NEOPLASIA 3

.

Molecular oncology

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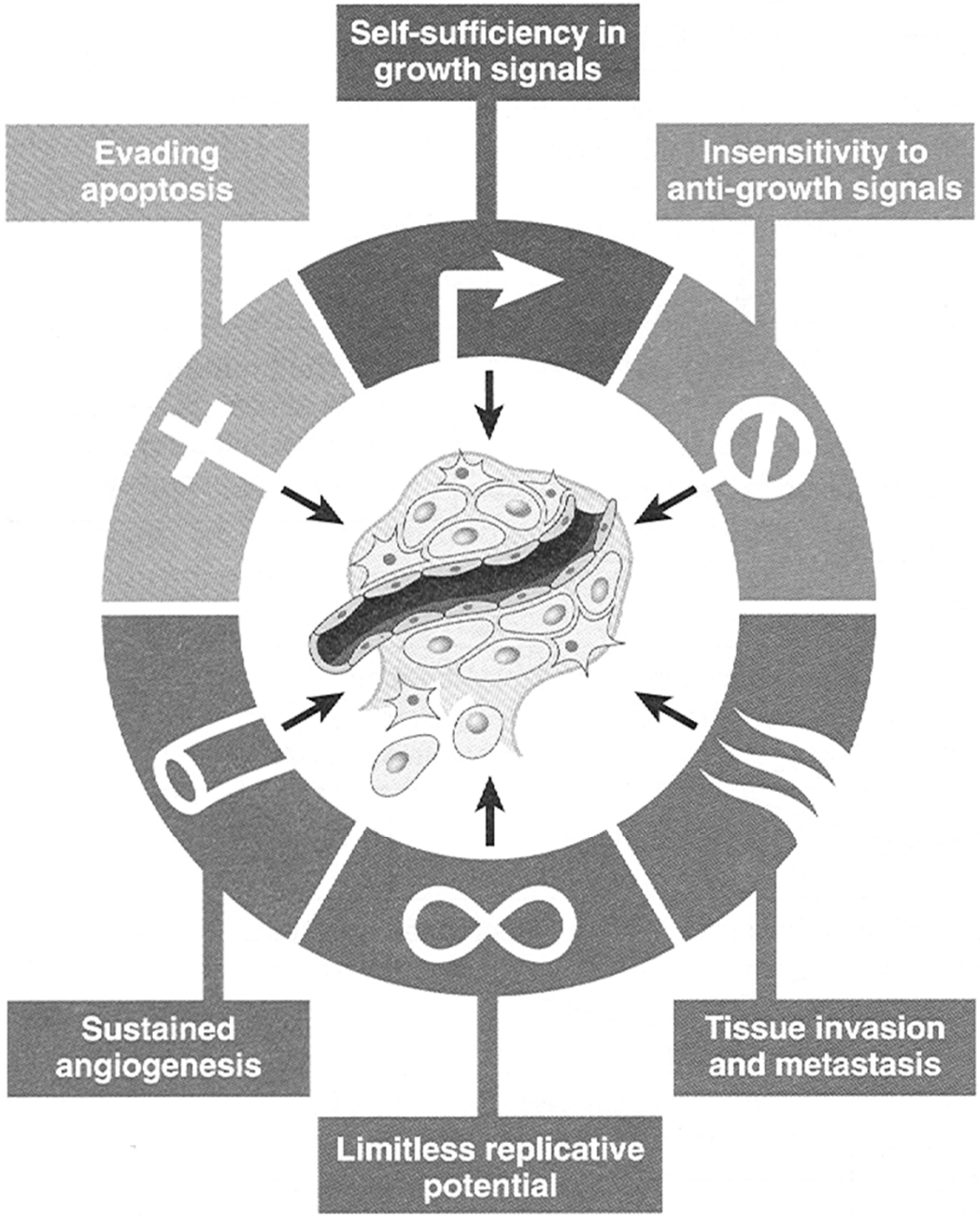
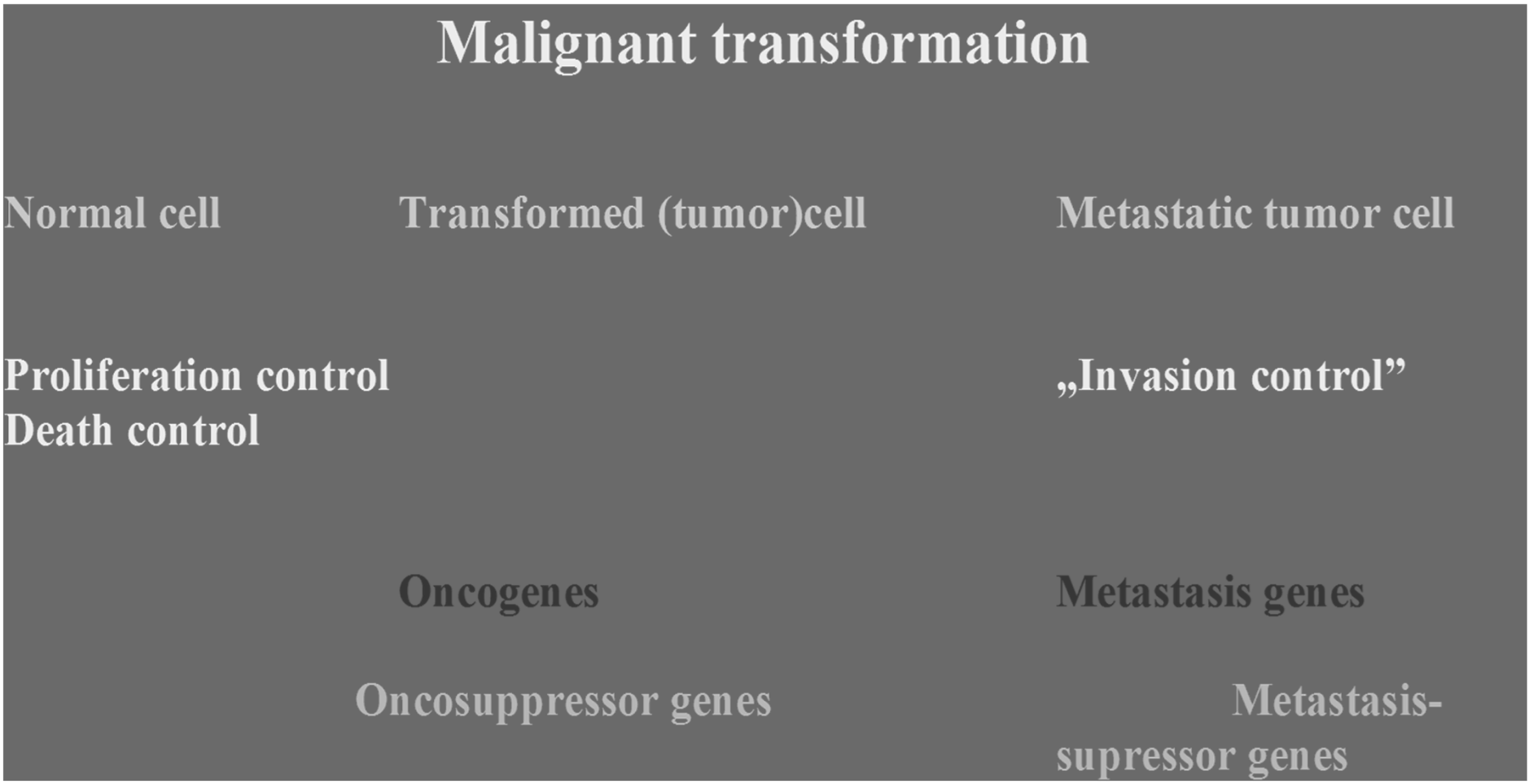
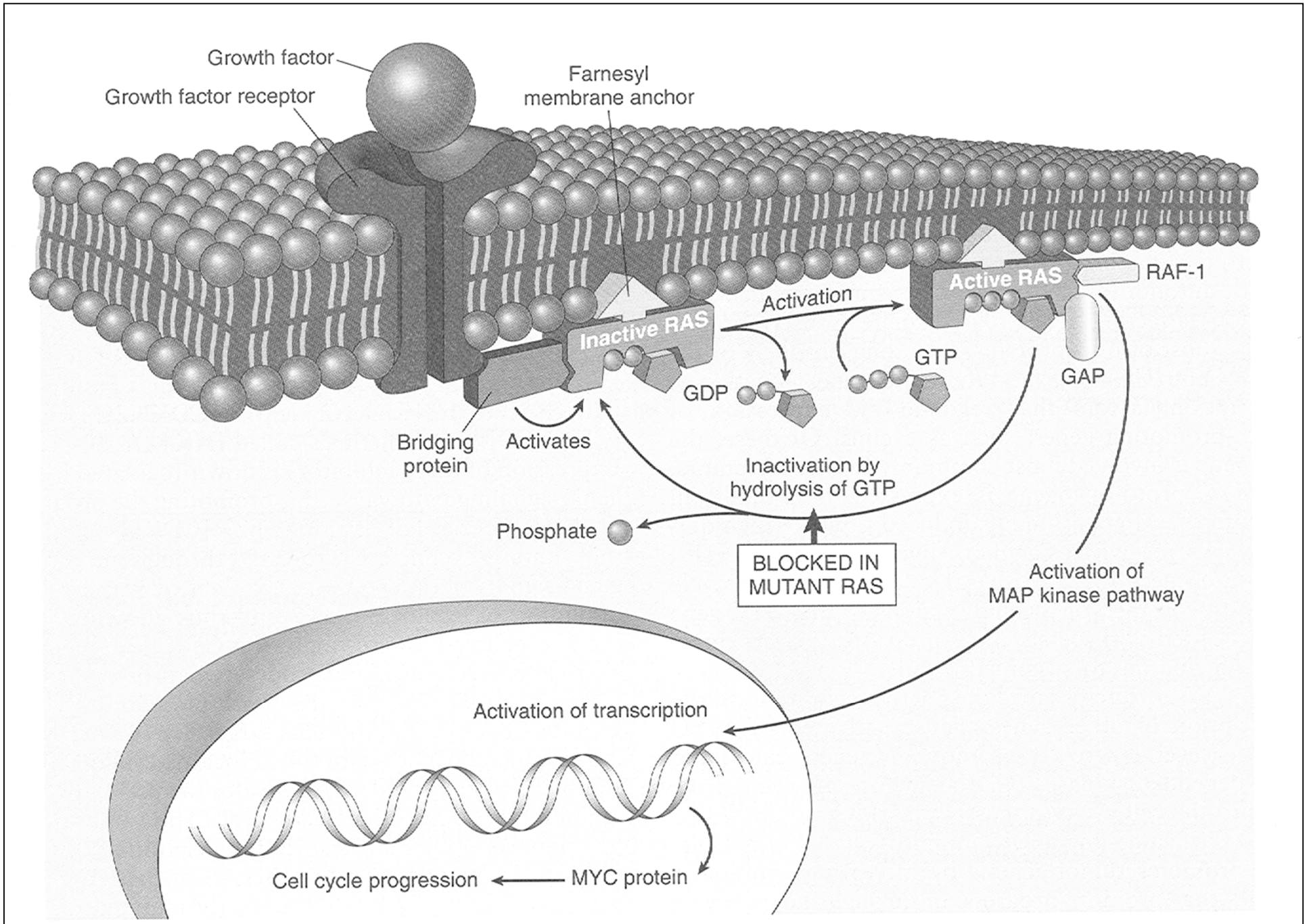
