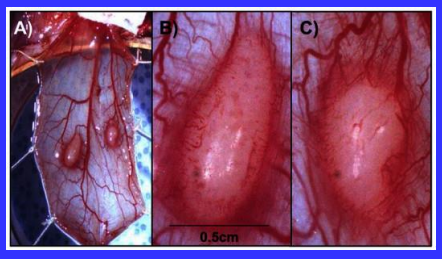
Cellular Composition of Tumors

Carcinomas: ***Epithelial cell-derived.***

Stroma:



Fibroblasts, Myofibroblasts, Fibrocytes
Inflammatory/Immune Cells
- Lymphocytes
- T-Cells, Dendritic cells
- NK cells
- Neutrophils
- Monocytes/Macrophages
- Mast cells

Vascular Cells
- Endothelial cells
- Endothelial Precursor Cells
- Pericytes/Smooth Muscle Cells

Sarcomas: ***Mesenchymal cell-derived***

Stroma: All the above!

Relationship Between Wound Healing and Carcinogenesis

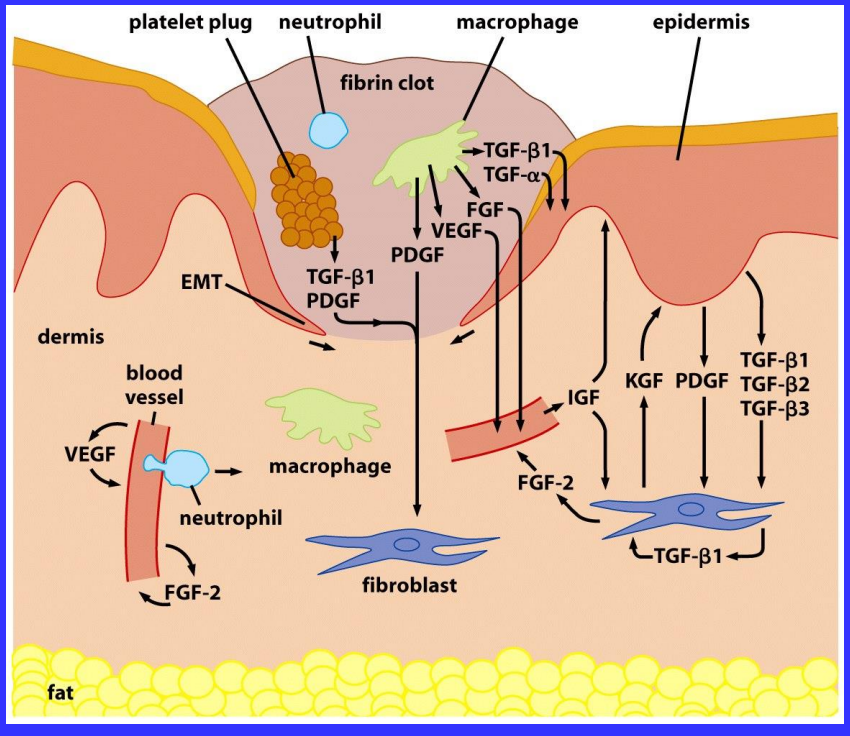


Figure 13.14 *The Biology of Cancer* (© Garland Science 2007)

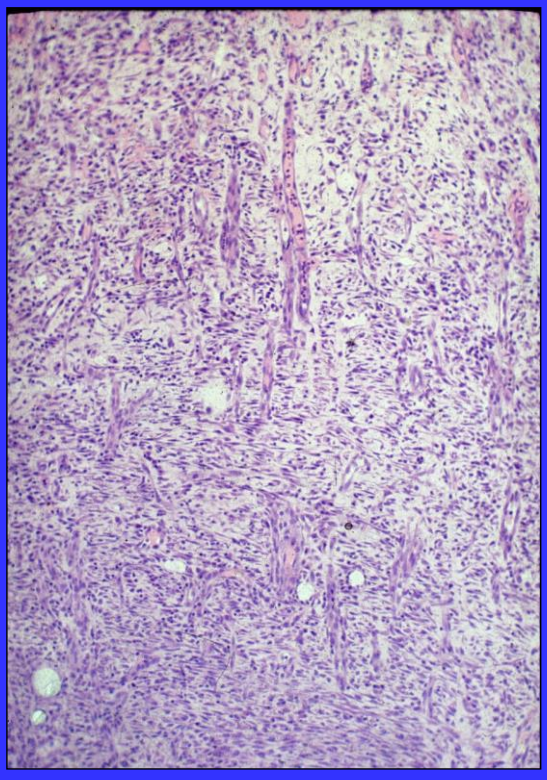
Moses Judah Folkman, 1933-2008

"Father of Angiogenesis"

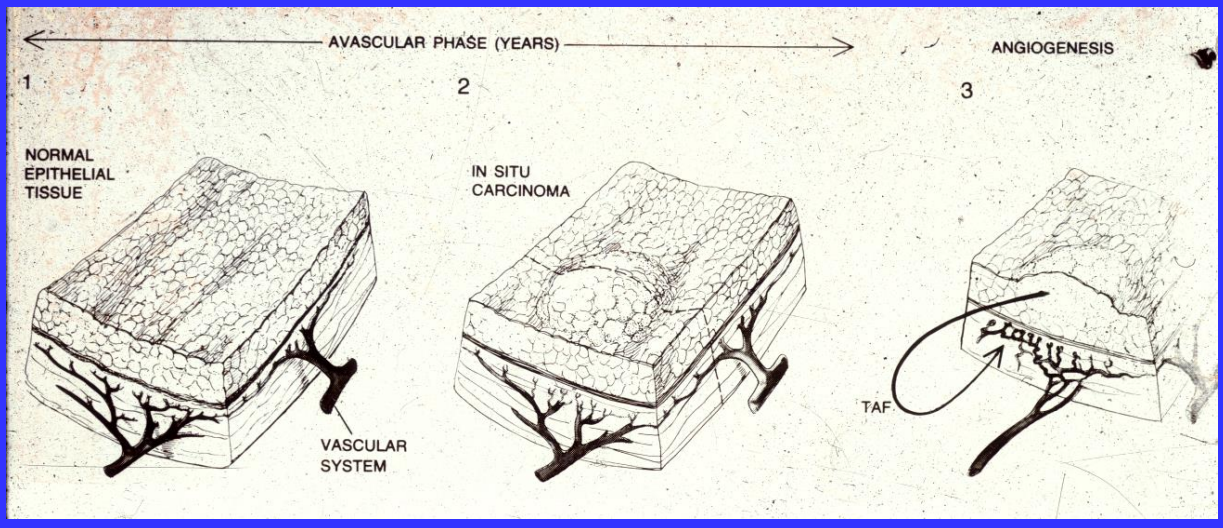
ANGIOGENESIS

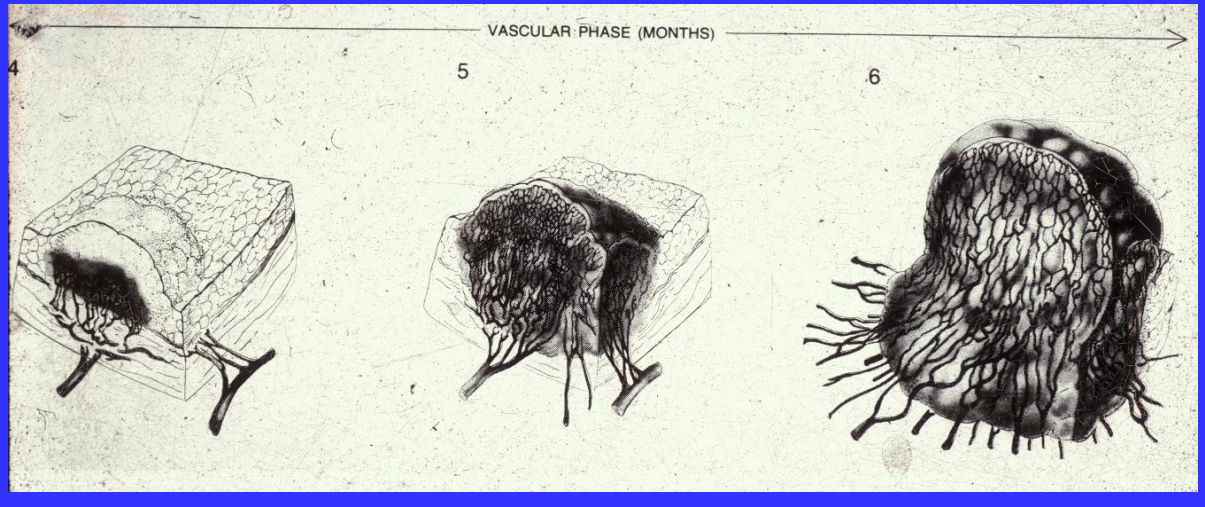
- Prominent during embryogenesis, development and growth
- Virtually absent in adults
- Prominent in ovulation, menstrual cycle and placental formation
- Critical in wound repair and granulation tissue formation
- Prominent in chronic inflammation and fibrosis
- Critical in solid tumor growth and development

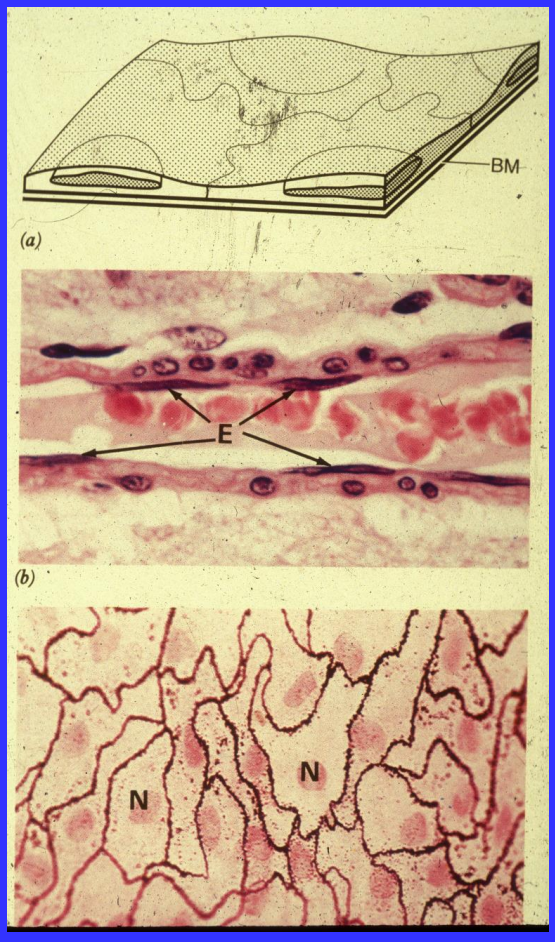
Granulation Tissue



Tumour Angiogenesis




VASCULAR PHASE (MONTHS)
4
5
6


BM
(a)
E
(b)
N
N

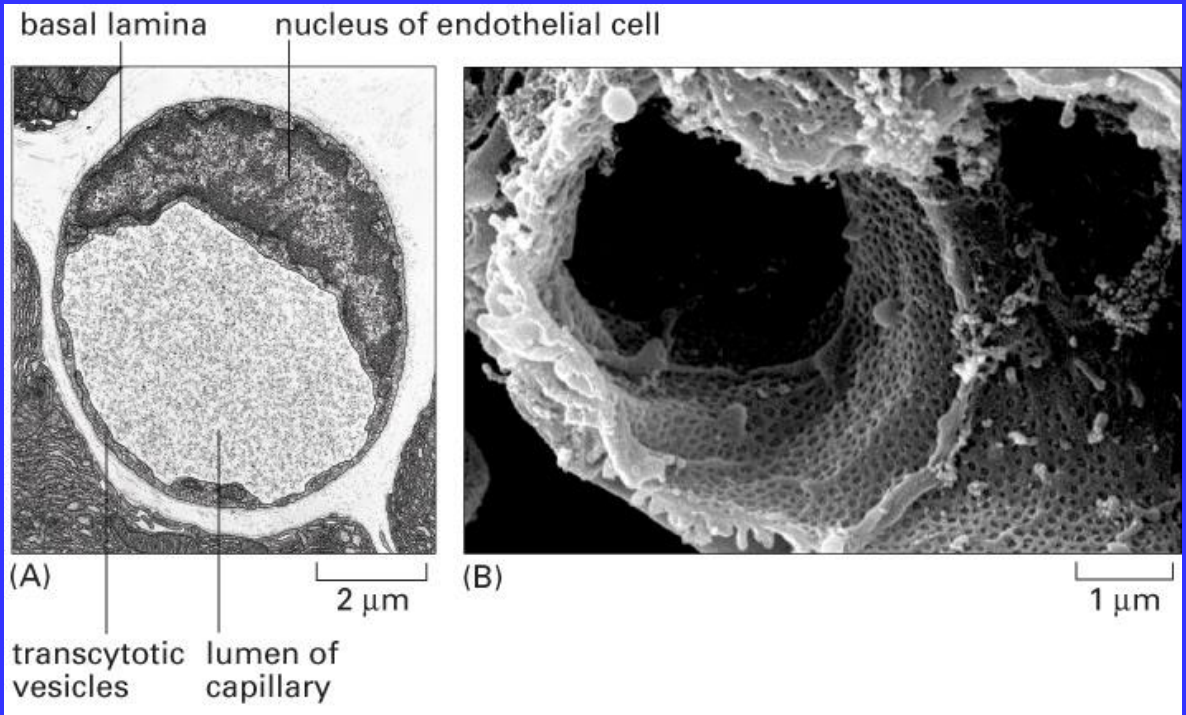


Figure 22–23. Molecular Biology of the Cell, 4th Edition.

