
بسم الله الرحمن الرحیم

Angiogenesis & VEGF Signaling

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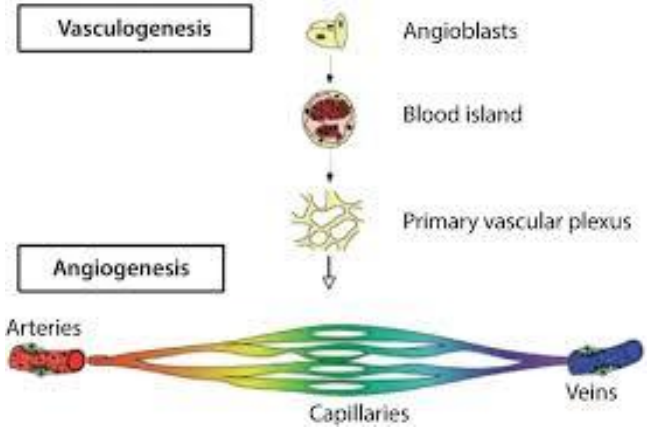
Definitions:

Angiogenesis: formation of new blood vessels from existing ones

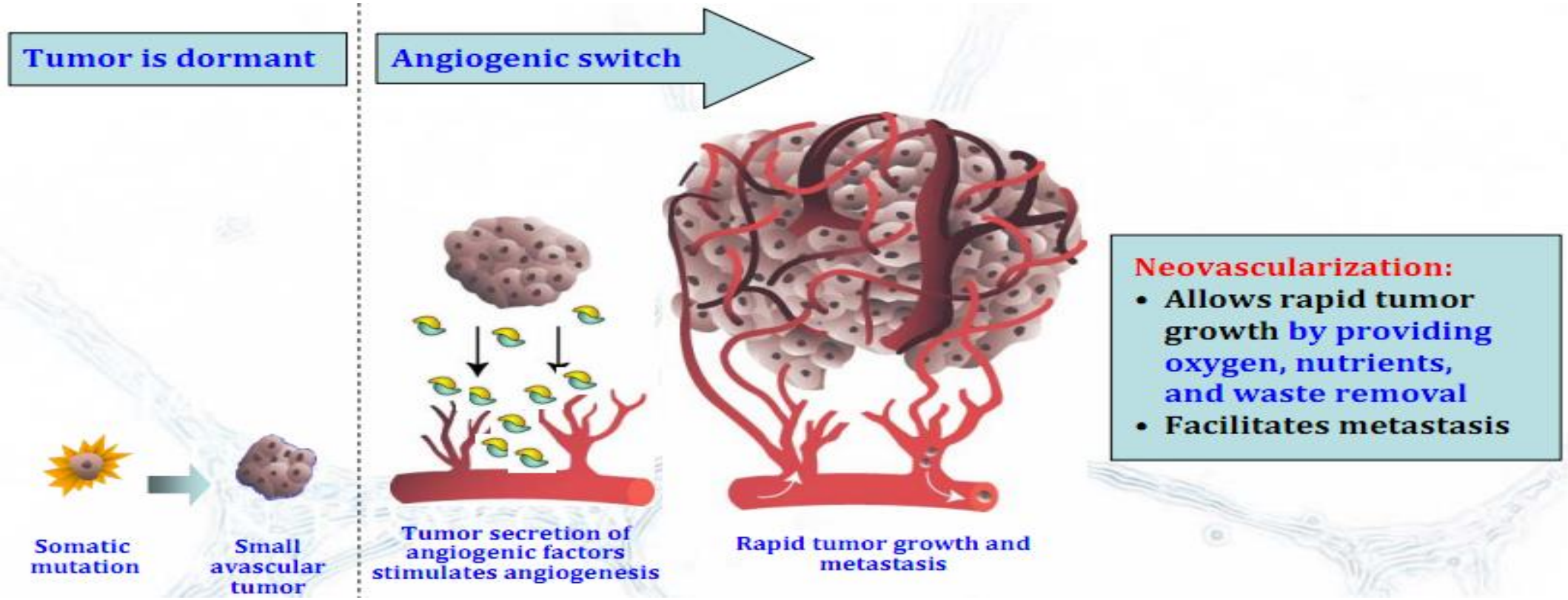
Vasculogenesis: formation of blood vessels from endothelial progintor cells



In adult: endothelial cells rarely divide

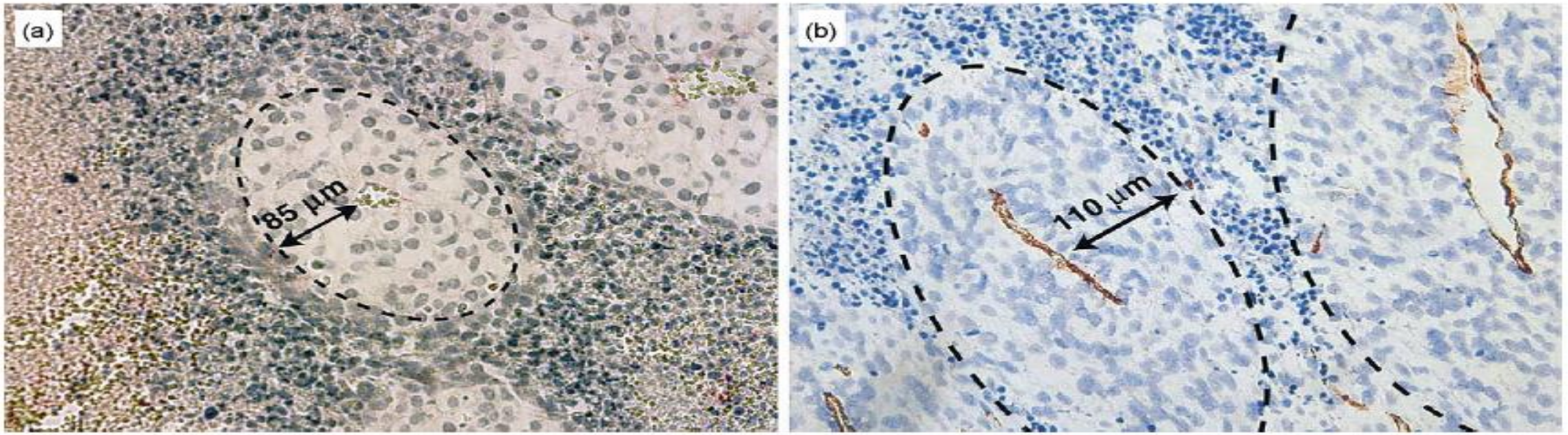
J. Folkman, M.D. February 24, 1933–January 14, 2008

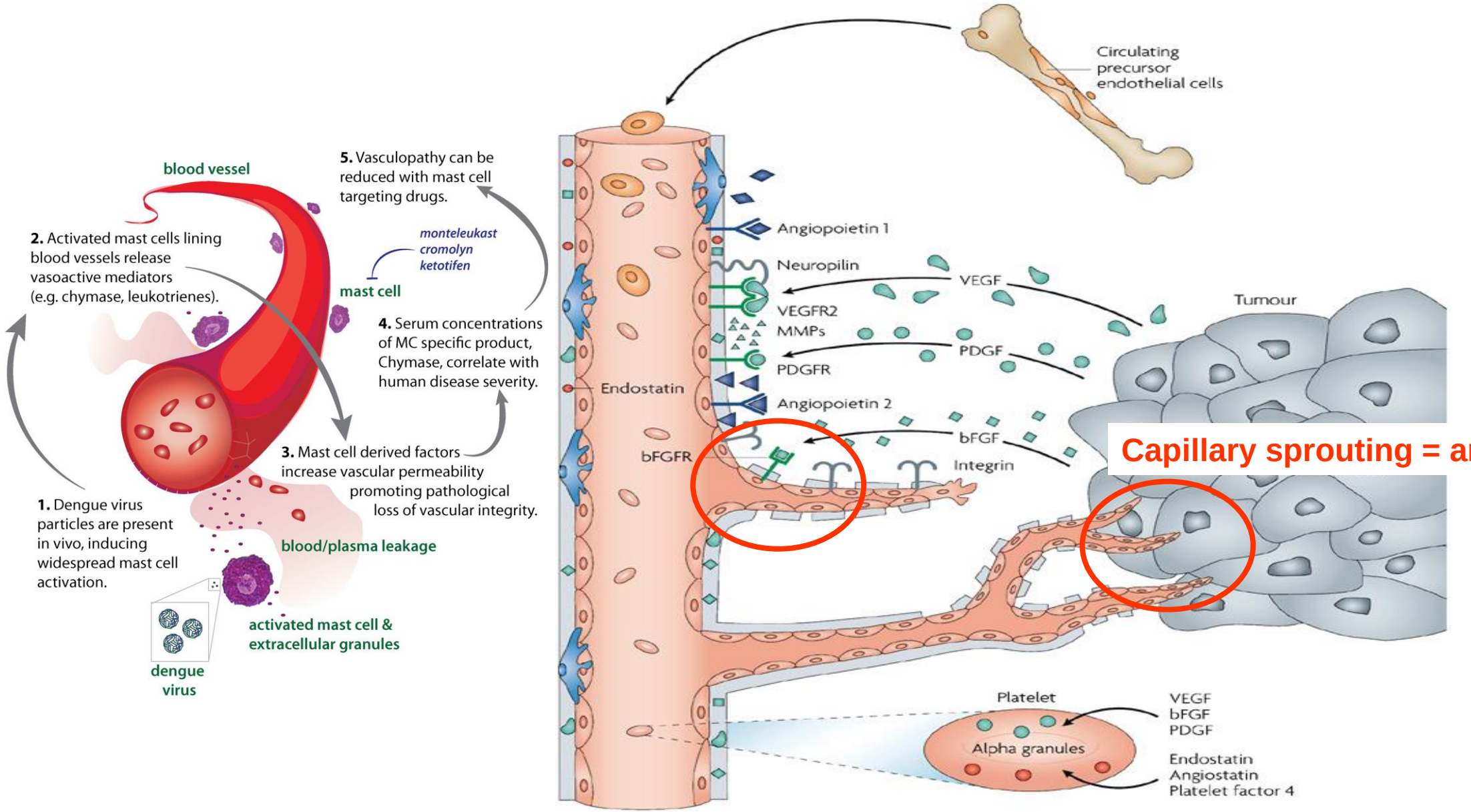
A tumor may persist in a diffusion-limited state, usually not more than 2 mm in diameter, with cell proliferation balanced by cell death, for many months or years. It rarely causes significant damage in this dormant phase, and often goes undetected.



Angiogenesis greatly improves the tumor's blood supply, providing it with an almost unlimited supply of oxygen and nutrients and a system for the removal of waste products, thus permitting rapid growth. In addition, the proximity of large numbers of blood vessels increases the likelihood of tumor cells entering the bloodstream and being transported to remote parts of the body.

Neoplastic tissue usually exceeds the oxygen diffusion limit when tumor cell layers accumulate to a thickness of approximately 150–200 mm from a nearest open microvessel. Tumor cells beyond this limit undergo apoptosis.
Therefore, almost any tumor that has reached a diameter of >10–100 mm, is probably already neovascularized.

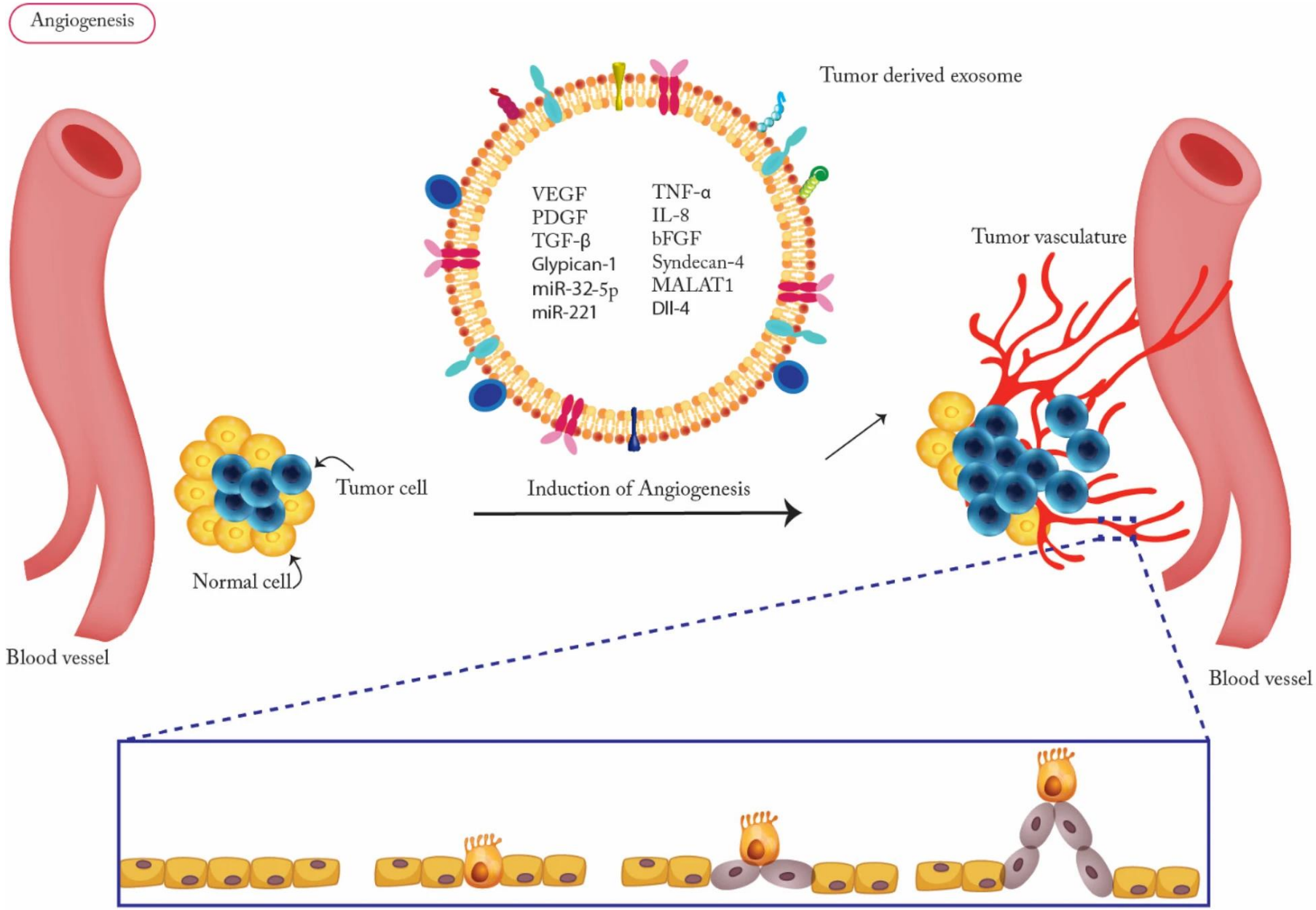




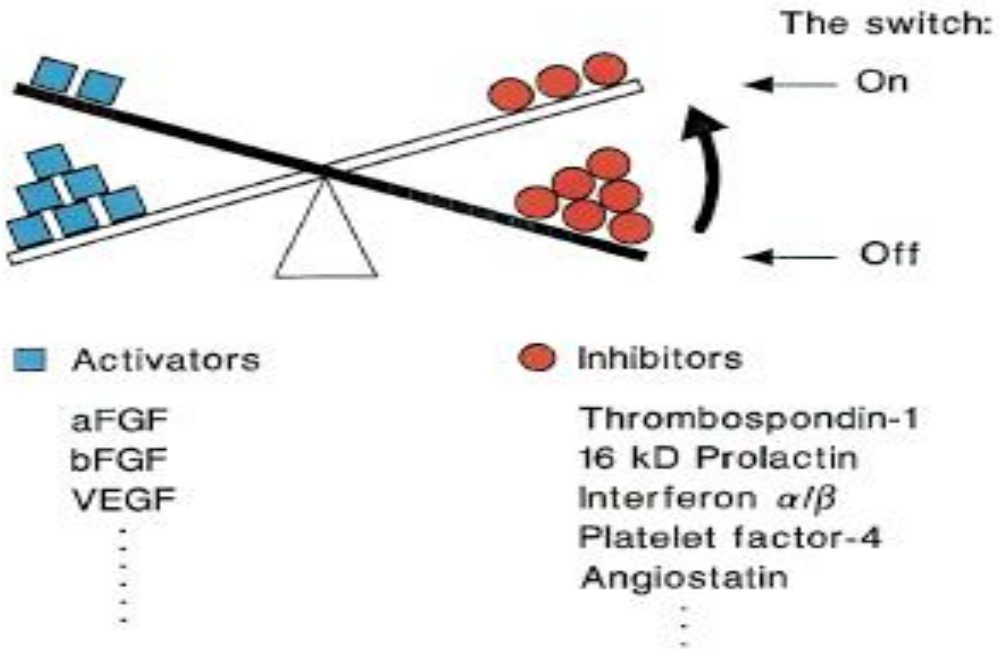
Capillary sprouting = angiogenesis

Folkman J (2007)

