Heterotypy and Angiogenesis

Tumors are perpetual wounds

1. Normally stroma and epithelia converse at a distance.

2. Juxtaposition of stroma and epithelia is indicative of tissue damage.

4. Activate strategies to fight infection and restore normal architecture.

5. Important strategy for wound-healing is to restore blood supply.

Program of wound healing

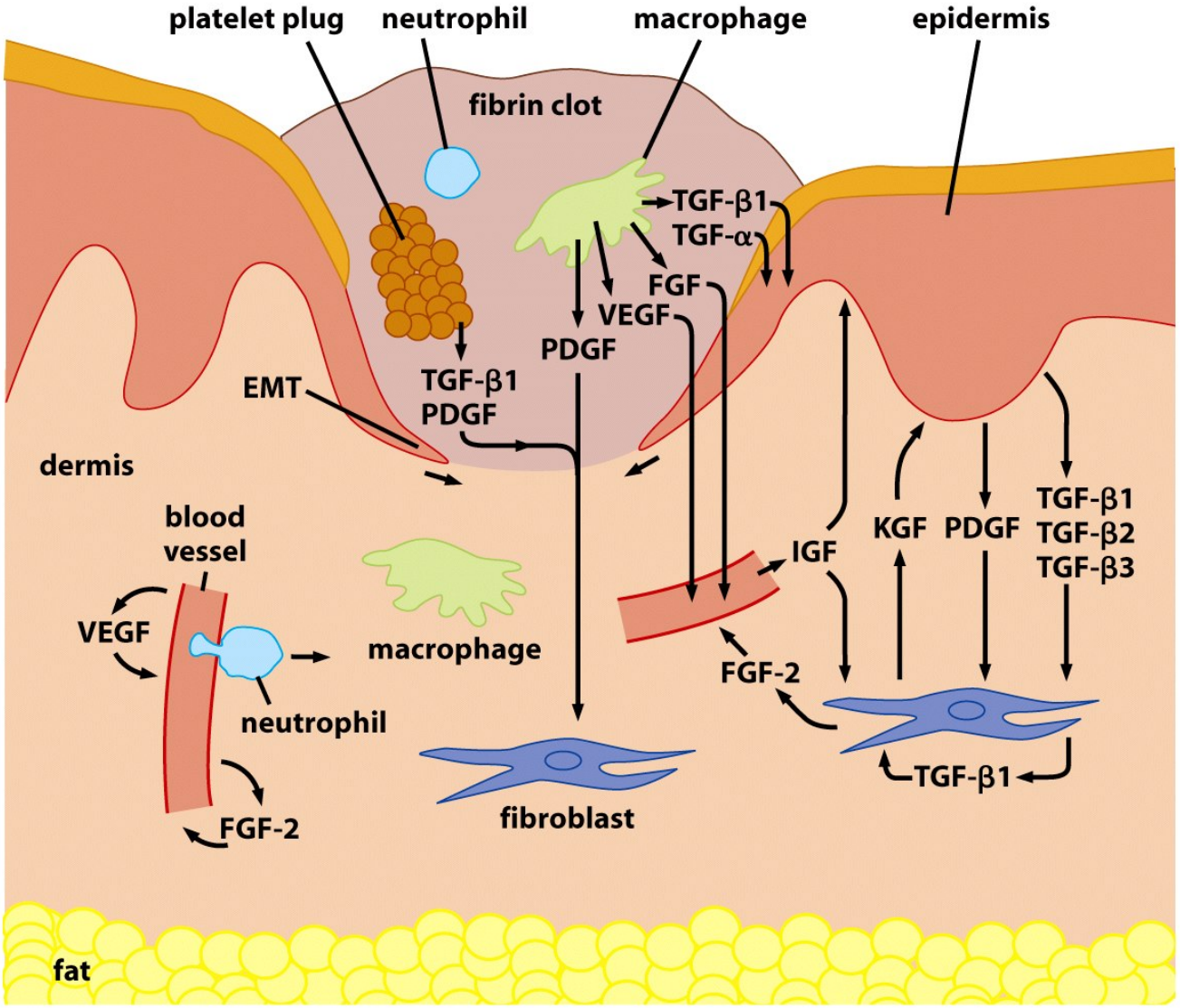


Figure 13-14 The Biology of Cancer (© Garland Science 2007)

The vasculature:

Capillaries supply every cell in the body with oxygen and nutrients.

One pound of fat contains one mile of capillary tubing.

Bone-marrow 6 billion cells divide per hour (whole marrow replaced every 5 days).

In contrast endothelium-
replacement 3-5 years (10 years in the retina).

However, in proximity to tumors endothelial cells turn over at rates similar to bone marrow suggesting tumors regulate their own blood supply.

Wounding and tumors have leaky vasculature that provides a “provisional matrix”

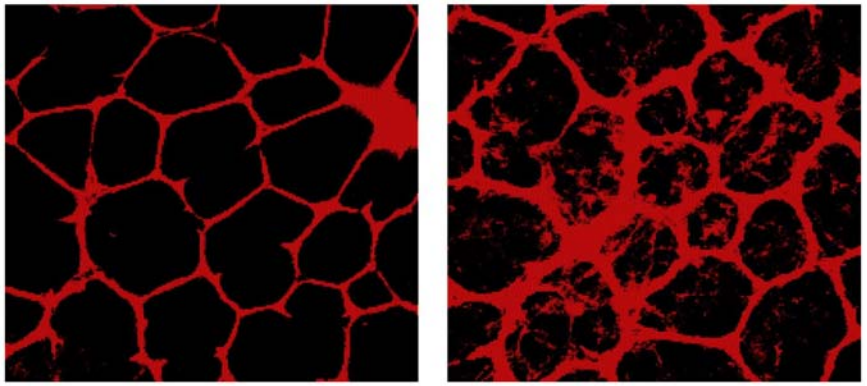


Figure 13-15a The Biology of Cancer (© Garland Science 2007)

A. Tumor leakiness (red dextran dye)

B. Leakage thrombin and fibrinogen into parenchyma-fibrin deposition

C. Fibrin bundles form ECM - support tumor growth, movement but also revascularization

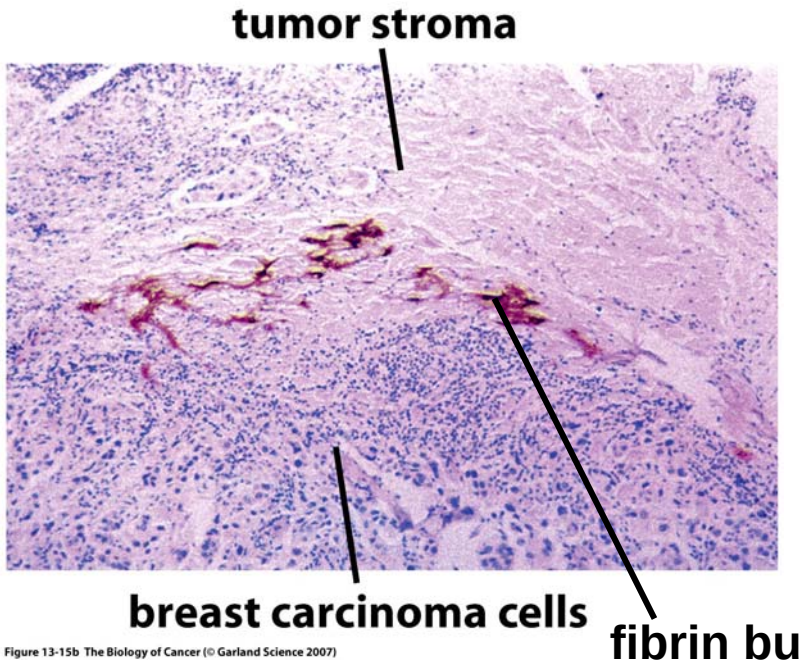


Figure 13-15b The Biology of Cancer (© Garland Science 2007)

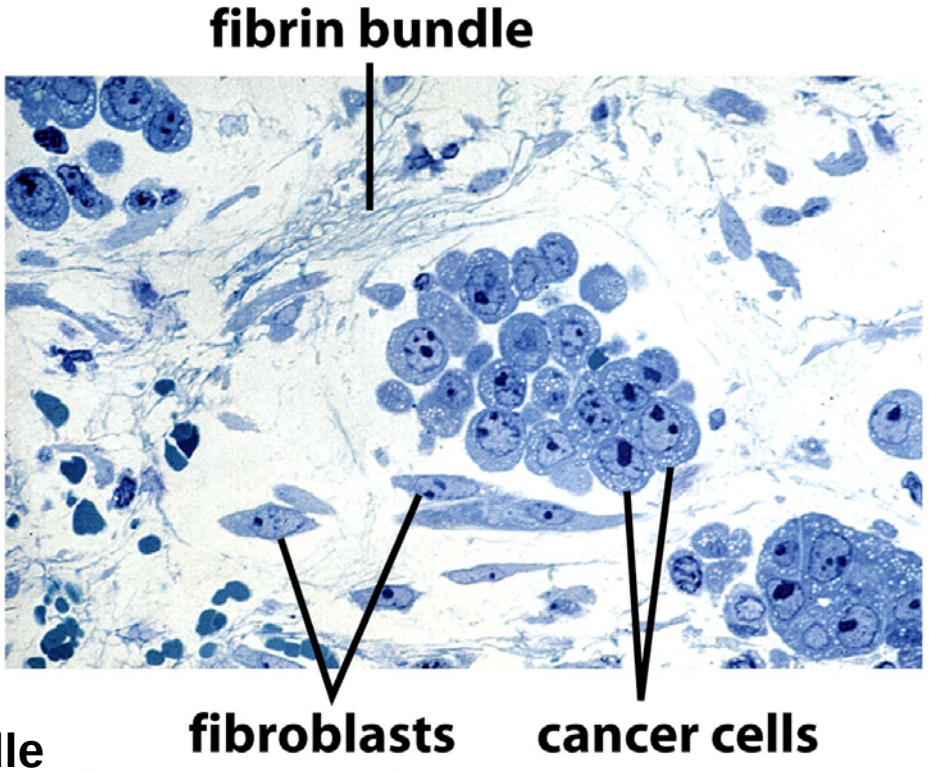


Figure 13-15c The Biology of Cancer (© Garland Science 2007)

Myofibroblasts are characteristic of wound tissue

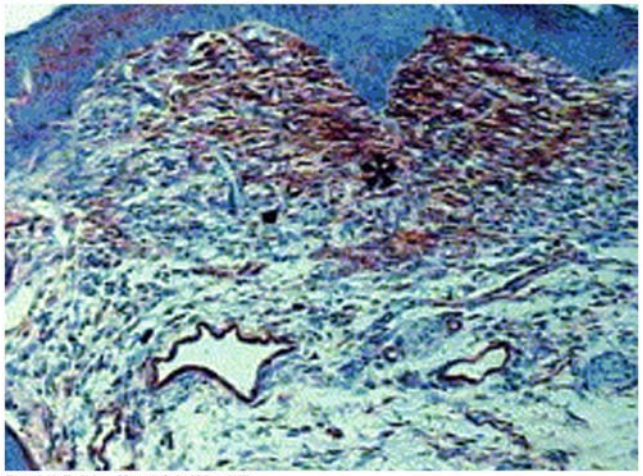


Figure 13-16a The Biology of Cancer (© Garland Science 2007)

Myofibroblasts – identified by smooth muscle actin (SMA) red infiltrate wound at 3 days.

Chronically inflamed tissue-

cirrhotic liver-myofibroblasts (brown)

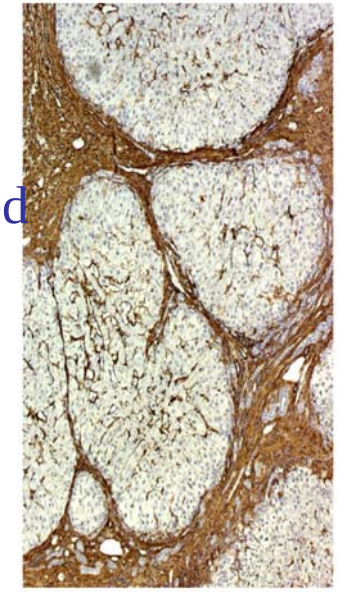


Figure 13-16b The Biology of Cancer (© Garland Science 2007)

Hepatocellular carcinoma – stained for SMA (very similar)

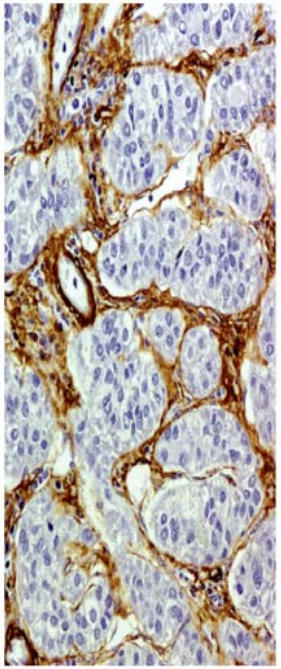


Figure 13-16c The Biology of Cancer (© Garland Science 2007)

Normal stroma becomes displaced by “desmoplastic” stroma in advanced carcinomas

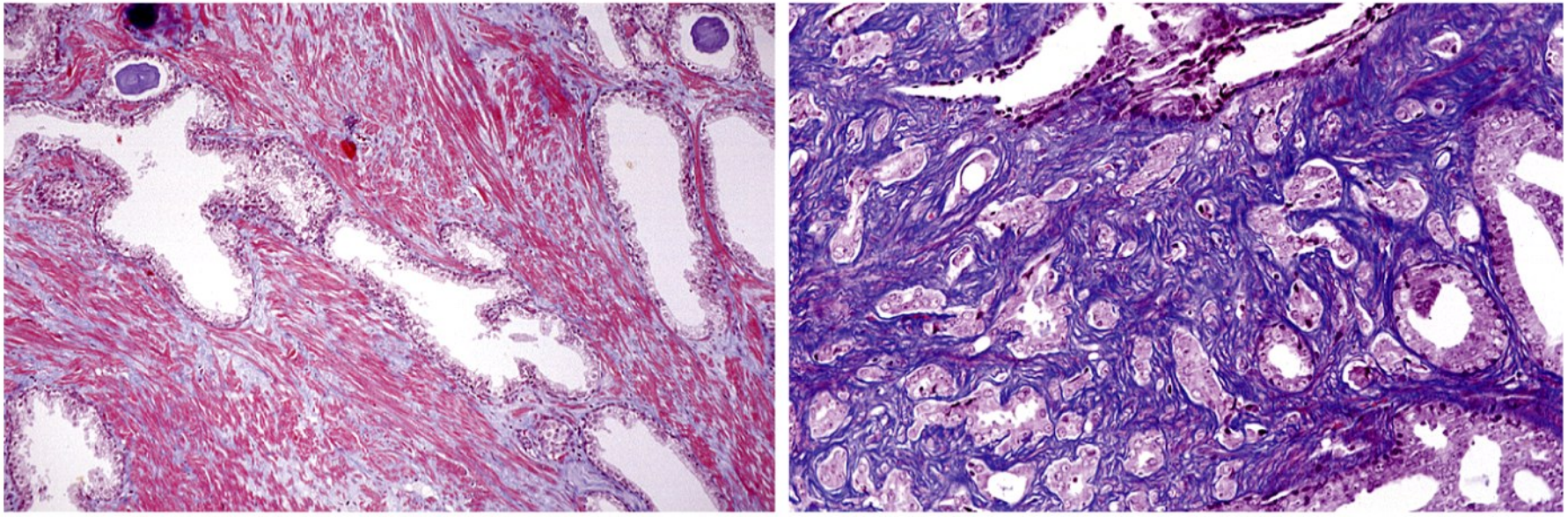


Figure 13-17 The Biology of Cancer (© Garland Science 2007)

Left: normal prostate (smooth muscle –pink).

Right: desmoplastic stroma rich in collagen I (purple) –myofibroblasts and fibroblasts rare.

