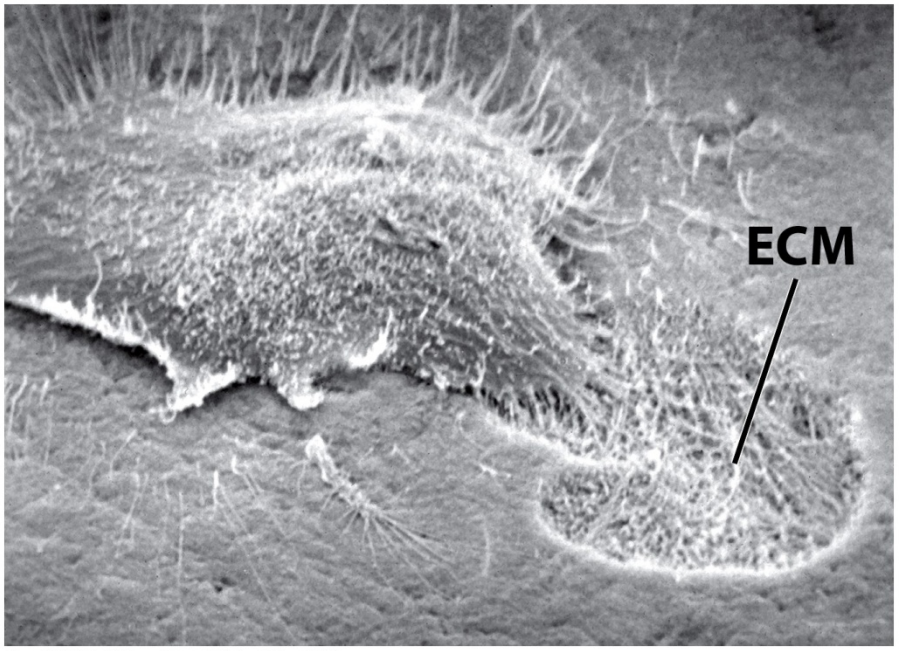


Figure 14-45b The Biology of Cancer (© Garland Science 2007)

Vicious cycle of osteoclast metastasis

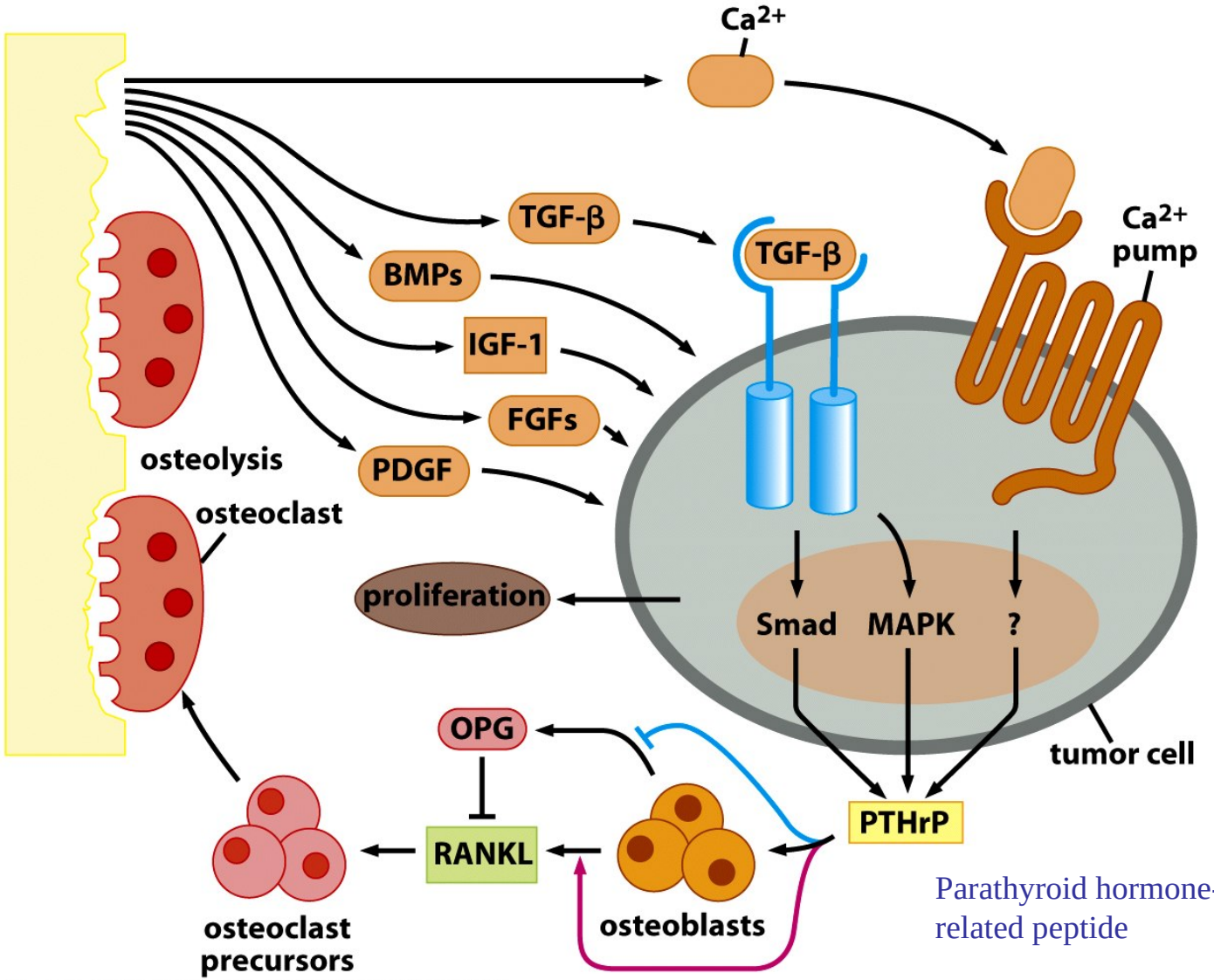


Figure 14-48 The Biology of Cancer (© Garland Science 2007)

Parathyroid hormone-related peptide

Are there metastasis genes?