Nature Reviews | Drug Discovery


Angiogenesis
Tumor derived exosome
VEGF
PDGF
TGF-β
Glypican-1
miR-32-5p
miR-221
TNF-α
IL-8
bFGF
Syndecan-4
MALAT1
Dll-4
Tumor vasculature
Tumor cell
Induction of Angiogenesis
Normal cell
Blood vessel
Blood vessel

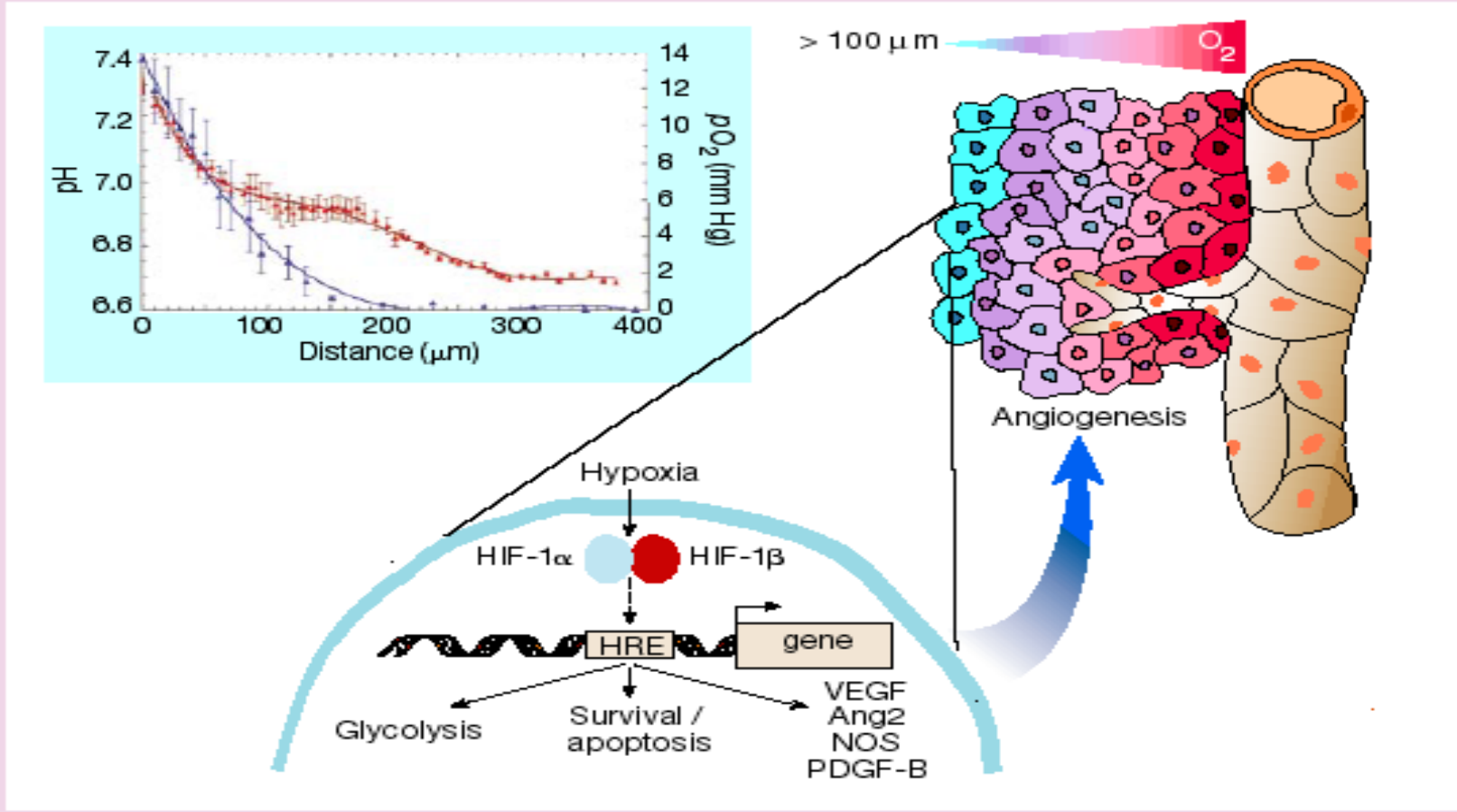
VEGF family
FGF family
PDGF
TGF family
Angiogenin
Angiopoietin-1/Tie2
TNF-a
HGF/scatter factor
IGF family
IL-8
Nitric oxide
Prostaglandins
Tissue factor
MMPs
.
.
.



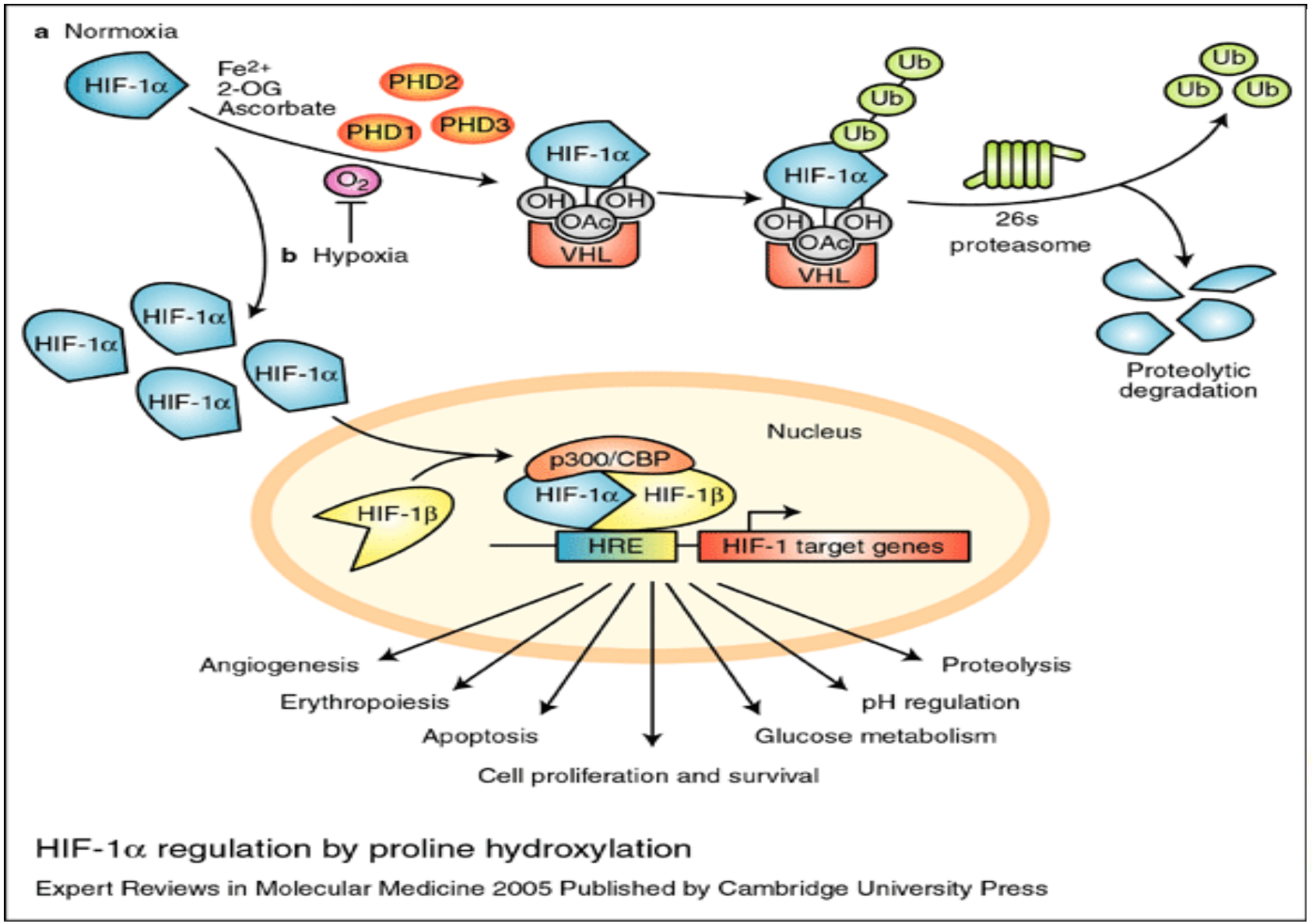
Angiostatin/other plasminogen kringles
Antithrombin (cleaved)
Endostatin
Fibronectin fragments
Prolactin
Prothrombin kringle-2
Vasostatin
IFNs
TIMPs
Angiopoietin-2
EMAP-II
.

Hypoxia - HIF - every cell must be within 50 to 100 μm of a capillary

Figure 2 Role of hypoxia in tumour angiogenesis. Because of the irregular pattern and organization of the tumour vasculature, some cells in tumours are located more than 100 μm (the diffusion limit for oxygen) away from blood vessels and become hypoxic (red-to-blue gradient indicates progressive hypoxia). Tumour cells survive fluctuations in oxygen tensions, in part because clones are selected in hypoxic tumours that switch to a proangiogenic phenotype. HIFs increase transcription of several angiogenic genes (for example, genes encoding VEGF, PDGF-BB and NOS). HIFs also affect cellular survival/apoptosis pathways. Inset: relationship between the distance of tumour cells from nearby vessels and their degree of hypoxia (blue symbols) and acidosis (red symbols)[24].

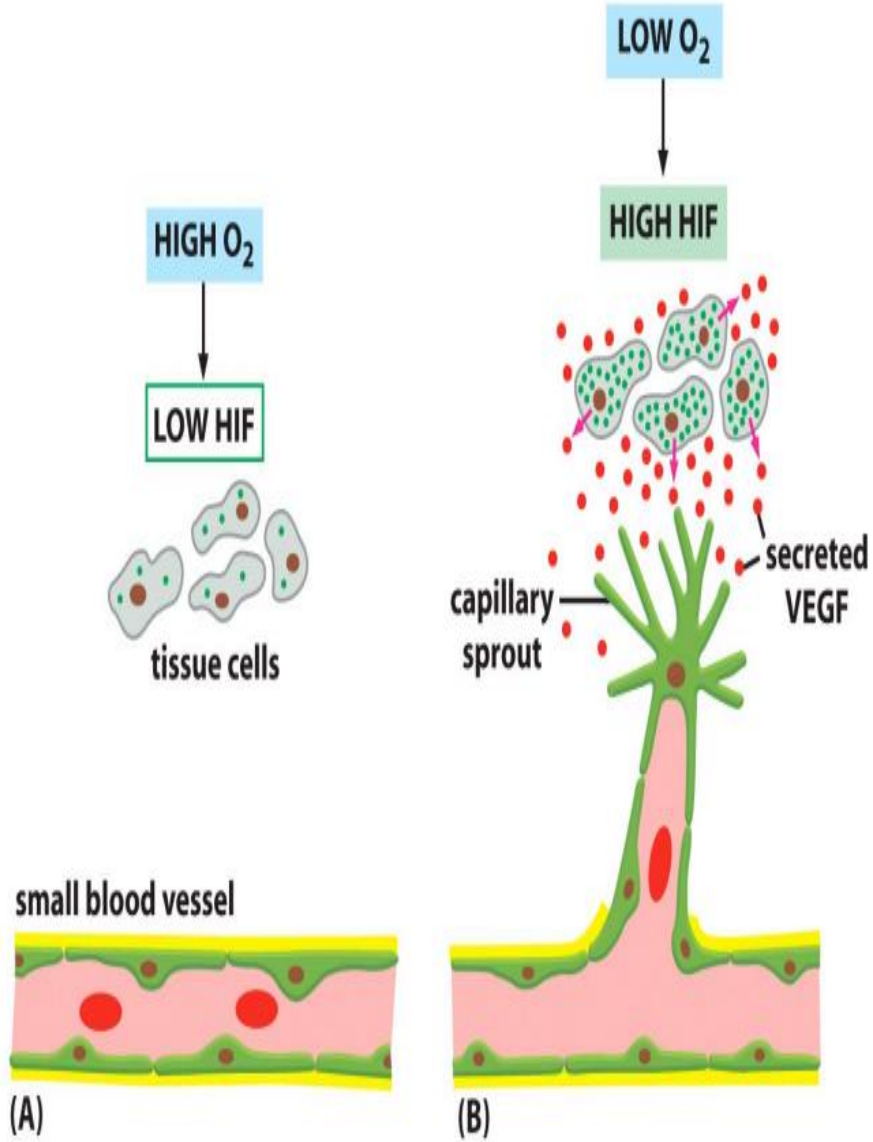


HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor



HIF-1α regulation by proline hydroxylation

Expert Reviews in Molecular Medicine 2005 Published by Cambridge University Press