Stromal cells contribute to tumorigenesis

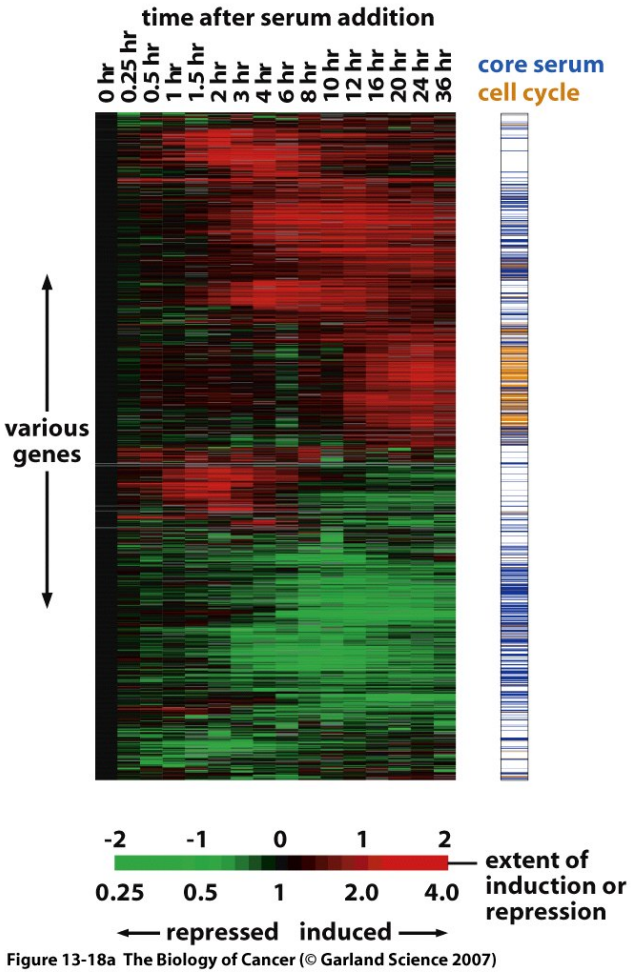


Figure 13-18a The Biology of Cancer (© Garland Science 2007)

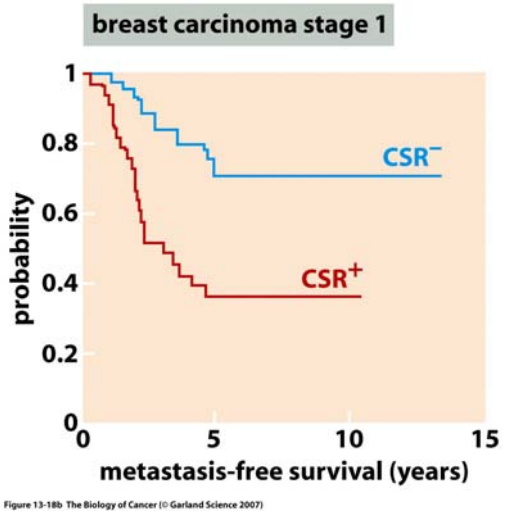


Figure 13-18b The Biology of Cancer (© Garland Science 2007)

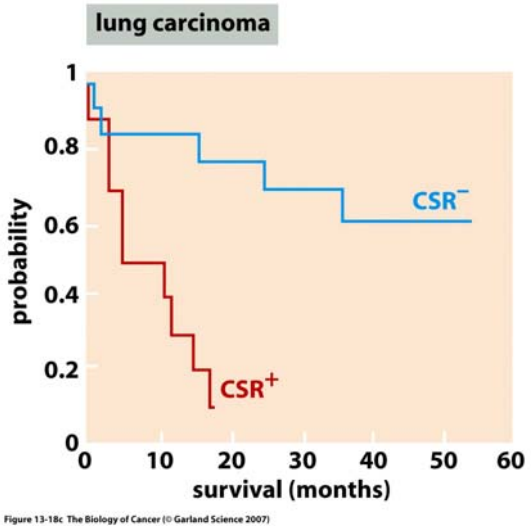
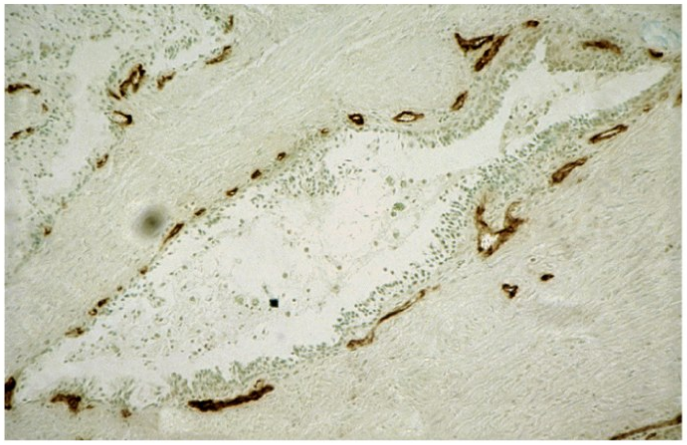


Figure 13-18c The Biology of Cancer (© Garland Science 2007)

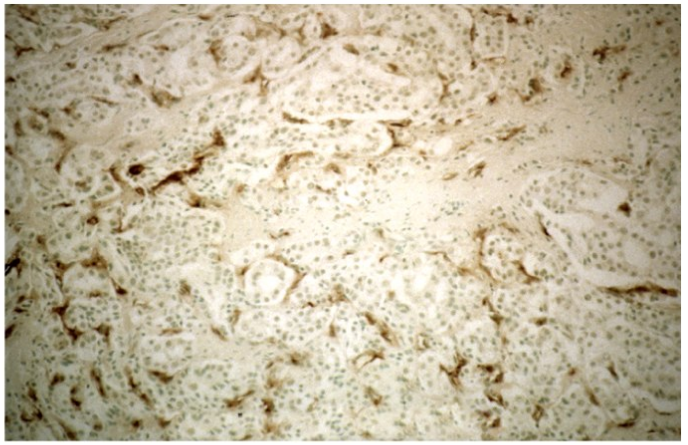
Changes in gene expression followed after addition of serum to serum-starved fibroblasts.

Core serum response (CSR) genes defined as genes that changed early and stably and were not associated with cell-cycle. CSR signatures in tumors indicative of CAF activity. The higher the activity the worse the prognosis.

Intensity of angiogenesis increases once cells breach basement membrane

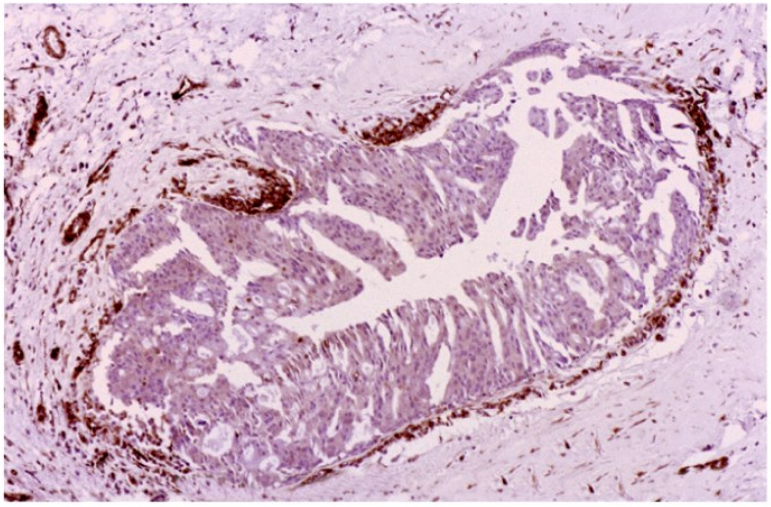


prostate cancer (PIN; *in situ*)

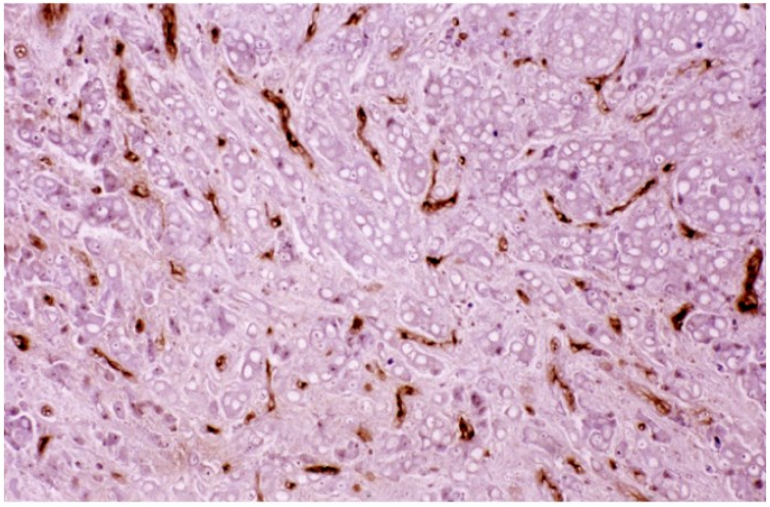


invasive prostate cancer

Figure 13-41a The Biology of Cancer (© Garland Science 2007)



human breast cancer (*in situ*)



invasive human breast cancer

Figure 13-41b The Biology of Cancer (© Garland Science 2007)

Prostatic tumors develop in mice with genetically altered fibroblasts

Selective KO of TGF-β type II receptor in fibroblasts in tissues releases fibroblasts from TGF-β growth inhibition. Resulting hyperplasia of stroma and epithelium suggests that stroma is releasing growth signals to epithelium. Production of Hepatocyte Growth Factor (HGF), a potent epithelial growth factor, by stroma is increased by 3x. Mice eventually develop gastric carcinomas. Stroma controls epithelial growth.

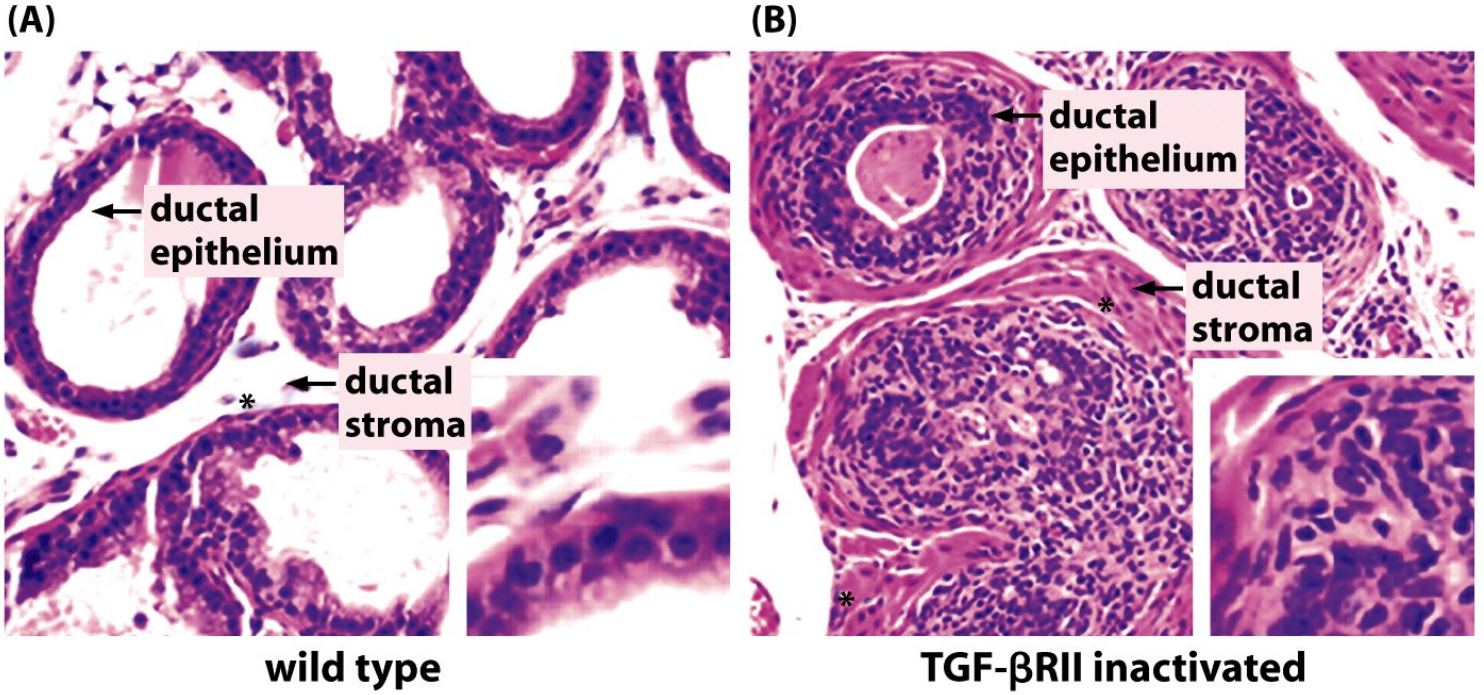


Figure 13-19 The Biology of Cancer (© Garland Science 2007)

Admixed normal fibroblasts promote tumor growth

Human mammary epithelial cells transformed by SV40, hTERT and activated *ras* form tumors with long lag time. Addition of matrigel accelerates tumor development. Normal mammary tissue fibroblasts stimulate tumor development. Fibroblast recruitment important rate-limiting step.

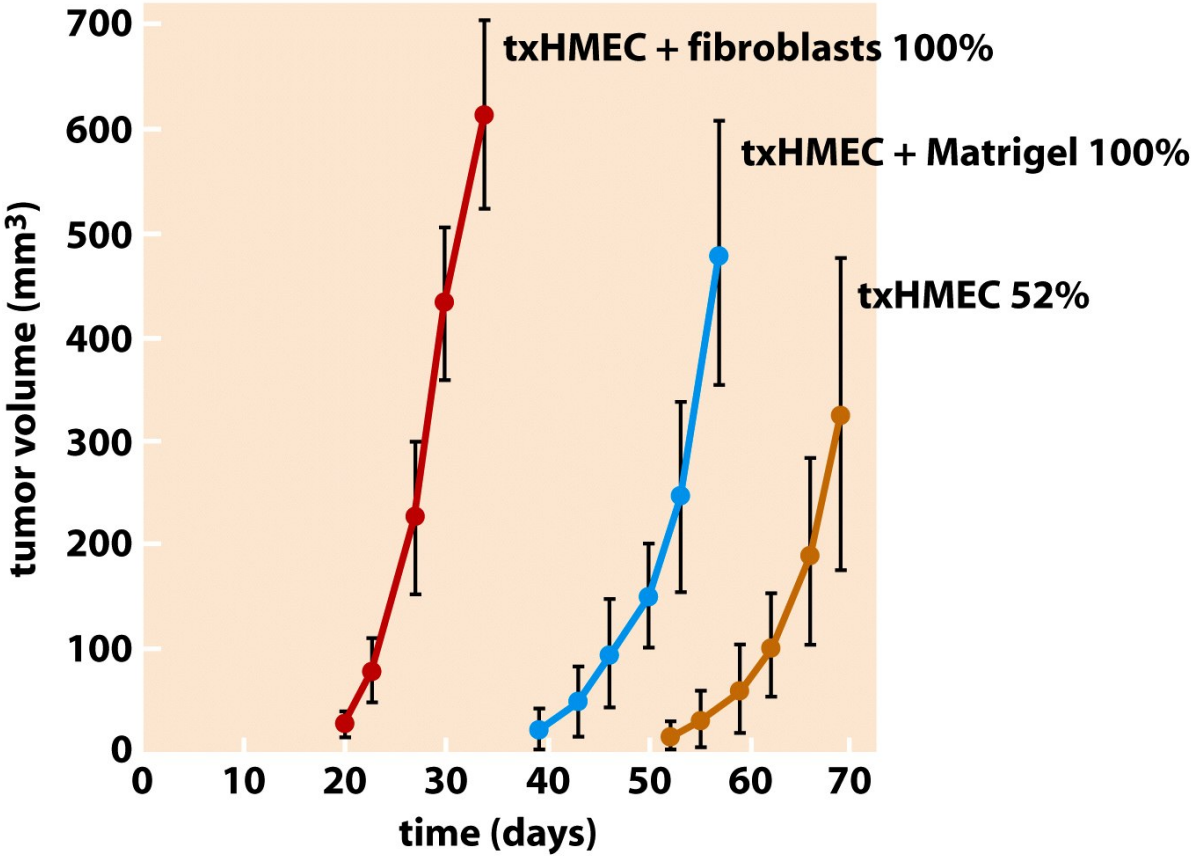


Figure 13-20 The Biology of Cancer (© Garland Science 2007)

Do carcinoma-associated fibroblasts promote tumor growth more efficiently?