ANGIOGENESIS

- Target Cells:
  - Endothelial Cells and Pericytes/Smooth Muscle Cells of Capillaries and Small Venules
- Processes Involved:
  - Disruption of Blood Vessel Continuity
  - Activation/De-Repression of Endothelial Cells
  - Degradation of Basement Membrane
  - Cell Migration
  - Cell Proliferation
  - Lumen Formation
  - Reformation of Basement Membrane
  - Cell Maturation
  - Capillary Loop Formation

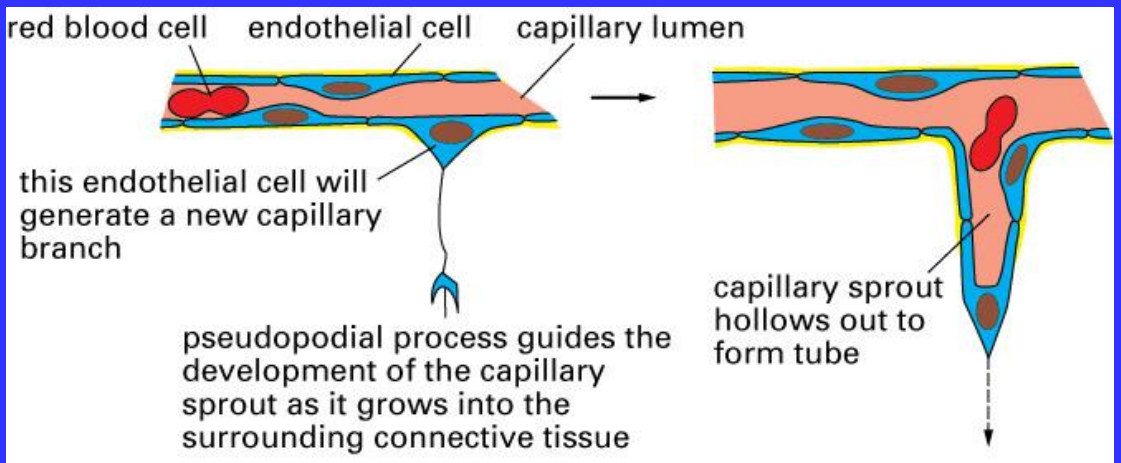
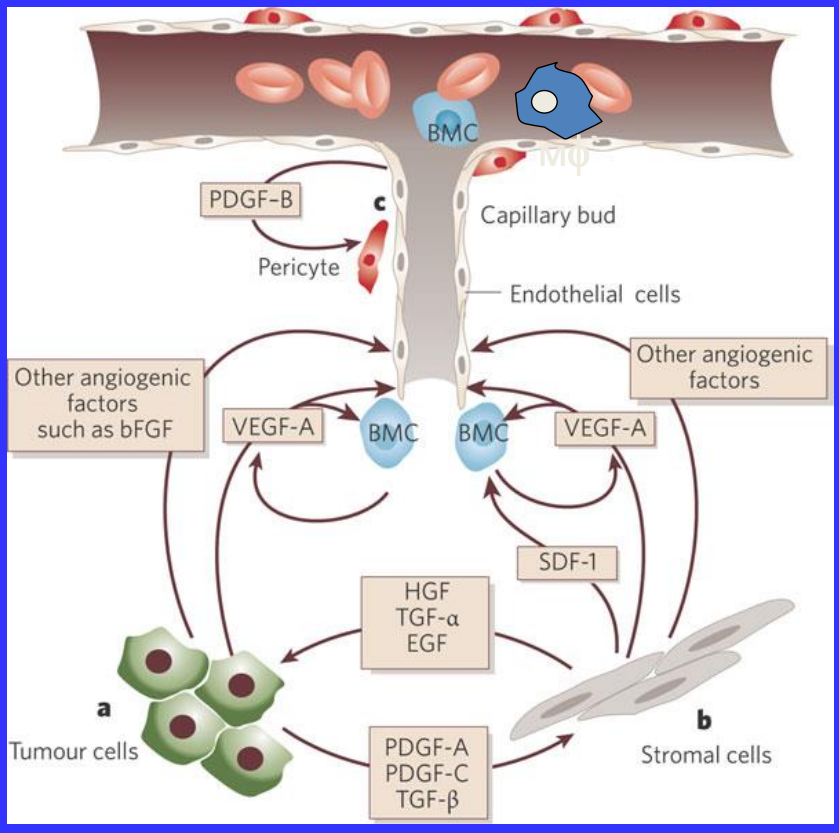


Figure 22–25. Molecular Biology of the Cell, 4th Edition.


BMC
PDGF-B
c
Pericyte
Capillary bud
Endothelial cells
Other angiogenic factors such as bFGF
VEGF-A
BMC
BMC
VEGF-A
Other angiogenic factors
SDF-1
HGF
TGF-α
EGF
a
Tumour cells
b
Stromal cells
PDGF-A
PDGF-C
TGF-β

ANGIOGENIC FACTORS

- Fibroblast Growth Factors (FGFs)
  - Two major forms:
    - FGF-1 (aFGF) and FGF-2 (bFGF)
    - M.Wt. 17kDa
    - Bind strongly to HEPARIN
    - No SECRETORY SIGNAL SEQUENCE
    - Found in BASEMENT MEMBRANES
  - Questions:
    - How are FGFs mobilized in Angiogenesis?
    - Are FGFs an autocrine control factor for endothelial cells?

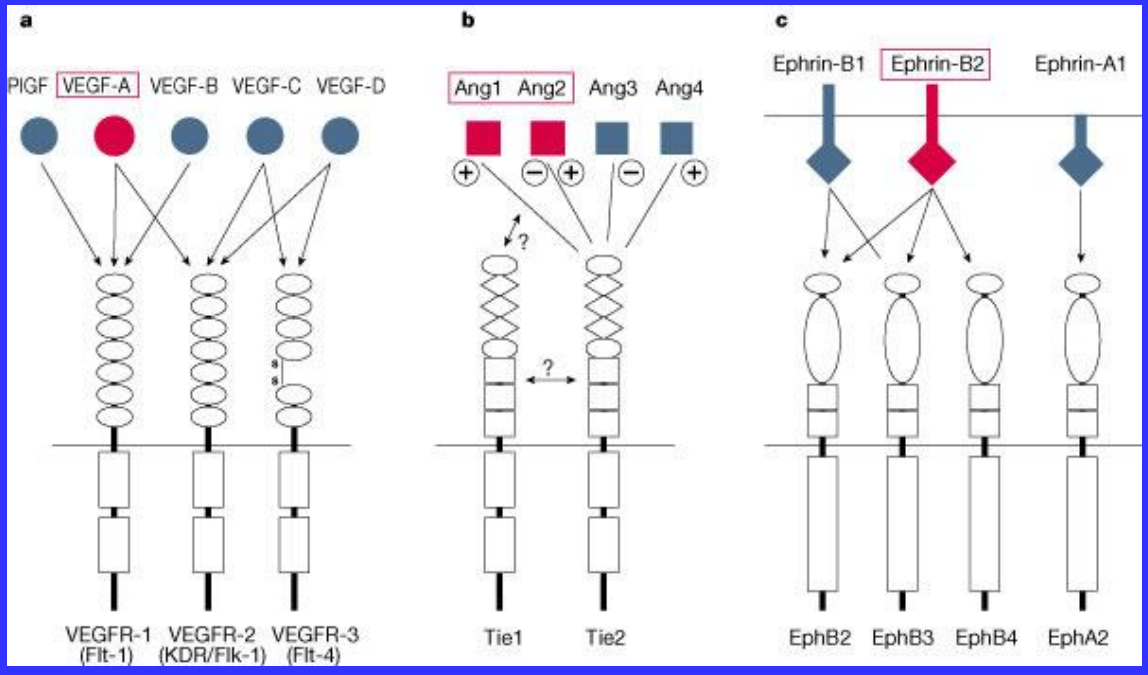
Vascular Endothelial Growth Factor (VEGF)

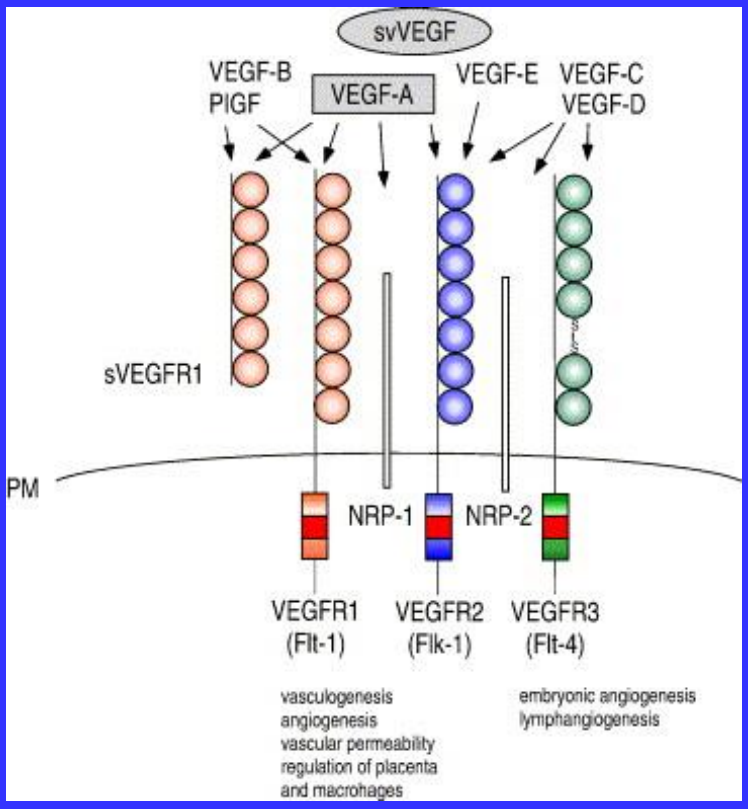
Vascular Permeability Factor

- Dimeric, heparin binding protein
- Potent regulator of vascular permeability
  - 50,000 X more potent than histamine
- Secreted by a wide range of tumor cells
  - carcinomas, sarcomas, glioblastomas, monocytic leukemia cells
- Secreted by macrophages
- VEGF = VPF
  - One gene, eight alternatively spliced exons
  - In human, four molecular species (121, 165, 189, 206 aa)
  - In mouse, three molecular species (121, 165, 189 aa)
- Hypoxia is a major regulator of expression via activation of HIF1.
- Adenosine signaling is also major regulator of VEGF expression.

Receptors & Ligands Involved in Regulation of Angiogenesis

LIGAND	RECEPTOR	FUNCTION
VEGF	VEGFR-2 (KDR, Flk-1) VEGFR-1 (Flt-1)	Endothelial Mitogen
Angiopoietin-1 Angiopoietin-2 (acts as antagonist)	TIE-2	Recruitment of accessory cells (SMCs, pericytes)
?	TIE-1	Endothelial cell-cell Interactions
Ephrin-B2 (Arterial)	Eph-B4 (Venous)	Differentiation of arterial vs venous microvasculature
Neuropilins	VEGF-R2	Patterning?


a
PlGF
VEGF-A
VEGF-B
VEGF-C
VEGF-D
VEGFR-1 (Flt-1)
VEGFR-2 (KDR/Flk-1)
VEGFR-3 (Flt-4)
b
Ang1
Ang2
Ang3
Ang4
Tie1
Tie2
c
Ephrin-B1
Ephrin-B2
Ephrin-A1
EphB2
EphB3
EphB4
EphA2

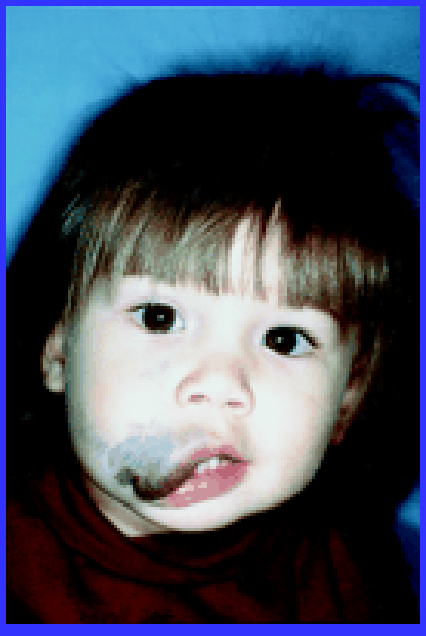

svVEGF
VEGF-B
PIGF
VEGF-A
VEGF-E
VEGF-C
VEGF-D
sVEGFR1
PM
NRP-1
NRP-2
VEGFR1
(Flt-1)
VEGFR2
(Flk-1)
VEGFR3
(Flt-4)
vasculogenesis
angiogenesis
vascular permeability
regulation of placenta
and macrohages
embryonic angiogenesis
lymphangiogenesis