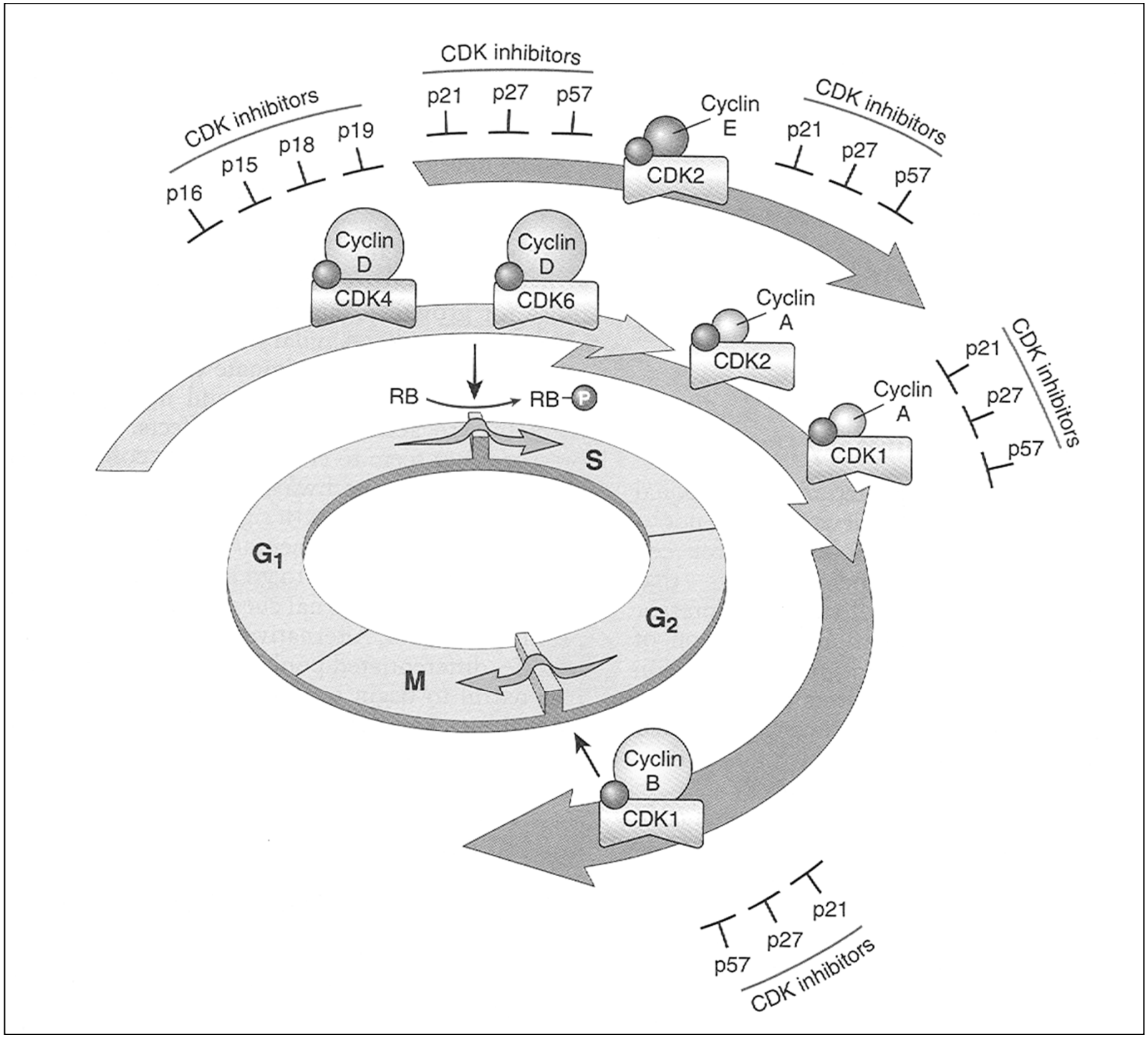
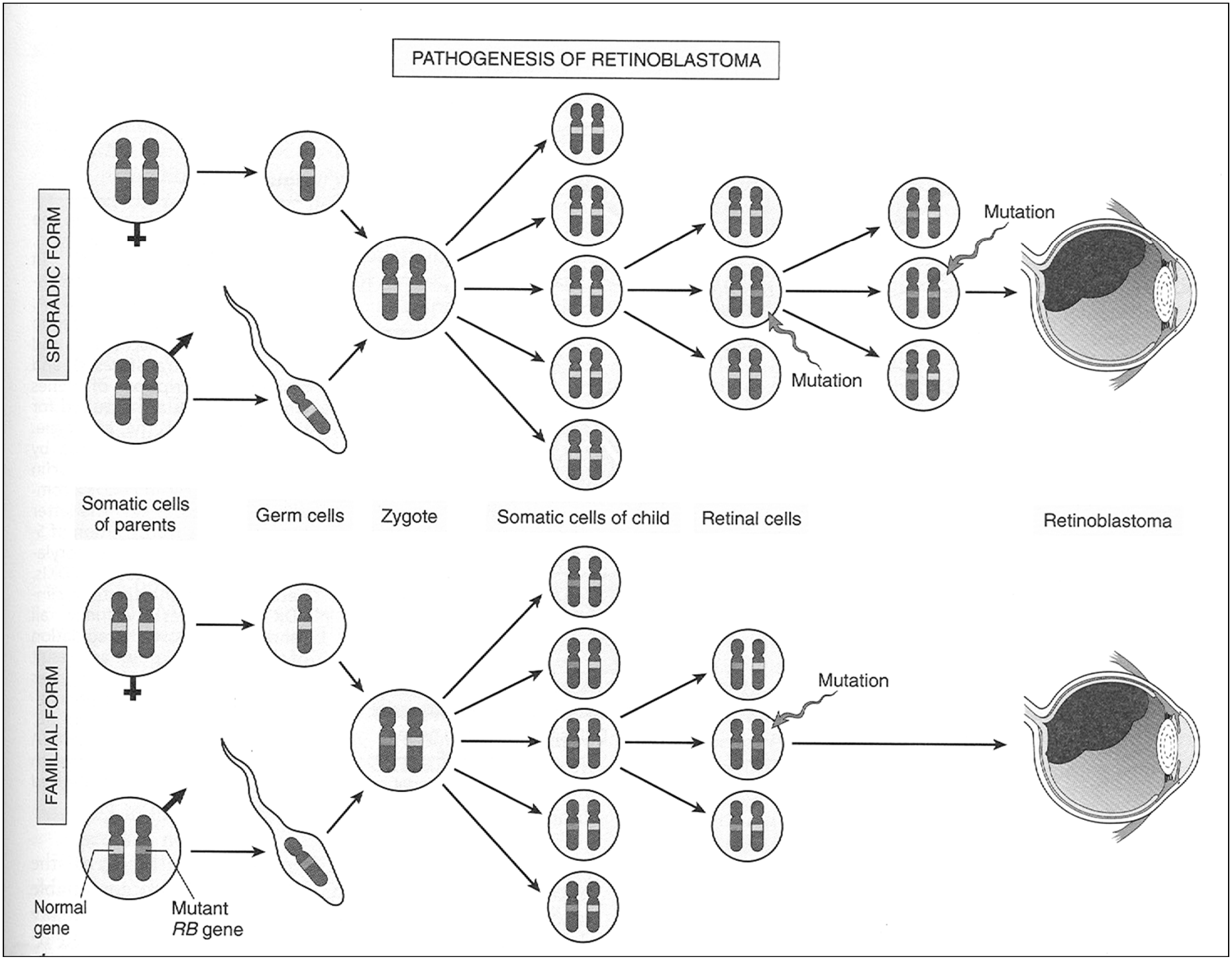