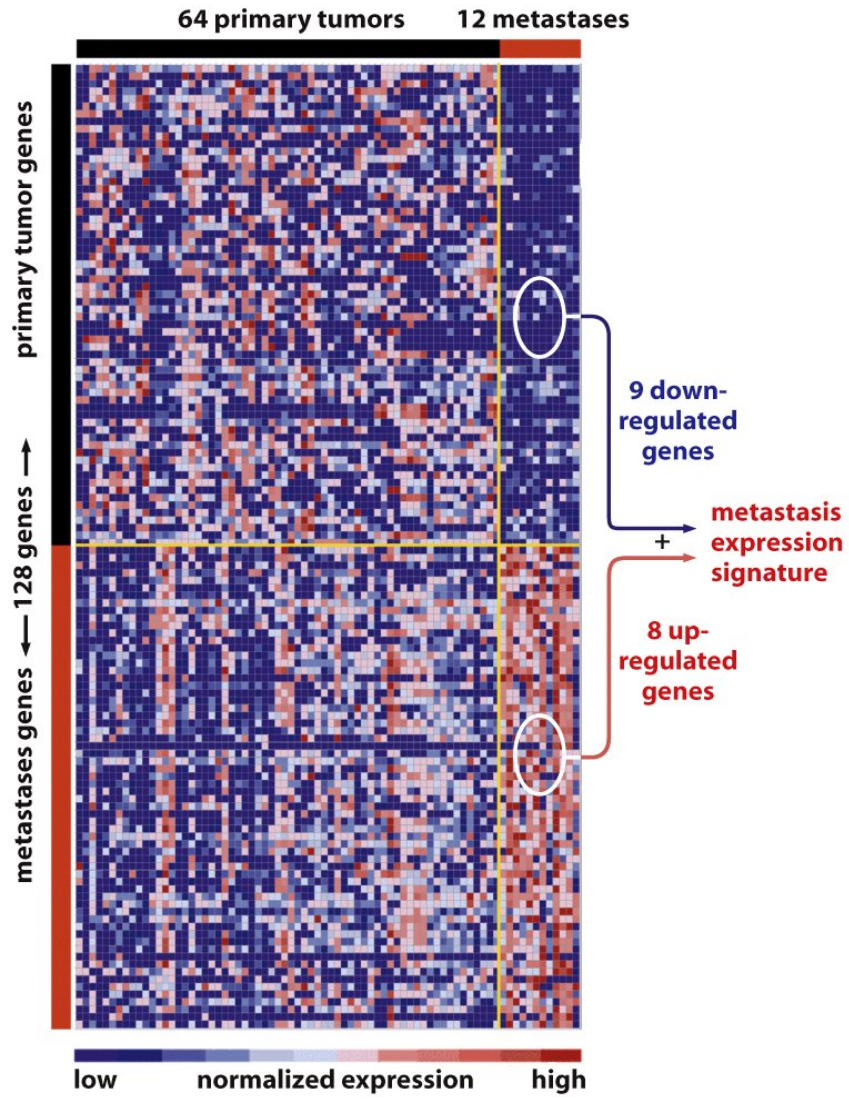


Figure 14-51a The Biology of Cancer (© Garland Science 2007)