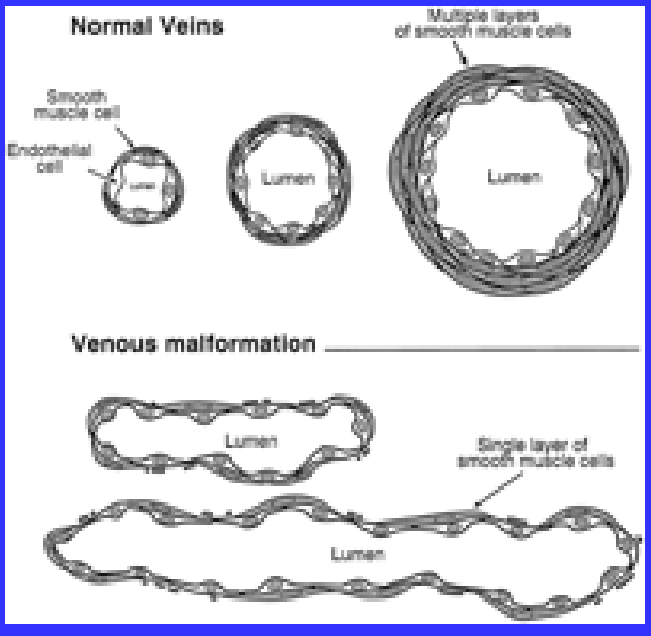
VEGF and VEGF RECEPTORS

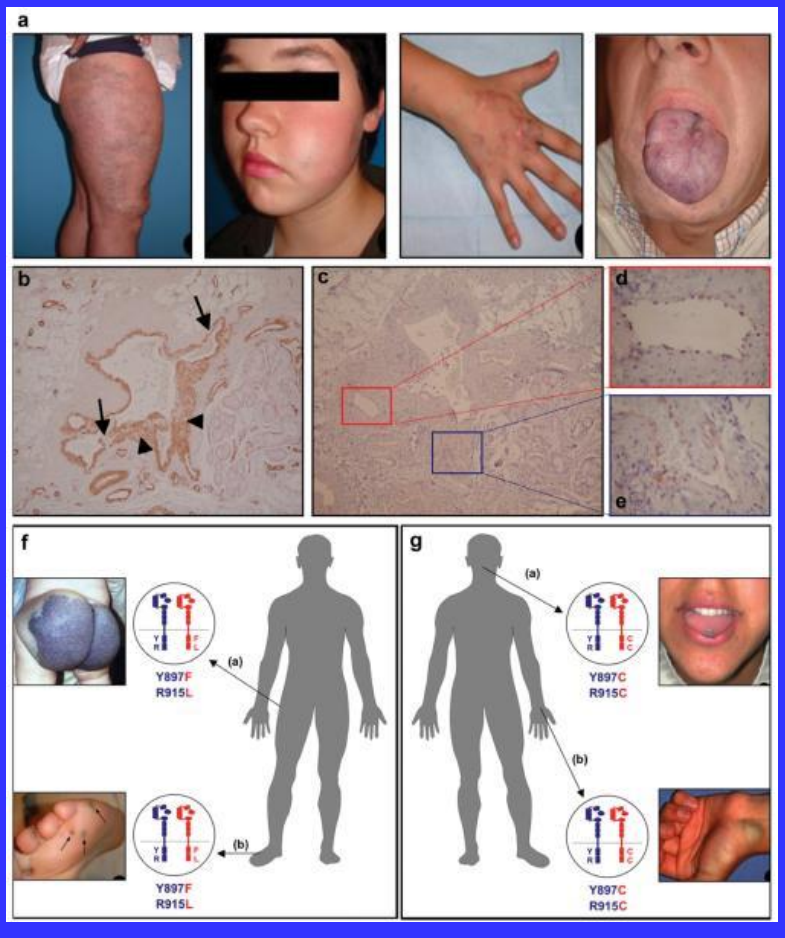
- VEGF
  - Essential for both vasculogenesis and angiogenesis
  - Knockouts:
    - Die at 8.5-9.5 days in utero.
    - Delayed differentiation of endothelial cells.
    - impairment of both angiogenesis and vasculogenesis.
- VEGF-R2 (Flk-1, KDR):
  - Restricted to endothelial cells and their embryonic precursors.
  - Knockouts:
    - Die in utero between 8.5 and 9.5 days.
    - Yolk sac blood islands do not form.
    - No organized blood vessels in embryo yolk sac..
    - Required for hemangioblast to endothelial cell differentiation
- VEGF-R1 (Flt-1):
  - Restricted to endothelial cells and their embryonic precursors.
  - Knockouts:
    - Die in utero at mid-somite stage (Day 9).
  - Essential for organization of embryonic vasculature (EC cell-cell or cell-matrix interaction).
  - Not essential for endothelial cell differentiation.

Endothelial Cell-Specific Tyrosine Kinase Receptors

- TIE-1:
  - Knockouts:
    - Form a primitive vasculature
    - Fail to develop structural integrity of vascular endothelial cells.
    - Develop edema and localized hemorrhage.
    - Die in utero at 9-10 days.
    - Ligand unknown
- TIE-2:
  - Knockouts:
    - Fail to recruit smooth muscle cells and pericytes precursors to primitive vasculature.
    - Poorly developed pericardium in heart.
    - Die in utero at 9-10 days.
  - Important in angiogenesis for vascular network formation.
  - Ligand is ANGIOPOIETIN-1 (Ang-1) – EC chemoattractant.
    - Knockouts of Ang-1 have phenotype similar to Tie-2 knockouts.
  - Mutation of TIE-2 in patients (Arg - Tryp):
    - Venous malformations.
    - Develop vein-like structures deficient in non-endothelial cells. Mainly lack smooth muscle cells and pericytes).
    - Angiopoietin-2 (Ang-2): Competitive inhibitor of Ang-1.
-




Normal Veins
Multiple layers of smooth muscle cells
Smooth muscle cell
Endothelial cell
Lumen
Lumen
Venous malformation
Lumen
Single layer of smooth muscle cells
Lumen


a
b
c
d
e
f
g
Y897F
R915L
(a)
(b)
Y897F
R915L
(a)
Y897C
R915C
(b)
Y897C
R915C

Endothelial Cell - Pericyte Interactions

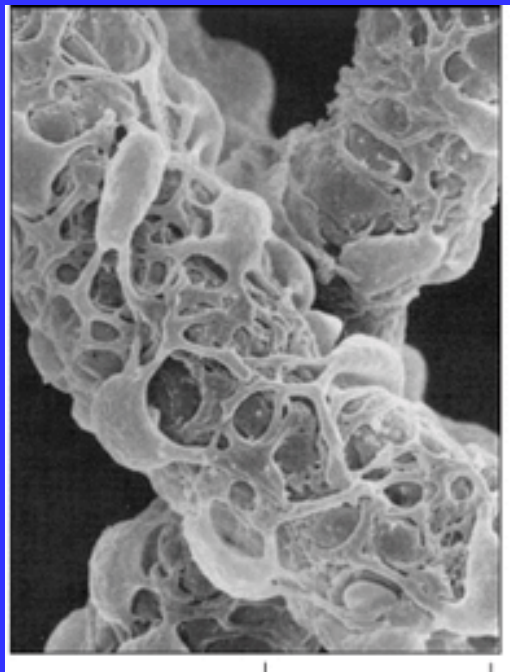
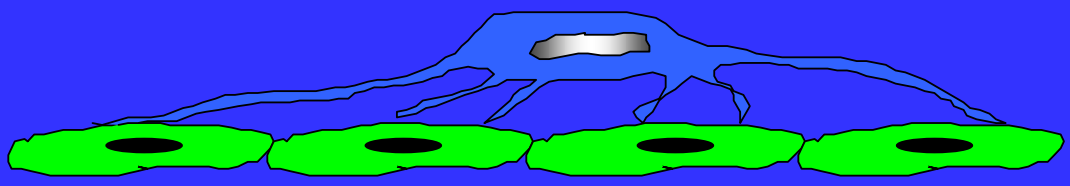


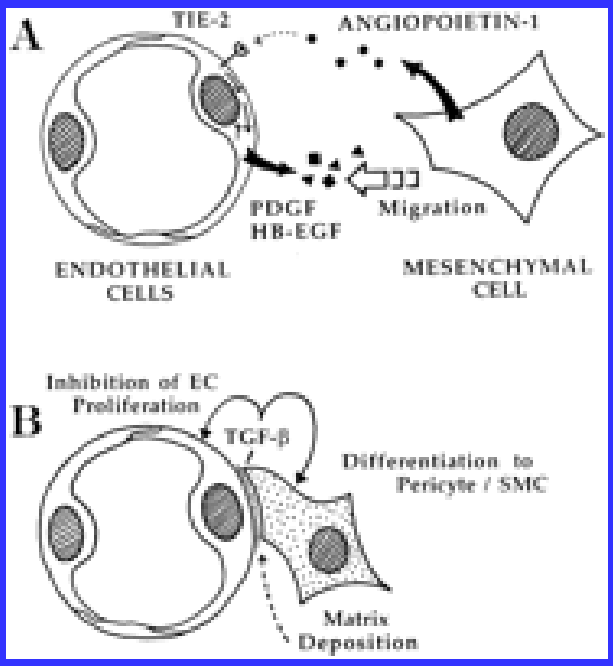
Figure 22–24. Molecular Biology of the Cell, 4th Edition.

Endothelial Cell - Pericyte Interactions

- **Recruitment of pericytes involves Tie-2, PDGF and Angiopoietin.**

- Pericytes are associated with the microvasculature, lying outside the endothelium, but within the basement membrane
- Pericytes extend long processes from their cell body, making contact with several endothelial cells
- Interaction is suppressive, resulting in quiescence of endothelial cells
- Activation of latent TGF-β to active TGF-β occurs at sites of pericyte-endothelial cell contact




A
TIE-2
ANGIOPOIETIN-1
PDGF
HB-EGF
Migration
ENDOTHELIAL CELLS
MESENCHYMAL CELL
B
Inhibition of EC Proliferation
TGF-β
Differentiation to Pericyte / SMC
Matrix Deposition

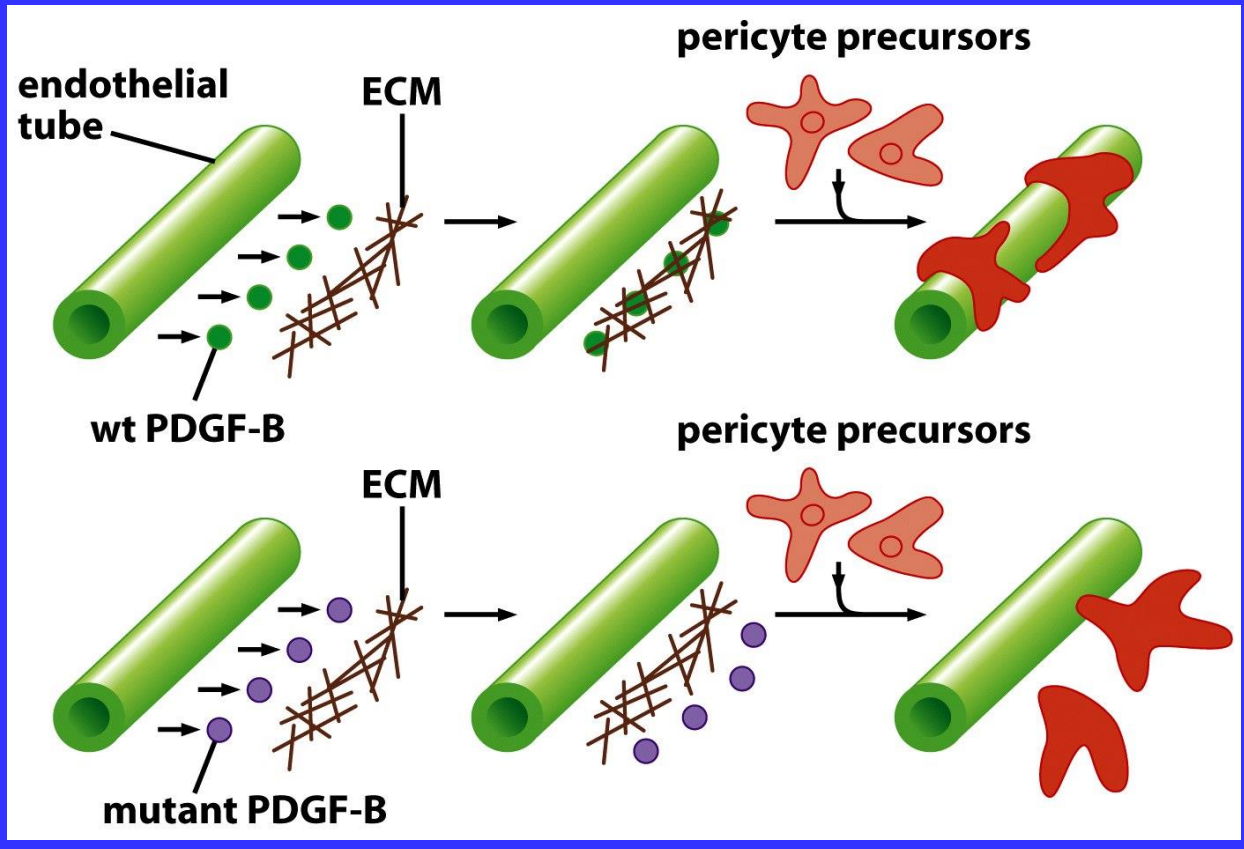


Figure 13.6d *The Biology of Cancer* (© Garland Science 2007)