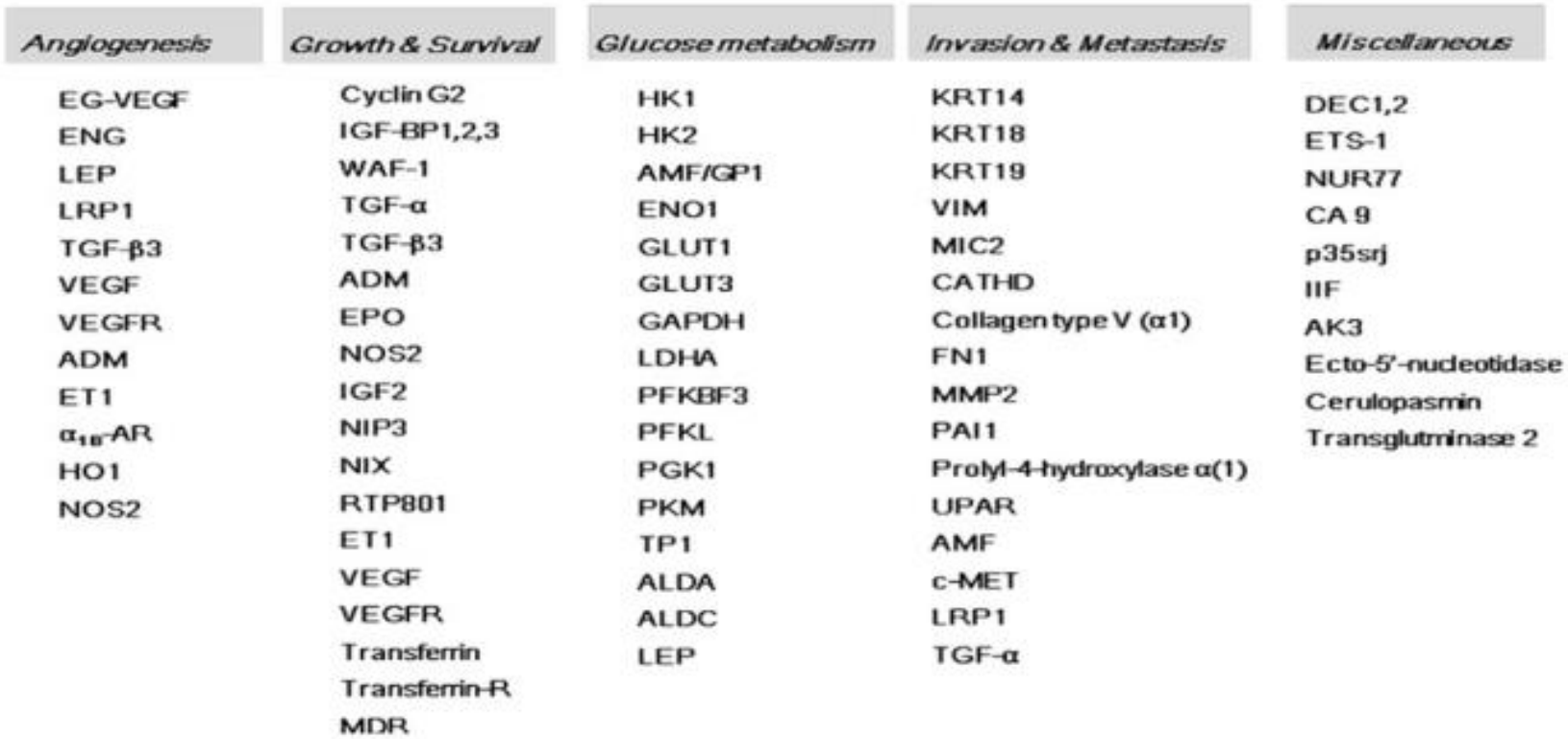
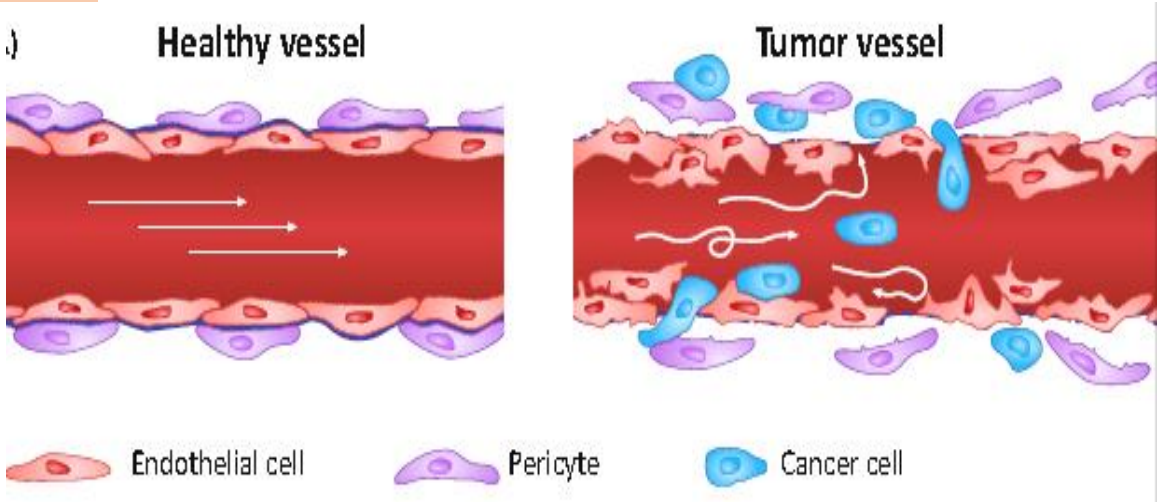


Fig. 2. HIF-1 target genes. α1B-AR: α1B-adrenergic receptor, ADM: adrenomedullin, AK3: adenylate kinase 3, ALDA: aldolase A, ALDC: aldolase C, AMF: autocrine motility factor, CA9: Carbonic anhydrase 9, CATHD: cathepsin D, ENG: endoglin, ET1: endothelin-1, ENO1: enolase 1, EPO: erythropoietin, FN1: fibronectin 1, GLUT1: glucose transporter 1, GLUT3: glucose transporter 3, GAPDH: glyceraldehyde-3-P-dehydrogenase, HK1: hexokinase 1, HK2: hexokinase 2, IGF2: insulin-like growth factor-2, IGF-BP1: IGF-factor-binding protein 1, IGF-BP2: IGF-factor-binding protein 2, IGF-BP3: IGF-factor-binding protein 3, ITF: intestinal trefoil factor, KRT14: keratin 14, KRT18: keratin 18, KRT19: keratin 19, LDHA: lactate dehydrogenase A, LEP: leptin, LRP1: LDL-receptor-related protein 1, MDR1: multidrug resistance 1, MMP2: matrix metalloproteinase 2, NOS2: nitric oxide synthase 2, PFKBF3: 6-phosphofructo-2-kinase/fructose-2:6-biphosphatase-3, PFKL: phosphor-fructo kinase L, PGK 1: phosphoglycerate kinase 1, PAI1: plasminogen-activator inhibitor 1, PKM: pyruvate kinase M, TGF-α: transforming growth factor-α, TGF-β3: transforming growth factor-β3, TPI: triosephosphate isomerase, VEGF: vascular endothelial growth factor, UPAR: urokinase plasminogen-activator receptor, VEGFR2: VEGF receptor-2, VIM: vimentin. The figure has been adapted from Ref. [30].

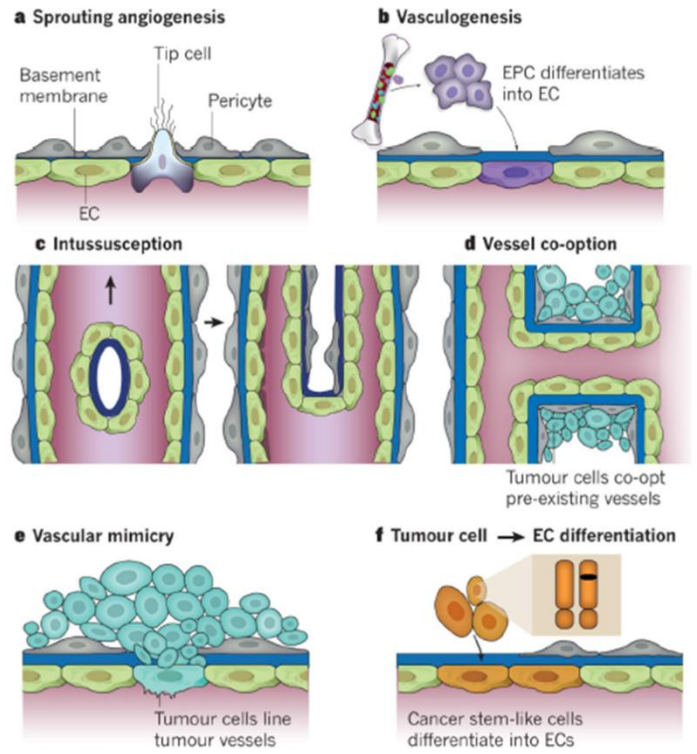
Tumor blood vessels are tortuous, highly permeable, and dilated, and show differential coverage and a loose association of perivascular cells along the vessels and weakened EC junctions

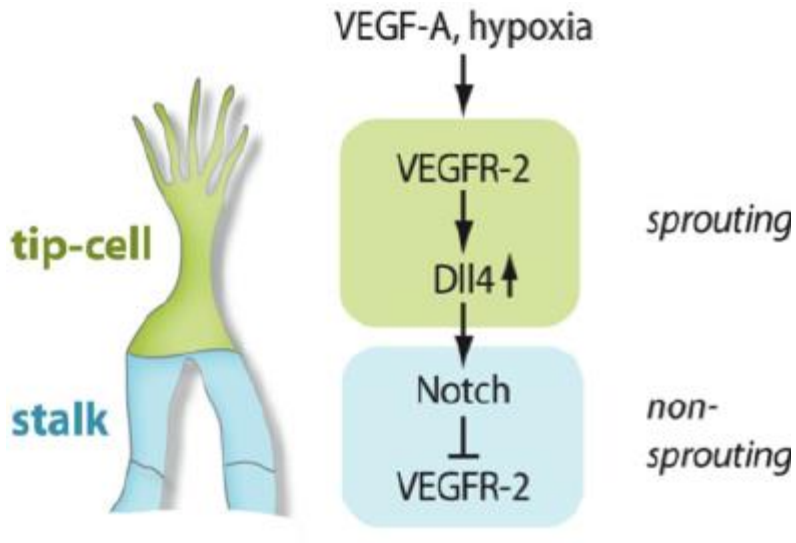


Heterogeneity of TECs – con.

2. Origin of TECs

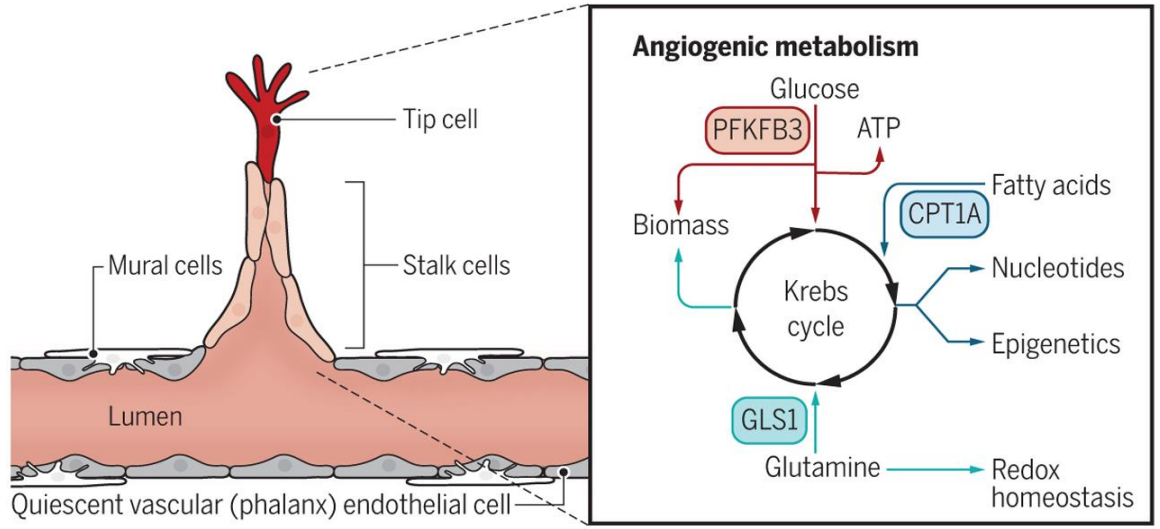
- EPCs or circulating endothelial precursor cells contribute to tumor angiogenesis
- ECs are heterogeneous
- Transdifferentiation to ECs