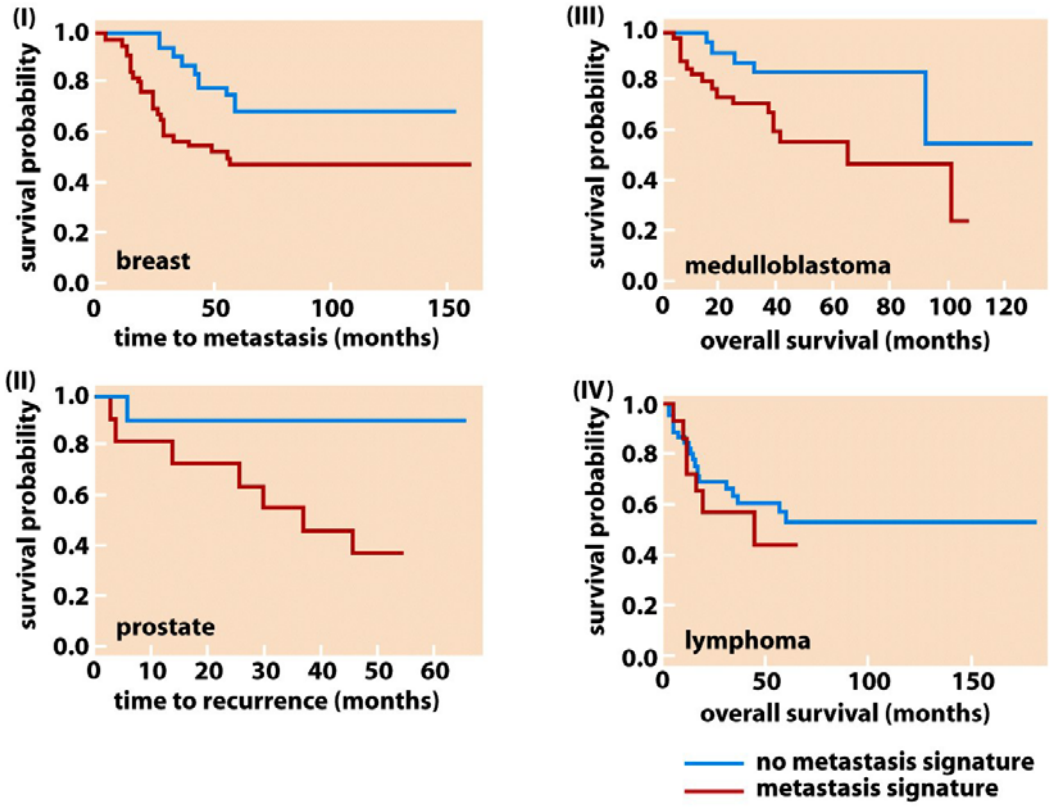
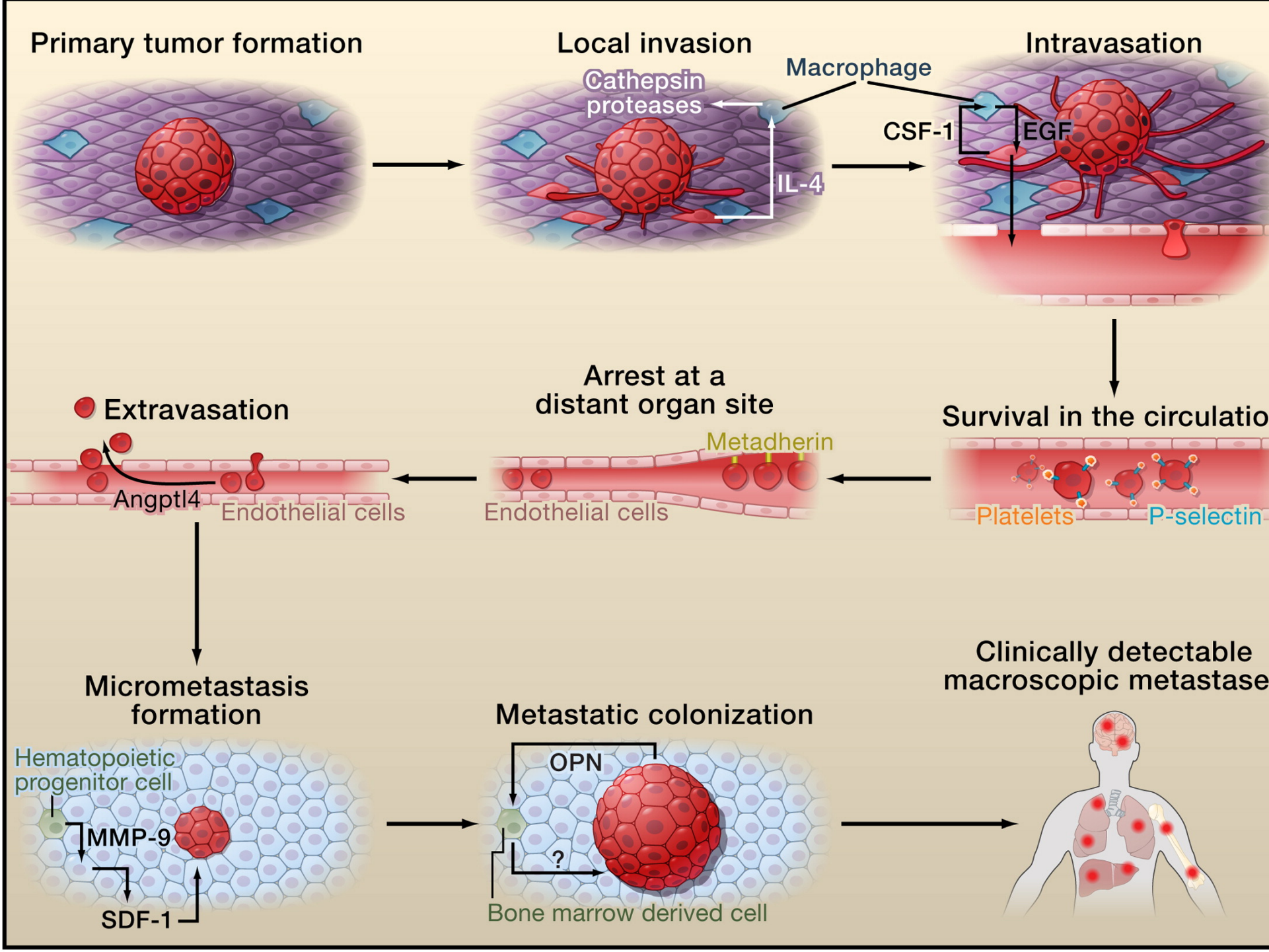