Self-sufficiency in growth signals
Evading apoptosis
Insensitivity to anti-growth signals
Sustained angiogenesis
Tissue invasion and metastasis
Limitless replicative potential

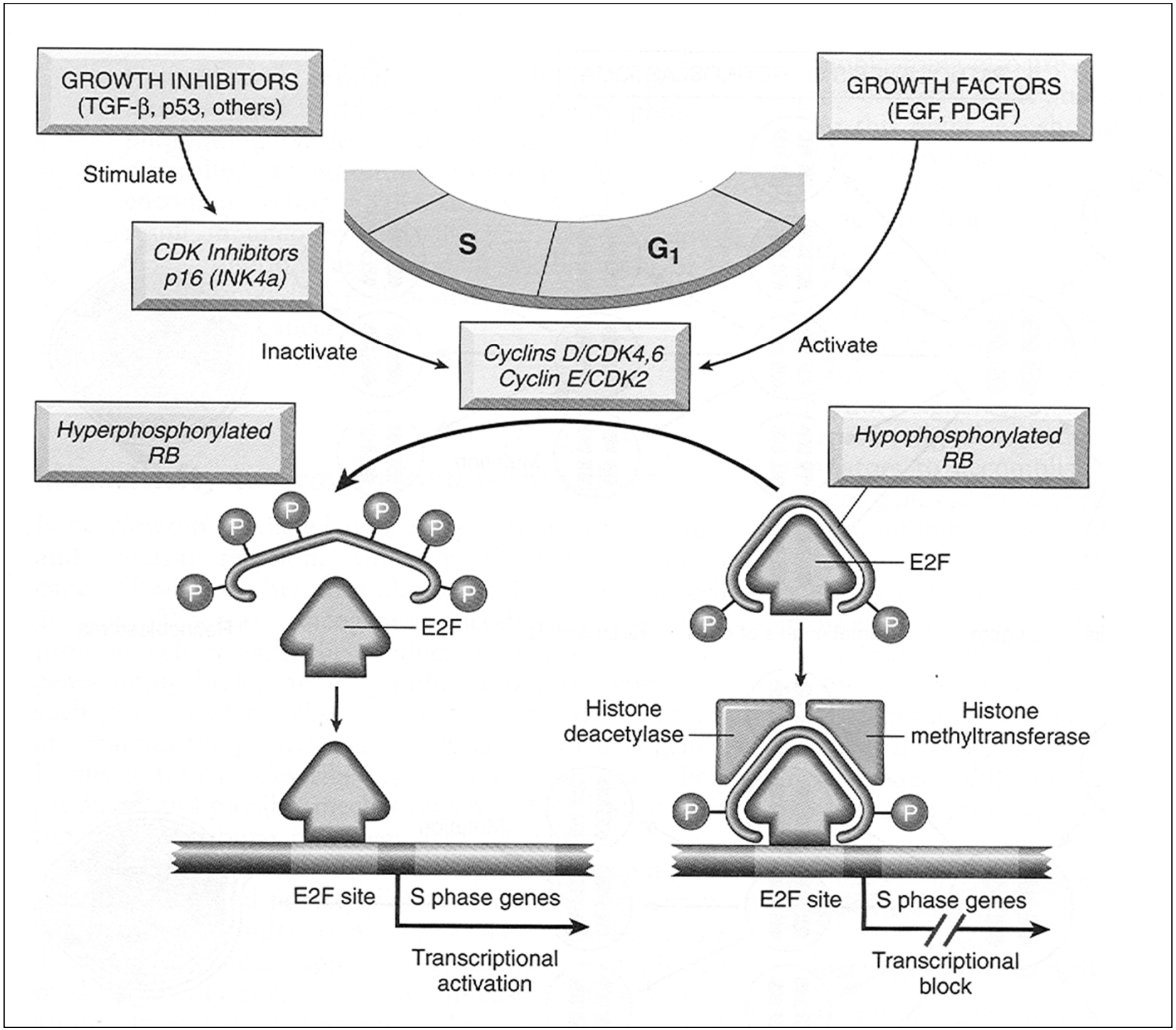


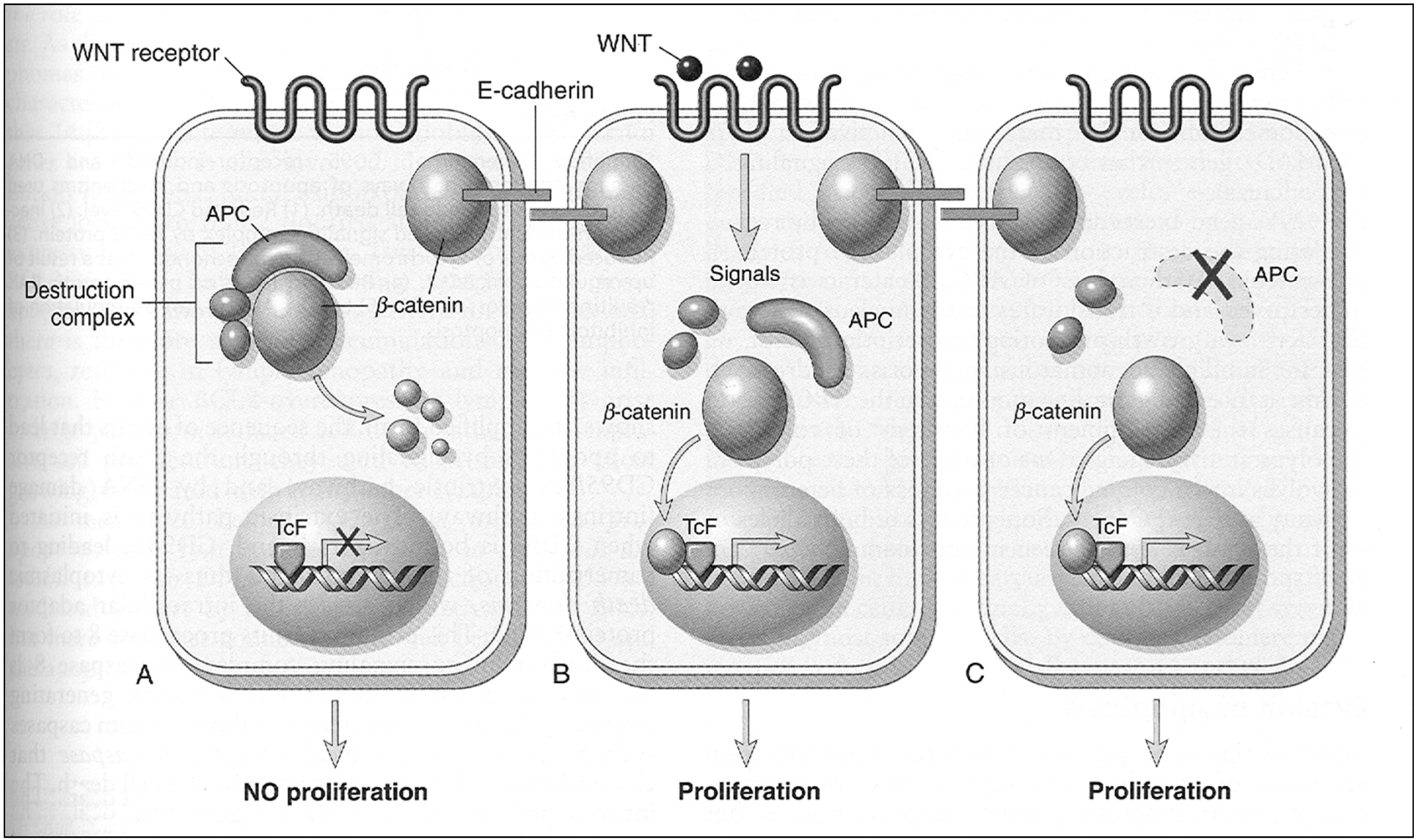
Oncogenic drivers

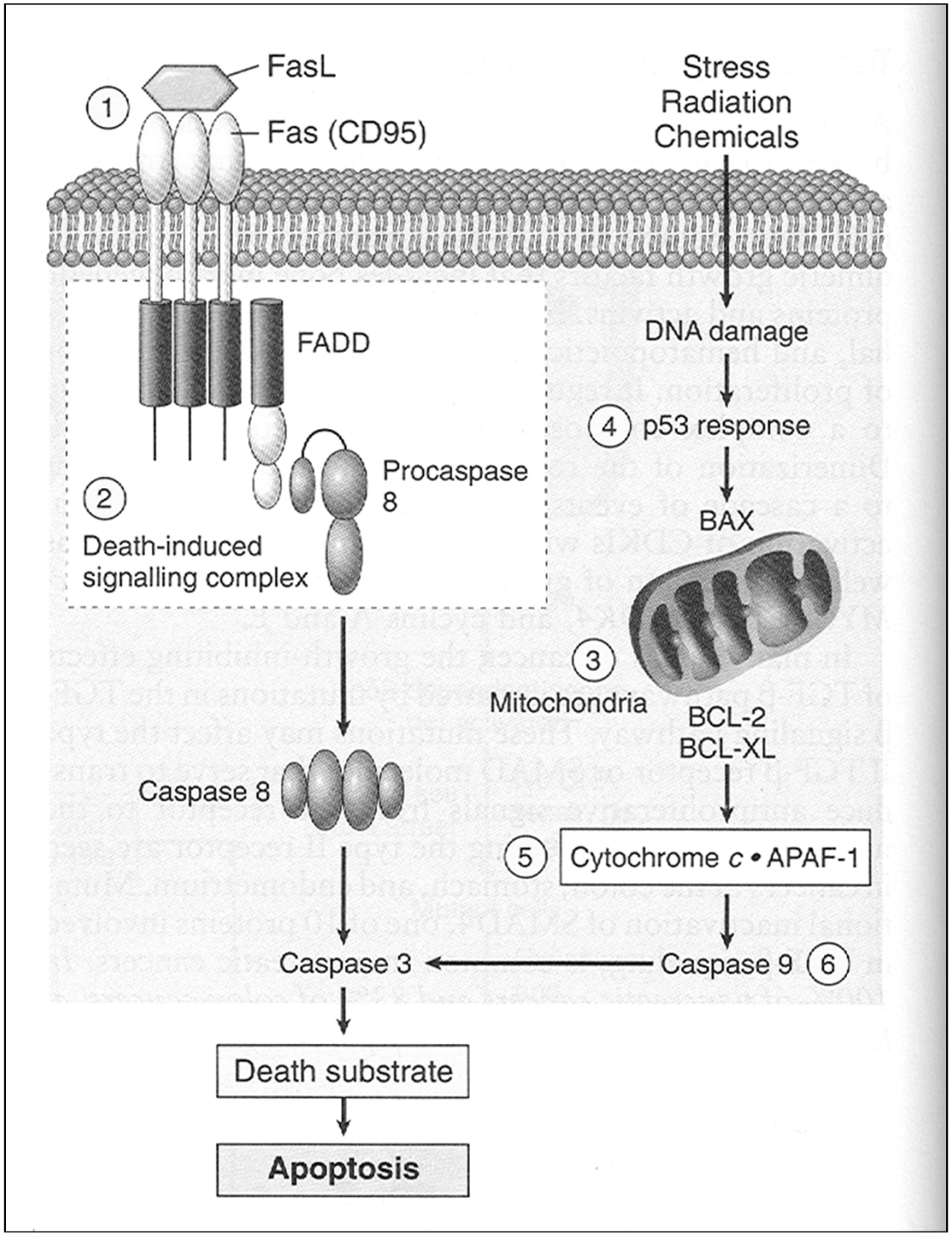

Growth factor
Growth factor receptor
Farnesyl membrane anchor
Inactive RAS
Activation
Active RAS
RAF-1
GTP
GDP
GAP
Bridging protein
Activates
Inactivation by hydrolysis of GTP
Phosphate
BLOCKED IN MUTANT RAS
Activation of MAP kinase pathway
Activation of transcription
Cell cycle progression
MYC protein


CDK inhibitors
p21
p27
p57
CDK inhibitors
p16
p15
p18
p19
Cyclin
E
CDK2
CDK inhibitors
p21
p27
p57
Cyclin
D
CDK4
Cyclin
D
CDK6
Cyclin
A
CDK2
RB
RB
P
S
Cyclin
A
CDK1
CDK inhibitors
p21
p27
p57
G1
G2
M
Cyclin
B
CDK1
p21
p27
p57
CDK inhibitors


PATHOGENESIS OF RETINOBLASTOMA
SPORADIC FORM
Mutation
Mutation
Somatic cells of parents
Germ cells
Zygote
Somatic cells of child
Retinal cells
Retinoblastoma
FAMILIAL FORM
Mutation
Normal gene
Mutant RB gene


GROWTH INHIBITORS