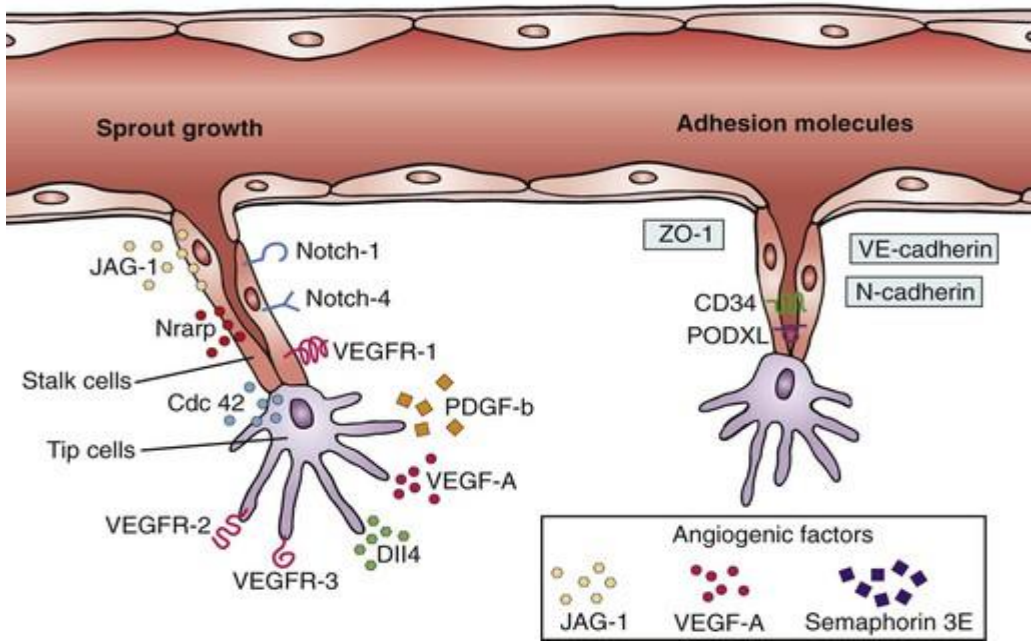


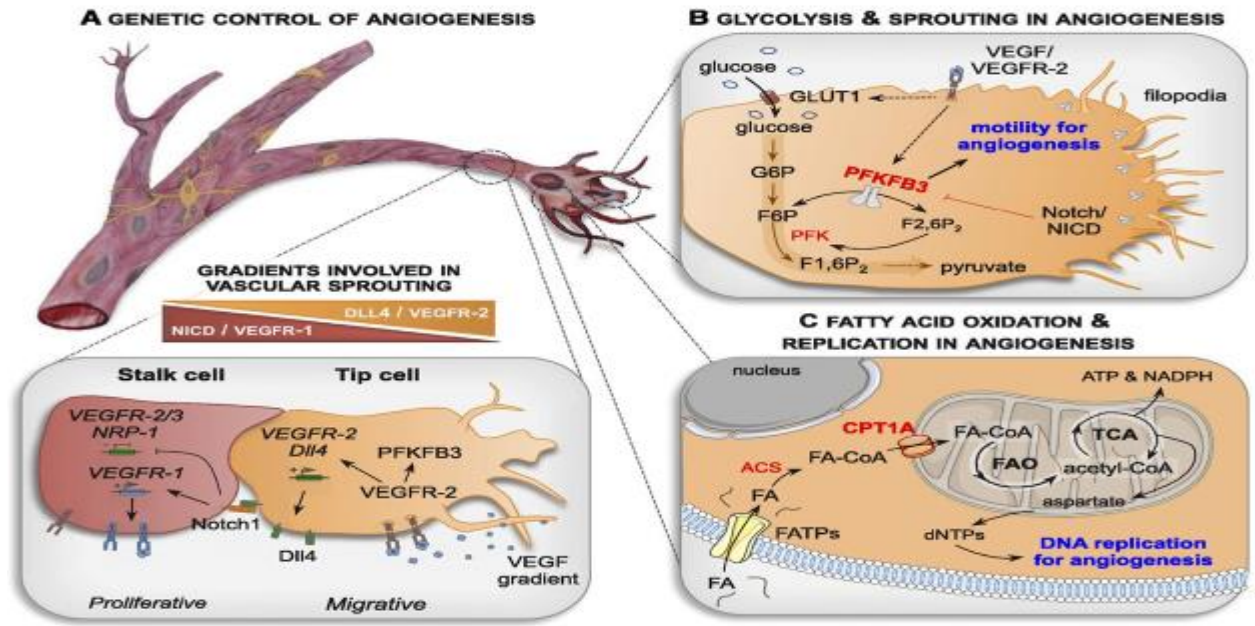


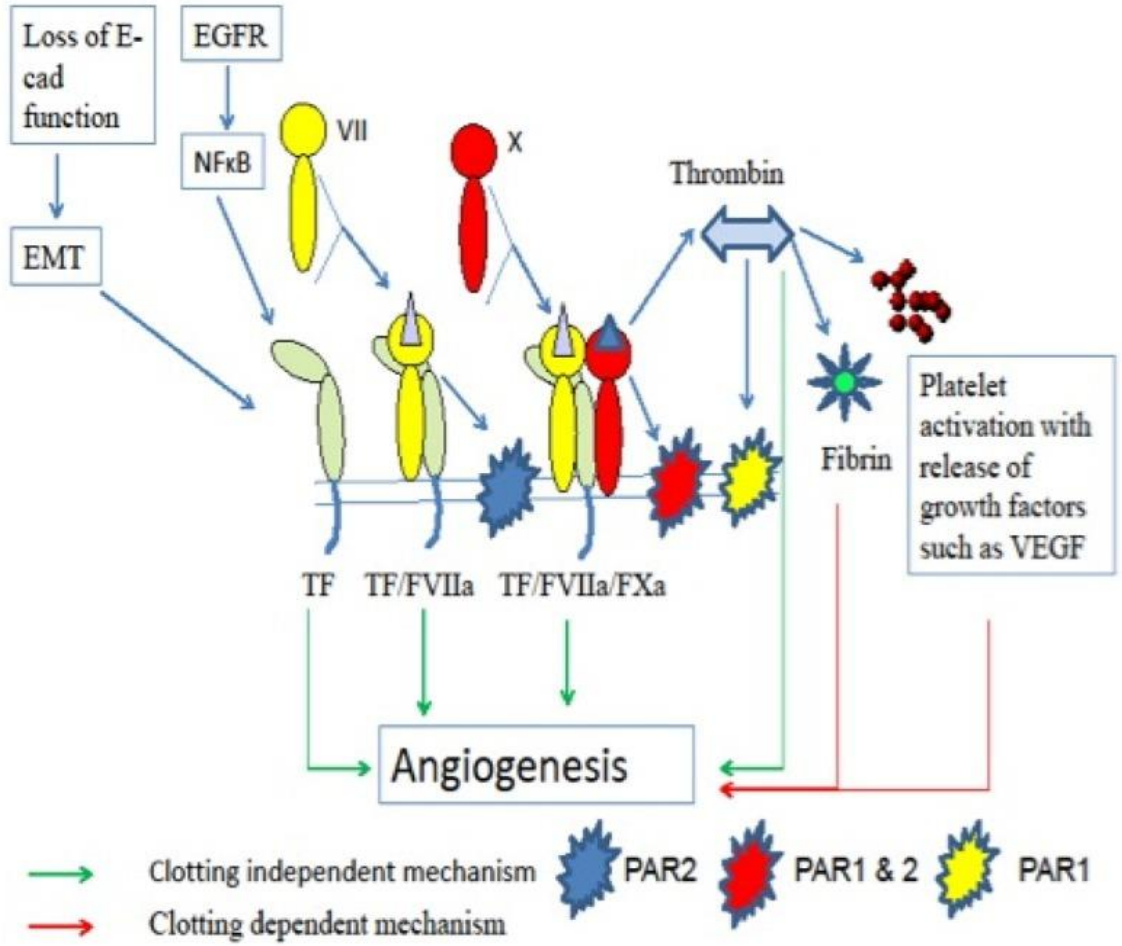
Metabolic pathways in angiogenesis

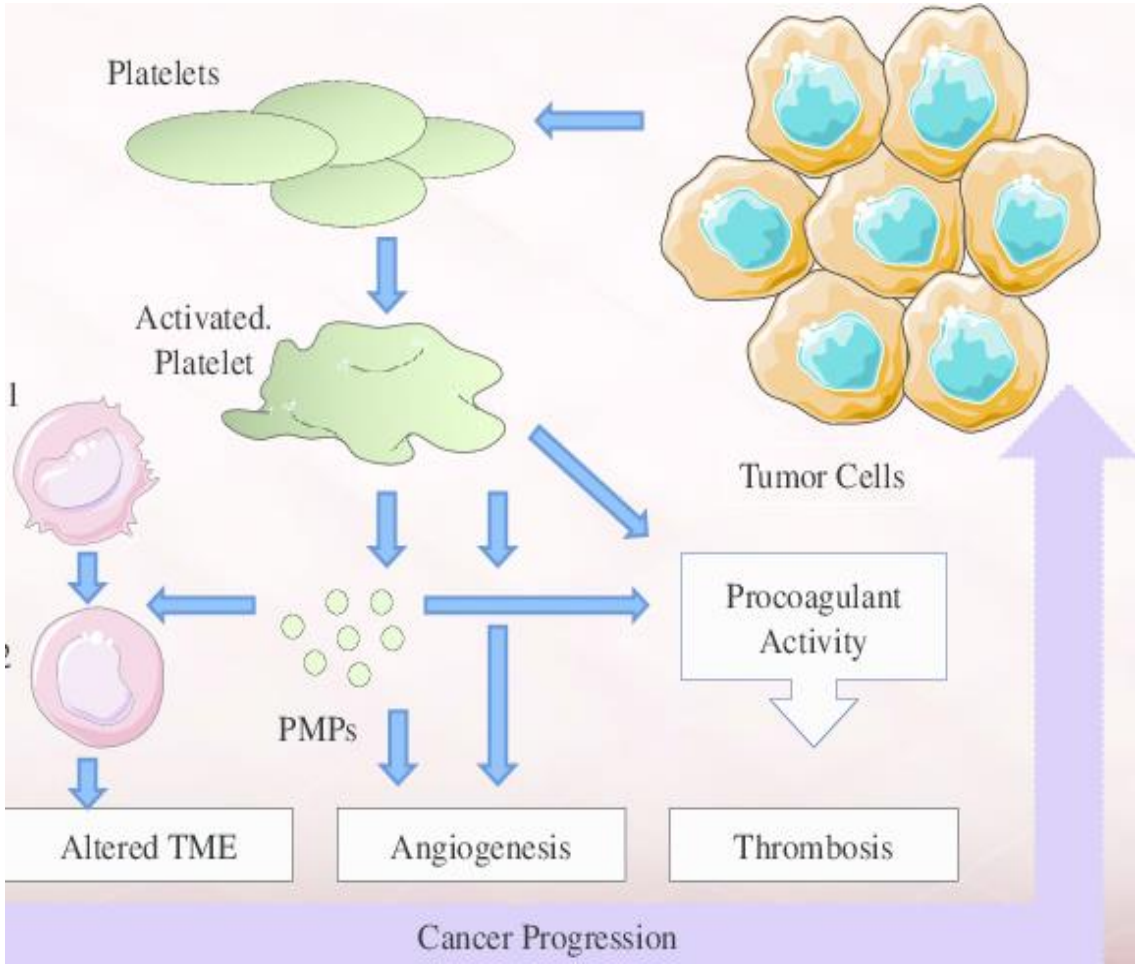
During angiogenesis, endothelial cells undergo metabolic changes that facilitate the formation of a sprout by stalk cells, which is directed by the tip cell. Key regulators of endothelial cell metabolism, PFKFB3, CPT1A, and GLS1, might be new therapeutic targets for various conditions.

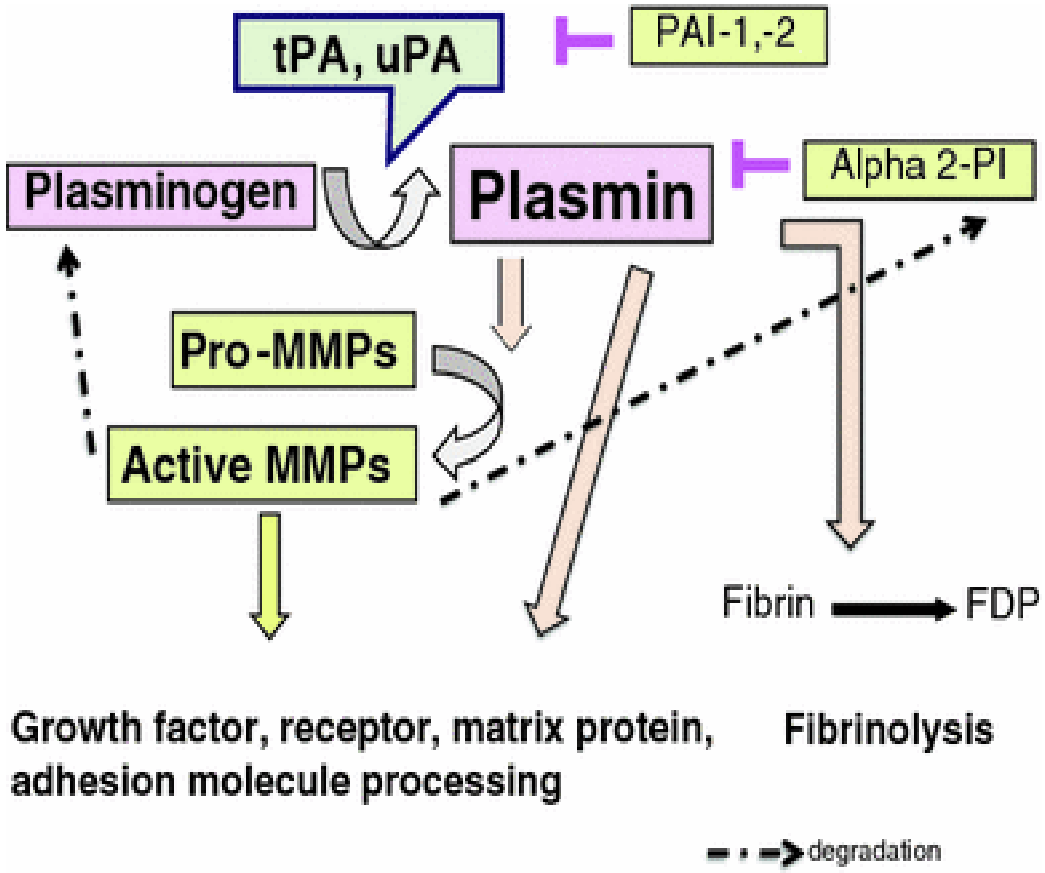


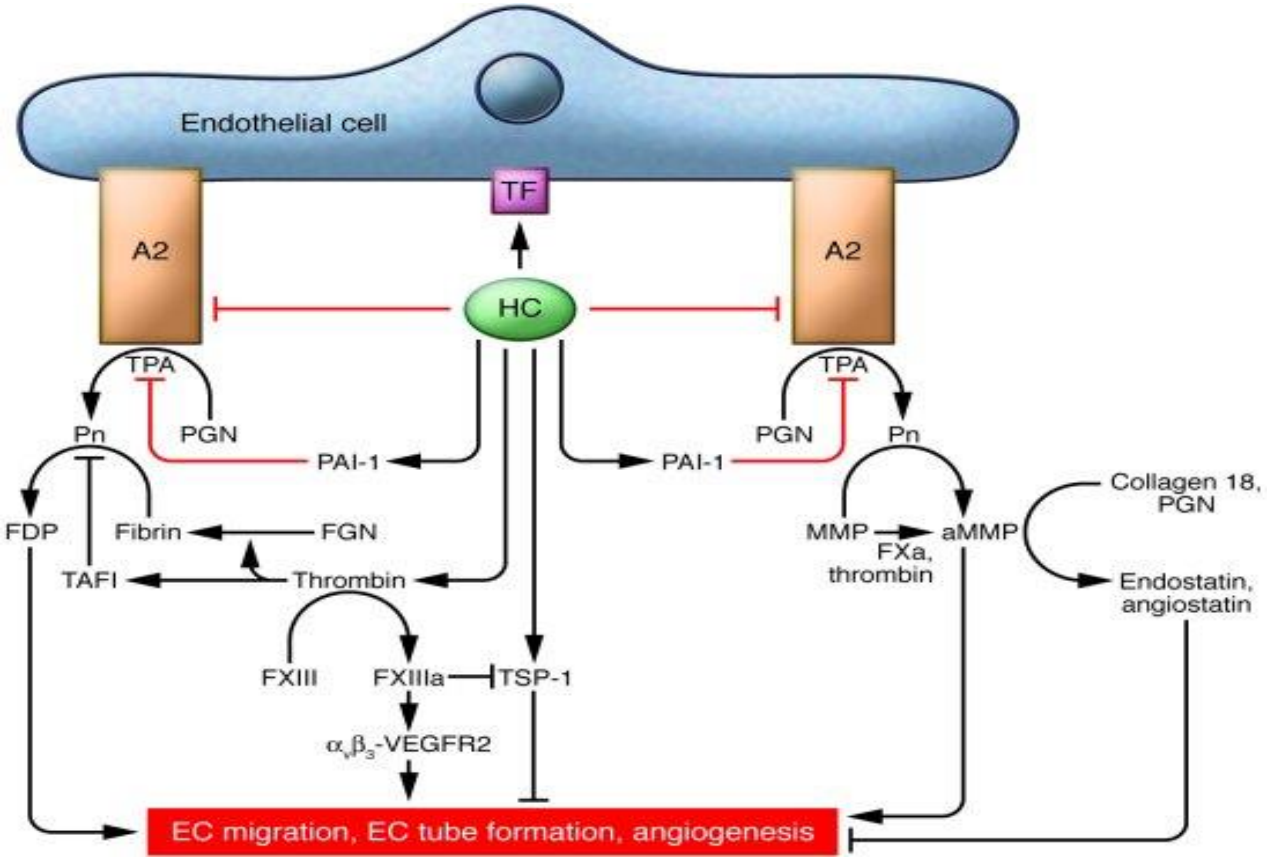





Loss of E-cad function
EGFR
NFκB
EMT
VII
X
Thrombin
Fibrin
Platelet activation with release of growth factors such as VEGF
TF
TF/FVIIa
TF/FVIIa/FXa
Angiogenesis
Clotting independent mechanism
Clotting dependent mechanism
PAR2
PAR1 & 2
PAR1


Platelets
Activated. Platelet
Tumor Cells
PMPs
Procoagulant Activity
Altered TME
Angiogenesis
Thrombosis
Cancer Progression


tPA, uPA
PAI-1,-2
Plasminogen
Plasmin
Alpha 2-PI
Pro-MMPs
Active MMPs
Fibrin
FDP
Growth factor, receptor, matrix protein, adhesion molecule processing
Fibrinolysis
degradation


Endothelial cell
TF
A2
A2
HC
TPA
PGN
PAI-1
PAI-1
PGN
TPA
Pn
Pn
FDP
Fibrin
FGN
TAFI
Thrombin
MMP
FXa, thrombin
aMMP
Collagen 18, PGN
Endostatin, angiostatin
FXIII
FXIIIa
TSP-1
$\alpha_v\beta_3$-VEGFR2
EC migration, EC tube formation, angiogenesis

Plasminogen (Plg)

PAI-1 ⊣ tPA → ← uPA

Plasmin

↓

Pro-MMPs → MMPs

Activation ←

Change of receptor specificity ←

Inactivation ←

Degradation ←

Cyto-/chemokine (KitL, MCP-1, CXCL5)
Cell receptors (e.g. uPAR)
matrix proteins (e.g. laminin)
Adhesion molecules (e.g. VCAM-1)