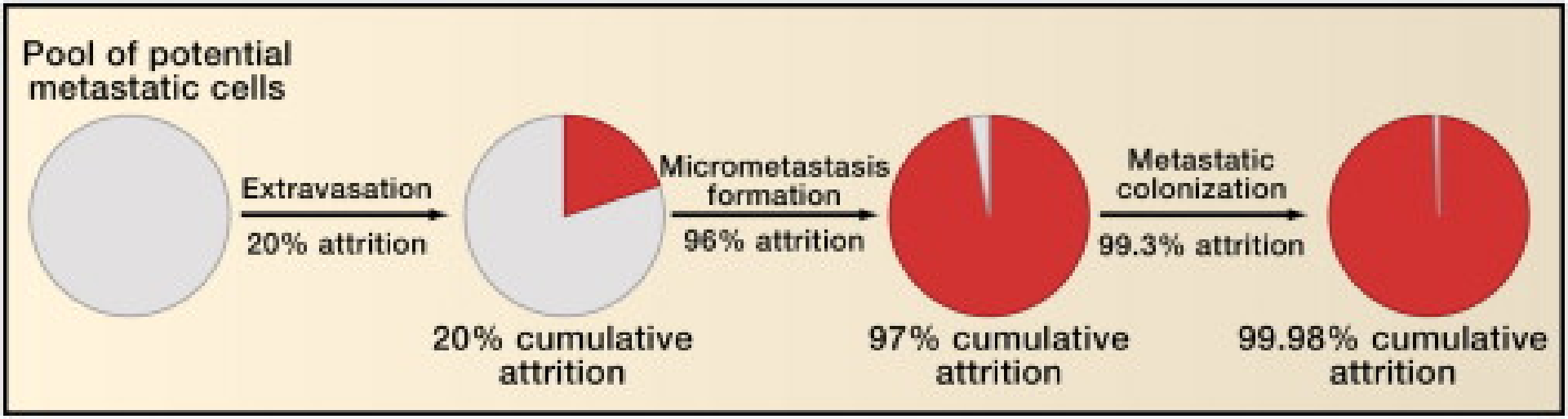
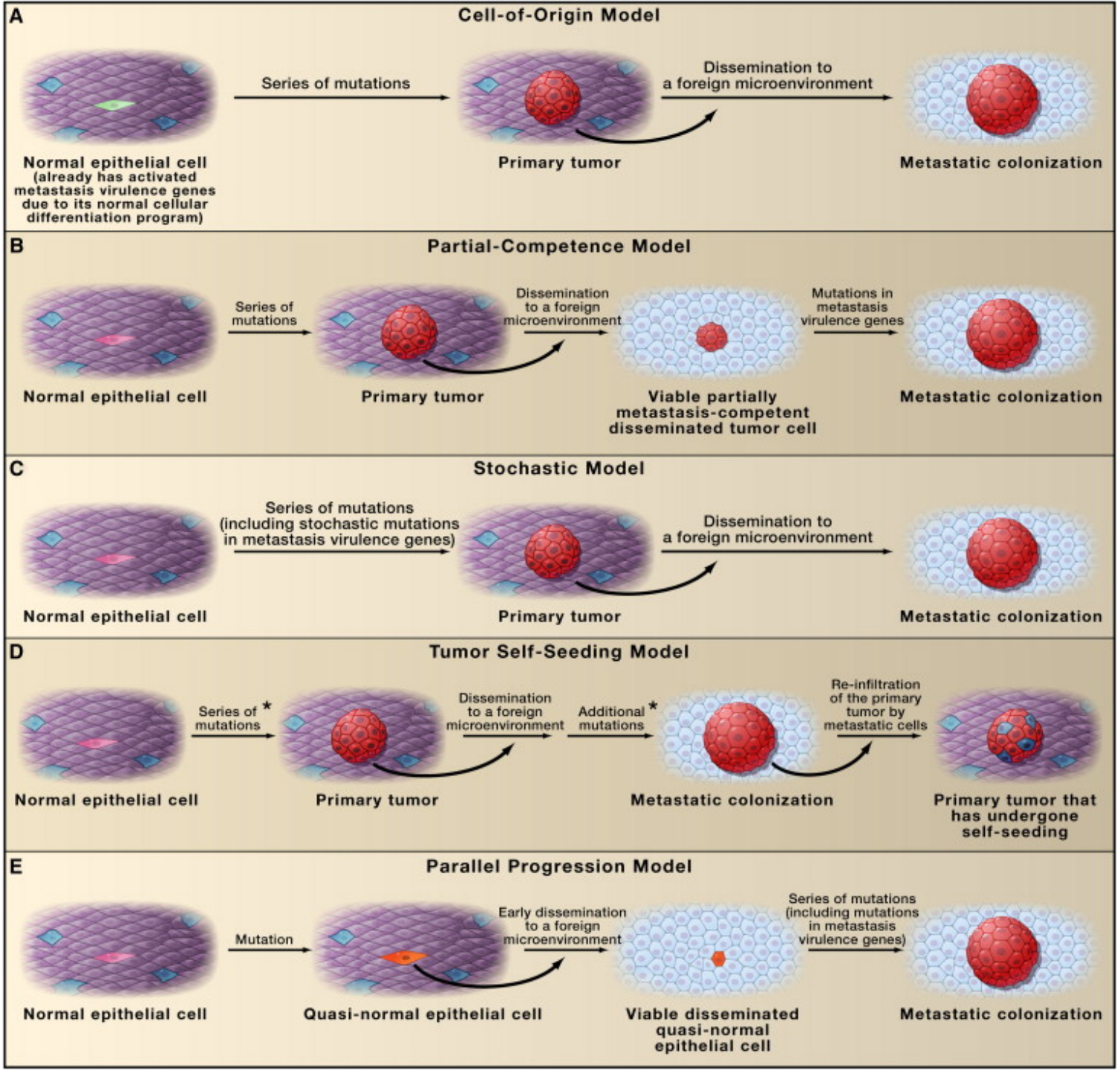