(TGF-β, p53, others)
GROWTH FACTORS
(EGF, PDGF)
Stimulate
CDK Inhibitors
p16 (INK4a)
S
G1
Inactivate
Activate
Cyclins D/CDK4,6
Cyclin E/CDK2
Hyperphosphorylated
RB
Hypophosphorylated
RB
P
E2F
Histone
deacetylase
Histone
methyltransferase
E2F site
S phase genes
Transcriptional
activation
E2F site
S phase genes
Transcriptional
block


WNT receptor
E-cadherin
WNT
APC
Destruction complex
β-catenin
Signals
APC
APC
β-catenin
β-catenin
TcF
TcF
TcF
A
B
C
NO proliferation
Proliferation
Proliferation


FasL
1
Fas (CD95)
Stress
Radiation
Chemicals
FADD
DNA damage
4
p53 response
BAX
Procaspase
8
2
Death-induced
signalling complex
3
Mitochondria
BCL-2
BCL-XL
Caspase 8
5
Cytochrome c • APAF-1
Caspase 3
Caspase 9
6
Death substrate
Apoptosis

Oncogenes in malignant tumors

1./ amplification (gencopy >4)

2./ translocation (kromosomal break/fusion, gene expression regulation)

3./ pointmutation (functional alteration in protein)

Result: **constitutive activity**

Alteration of oncosuppressor genes in cancer

1./ loss of function by mutation

2./ LOH: loss of heterozygosity (loss of one/2 breaks)