The proteolytic niche

ECM degradation →

Cell invasion

VEGF-A, FGF-2 →

Angiogenesis

Cyto-/chemokine (KitL, MCP-1, CXCL5 →

Myeloid cell recruitment

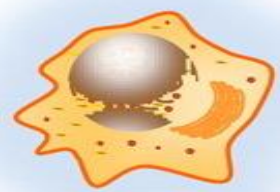
Extracellular Matrix

→

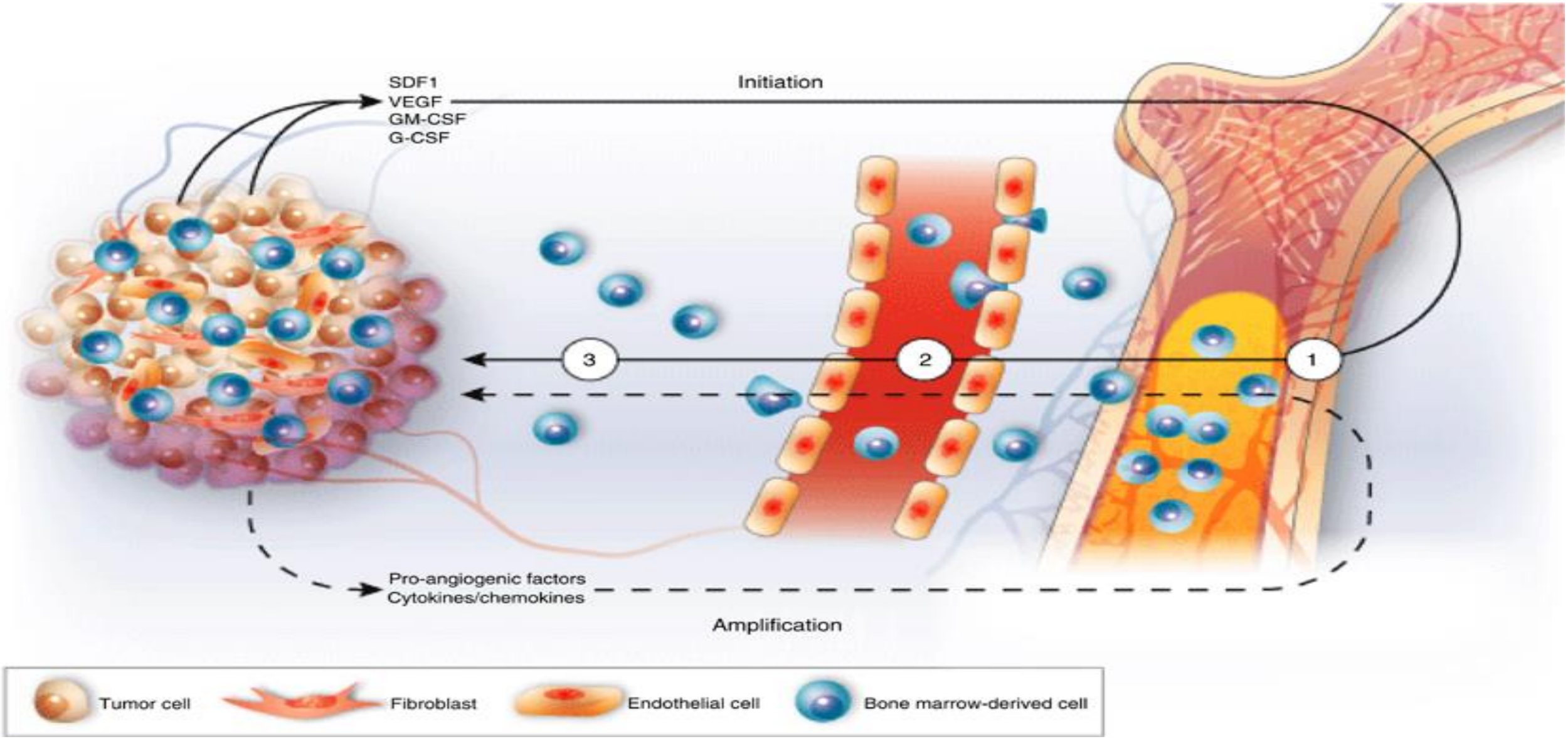
FDP

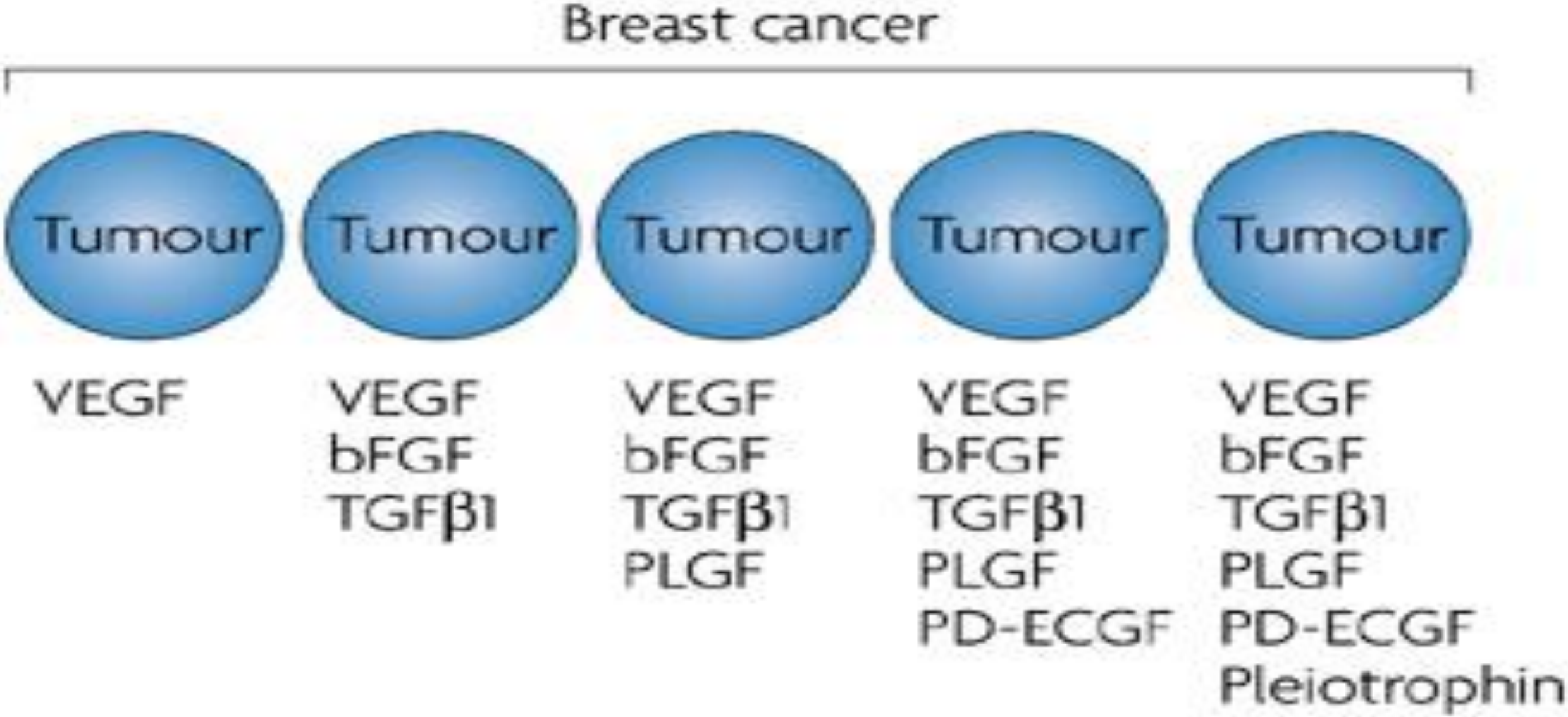
↓

Fibrin

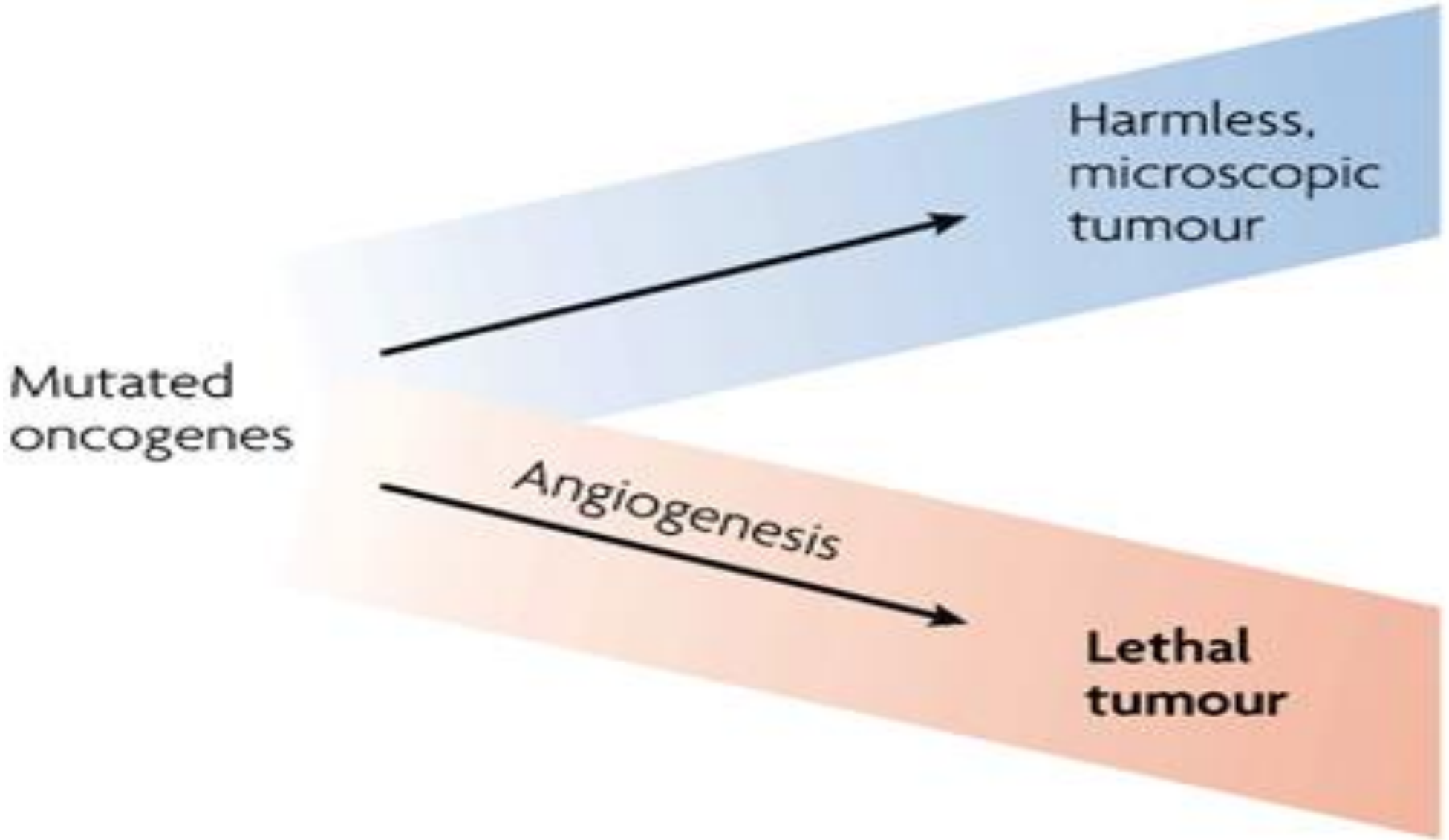


Cartoon


SDF1
VEGF
GM-CSF
G-CSF
Initiation
3
2
1
Pro-angiogenic factors
Cytokines/chemokines
Amplification
Tumor cell
Fibroblast
Endothelial cell
Bone marrow-derived cell



Nature Reviews | **Drug Discovery**



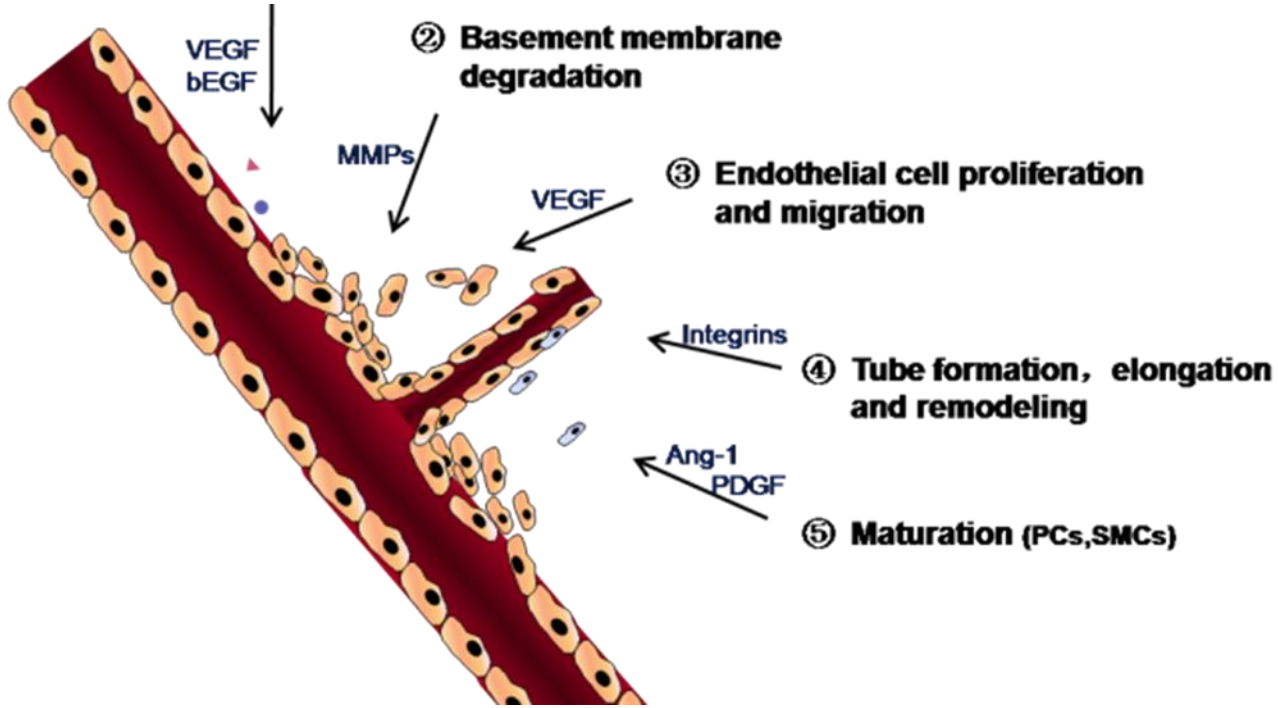
Folkman J (2007)

Nature Reviews | Drug Discovery

✖Tumors induce angiogenesis to obtain oxygen and nutrients

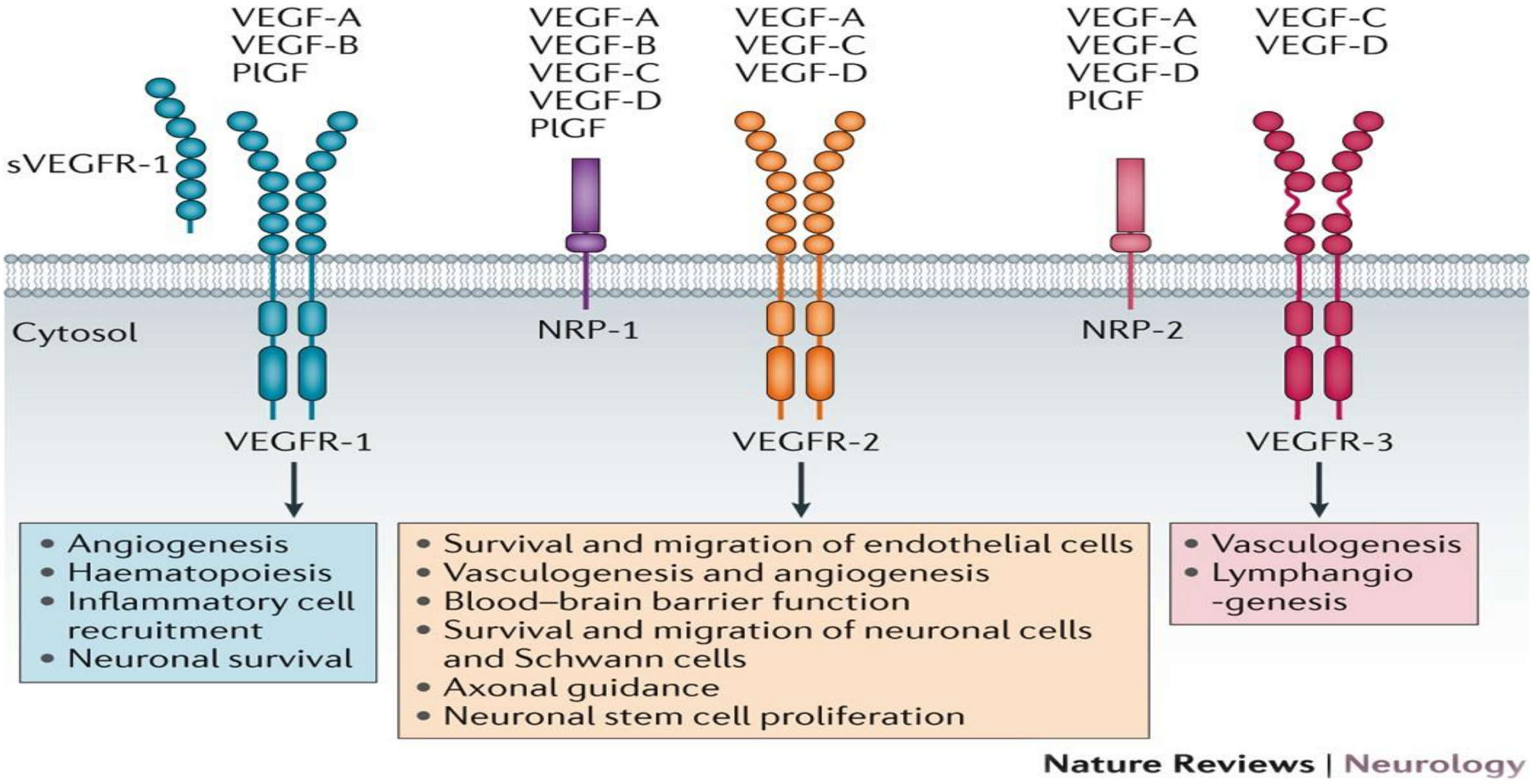
✖4 major steps of endothelial cells in angiogenesis