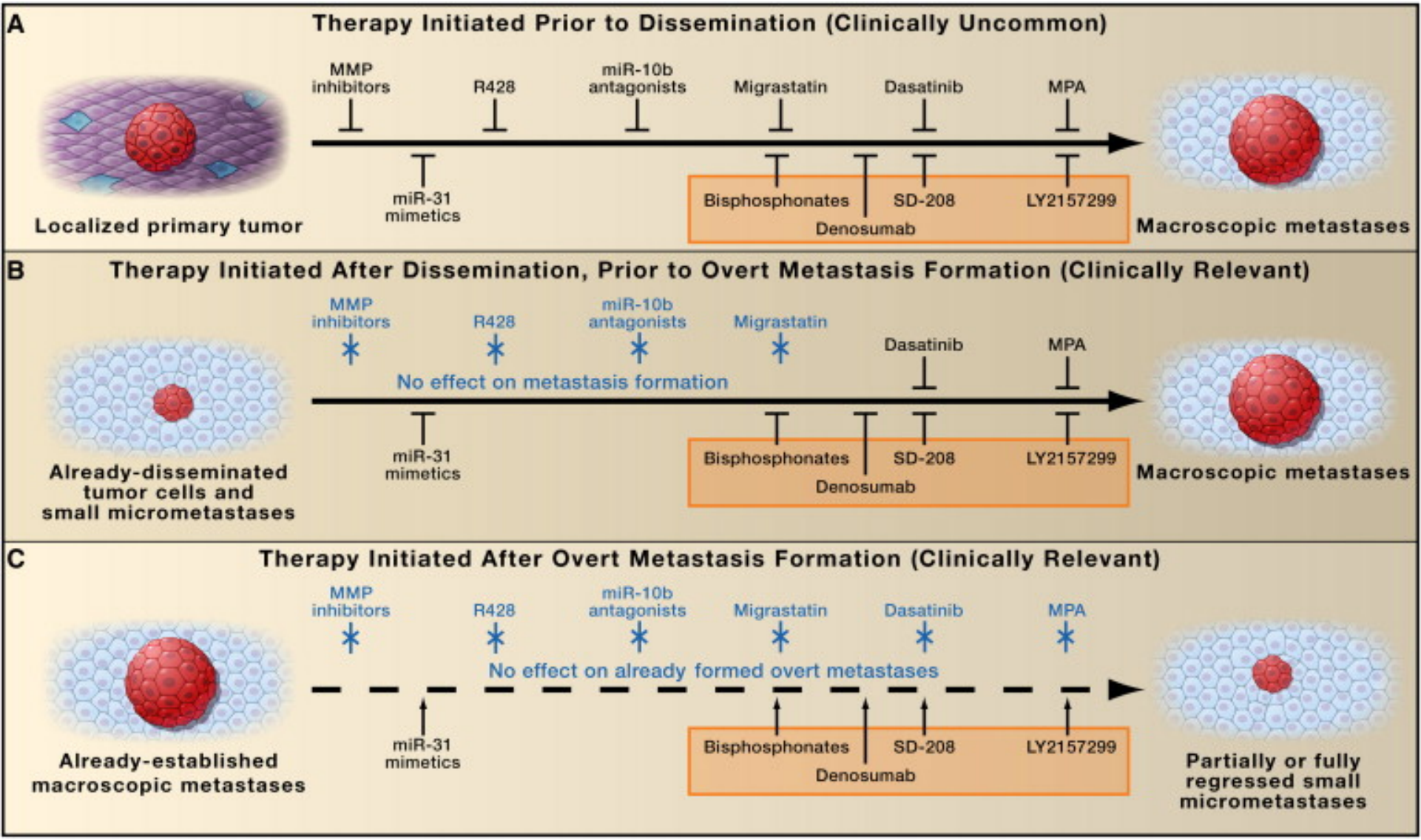
Figure 14-51b The Biology of Cancer (© Garland Science 2007)