3./ kromosomal deletion

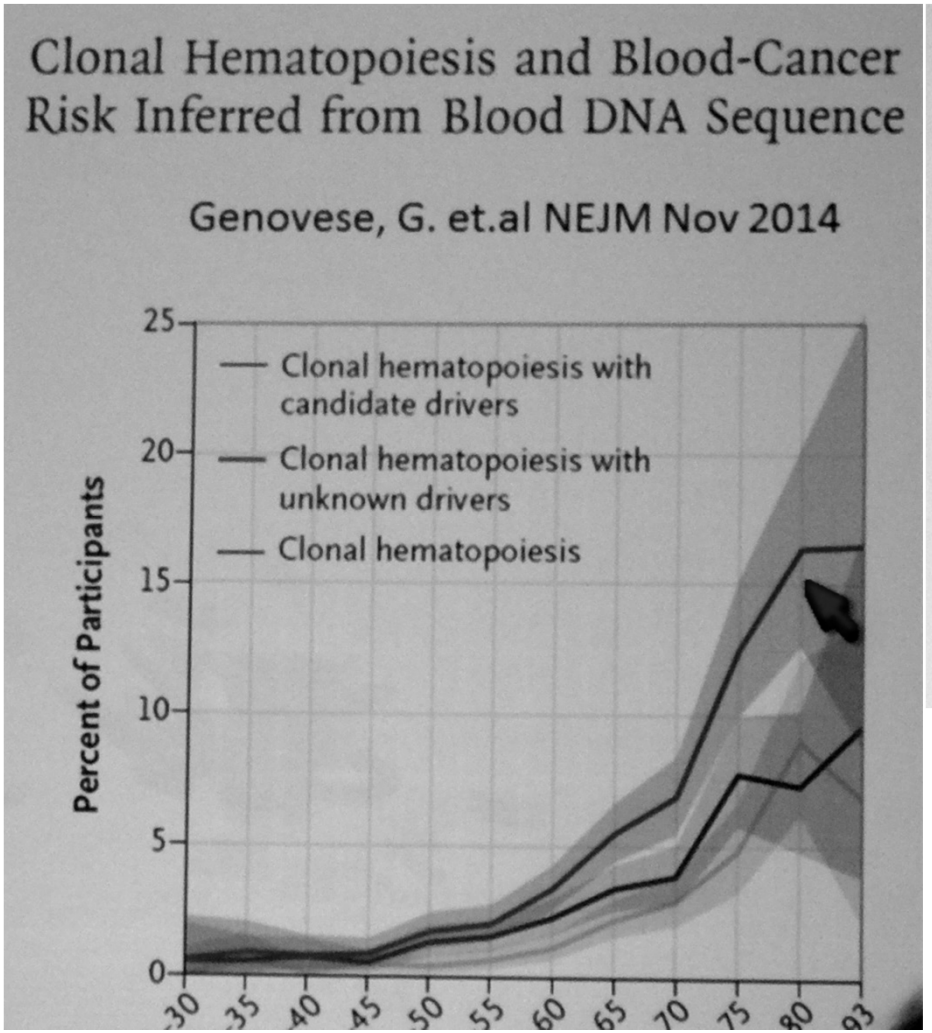
RESULT

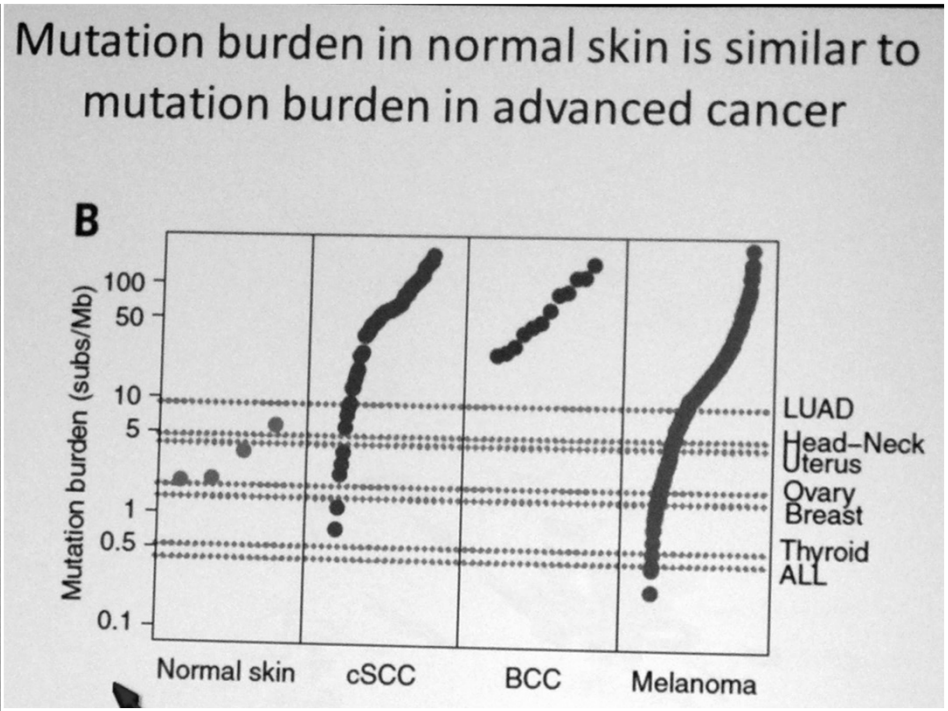
1./ decreased cell proliferation inhibition