ARTERIES vs VEINS

Role of Receptor Tyrosine Kinases of the EPHRIN-B / EphB Family

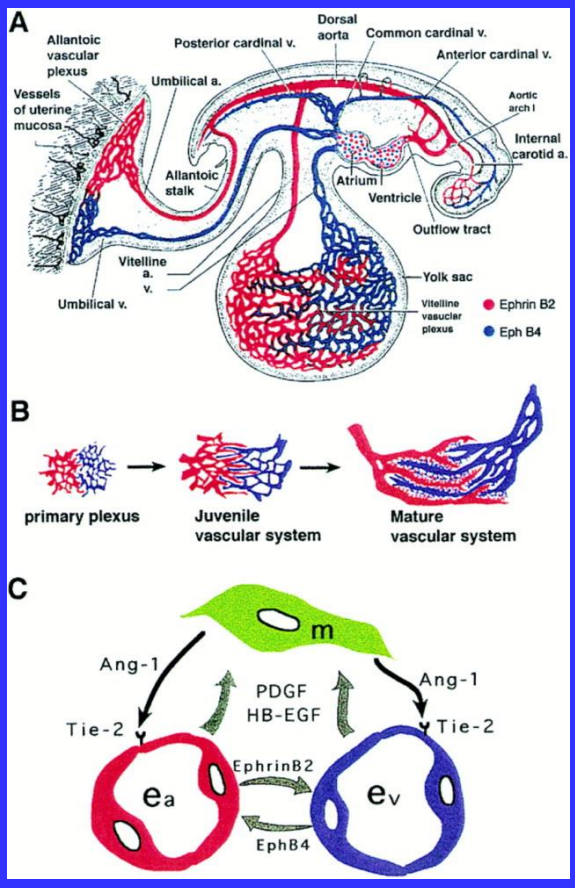
Eph Family: Has at least 14 members

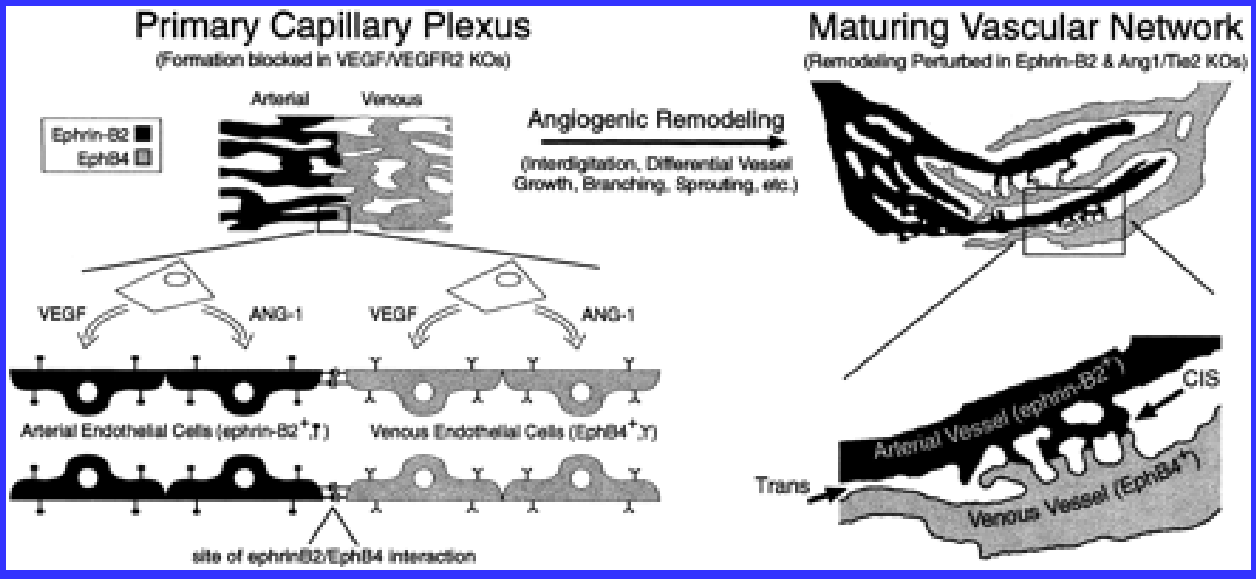
Ephrins: Ligands for Eph family proteins;
At least 8 family members
2 classes: A and B

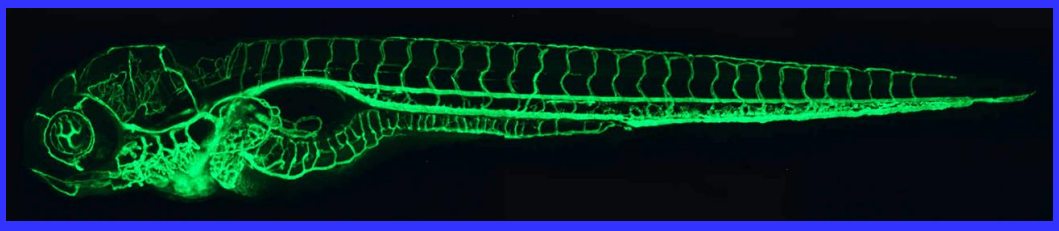
Ephrin-B ligands are trans-membrane proteins, that bind preferentially to receptors of the Eph-B sub-class. Ephrin-A ligands are GPI-linked membrane proteins.

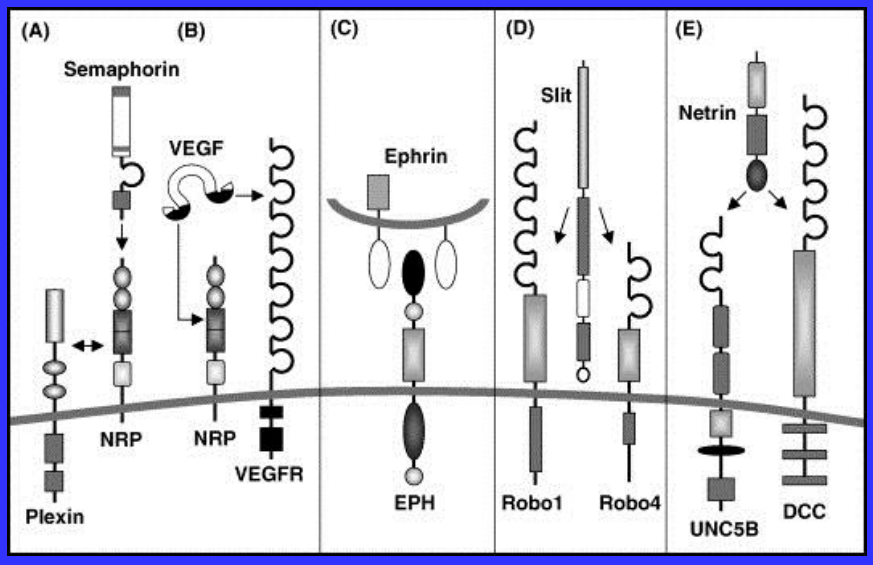
These molecules are NOT SOLUBLE mediators. They are membrane-bound, and activate cognate receptor on partner cells by cell-cell interactions. Signaling is RECIPROCAL, i.e. forward and reverse.

Ephrin-B2 is ARTERIAL. Eph-B4 is Venous

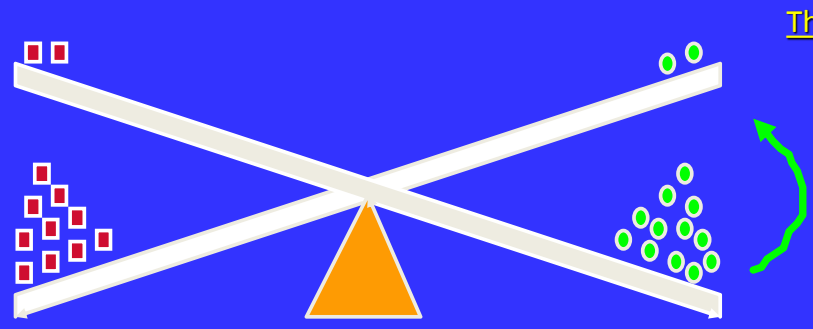

A
Allantoic vascular plexus
Posterior cardinal v.
Dorsal aorta
Common cardinal v.
Anterior cardinal v.
Vessels of uterine mucosa
Umbilical a.
Aortic arch I
Internal carotid a.
Allantoic stalk
Atrium
Ventricle
Outflow tract
Vitelline a.
v.
Yolk sac
Umbilical v.
Vitelline vascular plexus
Ephrin B2
Eph B4
B
primary plexus
Juvenile vascular system
Mature vascular system
C
m
Ang-1
PDGF
HB-EGF
Ang-1
Tie-2
Tie-2
EphrinB2
ea
ev
EphB4


Primary Capillary Plexus
(Formation blocked in VEGF/VEGFR2 KOs)
Arterial
Venous
Ephrin-B2
EphB4
Angiogenic Remodeling
(Interdigitation, Differential Vessel Growth, Branching, Sprouting, etc.)
VEGF
ANG-1
VEGF
ANG-1
Arterial Endothelial Cells (ephrin-B2+)
Venous Endothelial Cells (EphB4+)
site of ephrinB2/EphB4 interaction
Maturing Vascular Network
(Remodeling Perturbed in Ephrin-B2 & Ang1/Tie2 KOs)
CIS
Arterial Vessel (ephrin-B2+)
Trans
Venous Vessel (EphB4+)




(A)
(B)
(C)
(D)
(E)
Semaphorin
VEGF
Ephrin
Slit
Netrin
NRP
NRP
VEGFR
Plexin
EPH
Robo1
Robo4
UNC5B
DCC