Primary tumor formation
Local invasion
Cathepsin proteases
Macrophage
IL-4
Intravasation
CSF-1
EGF
Survival in the circulatio
Platelets
P-selectin
Arrest at a distant organ site
Metadherin
Endothelial cells
Extravasation
Angptl4
Endothelial cells
Micrometastasis formation
Hematopoietic progenitor cell
MMP-9
SDF-1
Metastatic colonization
OPN
?
Bone marrow derived cell
Clinically detectable macroscopic metastase

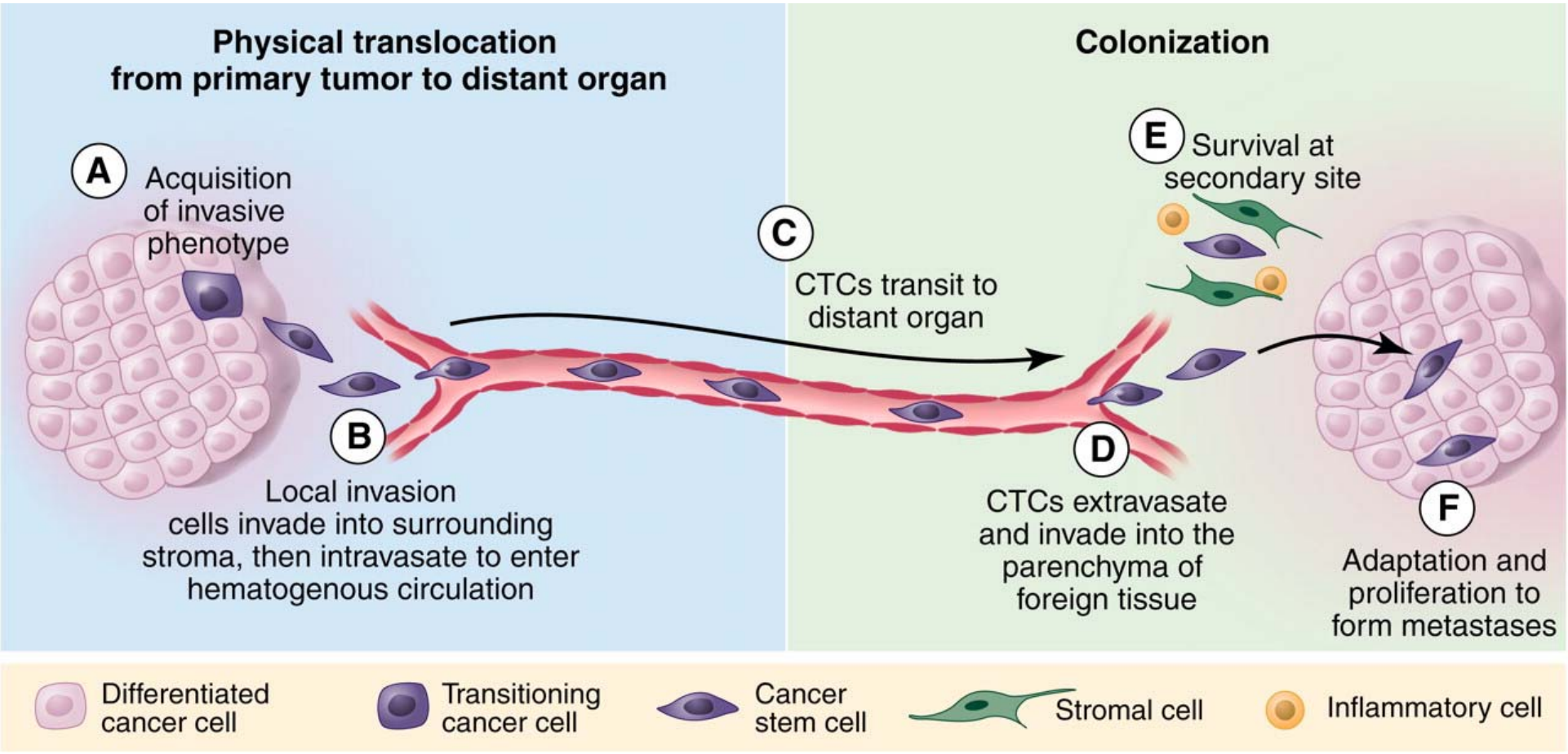


Cell 147: October 14 2011 p275-292

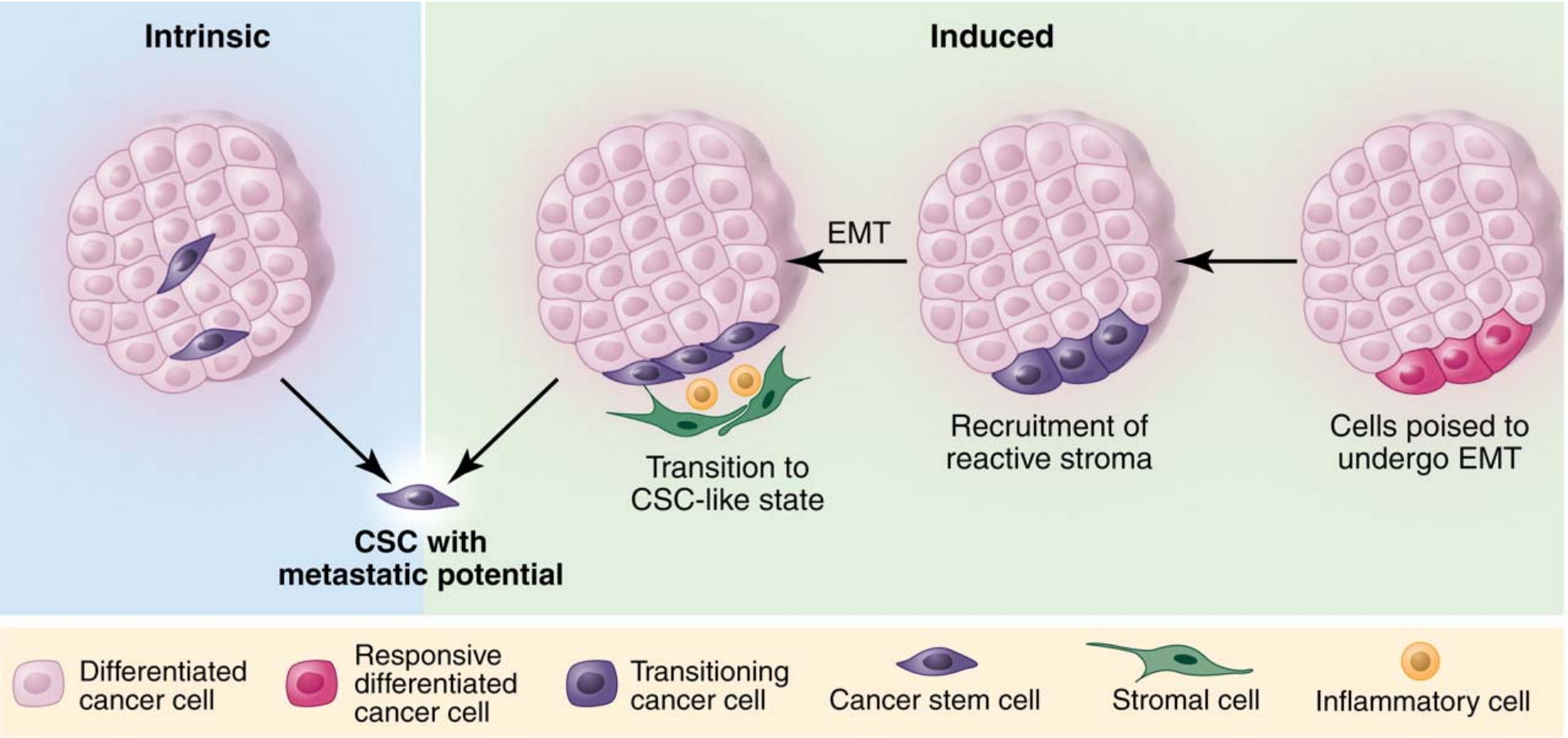

A
Cell-of-Origin Model
Series of mutations
Dissemination to a foreign microenvironment
Normal epithelial cell (already has activated metastasis virulence genes due to its normal cellular differentiation program)
Primary tumor
Metastatic colonization
B
Partial-Competence Model
Series of mutations
Dissemination to a foreign microenvironment
Mutations in metastasis virulence genes
Normal epithelial cell
Primary tumor
Viable partially metastasis-competent disseminated tumor cell
Metastatic colonization
C
Stochastic Model
Series of mutations (including stochastic mutations in metastasis virulence genes)
Dissemination to a foreign microenvironment
Normal epithelial cell
Primary tumor
Metastatic colonization
D
Tumor Self-Seeding Model
Series of mutations *
Dissemination to a foreign microenvironment
Additional mutations *
Re-infiltration of the primary tumor by metastatic cells
Normal epithelial cell
Primary tumor
Metastatic colonization
Primary tumor that has undergone self-seeding
E
Parallel Progression Model
Mutation
Early dissemination to a foreign microenvironment
Series of mutations (including mutations in metastasis virulence genes)
Normal epithelial cell
Quasi-normal epithelial cell
Viable disseminated quasi-normal epithelial cell
Metastatic colonization