Normal human prostate cells **immortalized** by SV40 T antigen form small tumors in nude mice. Carcinoma-associated fibroblasts from human prostate tumors (but not normal fibroblasts) enhance tumor formation. Indicates stromal fibroblasts become conditioned by tumors to support tumor growth. Dialogue.

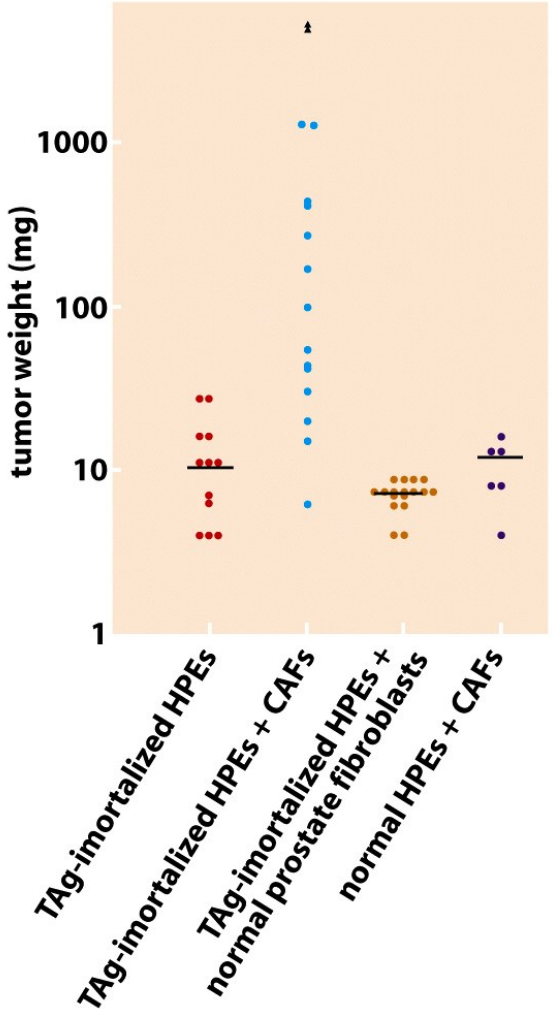
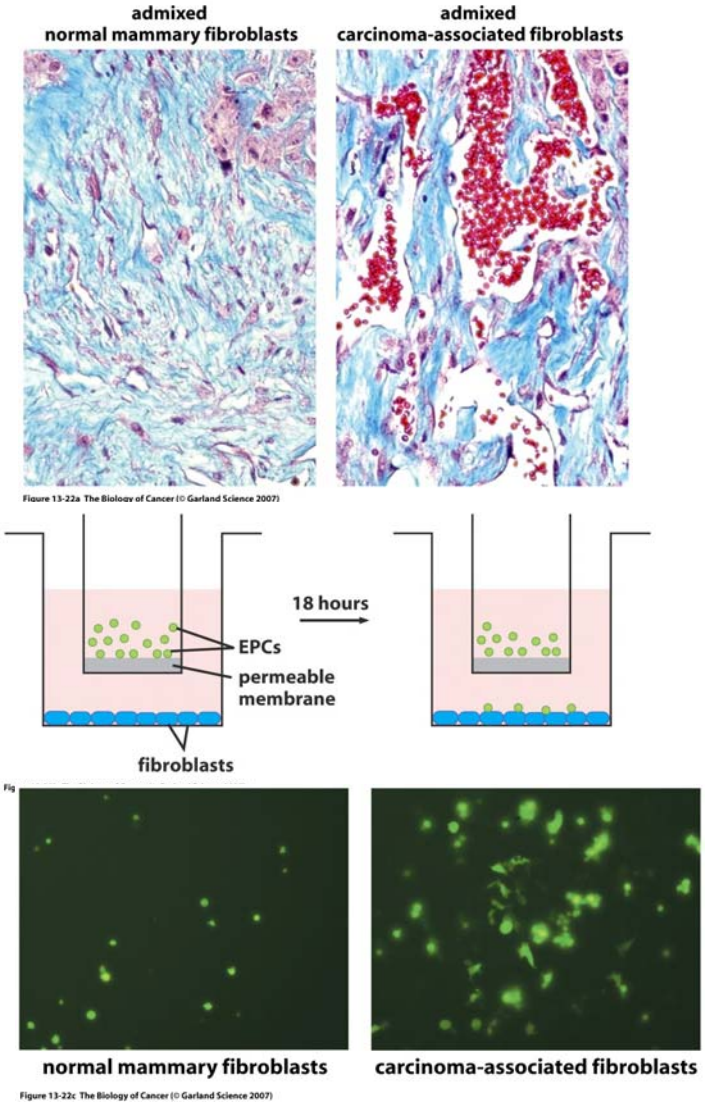


Figure 13-21 The Biology of Cancer (© Garland Science 2007)

Carcinoma-associated fibroblasts (CAF) initiate angiogenesis by recruiting endothelial cells



MCF-7 tumors form highly vascularised tumors if admixed with CAF (myofibroblasts).

CAF but not normal fibroblasts recruit GFP-labeled endothelial precursor cells (EPC) from marrow.

Recruitment reduced by blocking SDF-1 (chemokine).

VEGF secreted by myofibroblasts promotes differentiation EPC into endothelial cells.

Angiogenesis is the rate limiting step in tumor formation.

Tumor-stimulating properties of CAF in large part due to stimulation angiogenesis.

Stromal cells recruit macrophages which stimulate angiogenesis

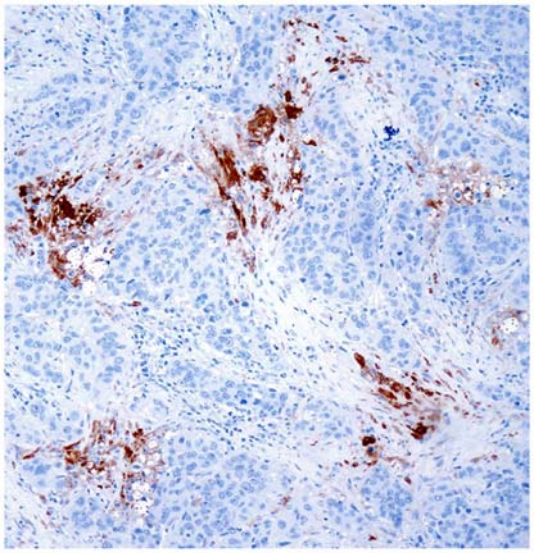


Figure 13-25a The Biology of Cancer (© Garland Science 2007)

Tumor associated macrophages (red) stained for Hypoxia Induced Factor 2 alpha (HIF2α) in human breast cancer.

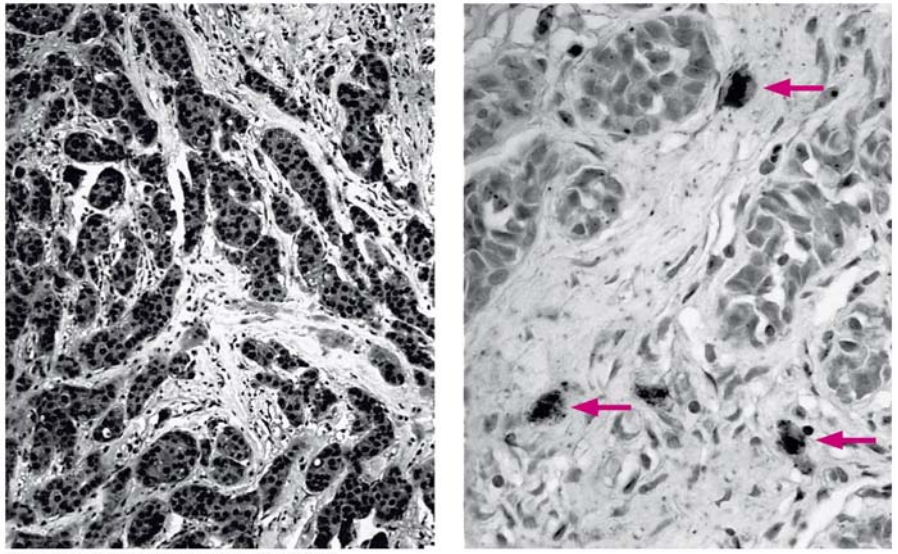


Figure 13-25b The Biology of Cancer (© Garland Science 2007)

Some breast cancers produce VEGF (left)

In others VEGF produced by macrophages within stroma (right).

Macrophages correlate with angiogenesis

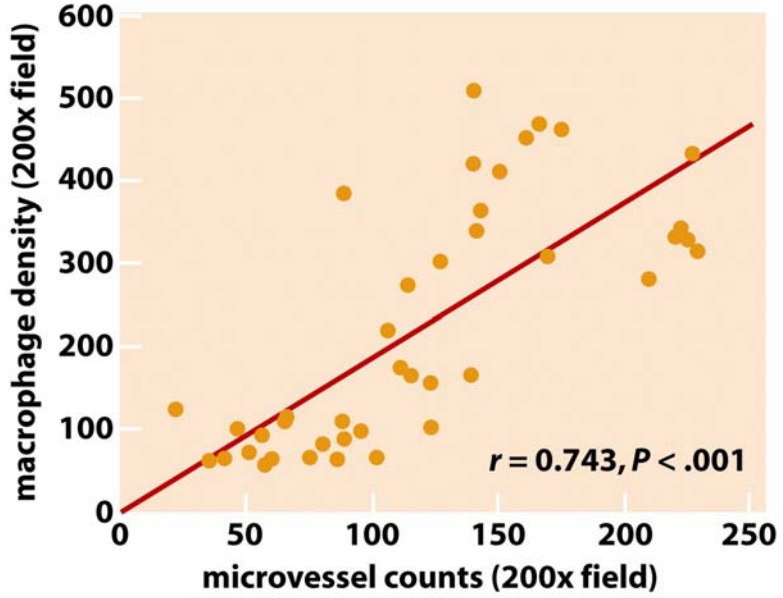


Figure 13-25c The Biology of Cancer (© Garland Science 2007)

Non-small cell lung cancer –density of TAMs v. density microvessels.

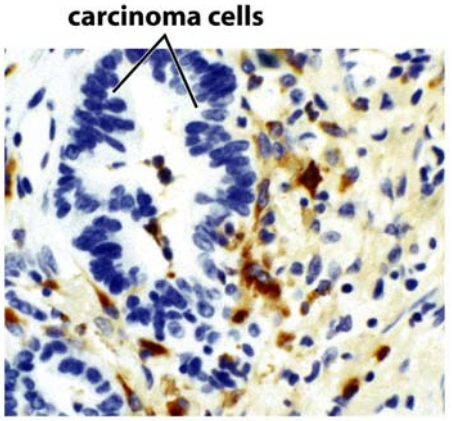


Figure 13-25d The Biology of Cancer (© Garland Science 2007)

Macrophages in human colorectal carcinoma produce MMP-9 (brown) a key enzyme in angiogenesis and invasion by releasing VEGF and other angiogenic factors from sequestration in the ECM

Role of macrophages in tumorigenesis

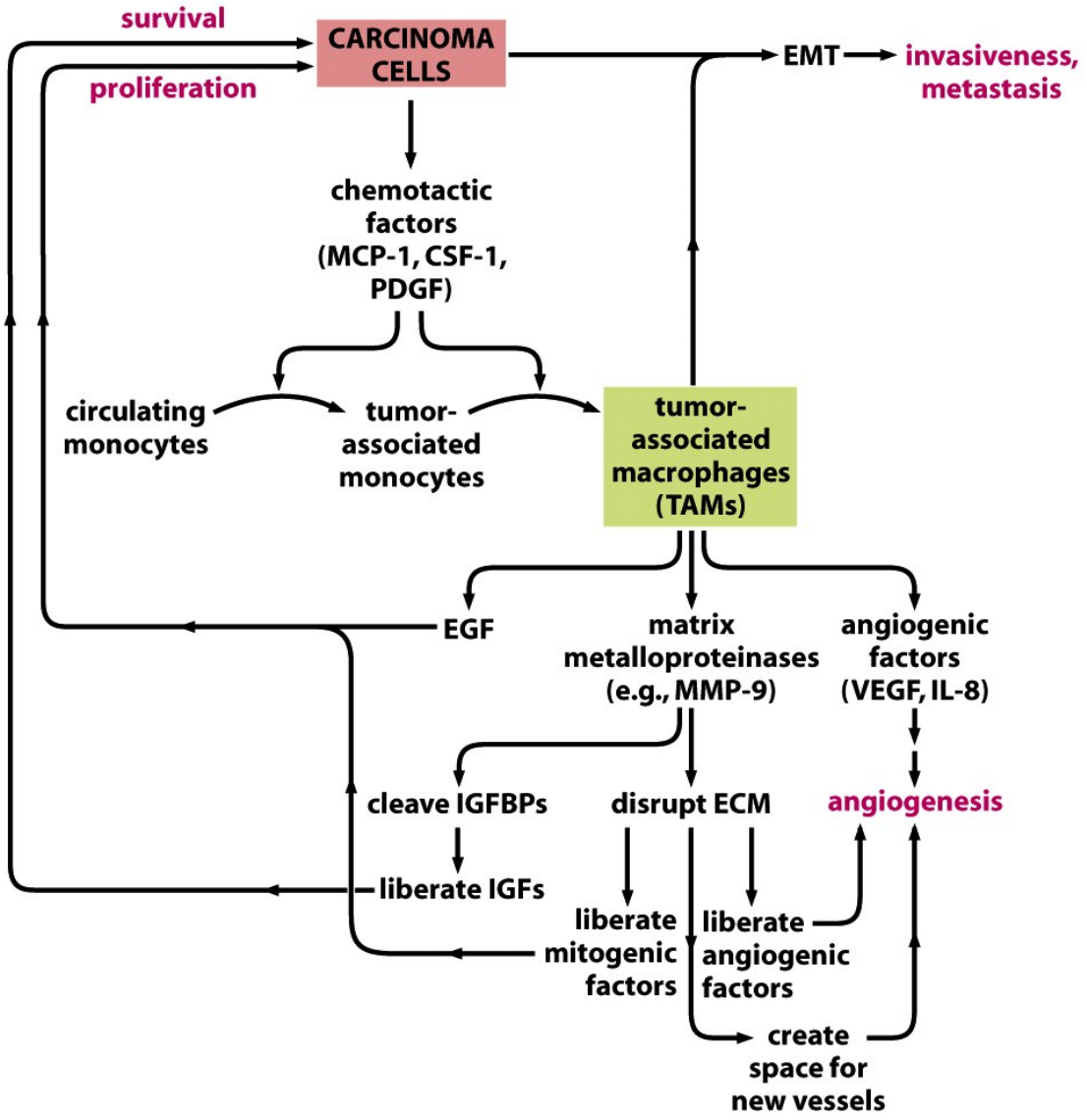


Figure 13-26 The Biology of Cancer (© Garland Science 2007)

Hypoxia and angiogenesis

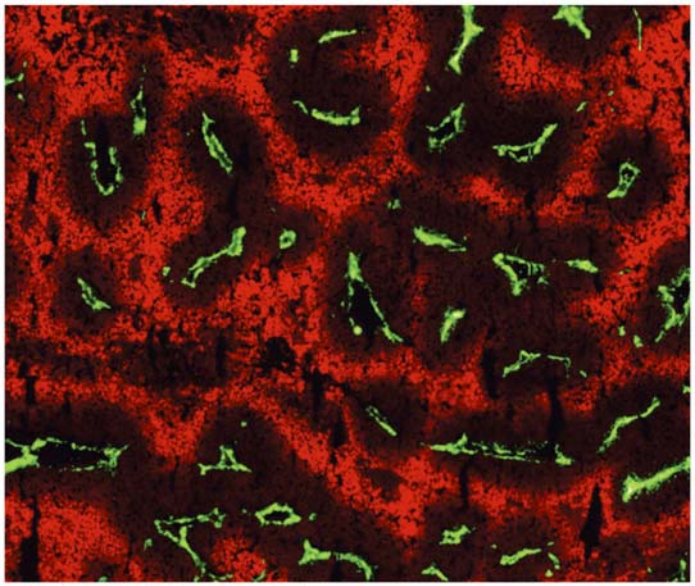


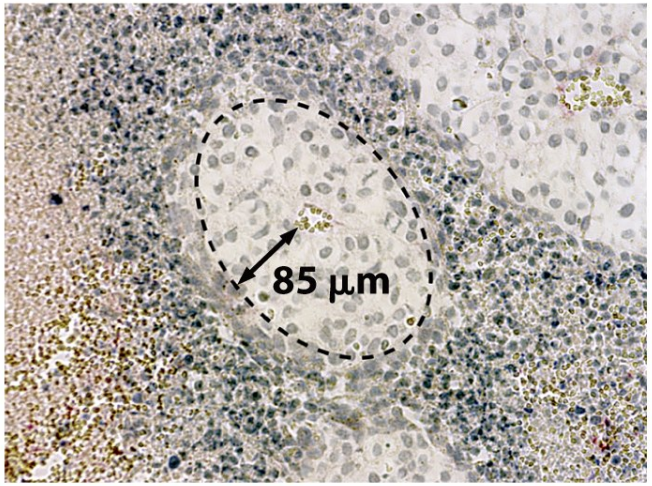
Figure 13-27a The Biology of Cancer (© Garland Science 2007)

Left: Capillaries (green). Hypoxic (red).