THE BALANCE HYPOTHESIS FOR THE ANGIOGENIC SWITCH



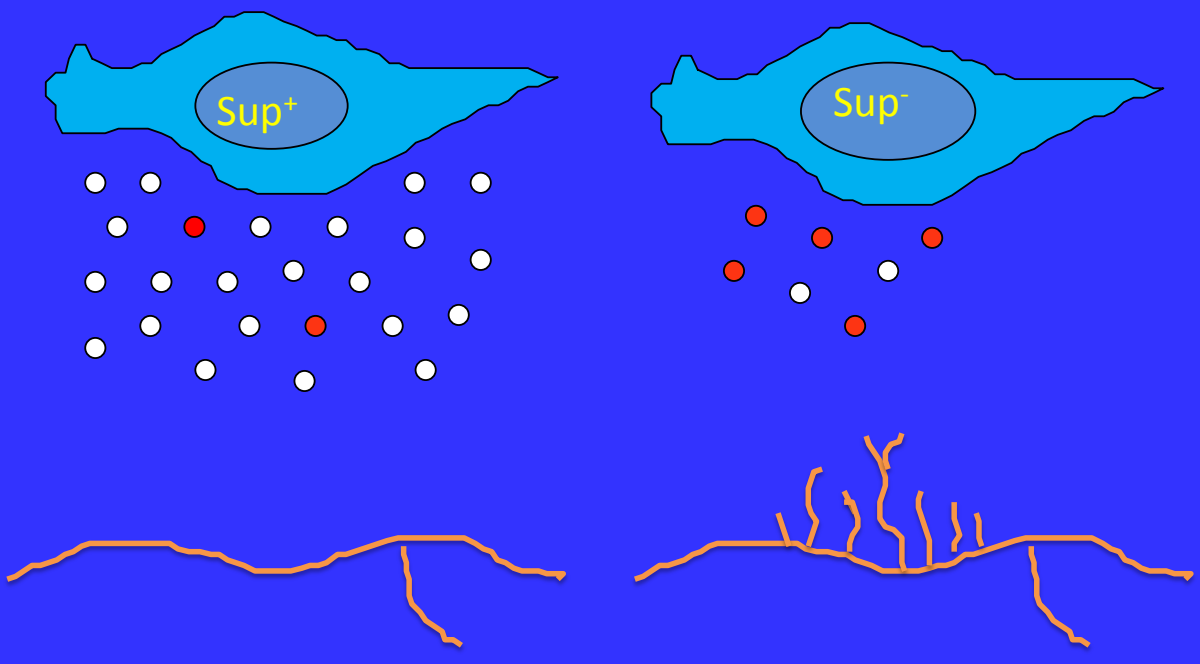
ACTIVATORS

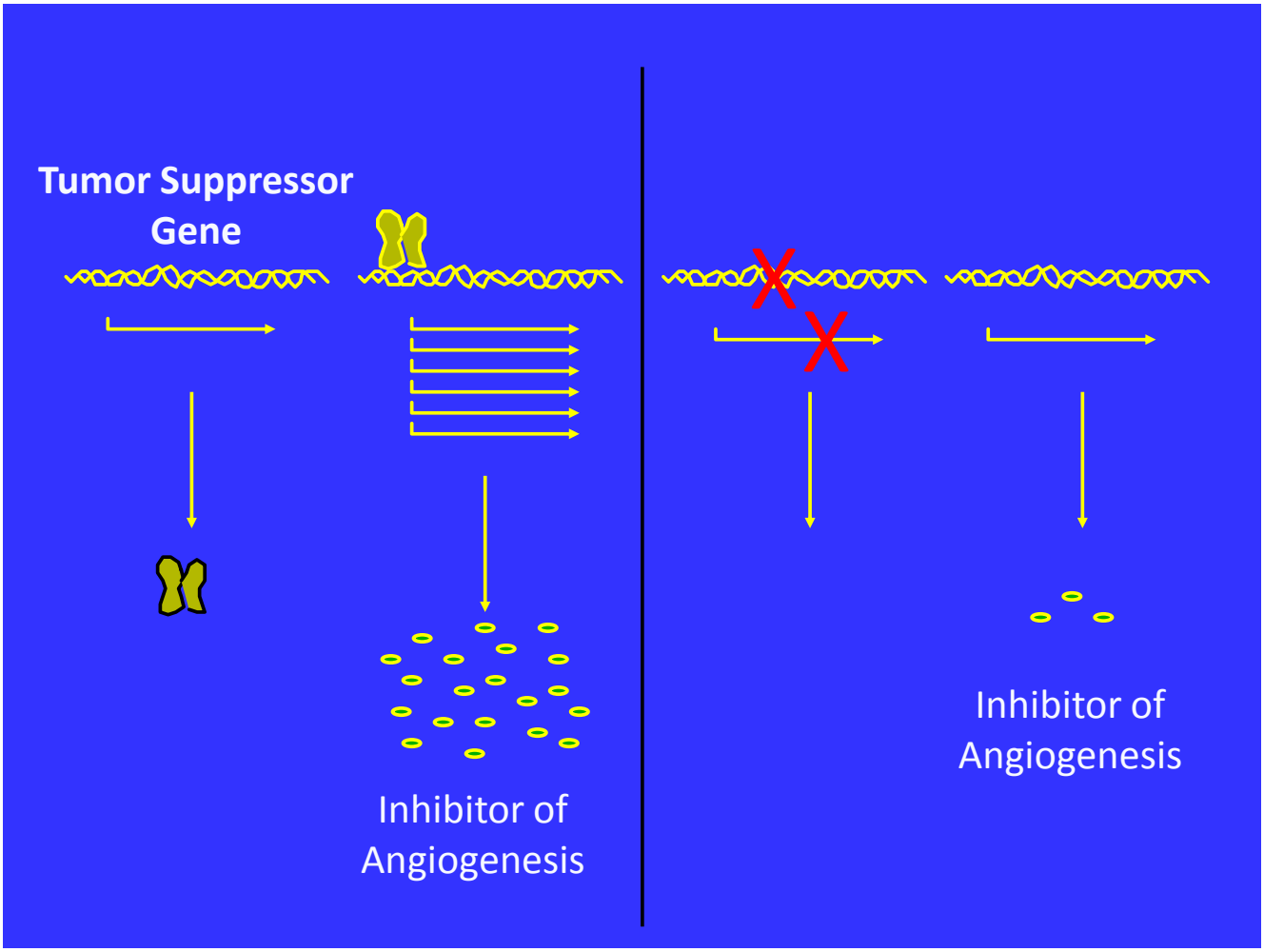
VEGF
aFGF
bFGF
Il-8

INHIBITORS

Thrombospondin
TGFβ
Angiostatin
Endostatin
TIMP
IL-12
γP10

Model for Tumor Suppressor Gene Control of Angiogenesis




Tumor Suppressor Gene
Inhibitor of Angiogenesis
Inhibitor of Angiogenesis

Thrombospondin

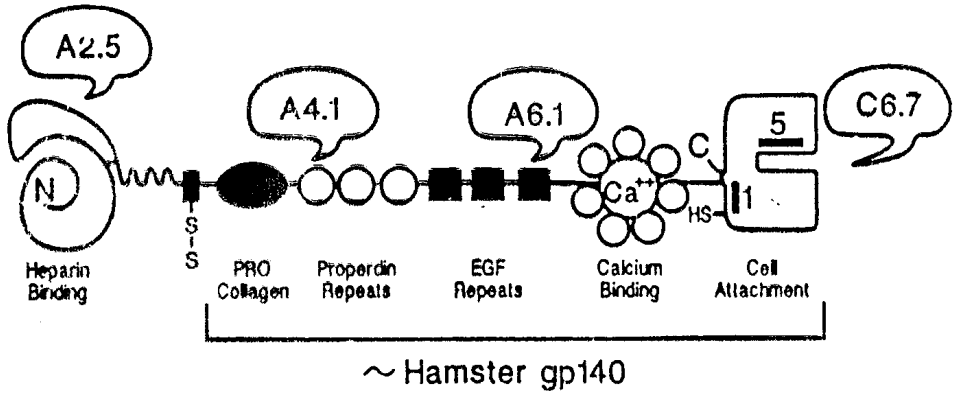


FIG. 1. Cartoon of the 180-kDa subunit of the homotrimeric human TSP molecule. Binding sites for individual monoclonal antibodies raised to TSP are indicated above the diagram; specific domains are below. The portion of the TSP molecule containing homology to the N-terminal amino acids of gp140 and shared antigenic sites is indicated by the bar. Numbers 1 and 5 mark the positions of the peptides used to make TSP anti-peptide serum. EGF, epidermal growth factor.

Li-Fraumeni Fibroblasts and Regulation of Angiogenesis by p53

- **LF patients show a greatly increased susceptibility to tumor development.**
- **Patients have 1 defective p53 allele and 1 wild-type allele.**
- **LF fibroblasts in culture lose remaining wt p53 allele over several generations.**

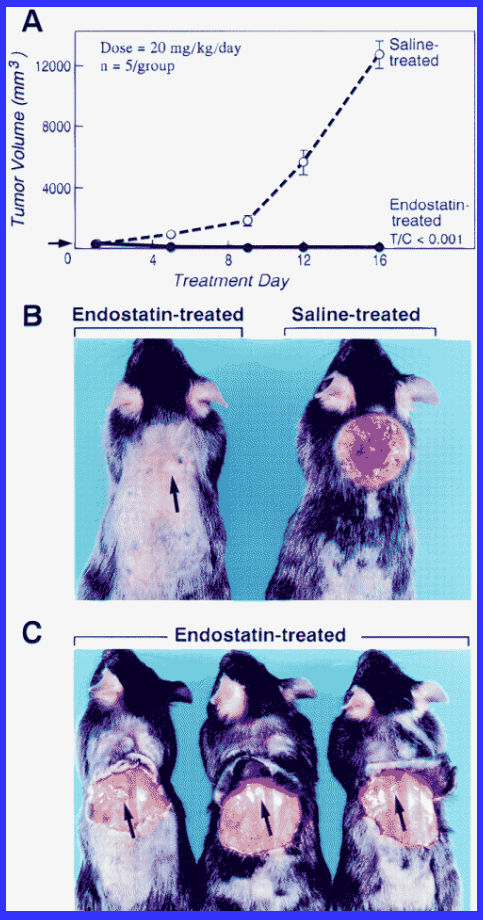
EARLY PASAGE		LATE PASSAGE
Intact	p53	Lost
Low	Angiogenic Activity	High
High	TSP-1	Low
Low	VEGF	High

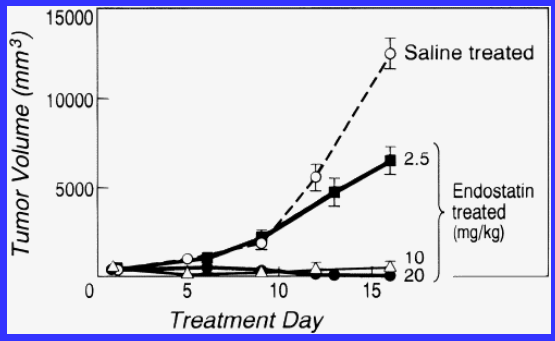
Transfected p53 T^0-sensitive mutant: WT at 32°C, Mutant at 38°C.

38°C.	TSP-1	Low
32°C.	TSP-1	High

Angiogenesis Inhibitors:

- *ANGIOSTATIN*:
  - Fragment of Plasminogen.
  - Produced by Primary Tumor Mass
  - Present in the Circulation
  - Suppresses Growth of Metastases
  - Removal of Primary Tumor de-suppresses Growth of metastases
- *ENDOSTATIN*:
  - Fragment of Type XVIII collagen


A
Dose = 20 mg/kg/day
n = 5/group
Saline-treated
Endostatin-treated
T/C < 0.001
Tumor Volume (mm^3)
12000
8000
4000
0
4
8
12
16
Treatment Day
B
Endostatin-treated
Saline-treated
C
Endostatin-treated


Tumor Volume (mm^3)
15000
10000
5000
0
5
10
15
Treatment Day
Saline treated
2.5
10
20
Endostatin treated (mg/kg)

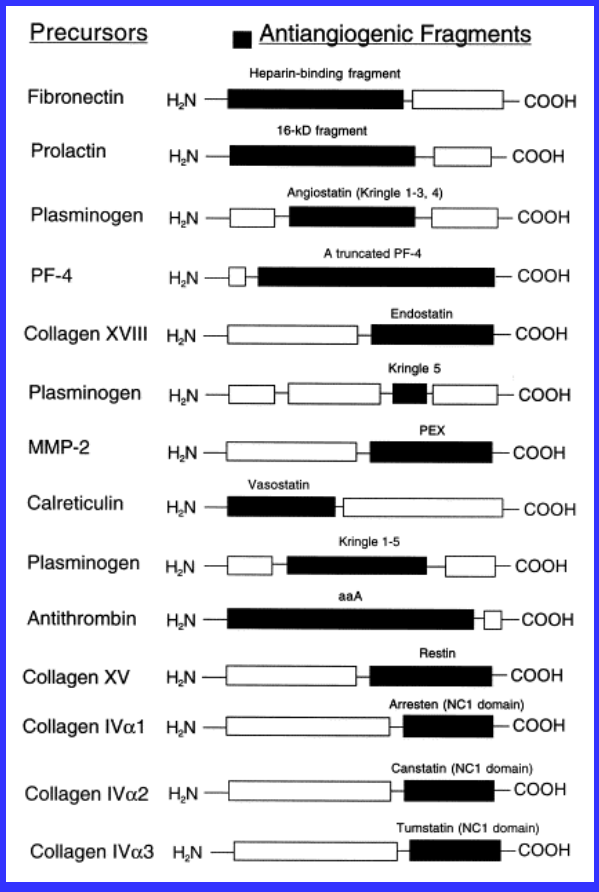

Precursors
Antiangiogenic Fragments
Fibronectin
Heparin-binding fragment
Prolactin
16-kD fragment
Plasminogen
Angiostatin (Kringle 1-3, 4)
PF-4
A truncated PF-4
Collagen XVIII
Endostatin
Plasminogen
Kringle 5
MMP-2
PEX
Calreticulin
Vasostatin
Plasminogen
Kringle 1-5
Antithrombin
aaA
Collagen XV
Restin
Collagen IVα1
Arresten (NC1 domain)
Collagen IVα2
Canstatin (NC1 domain)
Collagen IVα3
Tumstatin (NC1 domain)
H2N
COOH

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	

Table 13.4 *The Biology of Cancer* (© Garland Science 2007)

OXYGEN AND ANGIOGENESIS

- Oxygen Tension & Erythropoiesis
  - Low ppO_2 stimulates erythropoiesis
  - Low ppO_2 stimulates *erythropoietin production in kidney*
- *Oxygen Tension & Angiogenesis*:
  - High $pp0_2$ inhibits angiogenesis
  - Low $pp0_2$ stimulates angiogenesis

    Relationship to ALTITUDE PHYSIOLOGY:
    - Increased capillary density in muscle at high altitudes. Effect only up to certain elevation.
    - FAILURE of WOUND REPAIR above 15,000ft.
      - » (Himalayas Expeditions)

Hypoxia Inducible Factor (HIF1)
Hypoxia Response Element (HRE)

Hypoxia Inducible Factor (HIF)

Dimer of **HIF1-α** and **HIF1-β** (ARNT)

Unstable under Normoxia **Stabilized under hypoxia**	**Constitutively Expressed** **Stable**

Mammalian bHLH-PAS proteins

Class I	Class II
AHR	**ARNT (HIF-1β)**
CLOCK	ARNT2
HIF-1α	ARNT3 (BMAL1/MOP3)
HIF-2α (EPAS1/HLF/HRF/MOP2)	
HIF-3 α	
NPAS1 (MOP5)	
NPAS2 (MOP4)	
SIM1	
SIM2	

Hypoxia Inducible Genes

- Erythropoietin (Epo)
- VEGFs
- Glycolytic Enzymes
  - Lactate Dehydrogenase (LDH)
  - Pyruvate Kinase M (PKMP
  - Enolase 1
  - Phosphoglycerate Kinase 1 (PGK1)
  - Aldolase A (ALDA)
  - Phosphofructokinase (PFKL)
  - Glucose Transporter 1 (GLUT-1)
- Inducible Nitric Oxide Synthase (iNOS)
- Heme Oxygenase
- Ferritin Receptor and Ferritin
- Tyrosine Hydroxylase