Cell 147: October 14 2011 p275-292



Science 25 March 2011: Vol. 331 no. 6024 pp. 1559-1564



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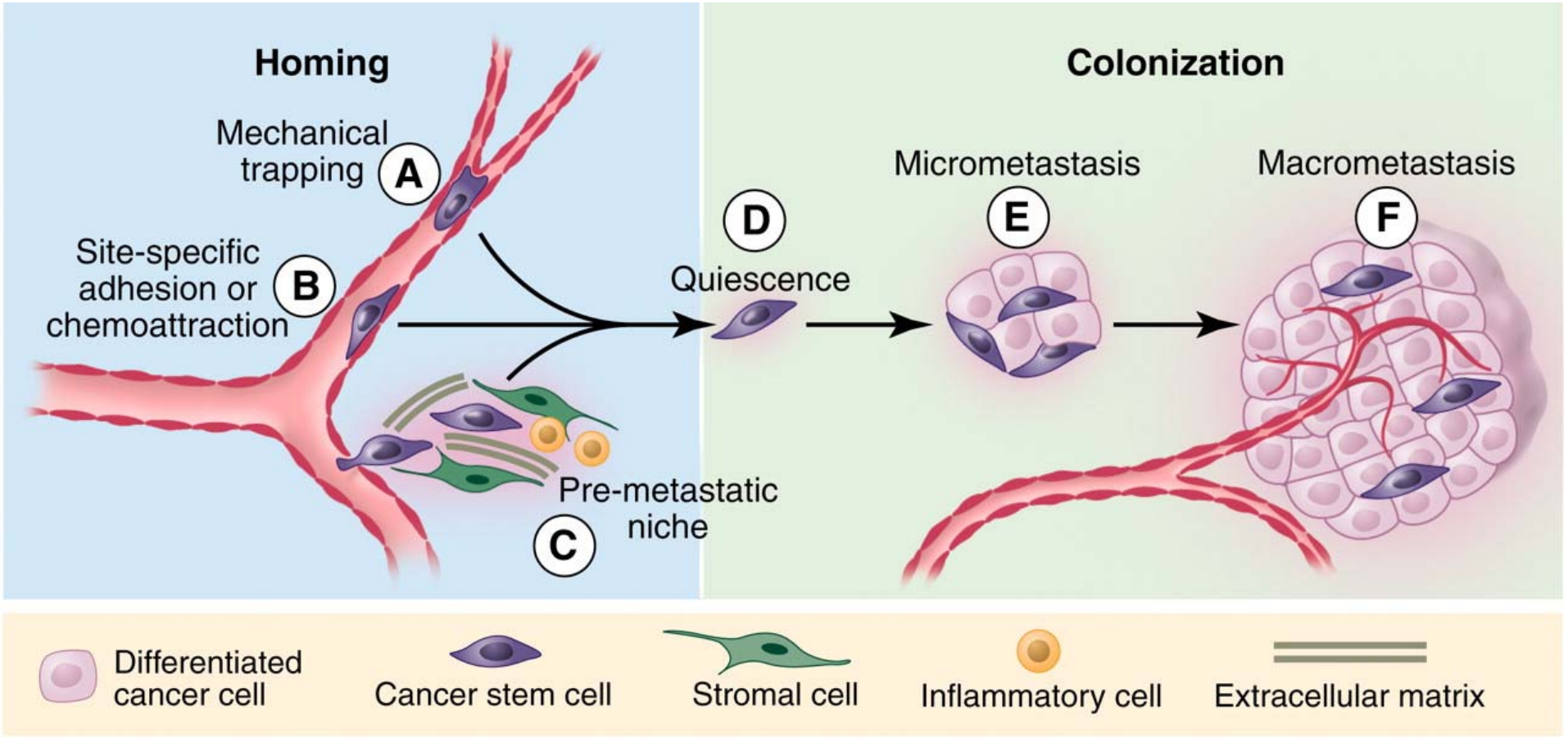

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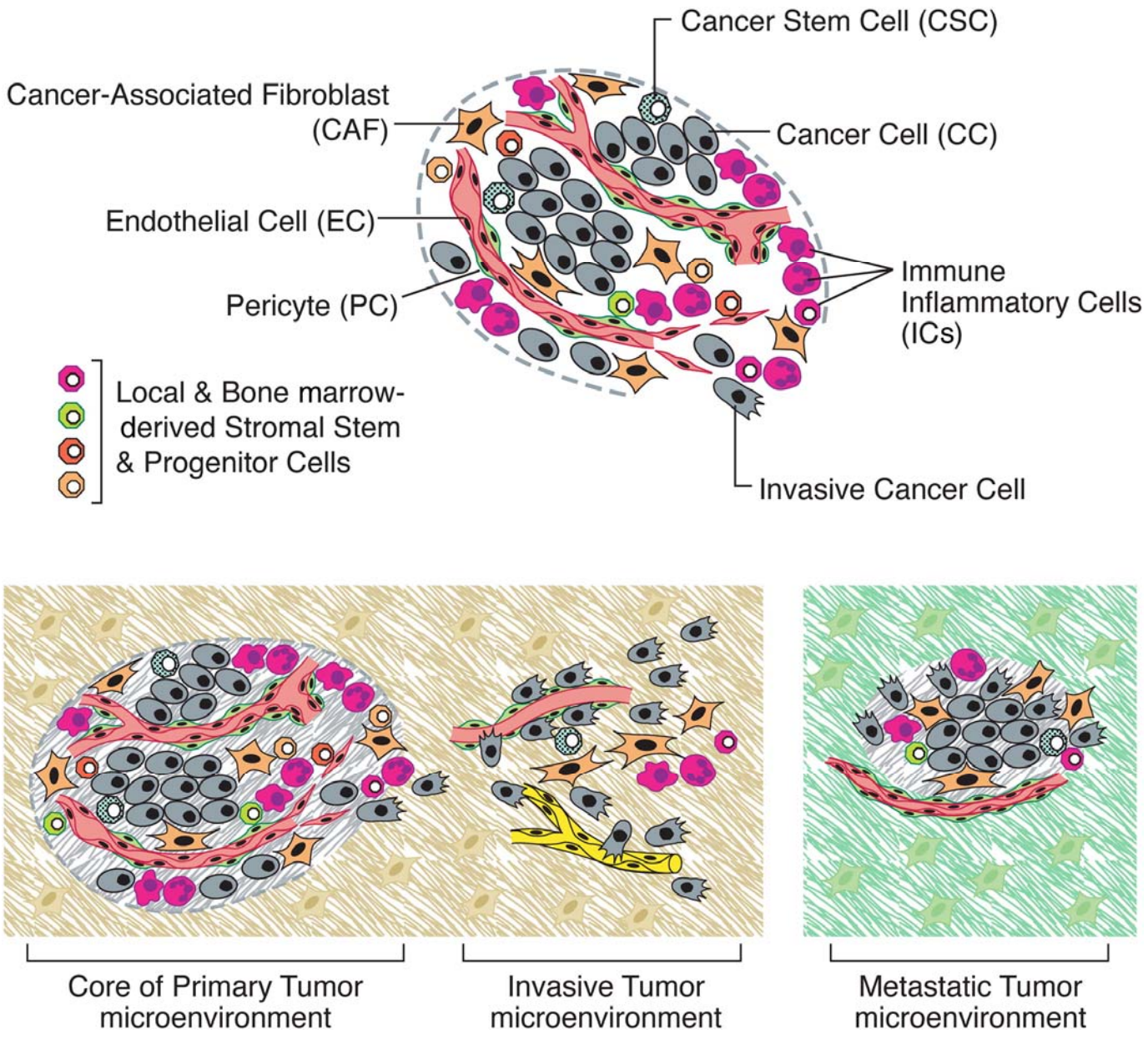