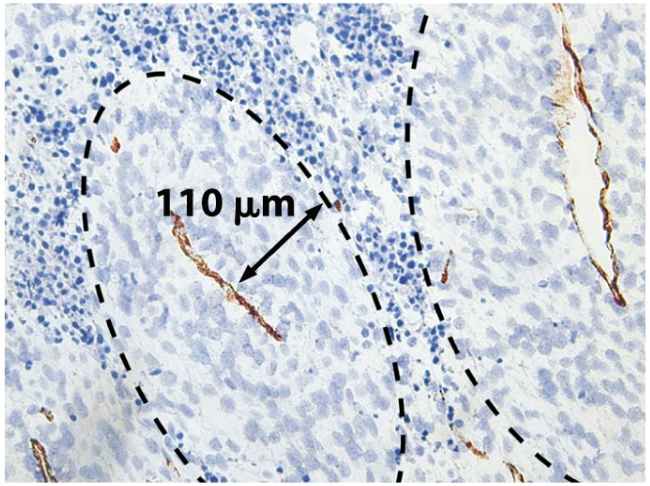
Below: Tumor cells become necrotic if too far from capillary identified by endothelial marker CD31 (brown).

Limitations of diffusion in conveying oxygen and nutrients – perivascular cuffs.

The more active the tumor the more blood vessels it needs to grow and survive.



human melanoma



rat prostate cancer

Figure 13-27b The Biology of Cancer (© Garland Science 2007)

Hypoxia and necrosis in squamous cell tumor

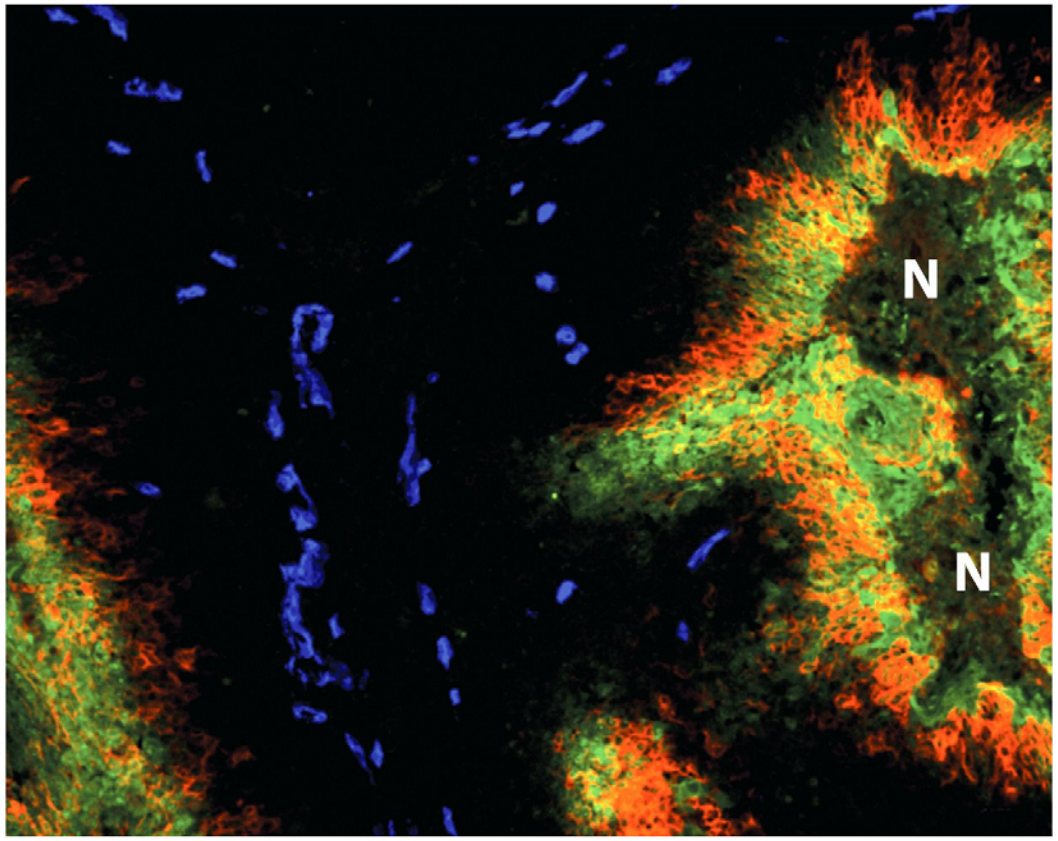


Figure 13-27c The Biology of Cancer (© Garland Science 2007)

Blood vessels (blue).

Areas of moderate hypoxia carbonic anhydrase (red).

Extreme hypoxia (green).

N: necrosis

VEGF

VEGF and notch cooperate to function as a “branching pattern generator”

VEGFR2 stimulates tip cell induction and filopodia
VEGFR3 embryonic – lymphatics .Tip cells re-express VEGFR3 .
VEGFR1 suppresses sprouting and vascularization

Feedback-loop between VEGF and Notch

Sequence of events that define angiogenesis

Myofibroblasts in tumor associated stroma release SDF-1 which recruits endothelial precursors.

VEGF assists development of ECP into mature endothelial cells.

Production of VEGF governed by availability of oxygen through VHL – dependent HIF-1 accumulation and transcription of VEGF.

VEGF produced by tumor cells, macrophages and myofibroblasts.

VEGF stimulates capillary formation

VEGF acts through tyrosine kinase receptors VEGF-RI and VEGF-RII to induce proliferation of endothelial cells.

Endothelial cells join thro' tight junctions (arrows) to form capillaries.

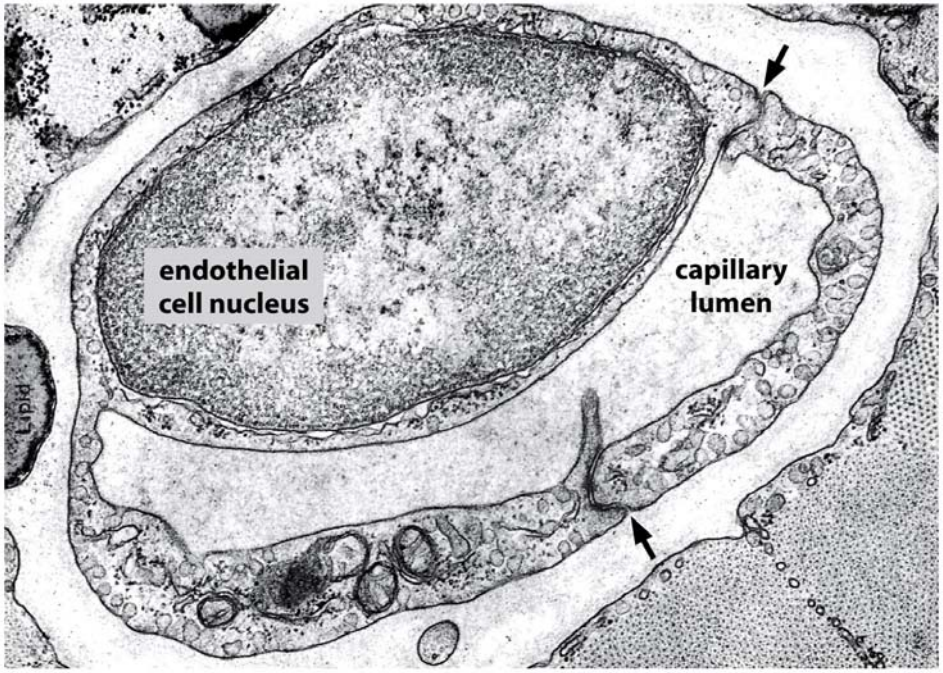
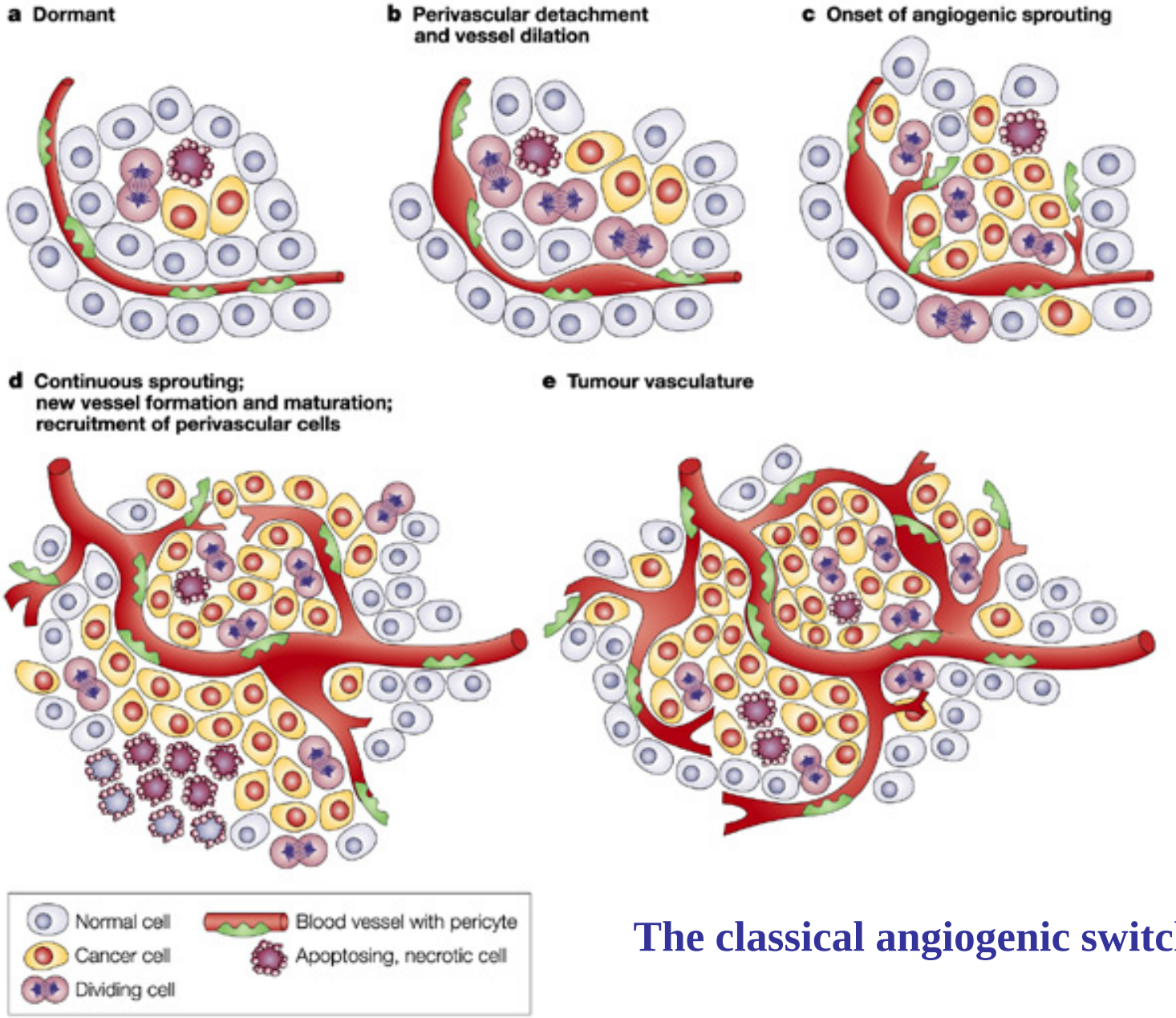


Figure 13-30 The Biology of Cancer (© Garland Science 2007)