1. Breaking through of basal lamina that envelops existing blood vessels
2. Migration towards source signal
3. Proliferation
4. Formation of tubes



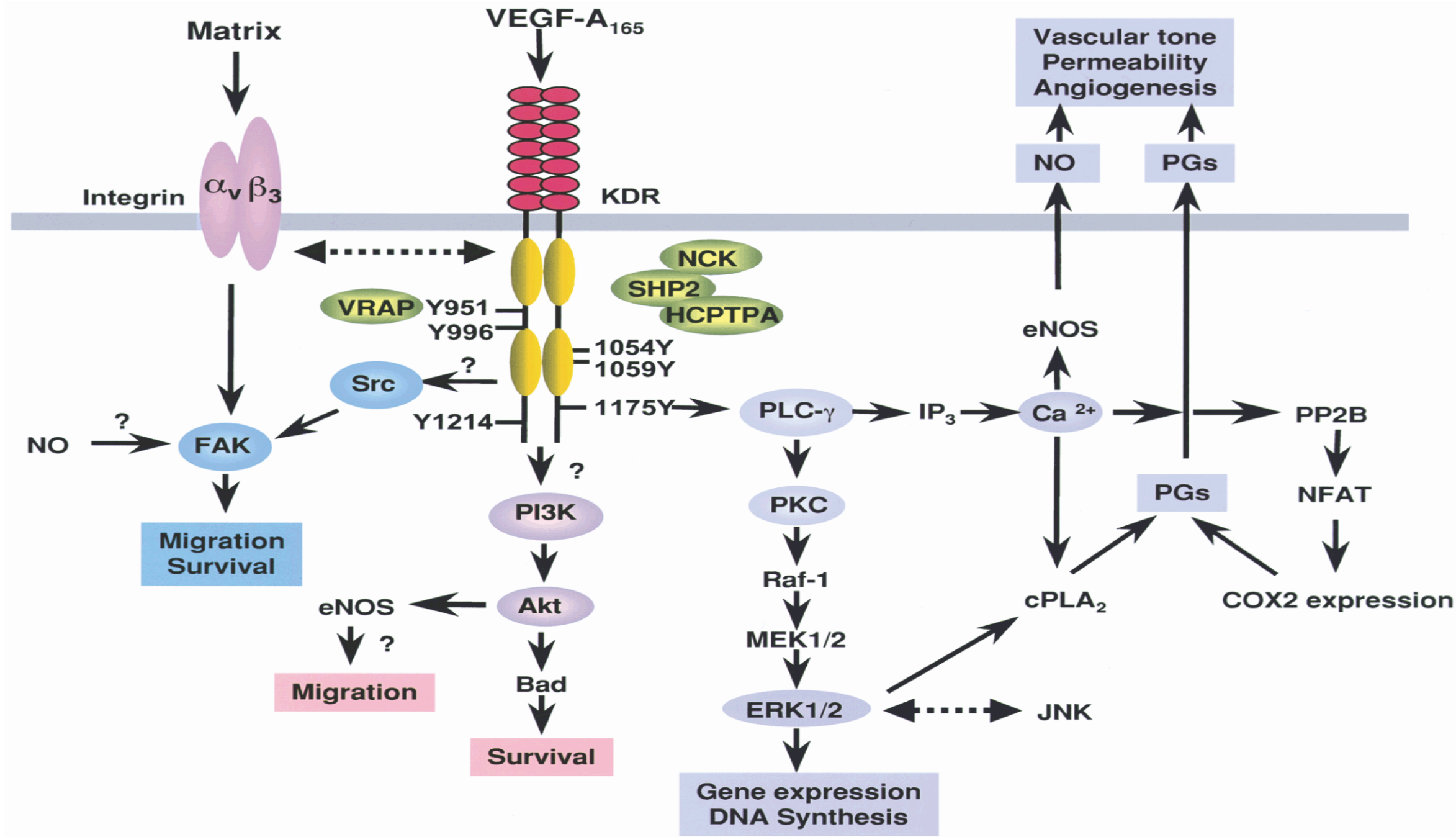
VEGF Family

- Consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E
- VEGF-A is the most potent angiogenic protein
- This isoform signal through VEGF receptor 2 (VEGFR-2), the major VEGF signaling receptor that mediates sprouting angiogenesis (called kinase-insert domain–containing receptor [KDR] in humans and fetal liver kinase 1 [flk-1] in mice).

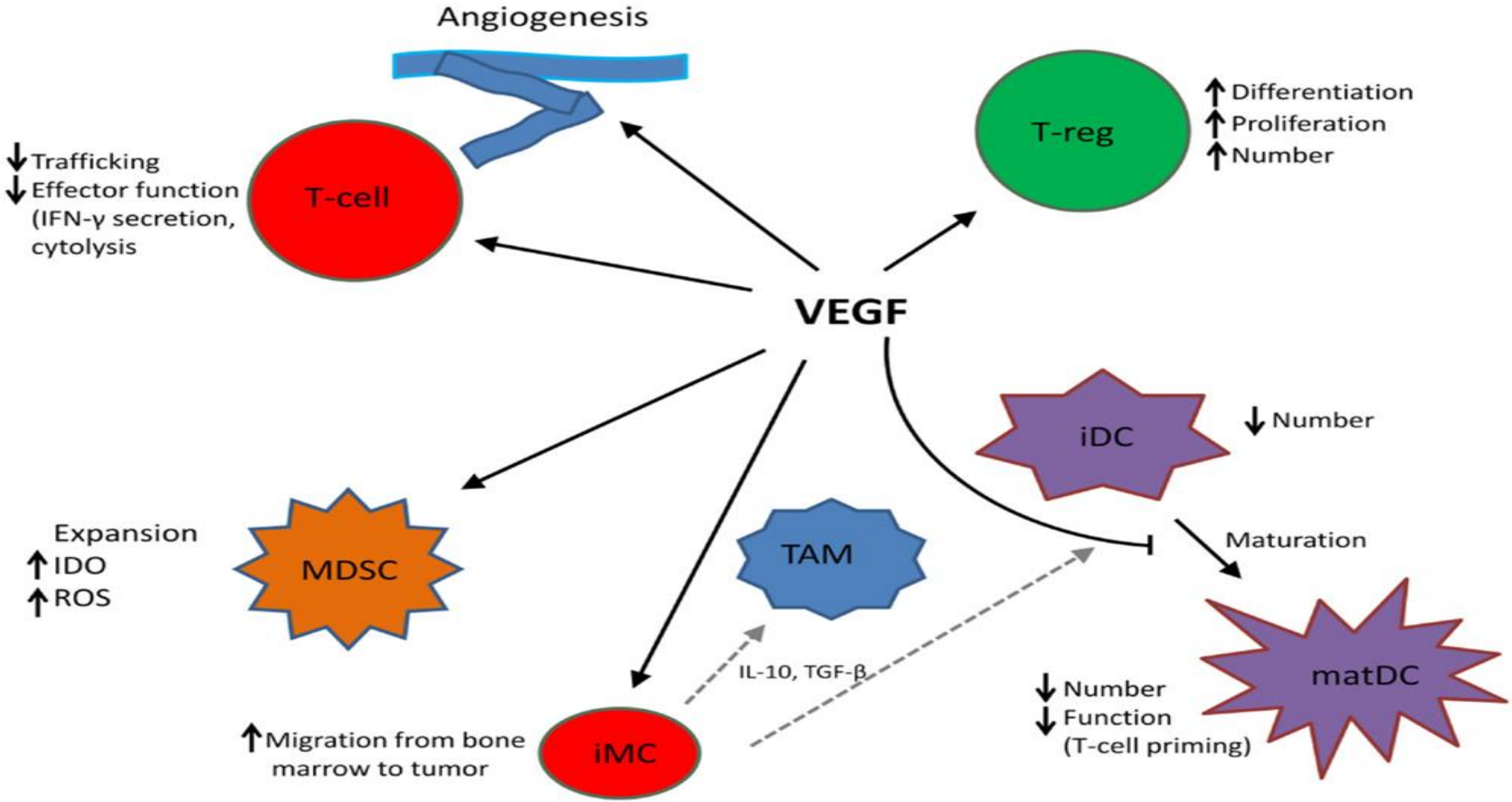

VEGF-A
VEGF-B
PlGF
sVEGFR-1
VEGF-A
VEGF-B
VEGF-C
VEGF-D
PlGF
VEGF-A
VEGF-C
VEGF-D
VEGF-A
VEGF-C
VEGF-D
PlGF
VEGF-C
VEGF-D
Cytosol
NRP-1
NRP-2
VEGFR-1
VEGFR-2
VEGFR-3
• Angiogenesis
• Haematopoiesis
• Inflammatory cell recruitment
• Neuronal survival
• Survival and migration of endothelial cells
• Vasculogenesis and angiogenesis
• Blood–brain barrier function
• Survival and migration of neuronal cells and Schwann cells
• Axonal guidance
• Neuronal stem cell proliferation
• Vasculogenesis
• Lymphangio -genesis
Nature Reviews | Neurology

Functions of the VEGF family of receptors

VEGFR-1[1,2]	Crucial to embryonic angiogenesis Does not appear to be critical in pathogenic angiogenesis
VEGFR-2[1,3]	Most important VEGF receptor in tumor angiogenesis Mediates the majority of VEGF angiogenic effects
VEGFR-3[1,4]	Found only in lymphatic endothelial cells Associated with lymph node metastasis


Matrix
VEGF-A165
Integrin
αv β3
KDR
NCK
SHP2
HCPTPA
VRAP
Y951
Y996
1054Y
1059Y
Src
?
Y1214
1175Y
NO
?
FAK
Migration
Survival
PI3K
?
PLC-γ
IP3
Ca 2+
eNOS
Vascular tone
Permeability
Angiogenesis
NO
PGs
PP2B
NFAT
PGs
COX2 expression
cPLA2
PKC
Raf-1
MEK1/2
ERK1/2
JNK
Gene expression
DNA Synthesis
eNOS
?
Migration
Akt
Bad
Survival

Function[1,2]	**Mechanism**
Proliferation	Activation of mitogen-activated protein kinases
Permeability	Vesicovascular organelles Endothelial fenestrations Opening of junctions between adjacent endothelial cells
Invasion	Induction of metalloproteinases uPA, uPAR, TTPA
Migration	Activation of FAK, p38, nitric oxide
Survival	Induction of PI3K/Akt, Bcl2, A1, survivin, XIAP, or FAK Inhibition of caspases
Activation	Upregulation of integrin expression Alteration of cell cytoskeleton


Angiogenesis
↓Trafficking
↓Effector function
(IFN-γ secretion,
cytolysis
T-cell
T-reg
↑Differentiation
↑Proliferation
↑Number
VEGF
iDC
↓Number
Expansion
↑IDO
↑ROS
MDSC
TAM
Maturation
IL-10, TGF-β
matDC
↓Number
↓Function
(T-cell priming)
↑Migration from bone
marrow to tumor
iMC

VEGF signaling pathways

Ras/MAP kinase pathway – Gene expression and proliferation

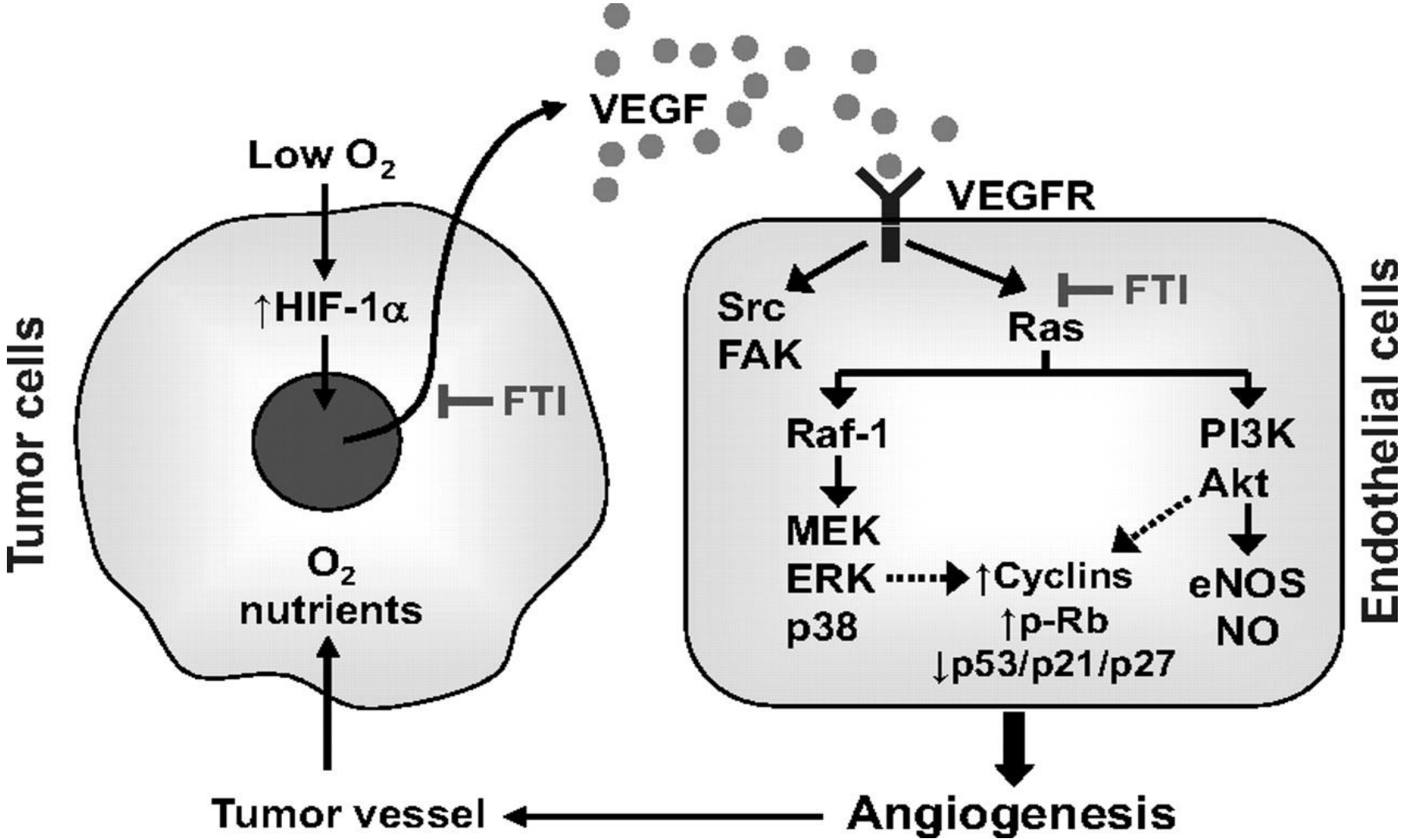
Akt/PI3K pathway – cell survival

NOS pathway – vascularpermeability

PKC pathway – cell proliferation and vascularpermeability

FAK/Paxillin pathway – cytoskeletal rearrangement and cell migration

Angiogenesis


VEGF
Low O_2
VEGFR
↑HIF-1α
FTI
Src
FAK
FTI
Ras
Raf-1
PI3K
Akt
MEK
ERK
p38
↑Cyclins
↑p-Rb
↓p53/p21/p27
eNOS
NO
O_2
nutrients
Tumor cells
Endothelial cells
Tumor vessel
Angiogenesis