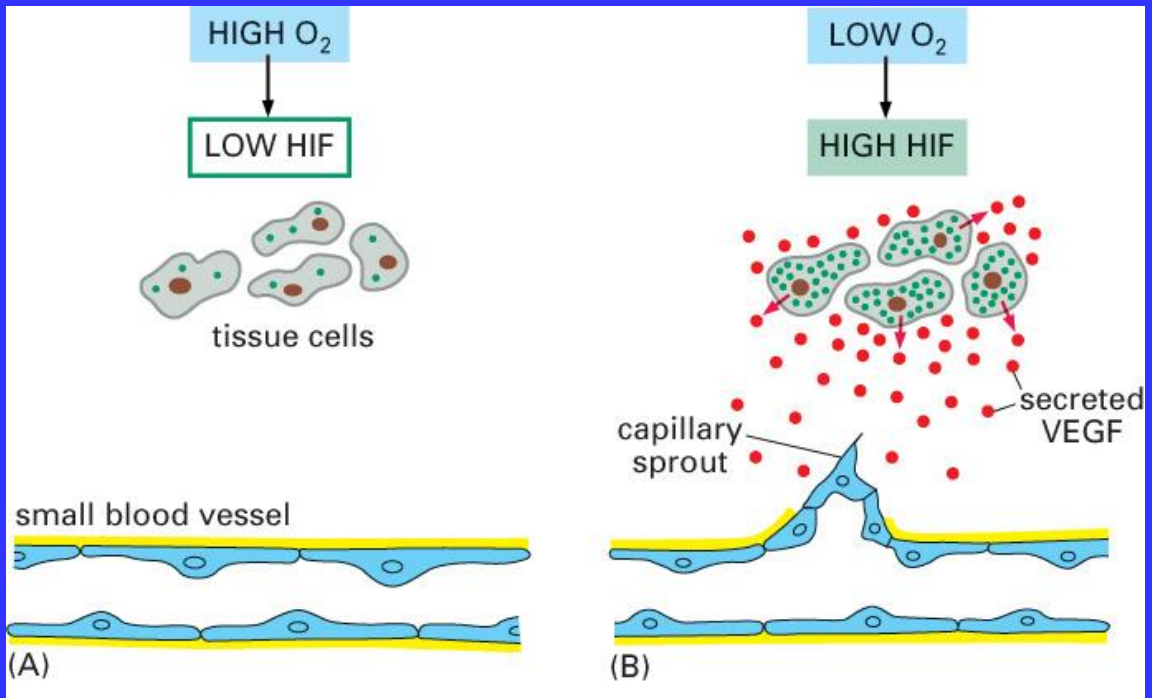
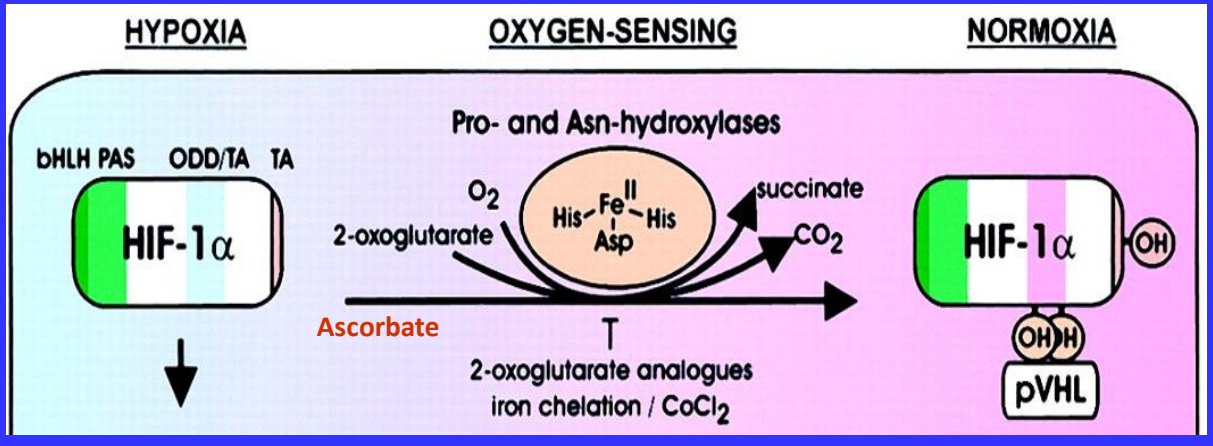
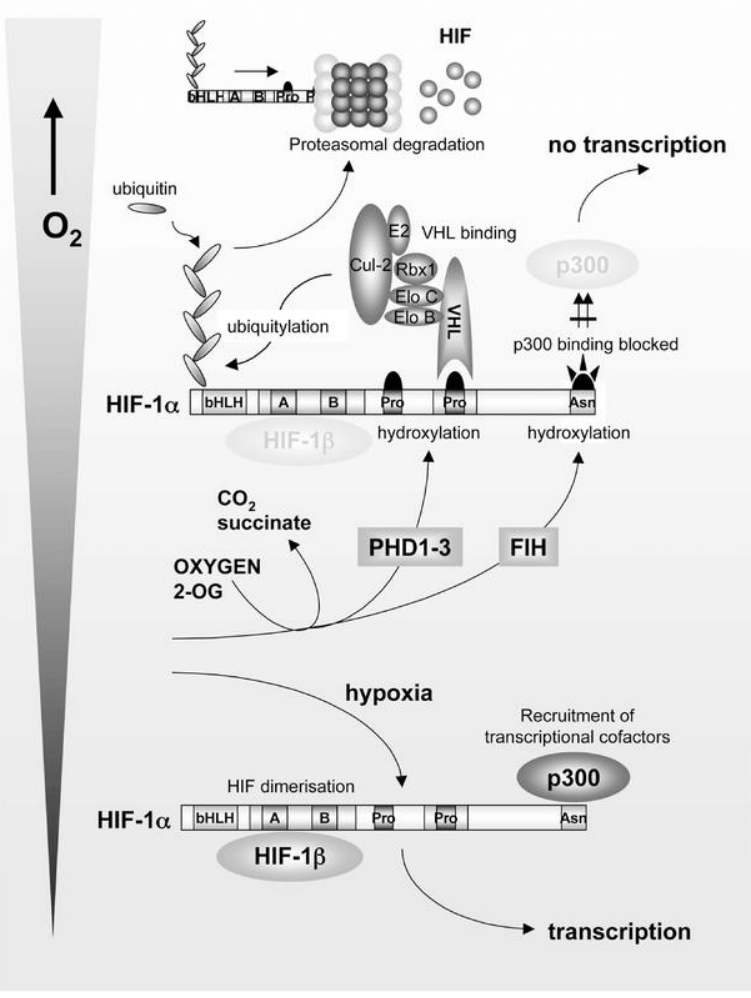


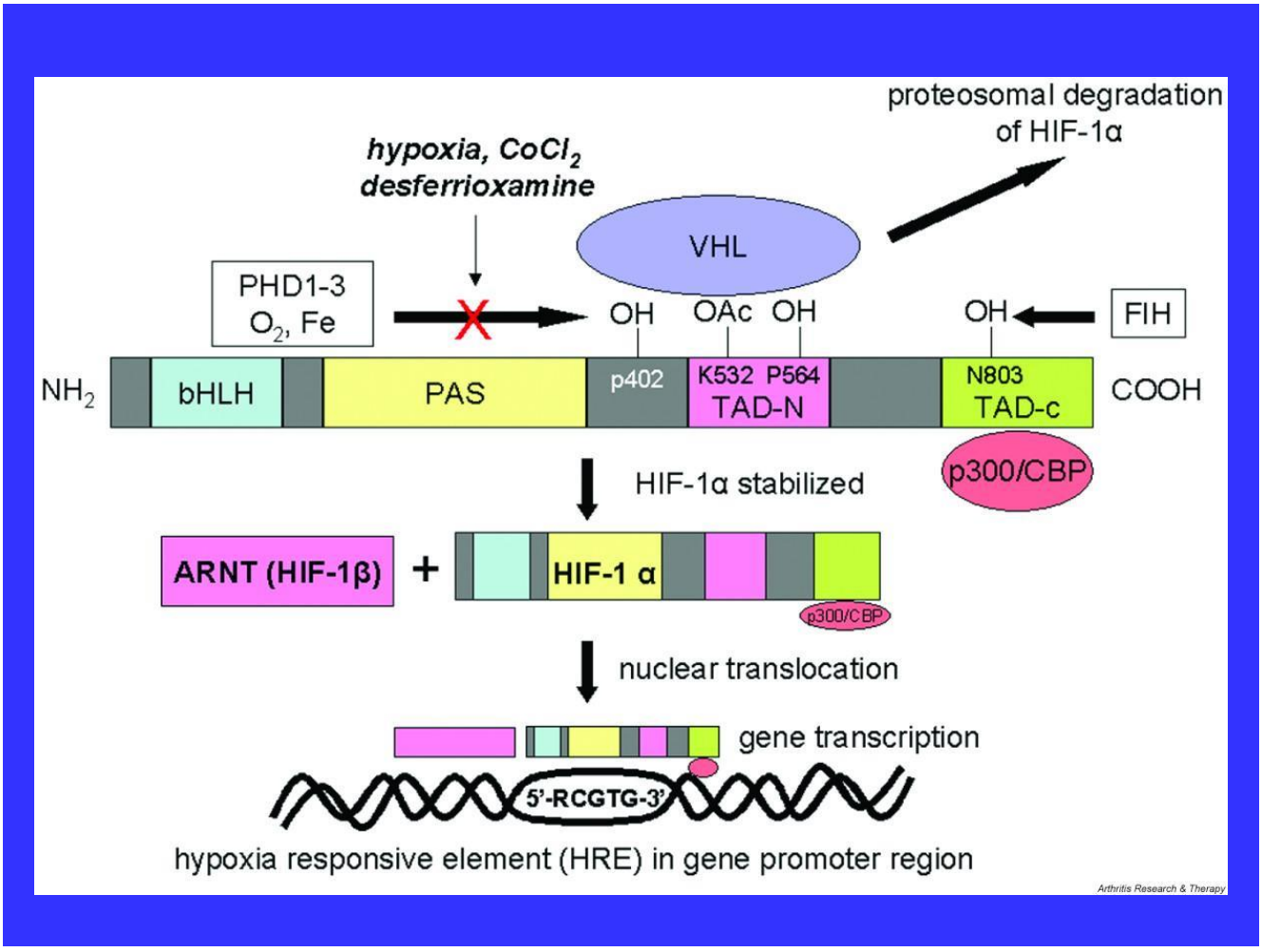
Figure 22–28. Molecular Biology of the Cell, 4th Edition.

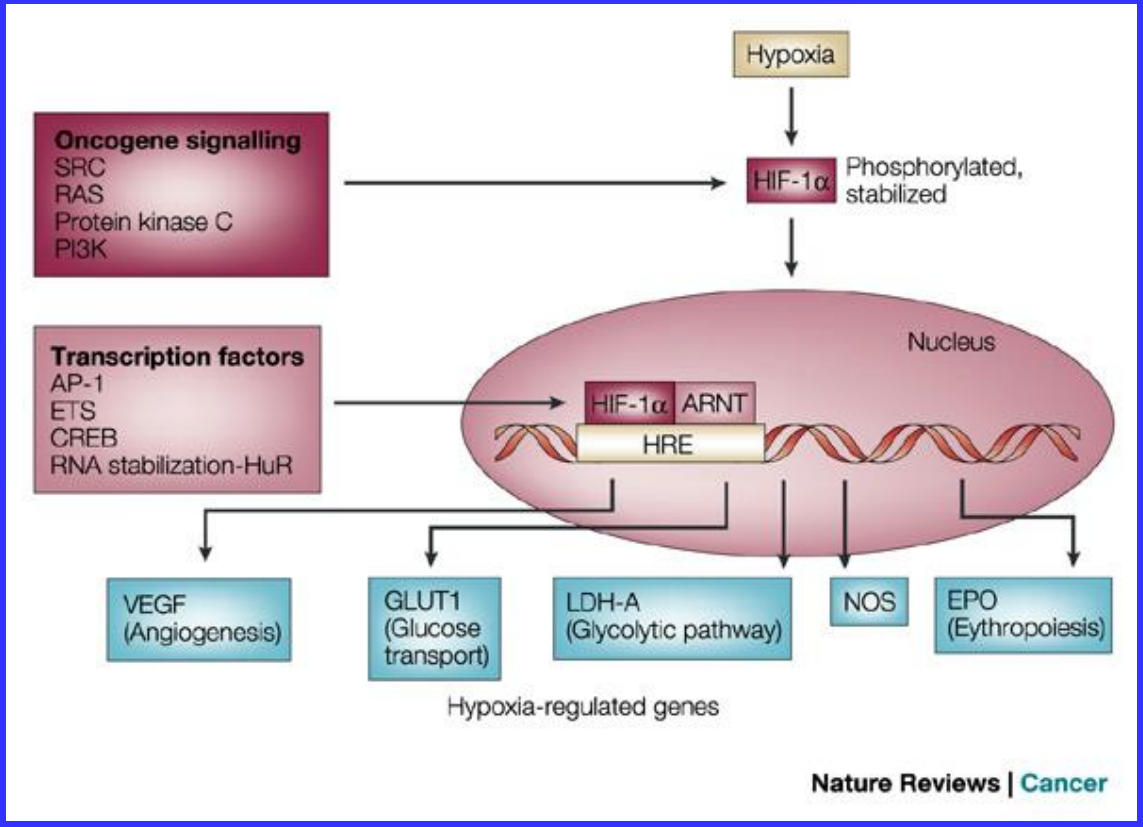
O_2 Sensors

- HIF-1 Prolyl-Hydroxylases 1-3
- Factor Inhibiting HIF-1(FIH1, Asn-hydroxylase)