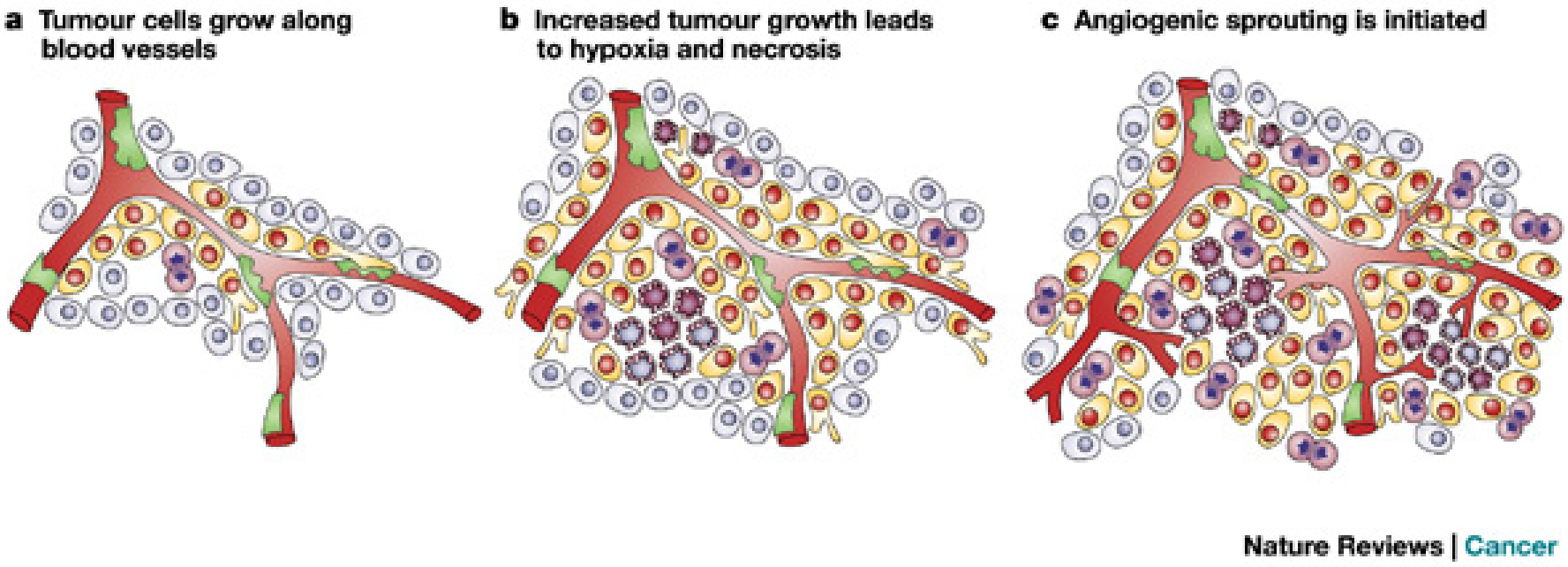


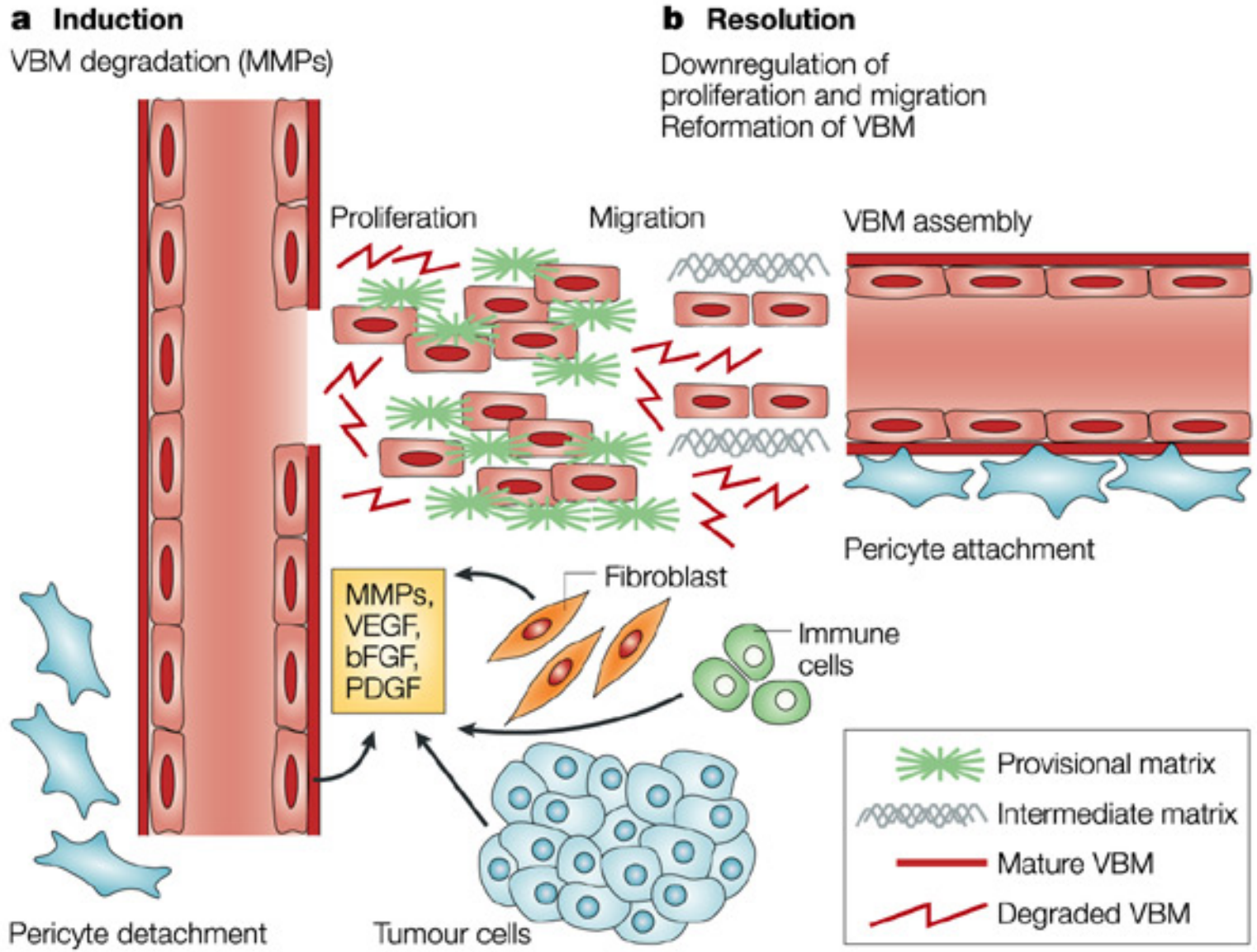
The classical angiogenic switch

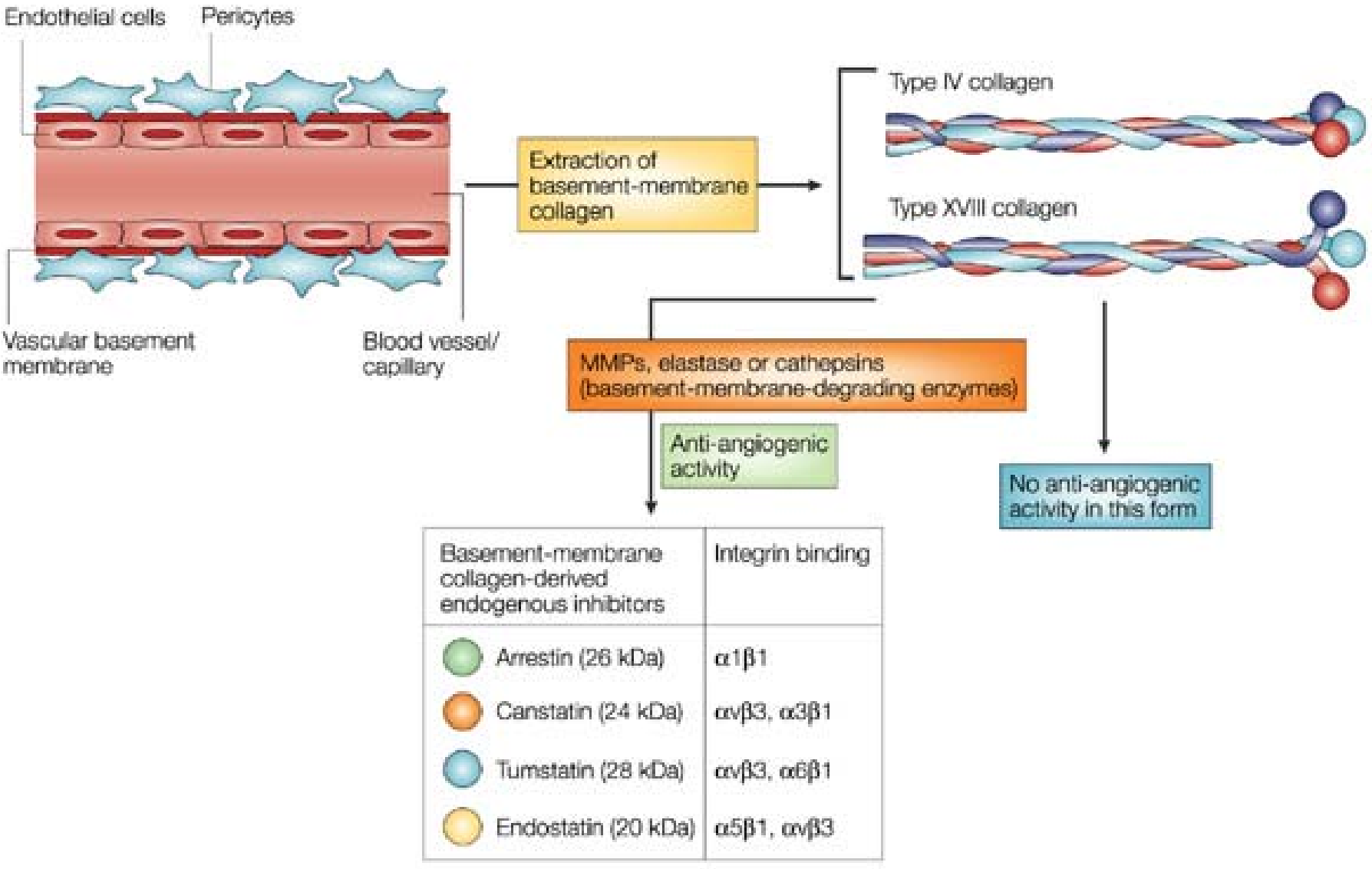
Blood vessel co-option precedes angiogenesis in astrocytoma progression

Low grade astrocytoma

Grade III astrocytomas progress to Grade IV they induce angiogenesis




a Induction
VBM degradation (MMPs)
b Resolution
Downregulation of
proliferation and migration
Reformation of VBM
Proliferation
Migration
VBM assembly
Pericyte attachment
MMPs,
VEGF,
bFGF,
PDGF
Fibroblast
Immune
cells
Pericyte detachment
Tumour cells
Provisional matrix
Intermediate matrix
Mature VBM
Degraded VBM


Endothelial cells
Pericytes
Vascular basement membrane
Blood vessel/ capillary
Extraction of basement-membrane collagen
Type IV collagen
Type XVIII collagen
MMPs, elastase or cathepsins (basement-membrane-degrading enzymes)
Anti-angiogenic activity
No anti-angiogenic activity in this form
Basement-membrane collagen-derived endogenous inhibitors
Integrin binding
Arrestin (26 kDa)
α1β1
Canstatin (24 kDa)
αvβ3, α3β1
Tumstatin (28 kDa)
αvβ3, α6β1
Endostatin (20 kDa)
α5β1, αvβ3

Endothelial cells also form lymph ducts

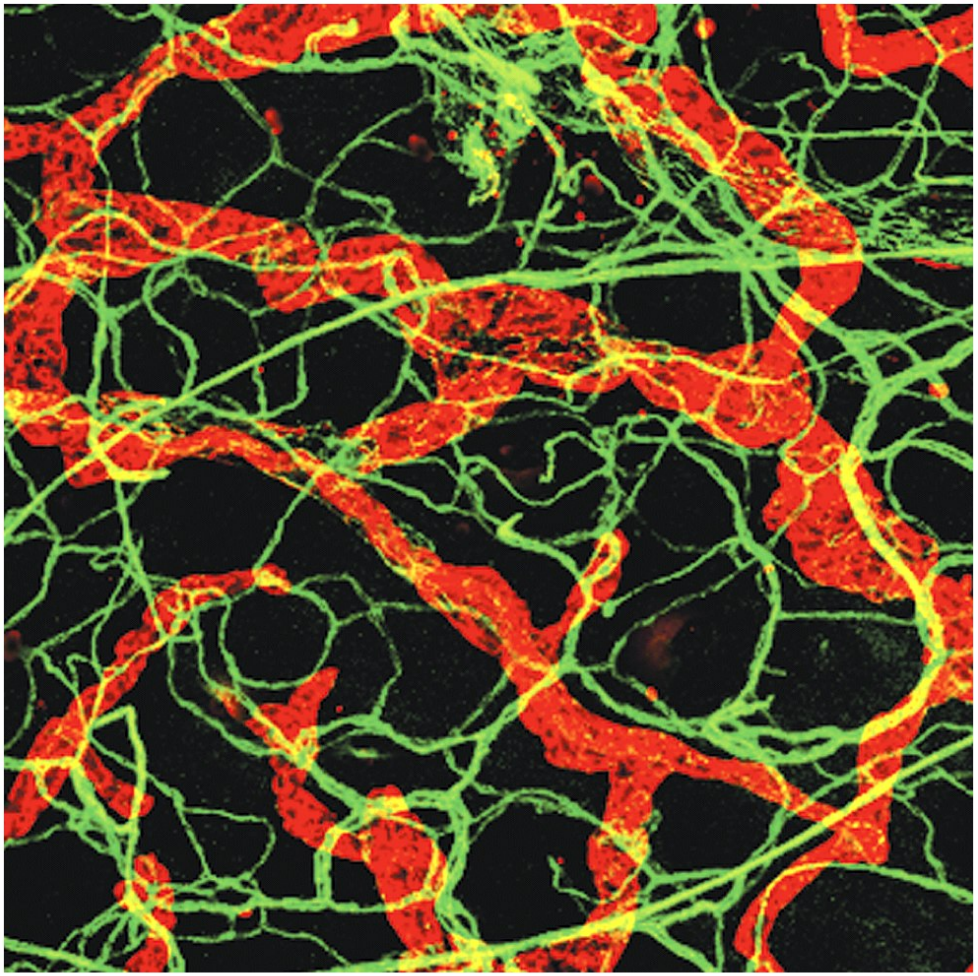


Figure 13-31 The Biology of Cancer (© Garland Science 2007)

Same embryonic endothelial cell population forms lymph ducts. Drain fluid from interstices between cells and return to circulation. Serve to inform immune cells of local antigens by draining to lymph nodes.

Capillaries (green)

Lymphatics (orange)

VEGFA and B stimulate blood

VEGFC and D stimulate lymphatics

Recruitment of capillaries by implanted tumor

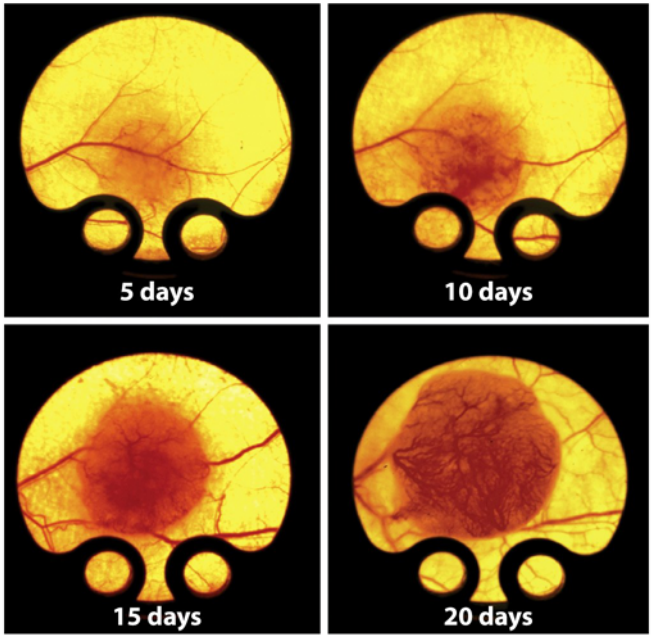


Figure 13-32a The Biology of Cancer (© Garland Science 2007)

Left: Growth of subcutaneous human colorectal ca cells over 20 days in mice

Right: Vascularization can be suppressed by ZD6474 (inhibitor of VEGF receptor)-bottom.