Ras/MAP kinase pathway

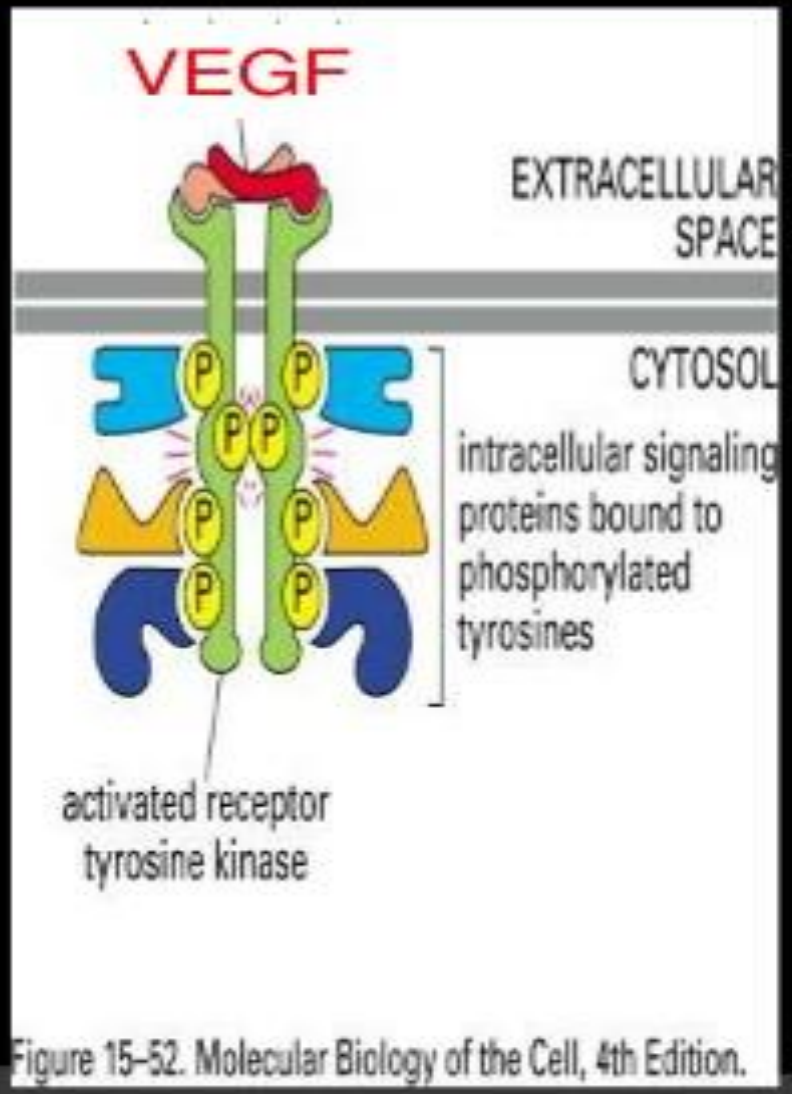


Figure 15–52. Molecular Biology of the Cell, 4th Edition.

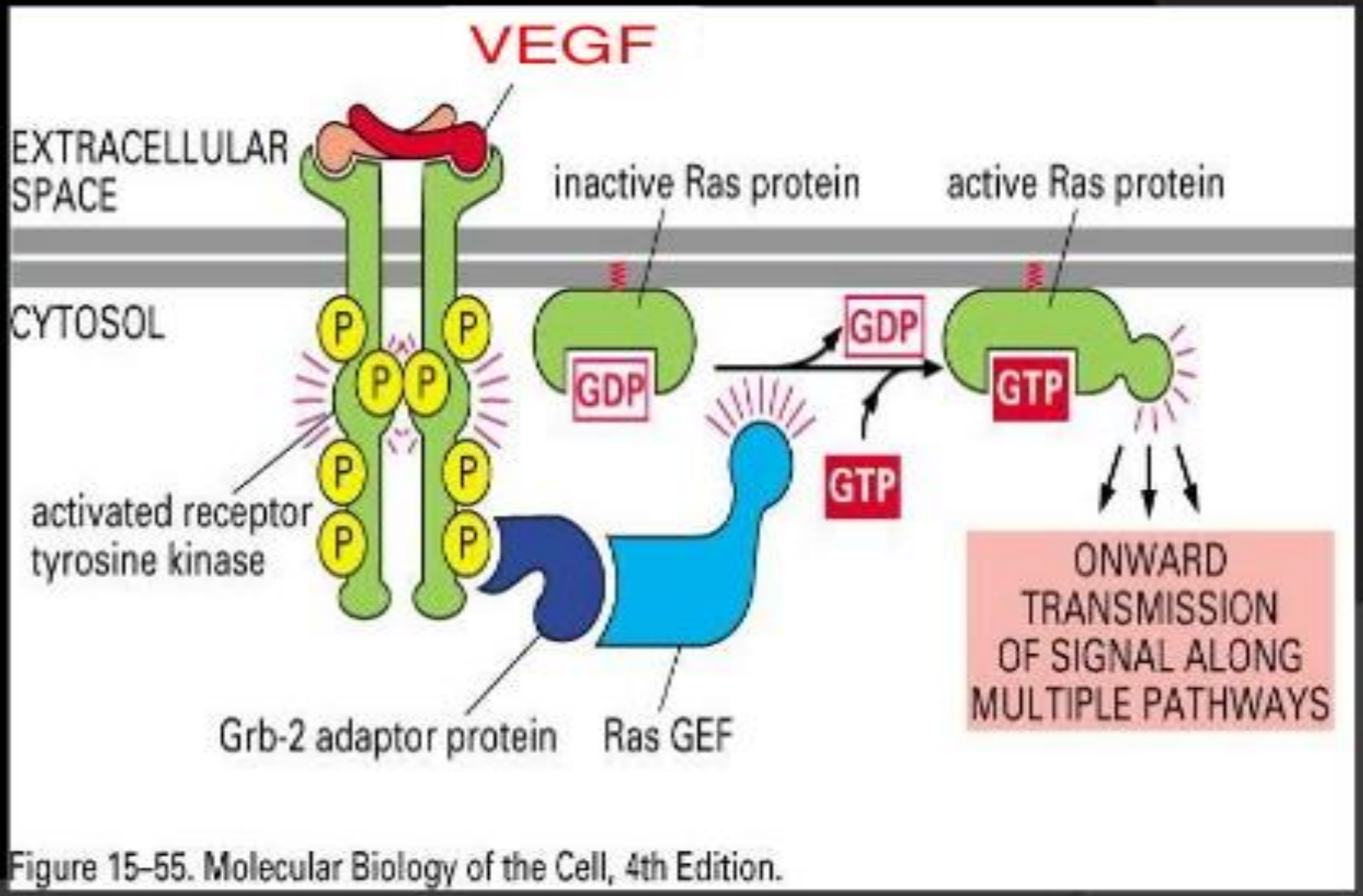
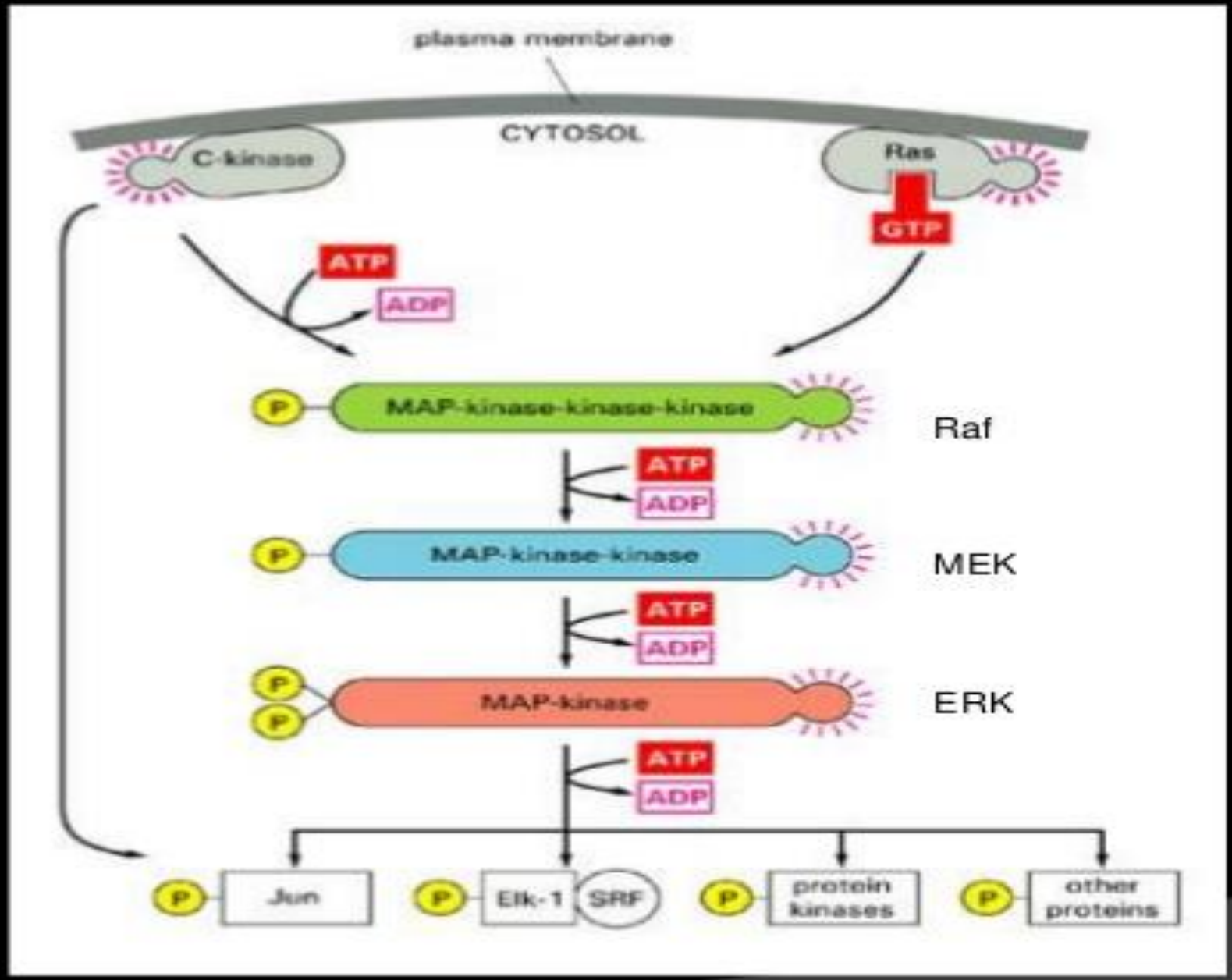


Figure 15–55. Molecular Biology of the Cell, 4th Edition.

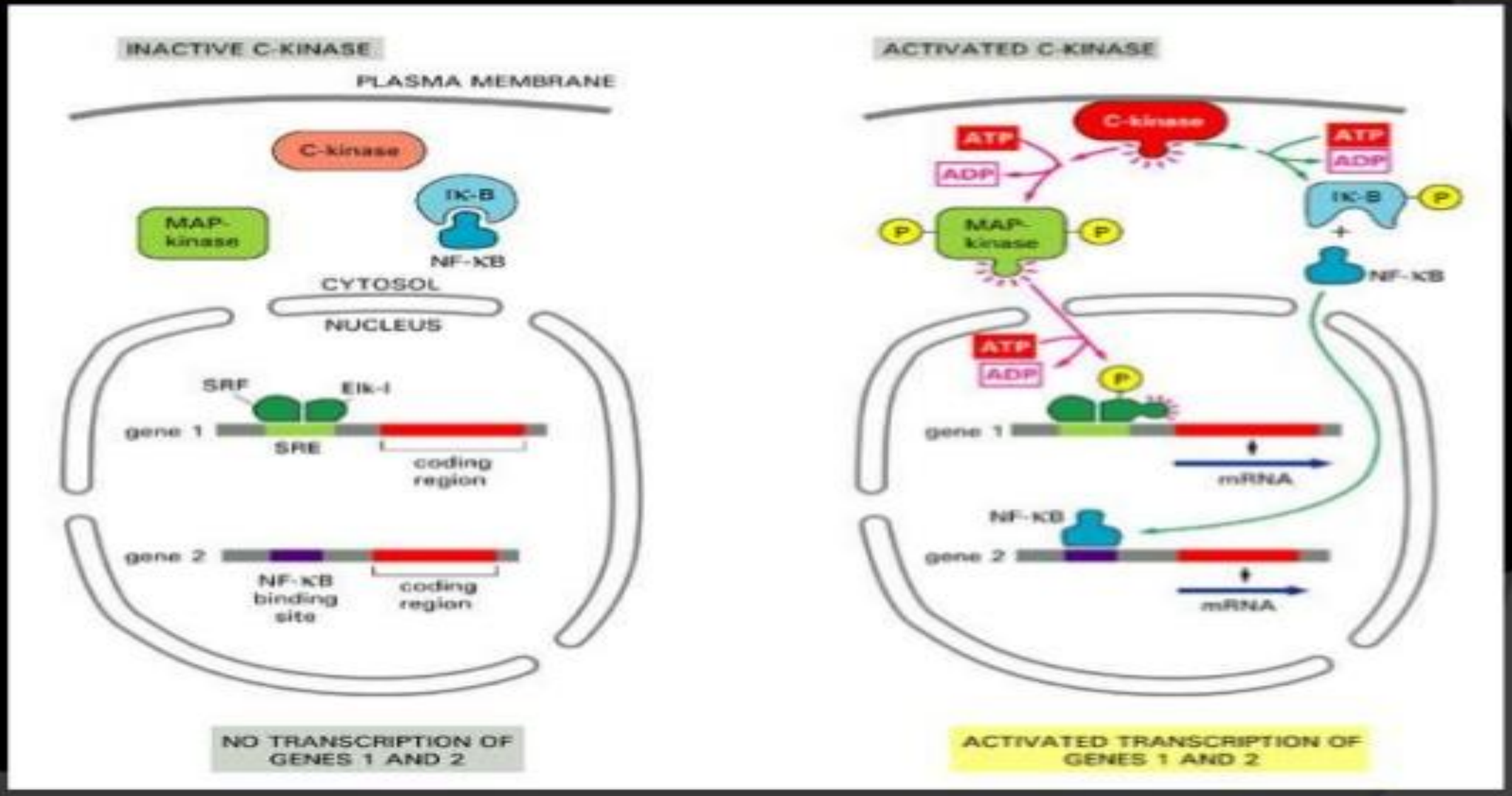


Ras activates MAP-Kinase Pathway

1- MAPKKK
2- MAPKK
3- MAPK

MAPK: Mitogen-activated Kinase

(there are three MAP-Kinase cascades:
MEK/ERK
P38
JNK)


INACTIVE C-KINASE
PLASMA MEMBRANE
C-kinase
IK-B
MAP-kinase
NF-KB
CYTOSOL
NUCLEUS
SRF
Elk-I
gene 1
SRE
coding region
gene 2
NF-κB binding site
coding region
NO TRANSCRIPTION OF GENES 1 AND 2
ACTIVATED C-KINASE
C-kinase
ATP
ADP
ATP
ADP
IK-B
P
P
MAP-kinase
P
NF-KB
ATP
ADP
P
gene 1
mRNA
NF-KB
gene 2
mRNA
ACTIVATED TRANSCRIPTION OF GENES 1 AND 2