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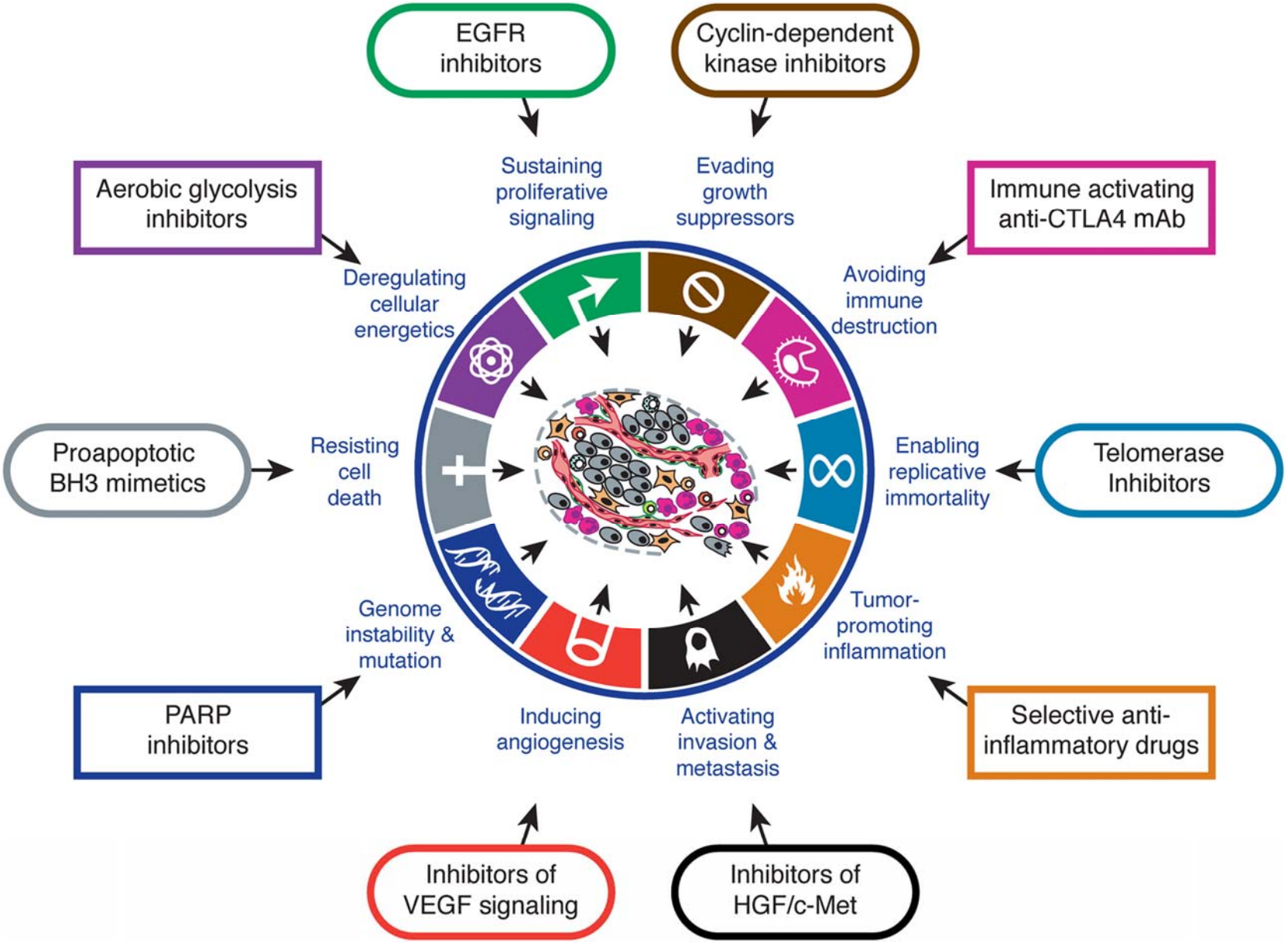

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Breast Cancer Res Treat (2009) 117:483–495
DOI 10.1007/s10549-008-0191-2

PRECLINICAL STUDY

Validation of 70-gene prognosis signature in node-negative breast cancer

J. M. Bueno-de-Mesquita · S. C. Linn · R. Keijzer · J. Wesseling · D. S. A. Nuyten · C. van Krimpen · C. Meijers · P. W. de Graaf · M. M. E. M. Bos · A. A. M. Hart · E. J. T. Rutgers · J. L. Peterse · H. Halfwerk · R. de Groot · A. Pronk · A. N. Floore · A. M. Glas · L. J. van't Veer · M. J. van de Vijver

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Leading Edge
Review

Cell

Epithelial-Mesenchymal Transitions in Development and Disease

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PERSPECTIVE
nature medicine

Cancer stem cells: mirage or reality?

Piyush B Gupta[1], Christine L Chaffer[2,3] & Robert A Weinberg[2–4]

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Leading Edge Review Tumor Metastasis: Molecular Insights and Evolving Paradigms

Scott Valastyan1,2,4,* and Robert A. Weinberg1,2,3,*

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REVIEW:

A Perspective on Cancer Cell Metastasis

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Hallmarks of Cancer: The Next Generation Douglas Hanahan1,2,* and Robert A. Weinberg3,*

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