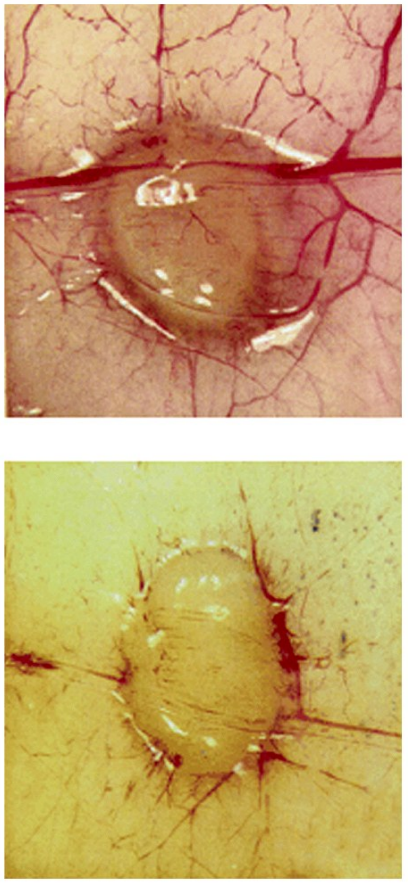


Figure 13-32b The Biology of Cancer (© Garland Scienc

Tumor blood vessels are leaky

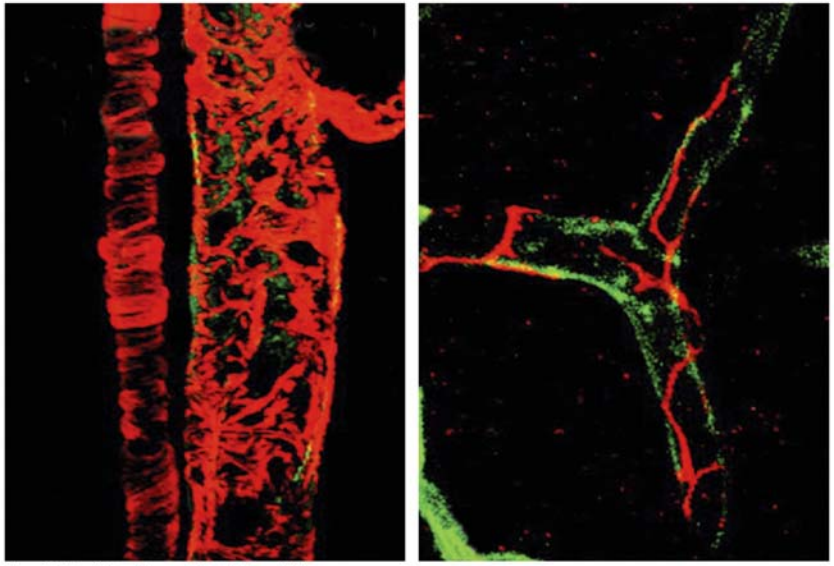
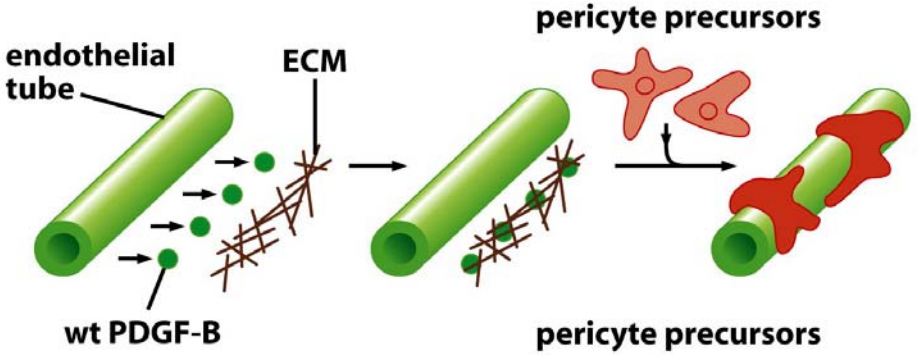


Figure 13-6b The Biology of Cancer (© Garland Science 2007)



Above: normal vessel

Below: tumor vessel

Pericytes only loosely attached -
walls of capillaries in tumors 10 leakier.

Leakiness also due to overproduction VEGF and imbalance between angiopoietin I and II

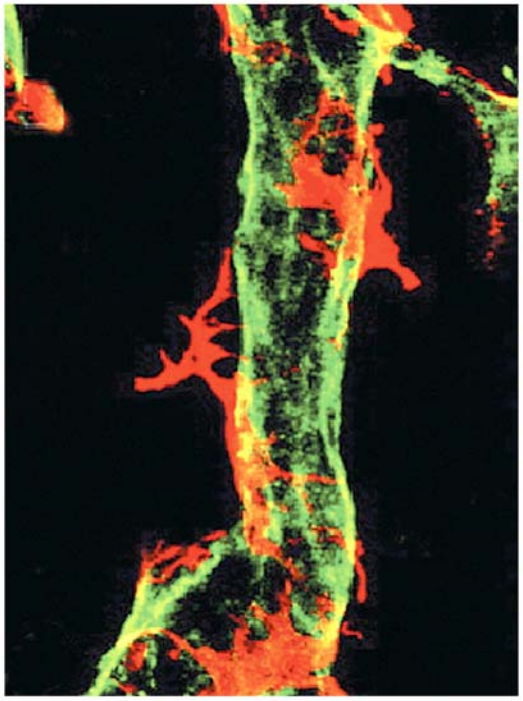


Figure 13-33 The Biology of Cancer (© Garland Science 2007)

Tumor vasculature is disorganized as well as leaky

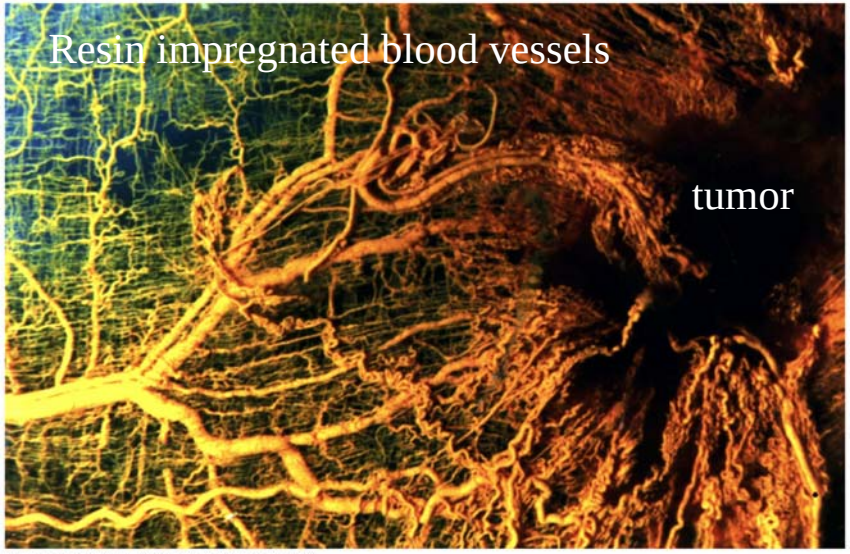


Figure 13-34a The Biology of Cancer (© Garland Science 2007)

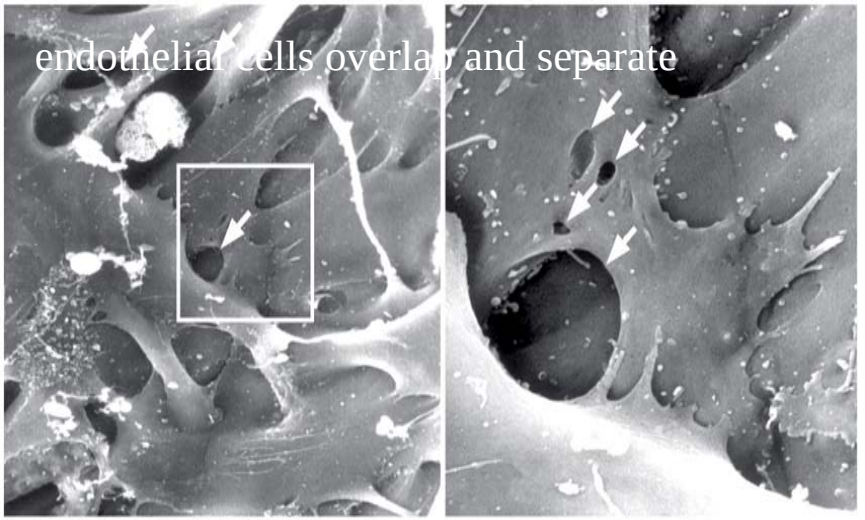


Figure 13-35 The Biology of Cancer (© Garland Science 2007)

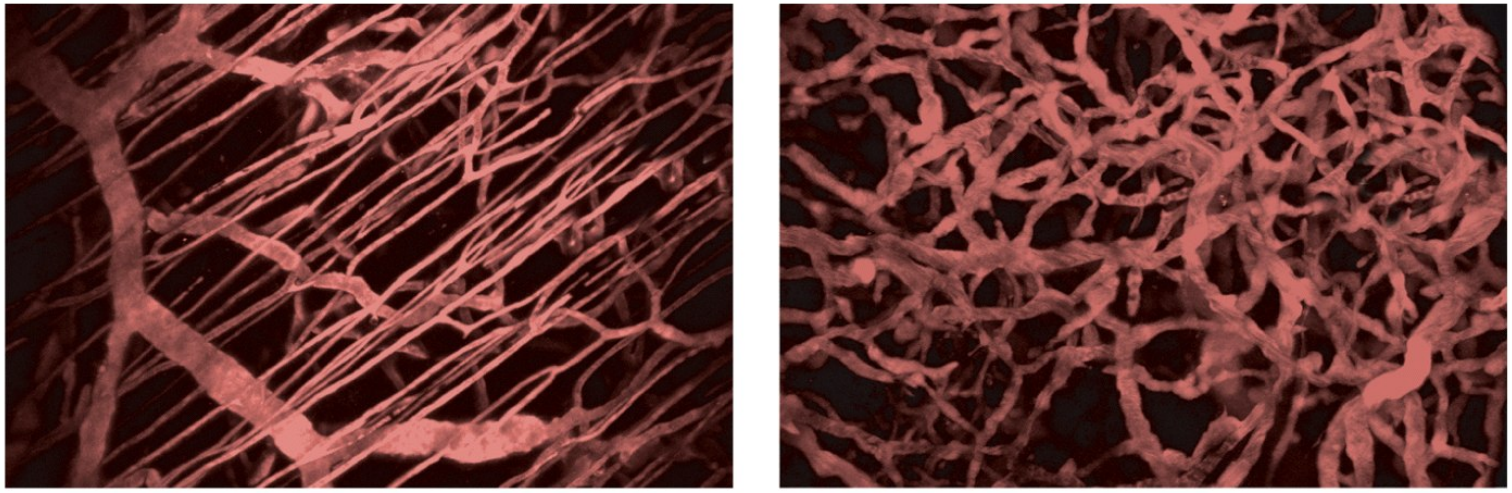


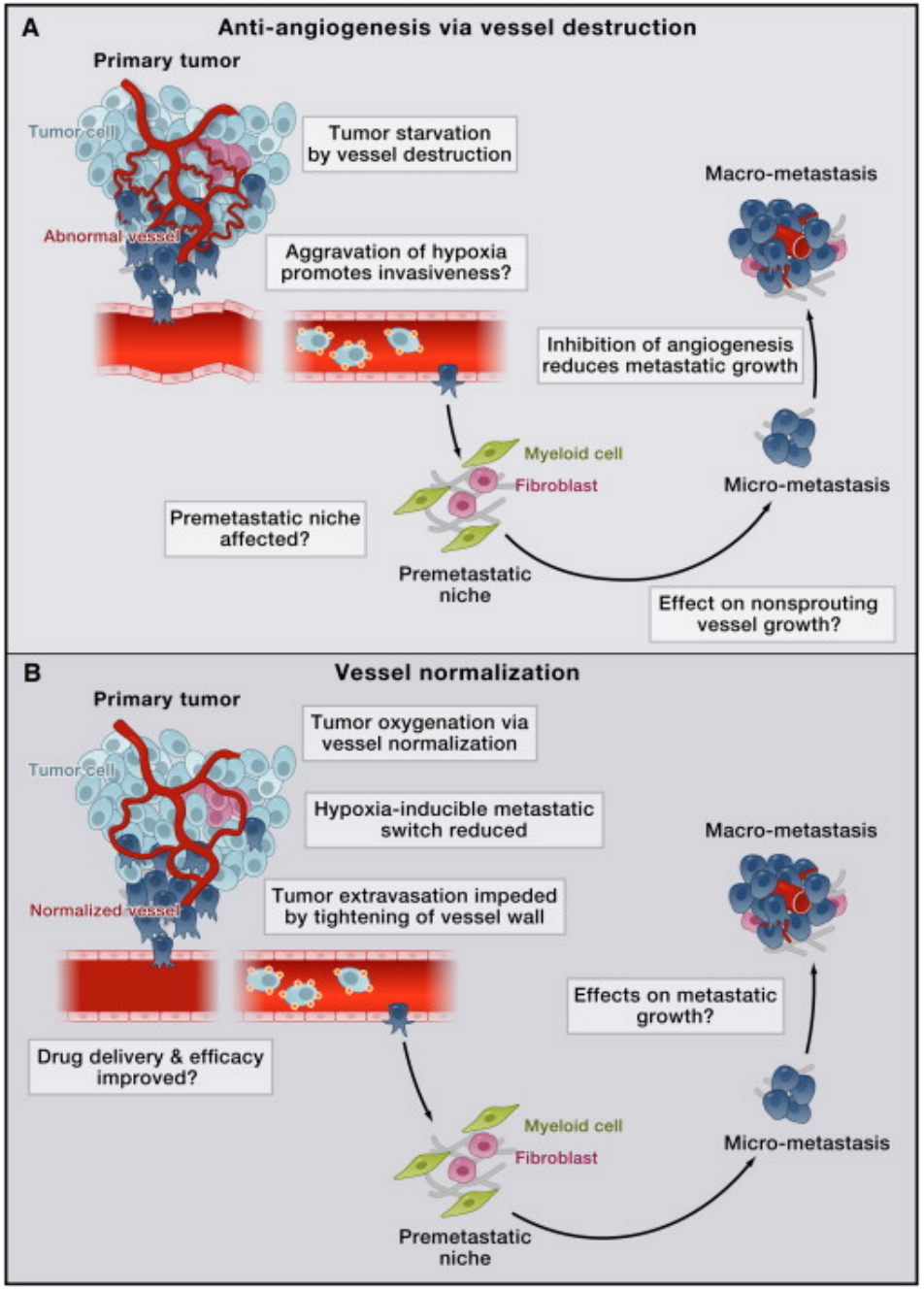
Figure 13-34b The Biology of Cancer (© Garland Science 2007)

Tumor vessels are chaotic

- EC’s lack cobblestone and may be multilayered
- Arterio-venous identity ill-defined- shunting
- Basement membrane irregular –fewer mural cells
- Uneven delivery of chemotherapeutics and reduced oxygenation decrease therapeutic efficacy
- Tumors may co-opt alternative vascular growth requirements by reducing dependence on VEGF.

Anti-angiogenesis versus vessel normalization as a therapeutic stratagem

Cell 146: 873-887 2011



Role of non tumor cells in tumor vascularization:

•Precursors of TIE2-expressing monocytes (TEM's) release growth factors. Tumor EC's express ANG2 activating TEMs to stimulate angiogenesis. Tumor associated macrophages release PIGF- vessel de-organization

•Mast cells secrete proteasesthat release pro-angiogenic factors from ECM.

•CXCR4 (SDF-1 R) +ve bone marrow derived cells are retained by tumor derived SDF-1 and release angiogenic factors into the tumor

•Myeloid cells believed to account for resistance to VEGF R inhibitors.

•Cancer associated fibroblasts originate from local mesenchyme or recruited from bone marrow

•Recruit endothelial progenitor cells and release pro-angiogenic factors

Tripping the angiogenic switch: insight from the Rip-Tag mouse

Most tumors initially lack the ability to attract blood vessels. The blood supply therefore limits growth of the primary tumor. Hypoxia is not sufficient to promote vascularization.

Rip-Tag transgenic mouse: insulin gene-dependent SV40 large T and small T antigen. Restricts expression of tumor to islets of Langherhans where tumor progression easily followed. Until tumors develop a blood supply hypoxia triggers p53-dependent apoptosis. Islets "suddenly" acquire the capacity to develop a blood supply and tumors start to grow.