Akt/PI-3 Kinase Pathway and Survival

PKB, PDK:
(PDK: PI-dependent kinase)
Ser/Thr kinases

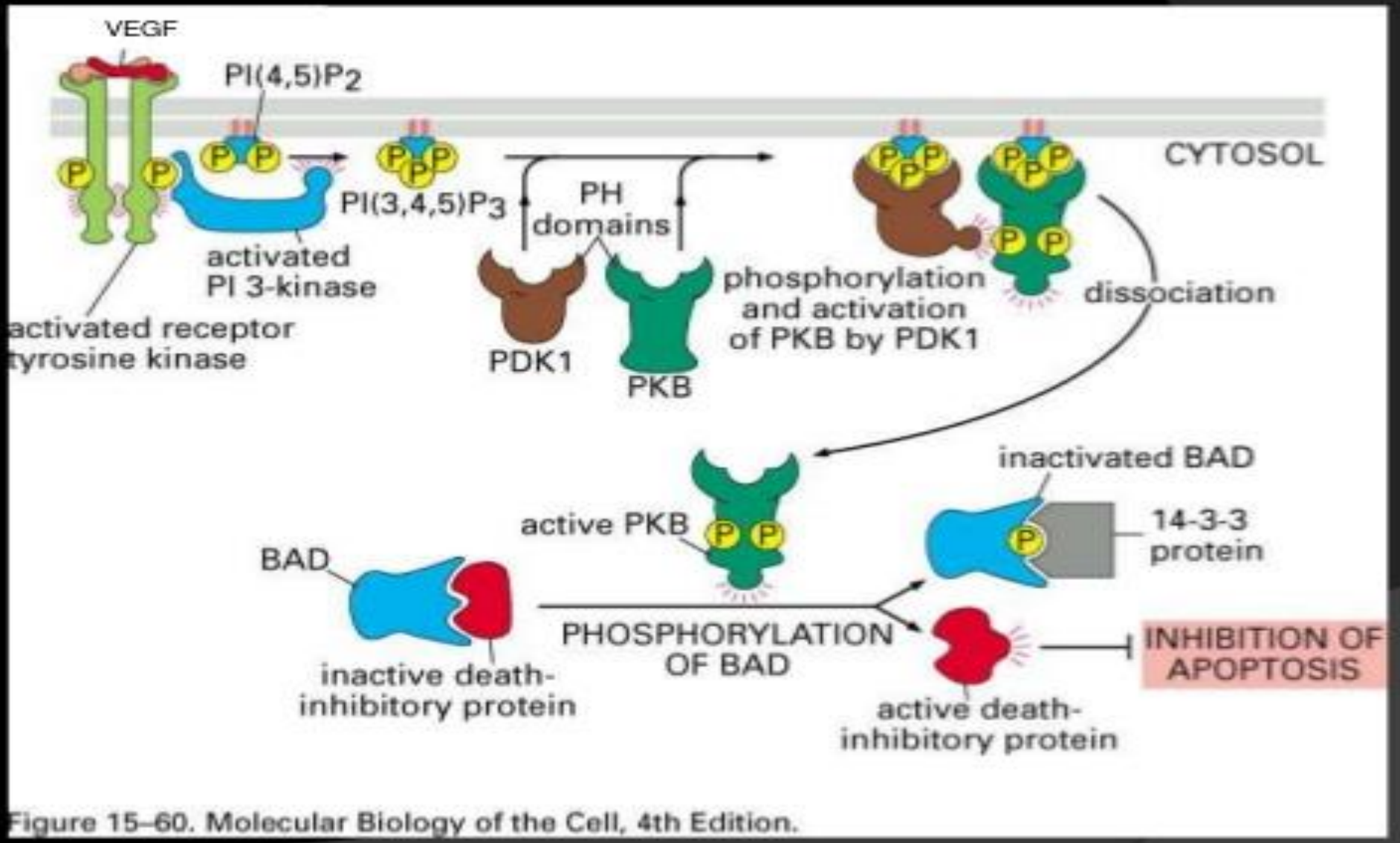
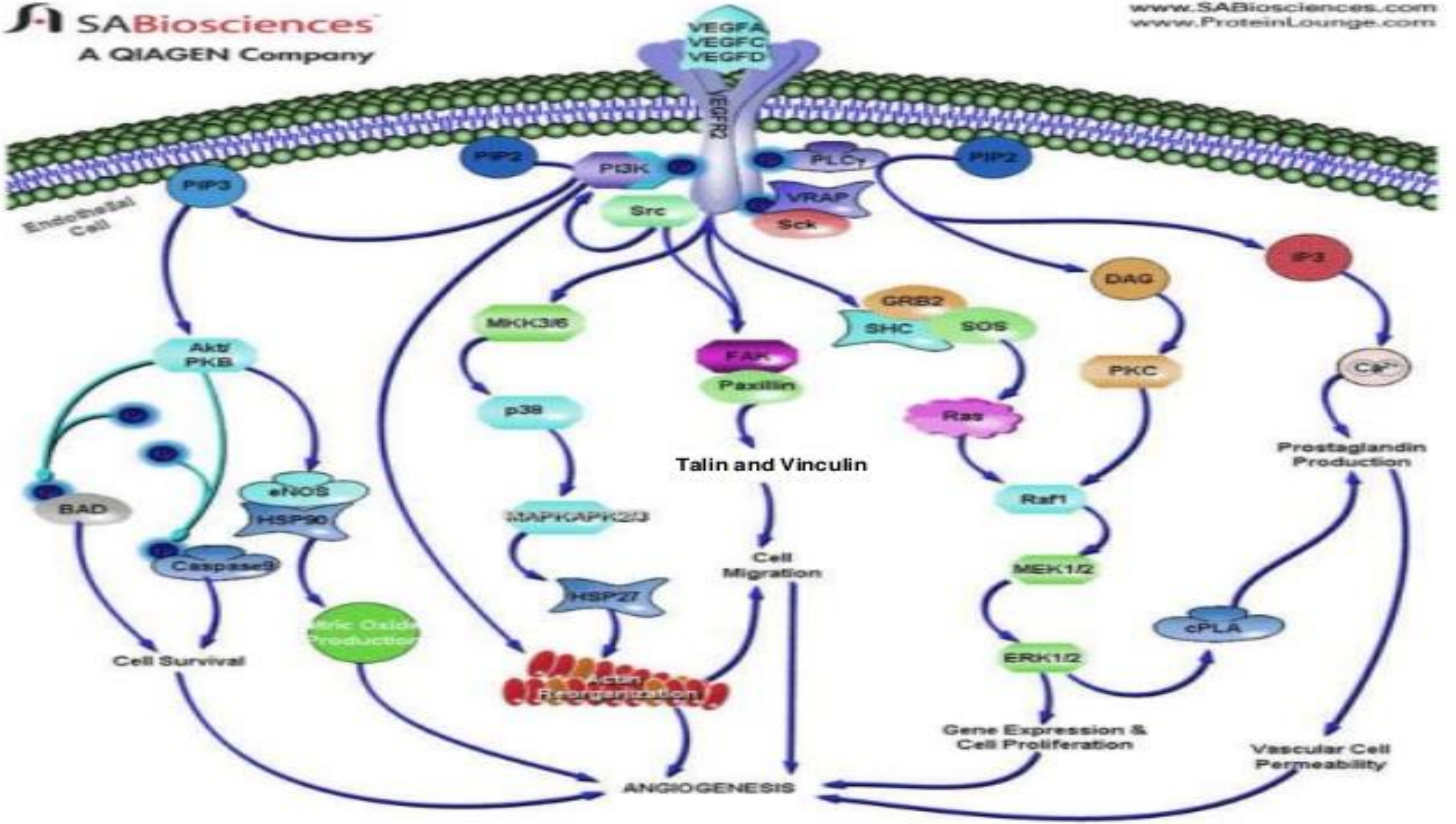
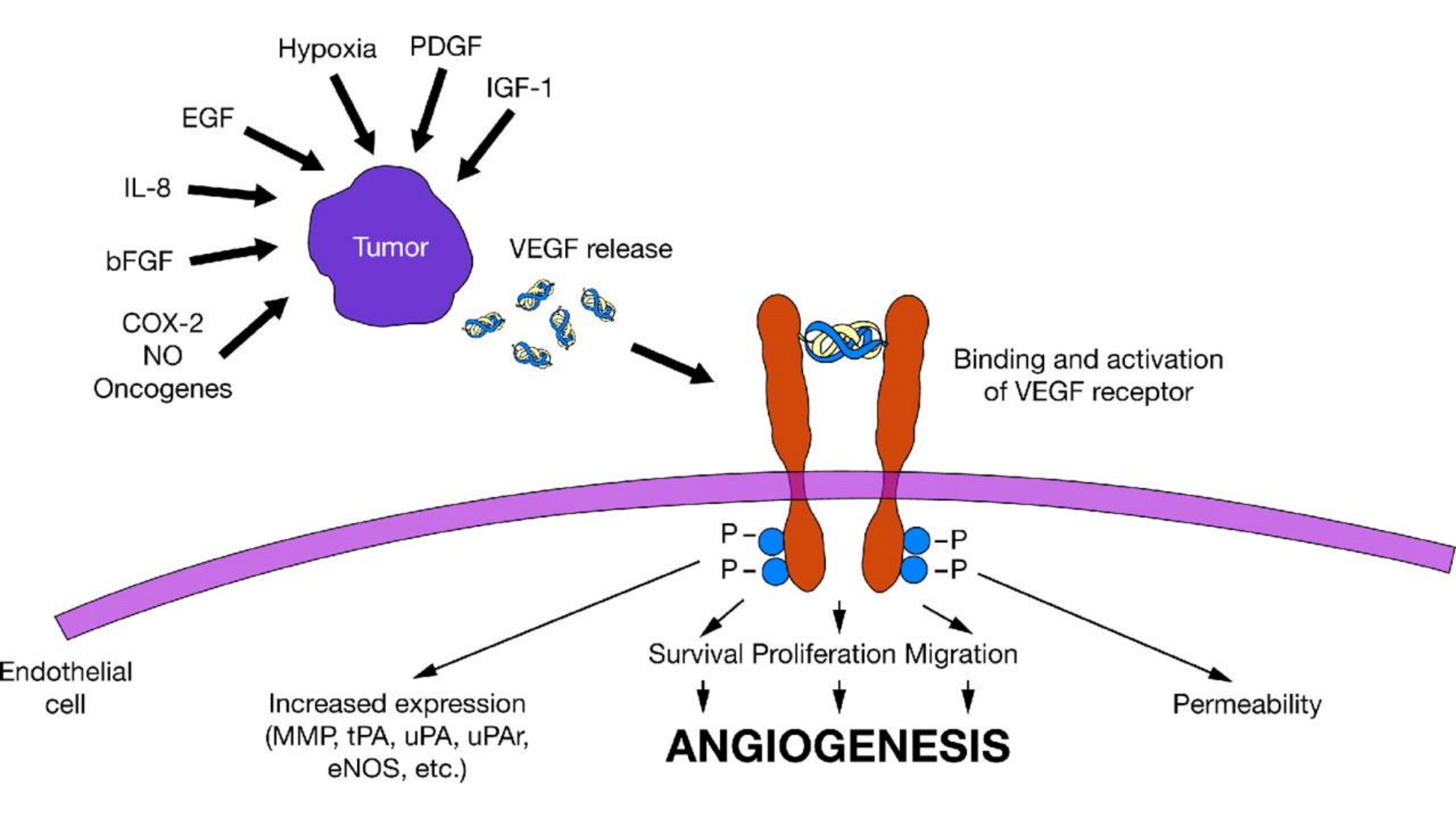
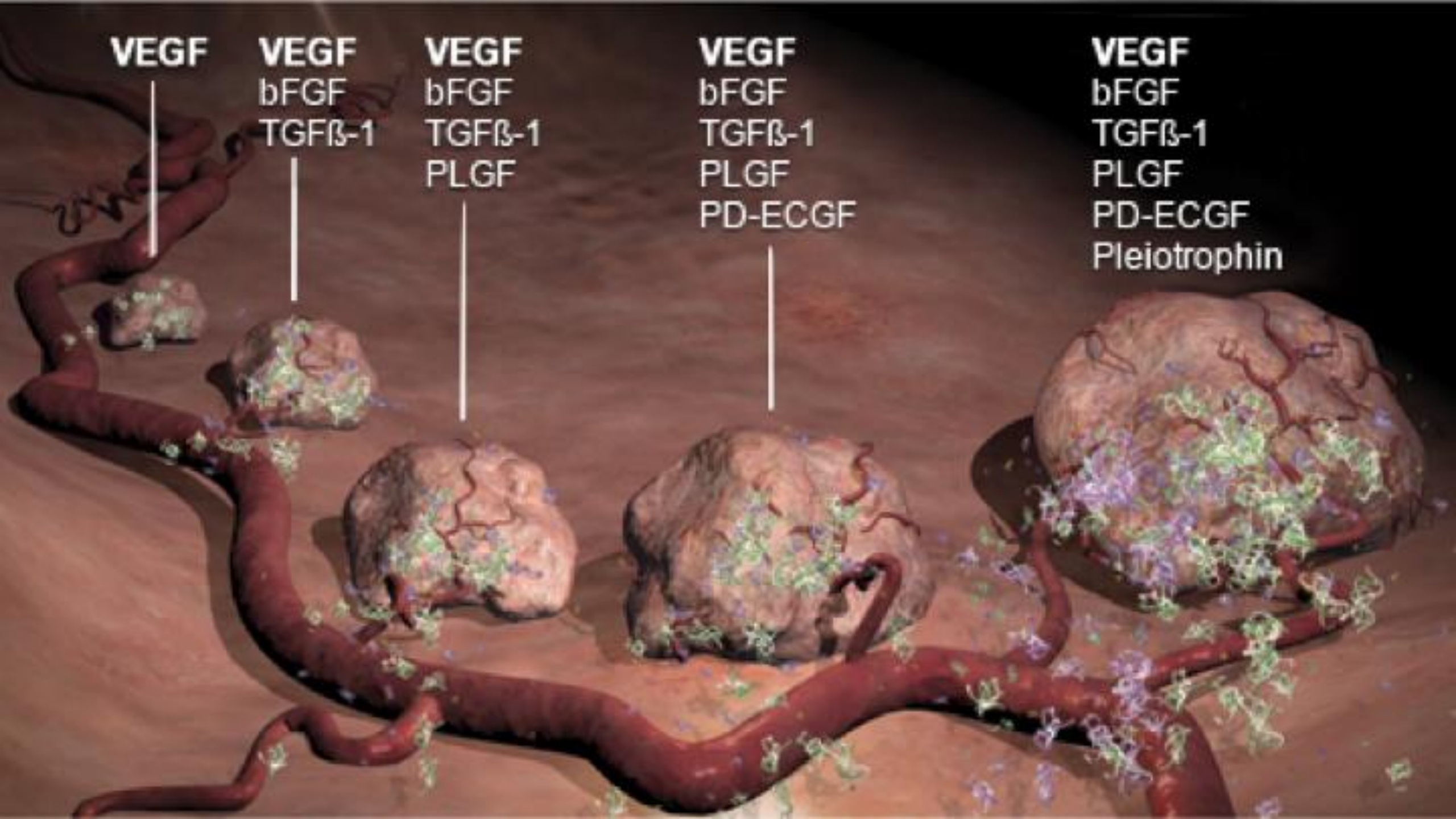


Figure 15–60. Molecular Biology of the Cell, 4th Edition.


SABiosciences
A QIAGEN Company
www.SABiosciences.com
www.ProteinLounge.com
VEGFA
VEGFC
VEGFD
VEGFR2
PIP2
PI3K
PLCγ
PIP2
PIP3
VRAP
Src
Sck
Endothelial Cell
IP3
DAG
GRB2
SHC
SOS
MKK3/6
FAK
Paxillin
PKC
Akt/ PKB
p38
Ras
Talin and Vinculin
Prostaglandin Production
BAD
eNOS
HSP90
MAPKAPK2/3
Raf1
Caspase9
HSP27
Cell Migration
MEK1/2
cPLA
Cell Survival
ERK1/2
Actin Reorganization
Gene Expression & Cell Proliferation
Vascular Cell Permeability
ANGIOGENESIS


Hypoxia
PDGF
IGF-1
EGF
IL-8
bFGF
COX-2
NO
Oncogenes
Tumor
VEGF release
Binding and activation
of VEGF receptor
P
P
P
P
Endothelial
cell
Increased expression
(MMP, tPA, uPA, uPAr,
eNOS, etc.)
Survival Proliferation Migration
ANGIOGENESIS
Permeability


VEGF
VEGF
bFGF
TGFß-1
VEGF
bFGF
TGFß-1
PLGF
VEGF
bFGF
TGFß-1
PLGF
PD-ECGF
VEGF
bFGF
TGFß-1
PLGF
PD-ECGF
Pleiotrophin


Thank You