2./ suppressed apoptotic ability

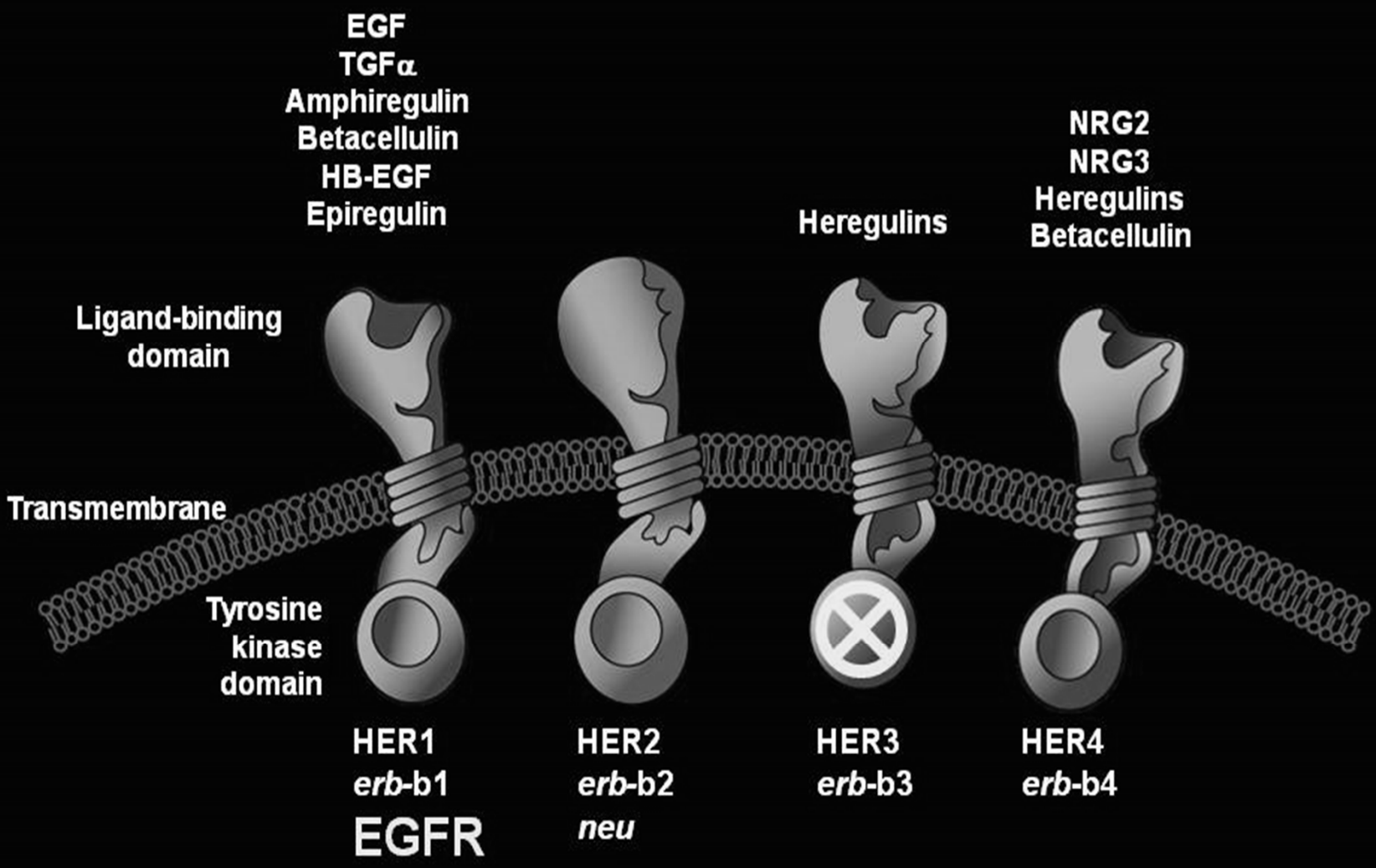
3./ decreased DNA repair

Accumulation of mutations during lifetime





The HER Family of Receptors

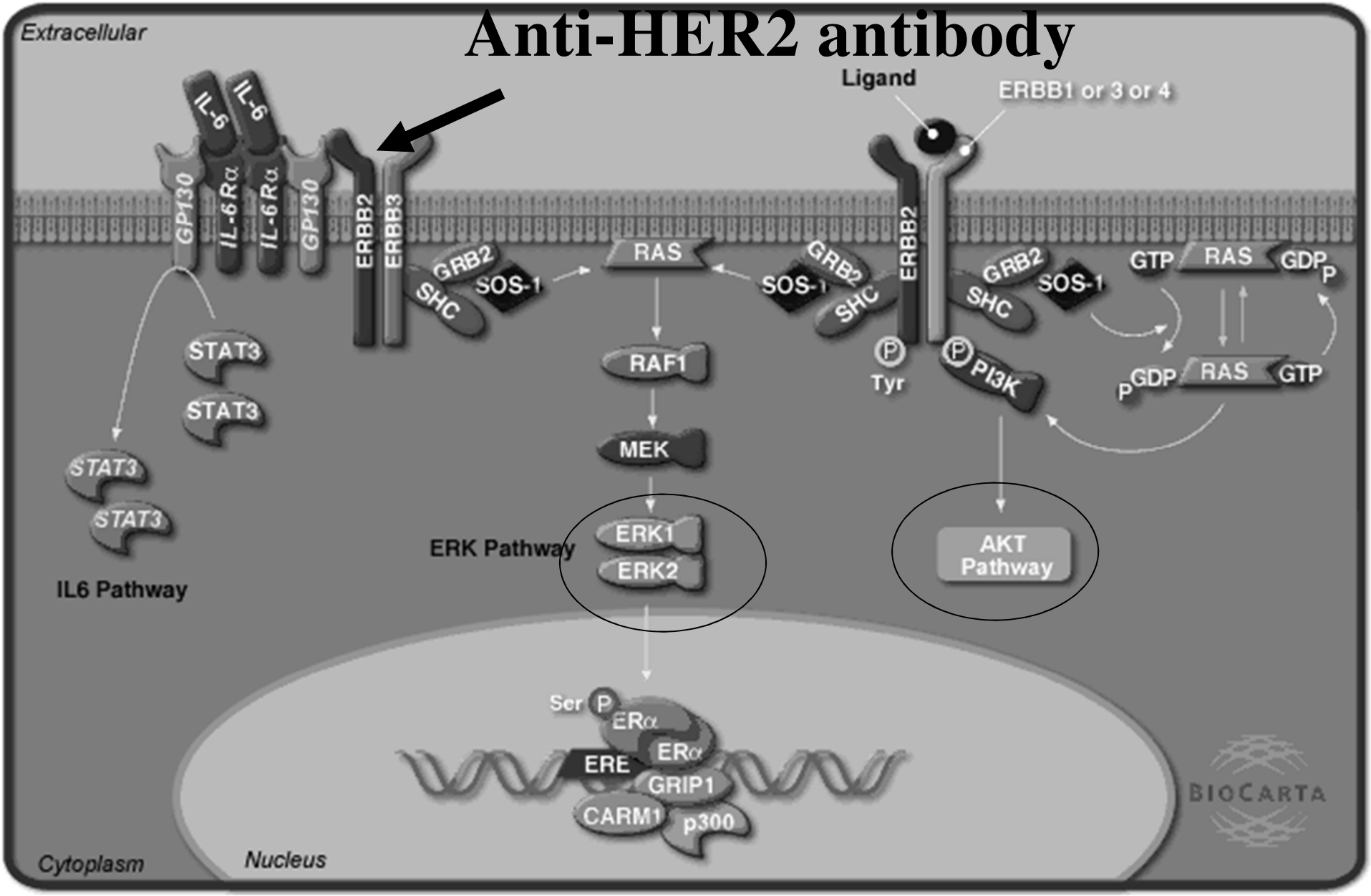


Roskoski. *Biochem Biophys Res Commun.* 2004;319:1; Rowinsky. *Annu Rev Med.* 2004;55:433.

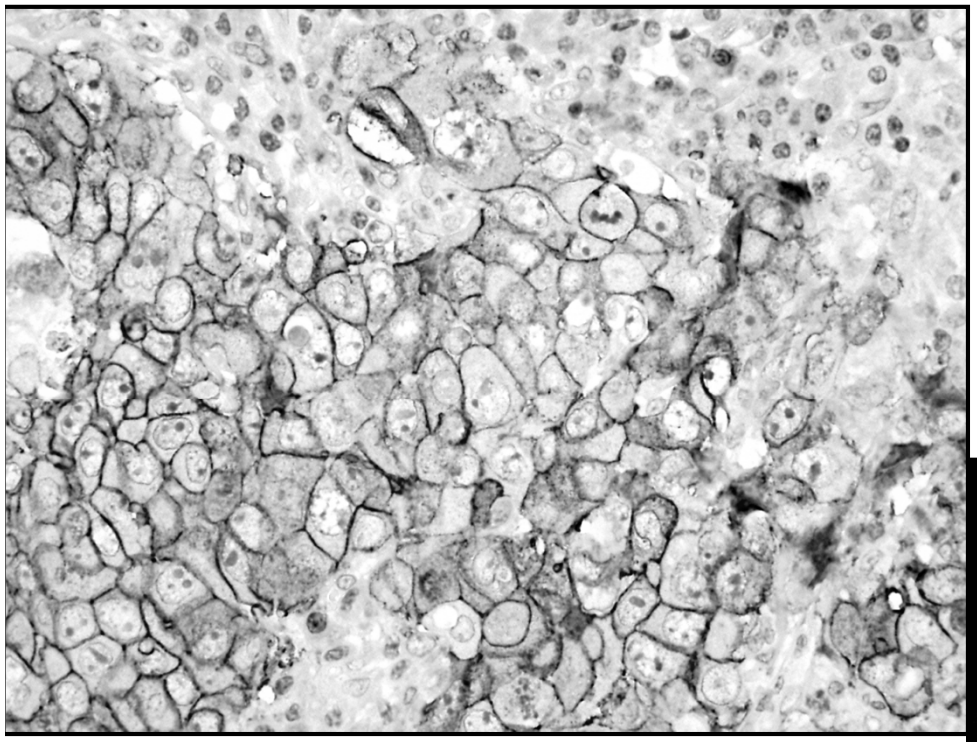
HER2 genetics in cancer

HER2	mutation	amplification
■ breast.:DIC	EC-delp95	+
■ Gastric cancer		+
■ Ovarian cancer		+
■ Endometrian cancer		+