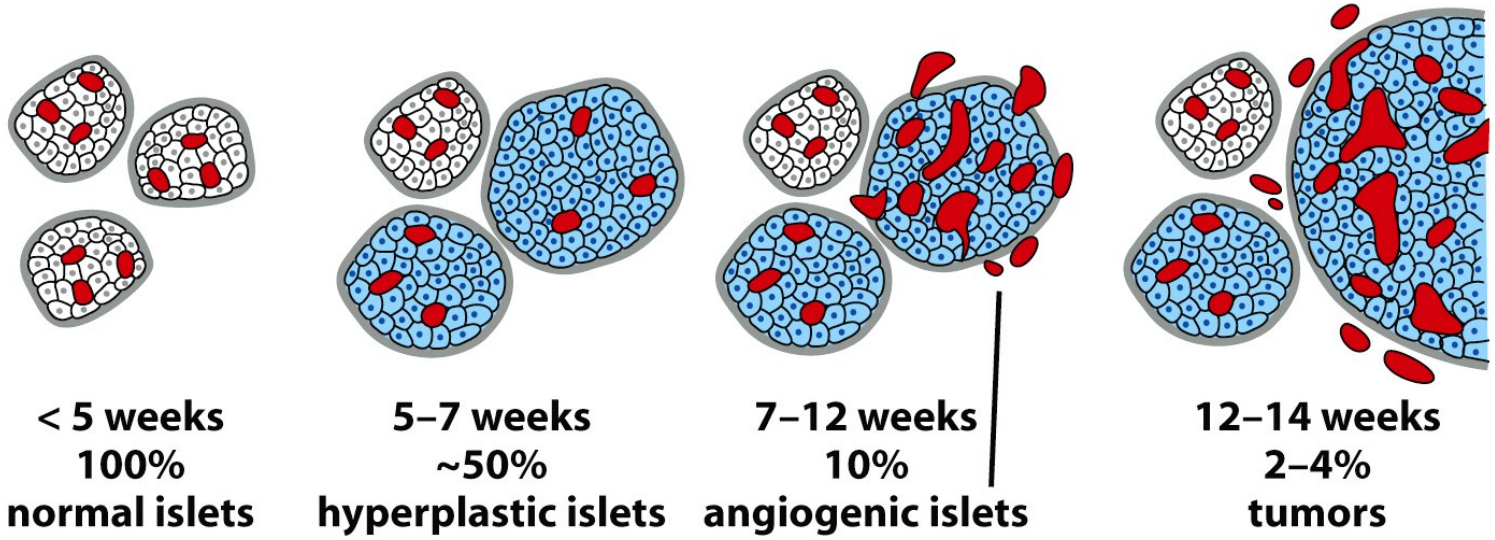
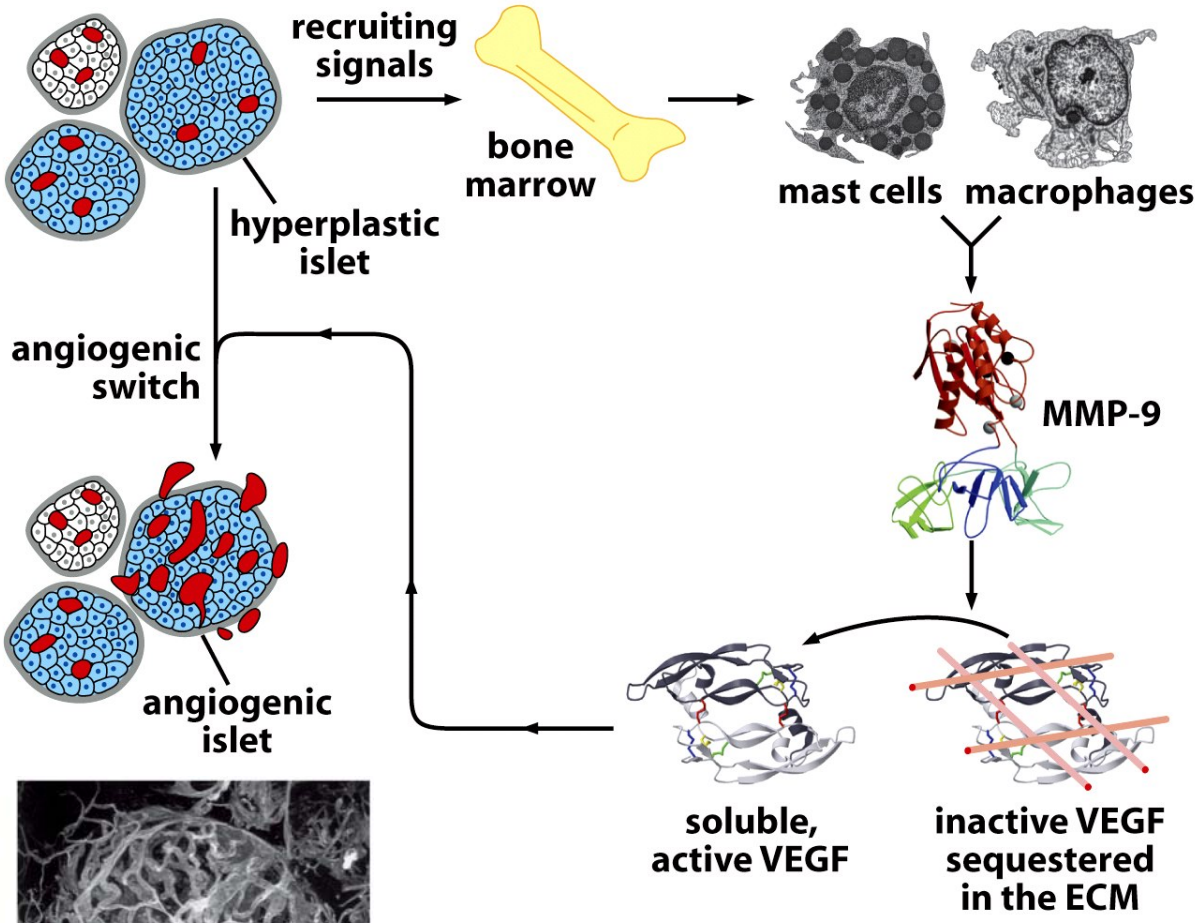
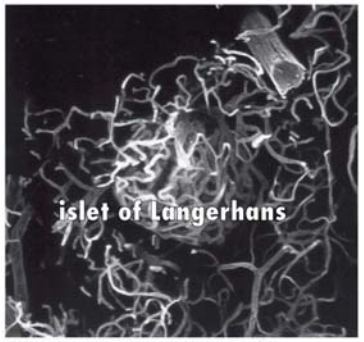
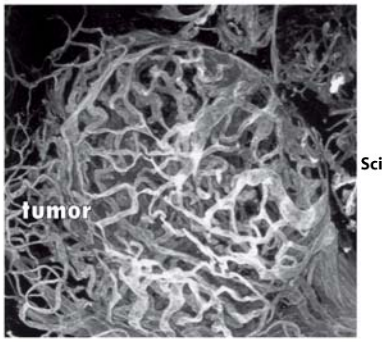


Figure 13-37 The Biology of Cancer (© Garland Science 2007)

Angiogenic switch and recruitment of inflammatory cells







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N.B. MMP9 not VEGF is limiting

Angiogenic switch is effected by recruitment and activation of macrophages

Different tumors depend on different angiogenic factors

Table 13.2 Important angiogenic factors

Name	Mol. wt. (kD)
Vascular endothelial GF (VEGF)	40–45
Basic fibroblast growth factor (bFGF)	18
Acidic fibroblast growth factor (aFGF)	16.4
Angiogenin	14.1
Transforming growth factor-α (TGF-α)	5.5
Transforming growth factor-β (TGF-β)	25
Tumor necrosis factor-α (TNF-α)	17
Platelet-derived growth factor (PDGF)	45
Granulocyte-colony–stimulating factor	17
Placental growth factor	25
Interleukin-8 (IL-8)	40
Hepatocyte growth factor (HGF)	92
Proliferin	35
Angiopoietin	70
Leptin	16

Table 13-2 The Biology of Cancer (© Garland Science 2007)

Clinical outcome and the intensity of angiogenesis

Breast cancer patients with low microvessel density exhibit better survival. VEGF production correlates negatively with survival.

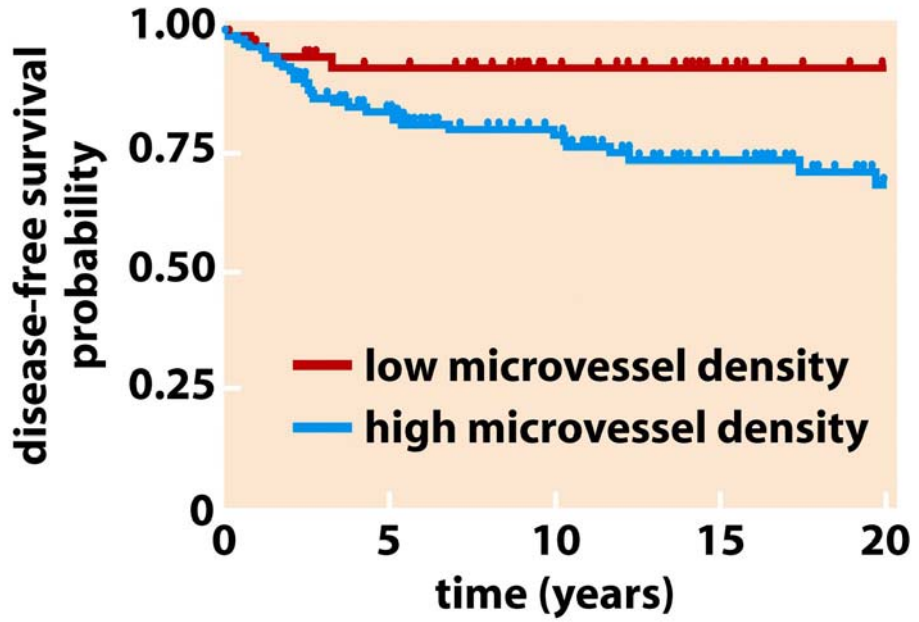


Figure 13-42a The Biology of Cancer (© Garland Science 2007)

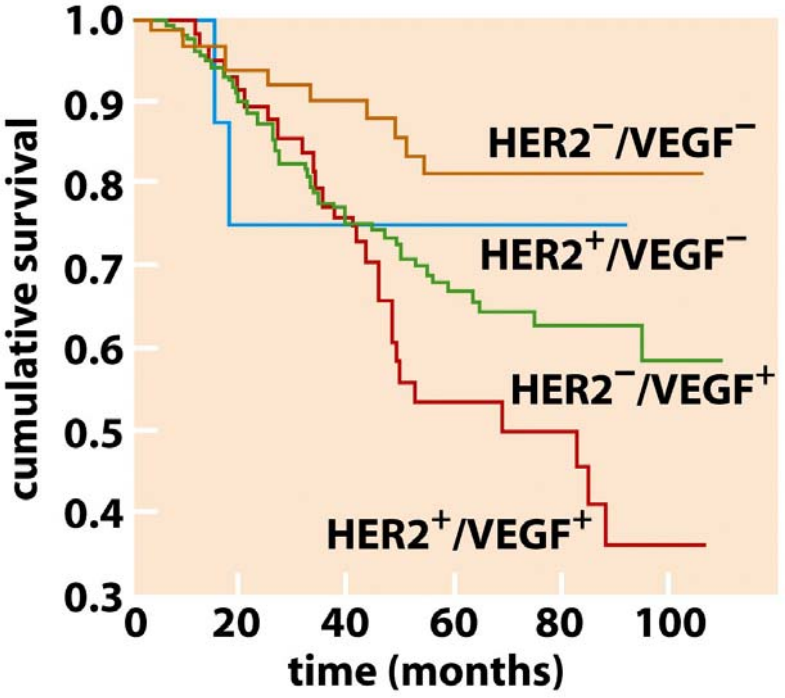
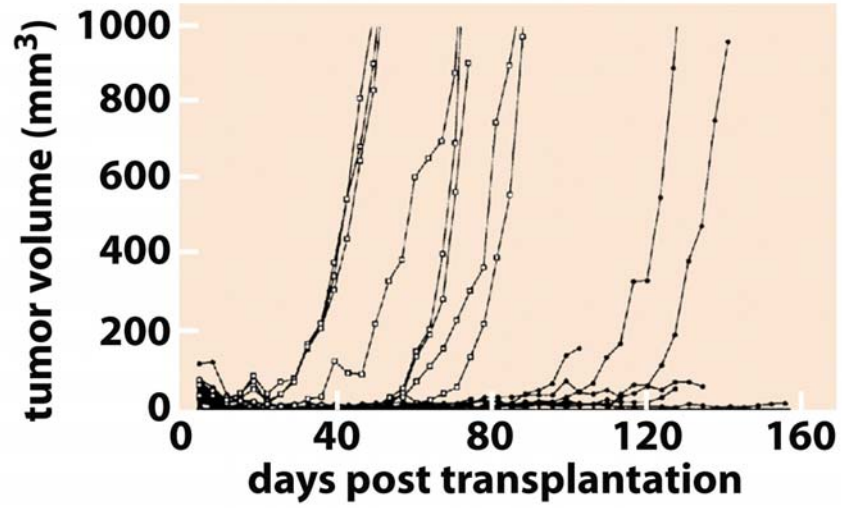
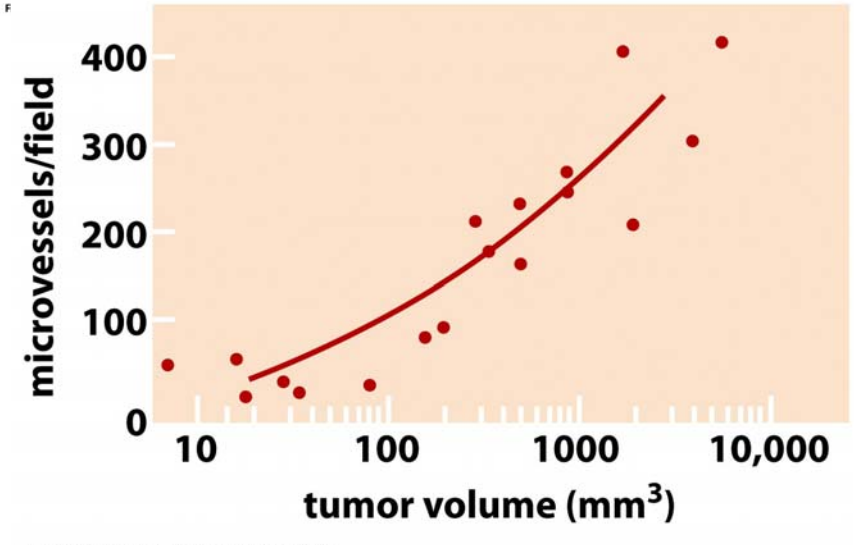


Figure 13-42b The Biology of Cancer (© Garland Science 2007)

Tumor cells vary greatly in angiogenic potential





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Liposarcoma cell line –subcloned and implanted into nude mice.

Because tumors are very heterogeneous in angiogenic potential the weakly angiogenic cells are supported by their highly angiogenic neighbors.

Angiogenesis is suppressed by physiological inhibitors

In wound healing important to shut down capillaries once reach density to support normal tissue (for example by suppressing HIF).

Components of ECM are ALSO important antagonists of angiogenesis.