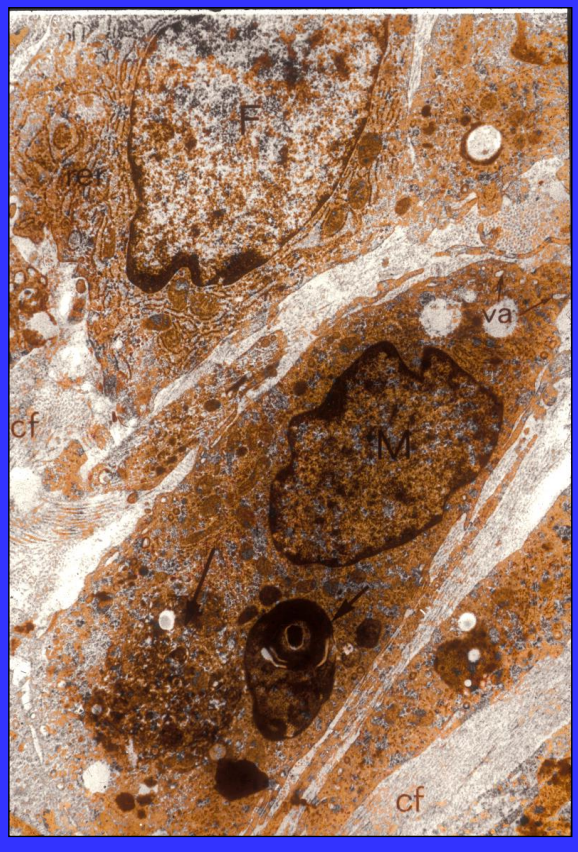


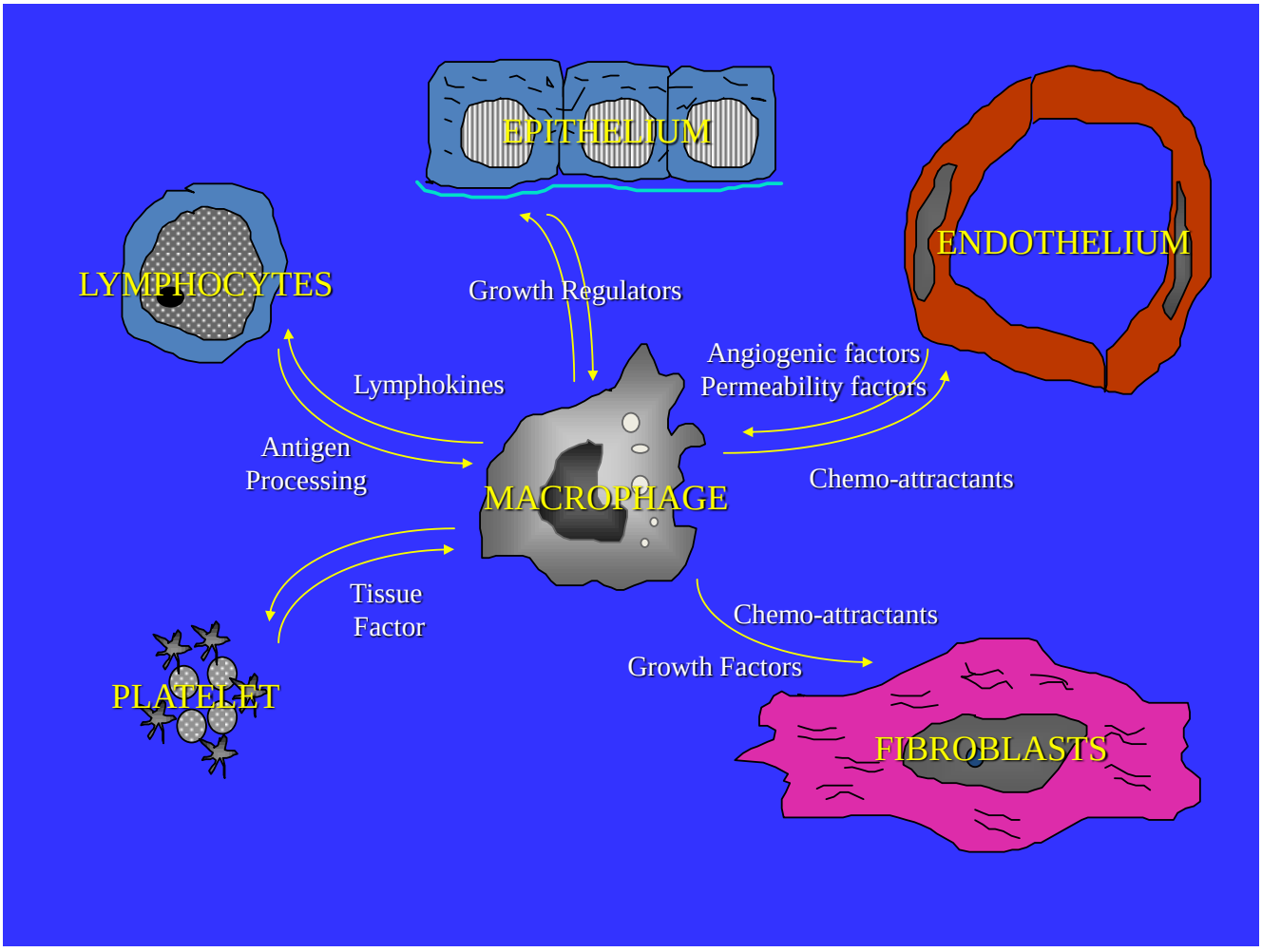

HIF
Proteasomal degradation
no transcription
ubiquitin
O_2
E2
VHL binding
Cul-2
Rbx1
Elo C
Elo B
VHL
p300
ubiquitylation
p300 binding blocked
HIF-1α
bHLH
A
B
Pro
Pro
Asn
HIF-1β
hydroxylation
hydroxylation
CO_2
succinate
PHD1-3
FIH
OXYGEN
2-OG
hypoxia
Recruitment of
transcriptional cofactors
p300
HIF dimerisation
HIF-1α
bHLH
A
B
Pro
Pro
Asn
HIF-1β
transcription


proteosomal degradation
of HIF-1α
hypoxia, CoCl2
desferrioxamine
VHL
PHD1-3
O2, Fe
OH
OAc
OH
OH
FIH
NH2
bHLH
PAS
p402
K532 P564
TAD-N
N803
TAD-c
COOH
p300/CBP
HIF-1α stabilized
ARNT (HIF-1β)
+
HIF-1 α
p300/CBP
nuclear translocation
gene transcription
5'-RCGTG-3'
hypoxia responsive element (HRE) in gene promoter region
Arthritis Research & Therapy

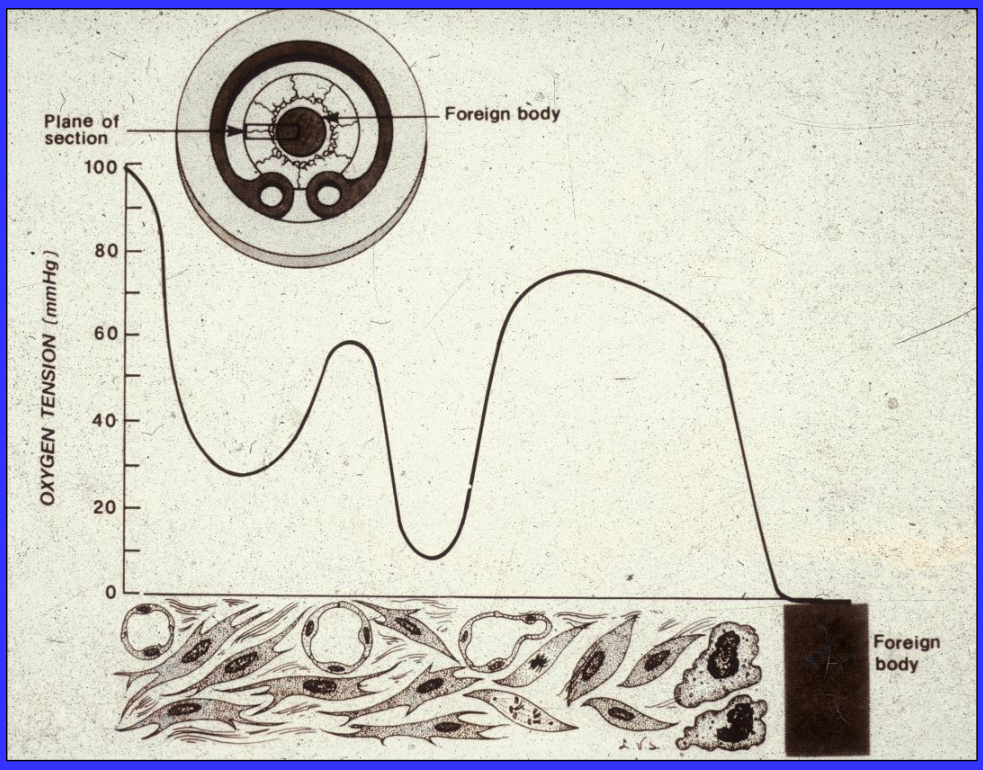

Hypoxia
Oncogene signalling
SRC
RAS
Protein kinase C
PI3K
HIF-1α
Phosphorylated, stabilized
Transcription factors
AP-1
ETS
CREB
RNA stabilization-HuR
Nucleus
HIF-1α
ARNT
HRE
VEGF (Angiogenesis)
GLUT1 (Glucose transport)
LDH-A (Glycolytic pathway)
NOS
EPO (Eythropoiesis)
Hypoxia-regulated genes
Nature Reviews | Cancer

Macrophage in Wound - EM




EPITHELIUM
ENDOTHELIUM
LYMPHOCYTES
Growth Regulators
Lymphokines
Angiogenic factors
Permeability factors
Antigen
Processing
MACROPHAGE
Chemo-attractants
Tissue
Factor
Chemo-attractants
Growth Factors
PLATELET
FIBROBLASTS

Role of Hypoxia in Angiogenesis



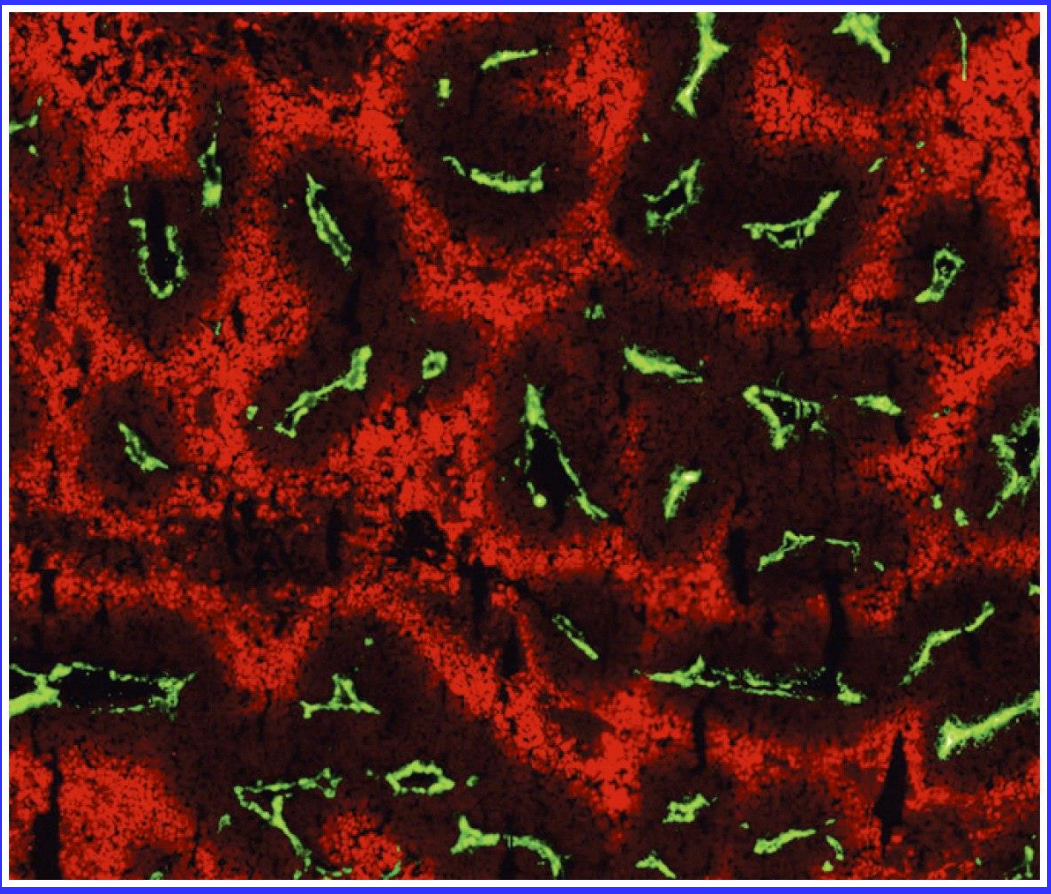


Figure 13.27a *The Biology of Cancer* (© Garland Science 2007)

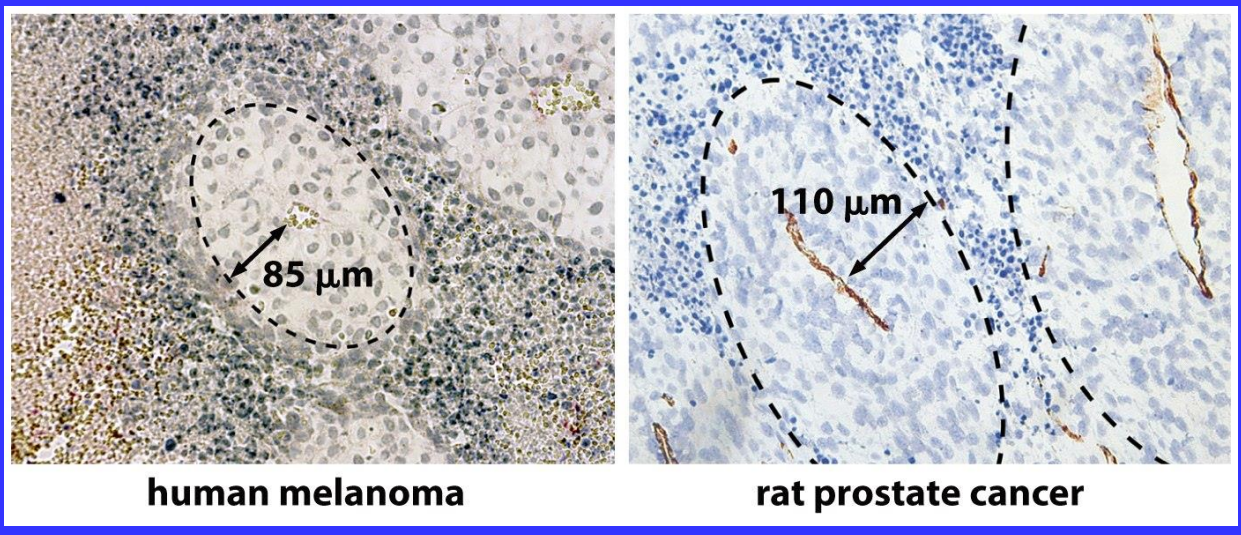
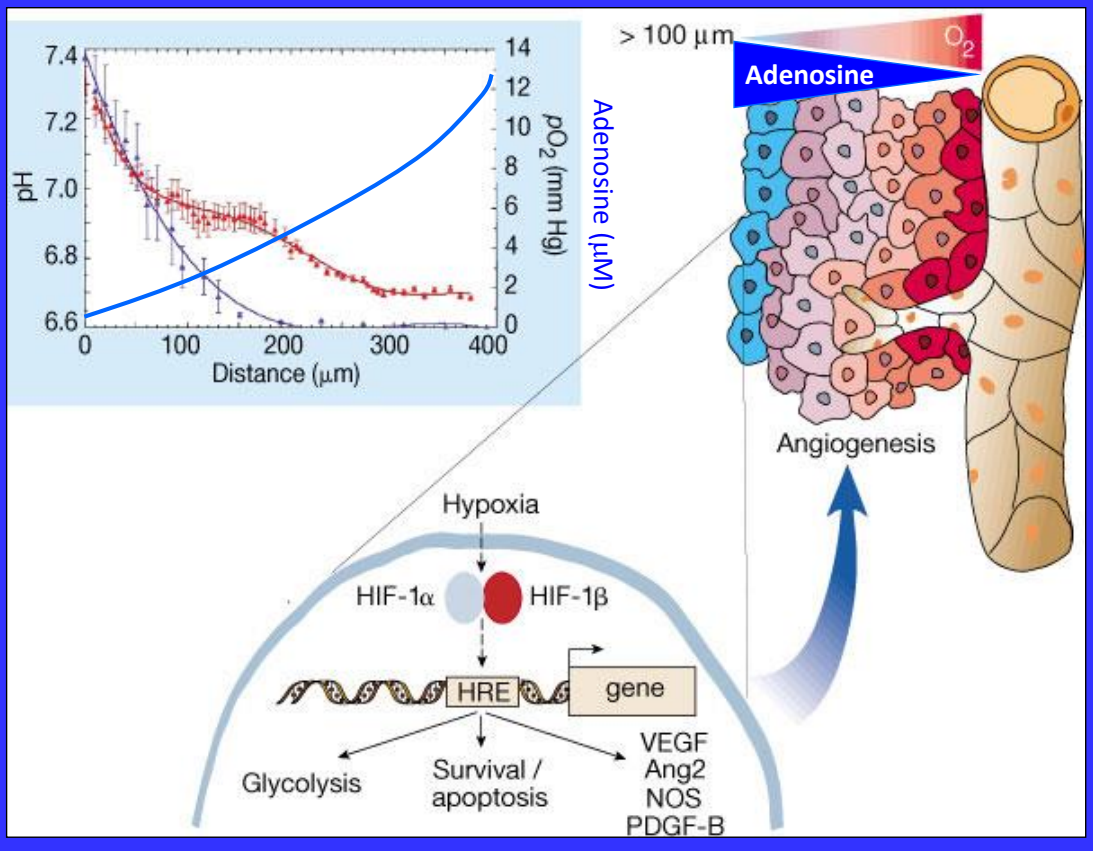
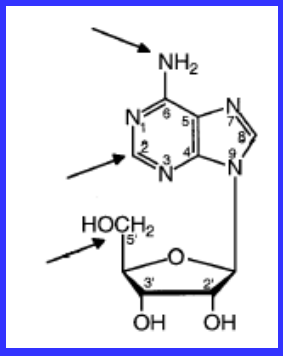
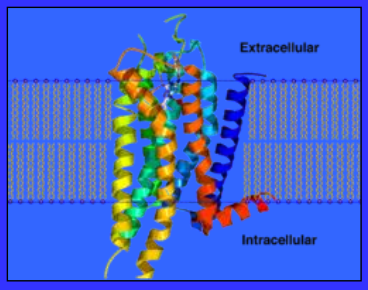