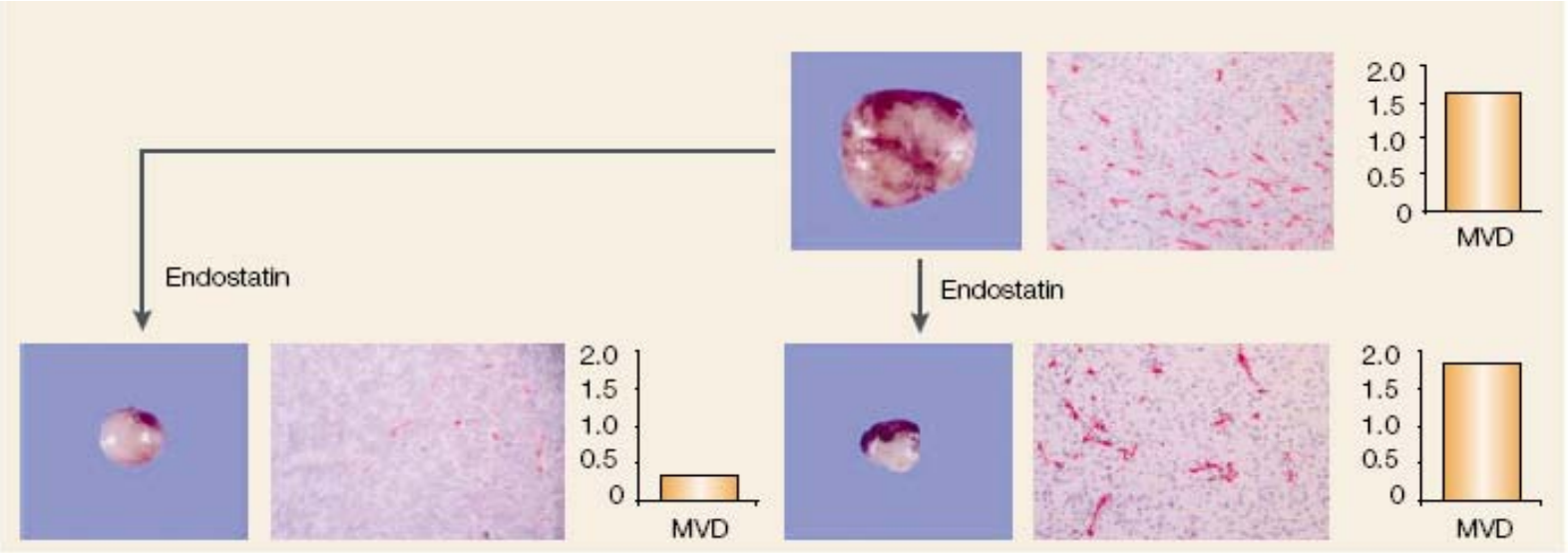
Table 13.3 Endogenous inhibitors of angiogenesis

Inhibitor	Description
A. Derived from extracellular matrix	
Arresten	fragment of type IV collagen α_1 chain of vascular basement membrane
Canstatin	fragment of type IV collagen α_2 chain of vascular basement membrane
EFC-XV	fragment of type XV collagen
Endorepellin	fragment of perlecan
Endostatin	fragment of collagen type XVIII
Anastellin	fragment of fibronectin
Fibulin	fragment of basement membrane protein
Thrombospondin-1 and -2	ECM glycoproteins
Tumstatin	fragment of type IV collagen α_3 chain
Chondromodulin-I	component of cartilage ECM
Troponin I	component of cartilage ECM
B. Non-matrix–derived	
Growth factors and cytokines	
Interferon-α (IFN-α)	cytokine
Interleukins (IL-1β, -12, -18)	cytokines
Pigment epithelium-derived factor (PEDF)	growth factor
Platelet factor-4	released by platelets during degranulation
Other types	
Angiostatin	fragment of plasminogen
Antithrombin III	fragment of antithrombin III
2-Methoxyestradiol	endogenous metabolite of estrogen
PEX	fragment of MMP-2
Plasminogen kringle 5	fragment of angiostatin
Prolactin fragments	specific cleavage fragment
Prothrombin kringle 2	fragment of prothrombin
sFlt-1	soluble form of VEGF-R1 (= Flt-1)
TIMP-2	inhibitor of metalloproteinase -2
TrpRS	fragment of tryptophan yl-tRNA synthetase
Vasostatin	fragment of calreticulin

Adapted from P. Nyberg, L. Xie and R. Kalluri, *Cancer Res.* 65:3967–3979, 2005.

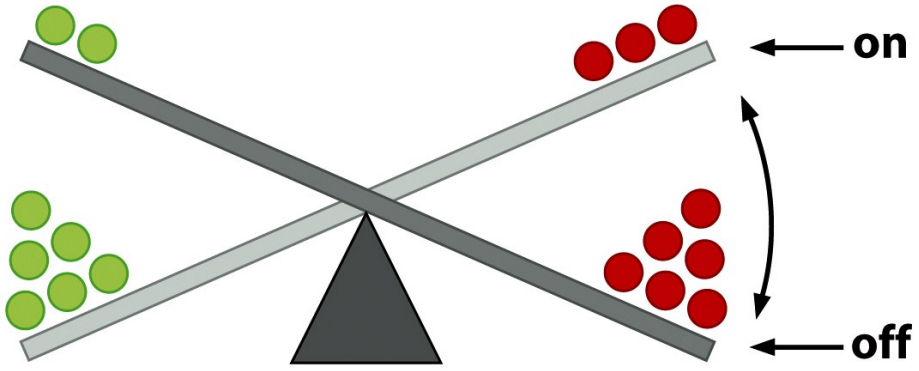
Table 13-3 The Biology of Cancer (© Garland Science 2007)

Treatment of tumors with endostatin decreases density of vascularity



However, some tumors regress without reduction in vascularity indicating that endostatin can also inhibit tumor growth through non-angiogenic mechanisms.

Balancing the angiogenic switch



activators
VEGF-A
VEGF-B, -C
FGF1 (aFGF)
FGF2 (bFGF)
other FGFs
etc.

inhibitors
thrombospondin-1, -2
interferon α/β
angiostatin
endostatin
collagen IV fragments
etc.

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Angiogenesis is an attractive therapeutic target because it involves the growth of normal cells which are genomically stable and therefore unlikely to rapidly develop resistance

Angiogenesis inhibitors and clinical trial

Table 13.4 Angiogenesis inhibitors and their development and use in clinical trials

Name	Status	Responses
A. Endogenous inhibitors of angiogenesis		
Endostatin	in clinical trial	scattered responses
Interferons-α and -β	effective in treating hemangioblastomas	Kaposi's sarcomas; limited efficacy against most other types of tumors
B. Agents that block VEGF and VEGF-R signaling		
Avastin anti-VEGF MoAb	in clinical trial	delayed progression 1–3 months in lung, 3–4 months in colon
SU5416 inhibitor of VEGF-R2 (Flk-1)	trial abandoned	severe vascular toxicities
ZD6474 inhibitor of VEGF-R2	under clinical test	
CP547, 632 inhibitor of VEGF-R2	in trial	
C. Miscellaneous other drugs		
Thalidomide	in trial	inhibits bFGF- and VEGF-dependent angiogenesis
Squalamine sterol from shark liver	in trial	strong anti-angiogenic activity
Celecoxib anti-inflammatory drug	in trial	multiple anti-neoplastic effects
ZD6126	in trial	antagonist of tubulin in endothelial cell cytoskeleton
Fumagillin and TNP-470	in trial; slowed tumor growth	antagonist of methionine aminopeptidase in endothelial cells
D. Inhibitors of ECM breakdown—MMP inhibitors		
Marimastat	in clinical trial	no delay of tumor progression
Prinomastat	in clinical trial	no slowing of tumor progression
BMS275291	in clinical trial	
BAY12-9566	in clinical trial	
Neovastat (shark cartilage MMPI)	in clinical trial	

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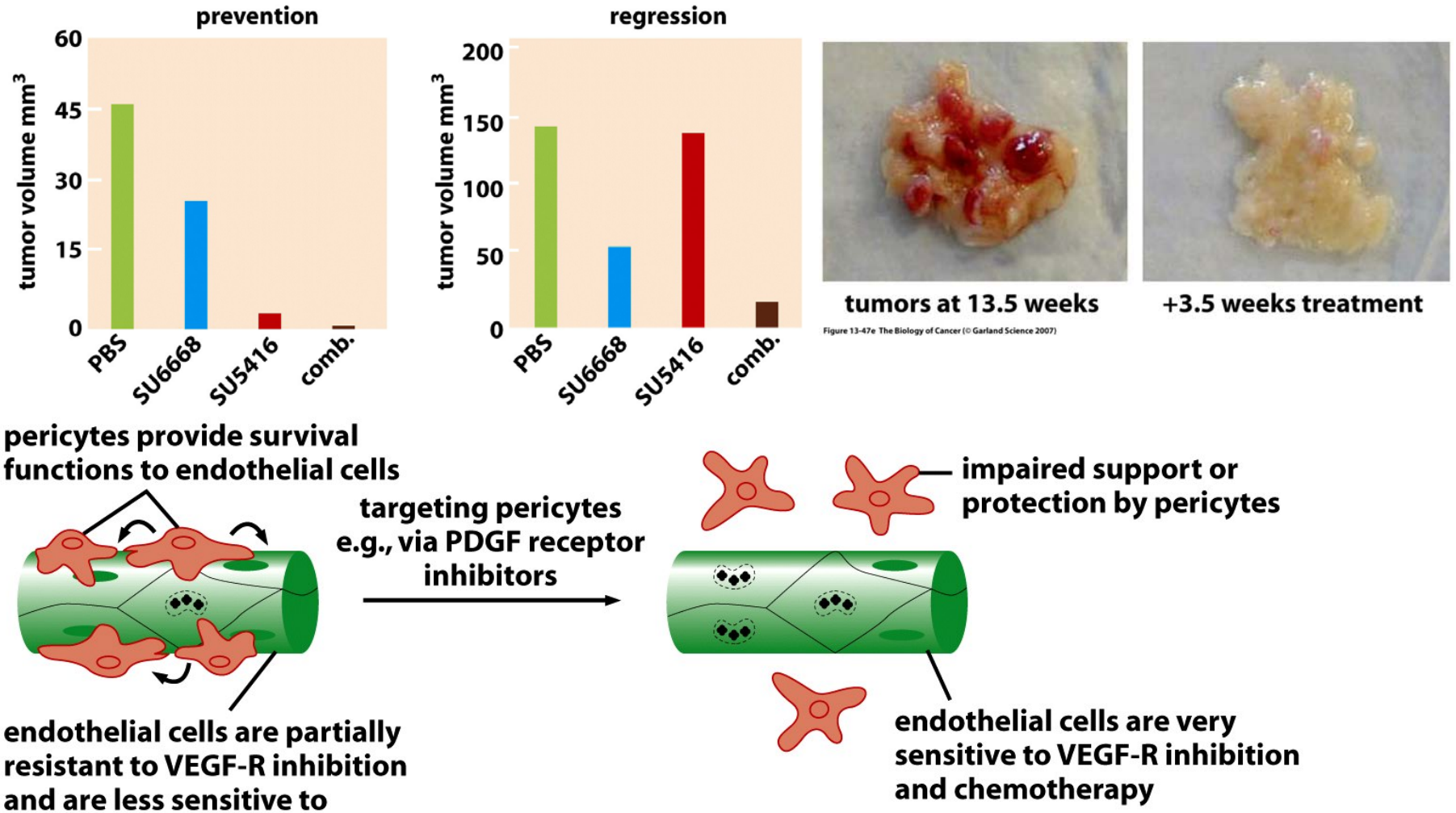
Clinically approved antiangiogenic therapy

- Avastin (anti-VEGF antibody) ~ combination with chemo/cytokine therapy treatment of metastatic cancers (non small cell lung cancer, colorectal cancer, renal cell, breast cancer).
- Pan VEGF- receptor tyrosine kinase inhibitors (Sunitinib) – metastatic RCC
- anti-VEGF R inhibitors block vascular branching and homing of BMDC's Deprive tumor vasculature of VEGF-survival. Prune immature pericyte devoid vessels.
- Clinical benefit limited. Months. Subsets of patients refractory and other acquire resistance.
- Some tumors produce other angiogenic factors Hypoxia upregulates proangiogenic molecules (PIGF, IL-8).
- VEGF receptor inhibitors induce hypoxia and create pro-inflammatory environment

Alternative anti-vascularization strategies

- VEGF inhibitors more effective on peri-cyte free vessels (capillaries). Targeting both EC and pericytes may increase efficacy but not promising
- Sustained vascular normalization – restore structure thereby increasing oxygenation and preventing hypoxia induction of vascularization genes.
- Role of VEGF (R) inhibitors in micrometastatic disease?
- Development of novel anti-angiogenic drugs to use in combination with VEGF R inhibitors.

Angiogenesis inhibitors as treatment of islet cell carcinogenesis



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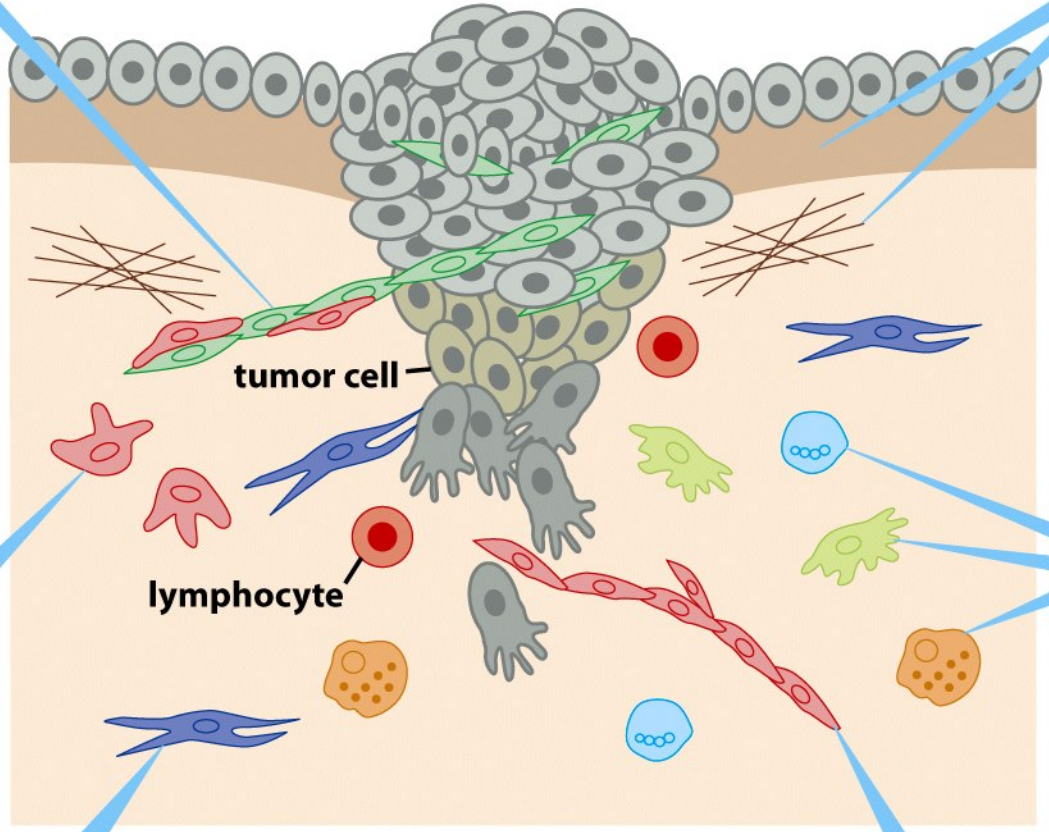
SU6668 (PDGF-R inhibitor) SU5416 (VEGF-R inhibitor). Treatment most effective when 2 inhibitors used in combination.



ENDOTHELIAL CELL

Inhibitors of VEGF, FGF, etc., signaling, e.g., anti-VEGF and anti-VEGF-R antibodies, small-molecule VEGF-R inhibitors, VEGF-Trap, Ang2/Tie2 blocking antibodies. Endogenous angiogenesis inhibitors, e.g., endostatin, tumstatin. Inhibitors of EPC recruitment.

PERICYTE

Inhibitors of PDGF signaling, e.g., anti-PDGF antibodies, PDGF-R inhibitors. Inhibitors of Ang-1/Tie2 signaling.

FIBROBLAST

Inhibitors of HGF or its receptor c-Met, inhibitors of CXCL12/ SDF-1, PDGF/PDGF-R, of fibroblast activation protein, e.g., sibrotuzumab.

BASEMENT MEMBRANE EXTRACELLULAR MATRIX

Inhibitors of matrix turnover, e.g. suramin, dalteparin and matrix-degrading enzymes, e.g., proteases (cathepsins, MMPs, uPA etc.), endoglycosidases (e.g., heparanase). Inhibitors of ECM contact, e.g., integrin $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha 5\beta 1$, or $\alpha 6\beta 4$ antibodies.

NEUTROPHIL MACROPHAGE MAST CELL

Anti-inflammatory inhibitors, e.g., cytokine and chemokine inhibitors, NF-κB, IKK, TNF-α inhibitors.

LYMPHATIC CELL

Anti-lymphatic targeting: inhibitors of VEGF-C, VEGF-D, VEGF-R3, or PDGF/PDGF-R.

Figure 13-49 The Biology of Cancer (© Garland Science 2007)

Supplementary reading

1. Basic and Therapeutic Aspects of Angiogenesis: M. Potente, H. Gerhardt and P. Carmeliet Cell 146: 873-887 2011

1. Tumorigenesis and the angiogenic switch G. Bergers and L.E. Benjamin Nature Reviews Cancer 3, 401 -410 (2003).

2. Weinberg Chapter 13.

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