Figure 13.27b *The Biology of Cancer* (© Garland Science 2007)


7.4
7.2
7.0
6.8
6.6
pH
0
100
200
300
400
Distance (μm)
14
12
10
8
6
4
2
0
pO2 (mm Hg)
Adenosine (μM)
> 100 μm
O2
Adenosine
Angiogenesis
Hypoxia
HIF-1α
HIF-1β
HRE
gene
Glycolysis
Survival / apoptosis
VEGF
Ang2
NOS
PDGF-B

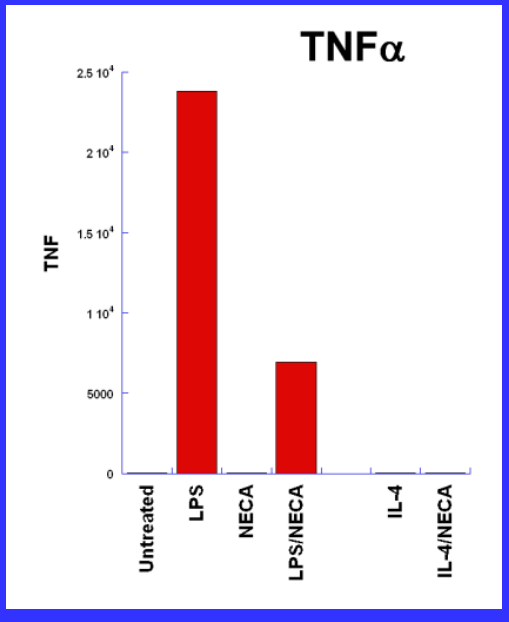
Adenosine and Adenosine Receptors

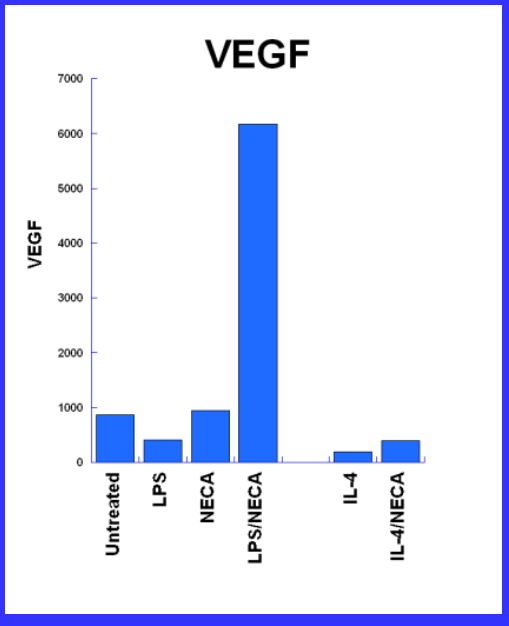




- Unstable and ubiquitous purine nucleoside produced by breakdown of ATP.
- Released in response to stressful stimuli. Binds receptors to modulate, and protect cells from the harmful consequences of stress
- Adenosine receptors (A_1R, $A_{2A}R$, $A_{2B}R$, A_3R) are expressed on many cell types: they bind a wide spectrum of natural and synthetic agonists and antagonists.
- Activation of ARs on immune cells generally exhibits anti-inflammatory effects:
  - Inhibition of phagocytosis.
  - Decreased expression of inflammatory cytokines and chemokines, ROS and NO.
  - Increased expression of anti-inflammatory cytokines.

Reciprocal Regulation of TNFα and VEGF in Macrophages In Response to LPS and Adenosine Receptor (AR) Agonists

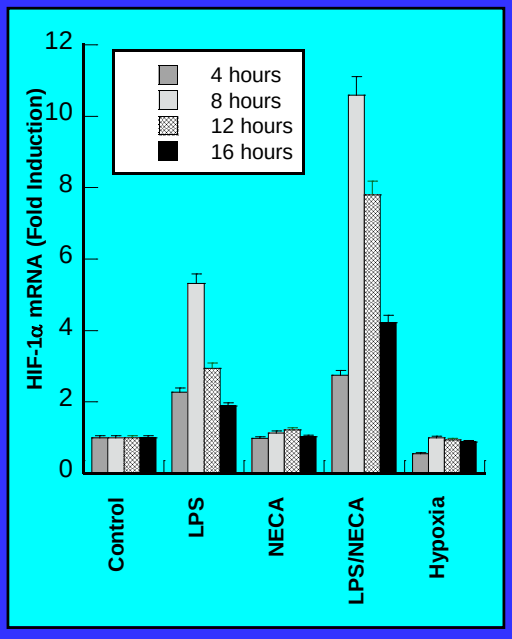




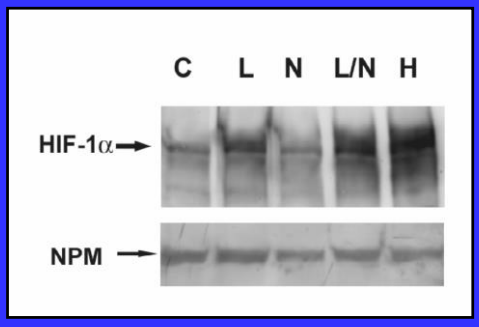
Adenosine synergizes with TLR signaling to switch macrophages from an "***inflammatory***" to an "***angiogenic***" phenotype

Regulation of HIF-1α Expression by LPS and NECA

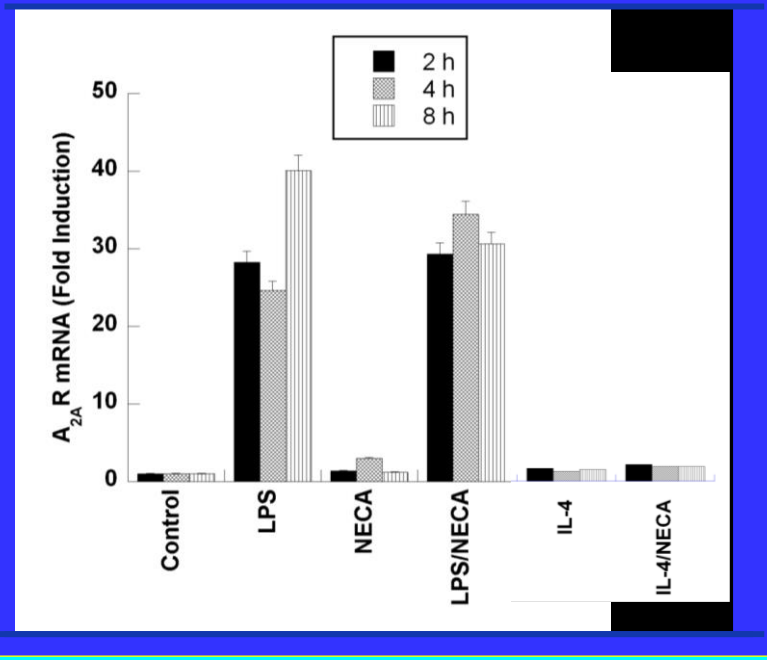
Q-RT-PCR Analysis of HIF-1α mRNA



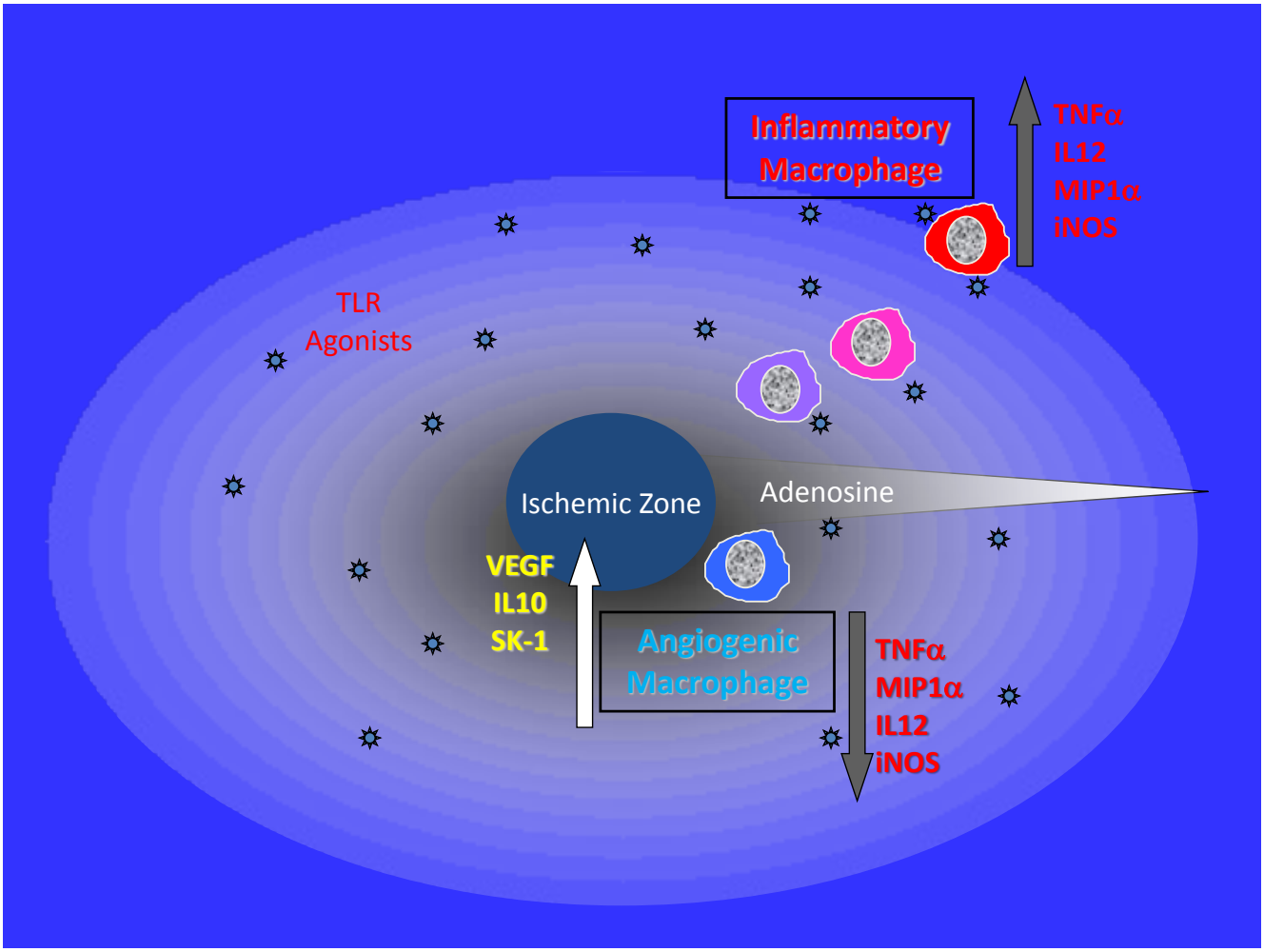
Western Blot Analysis of HIF-1α



Regulation of Adenosine A_{2A} Receptor Expression in Macrophages



- Induction is NF-κB and STAT1 dependent
- Agonists of TLR2, 7 and 9 also induce A_{2A}R expression


Inflammatory Macrophage
TNFα
IL12
MIP1α
iNOS
TLR Agonists
Ischemic Zone
Adenosine
VEGF
IL10
SK-1
Angiogenic Macrophage
TNFα
MIP1α
IL12
iNOS