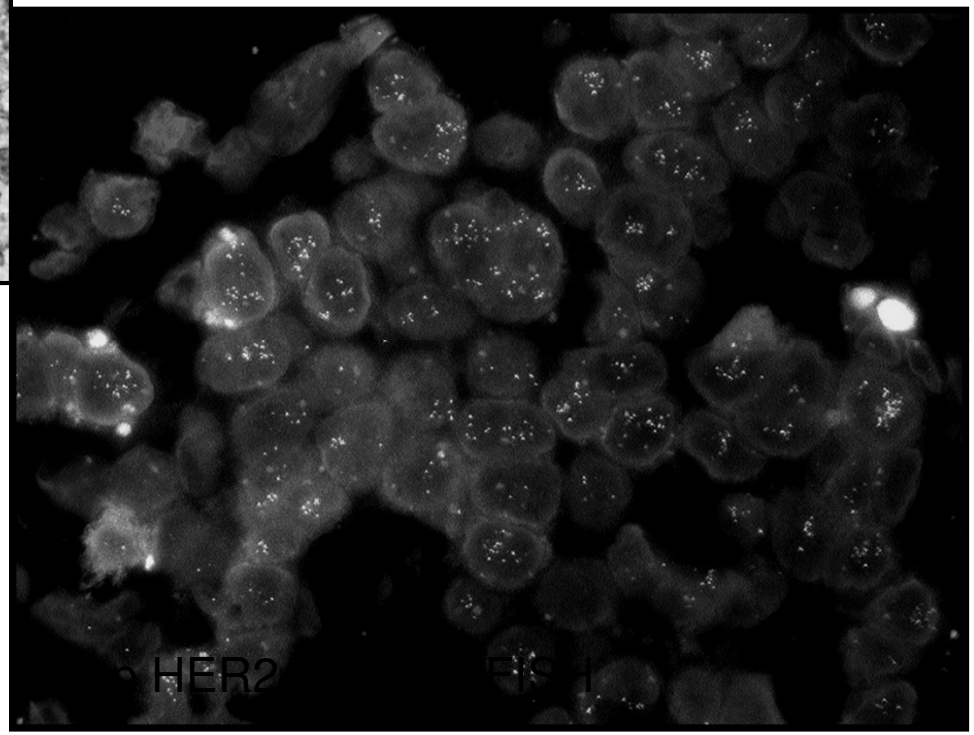
EGFR2/HER2 signaling



HER2 in breast cancer

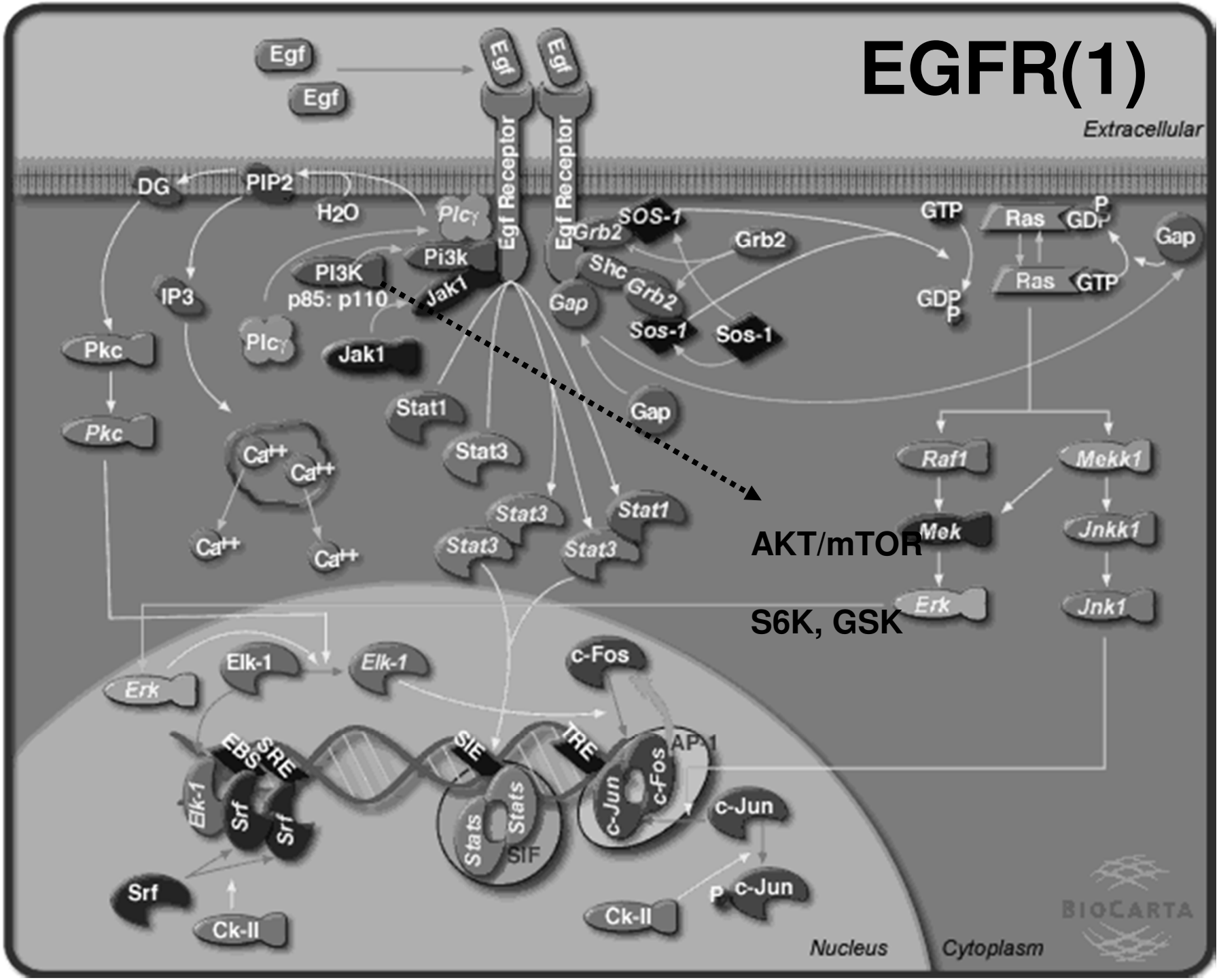


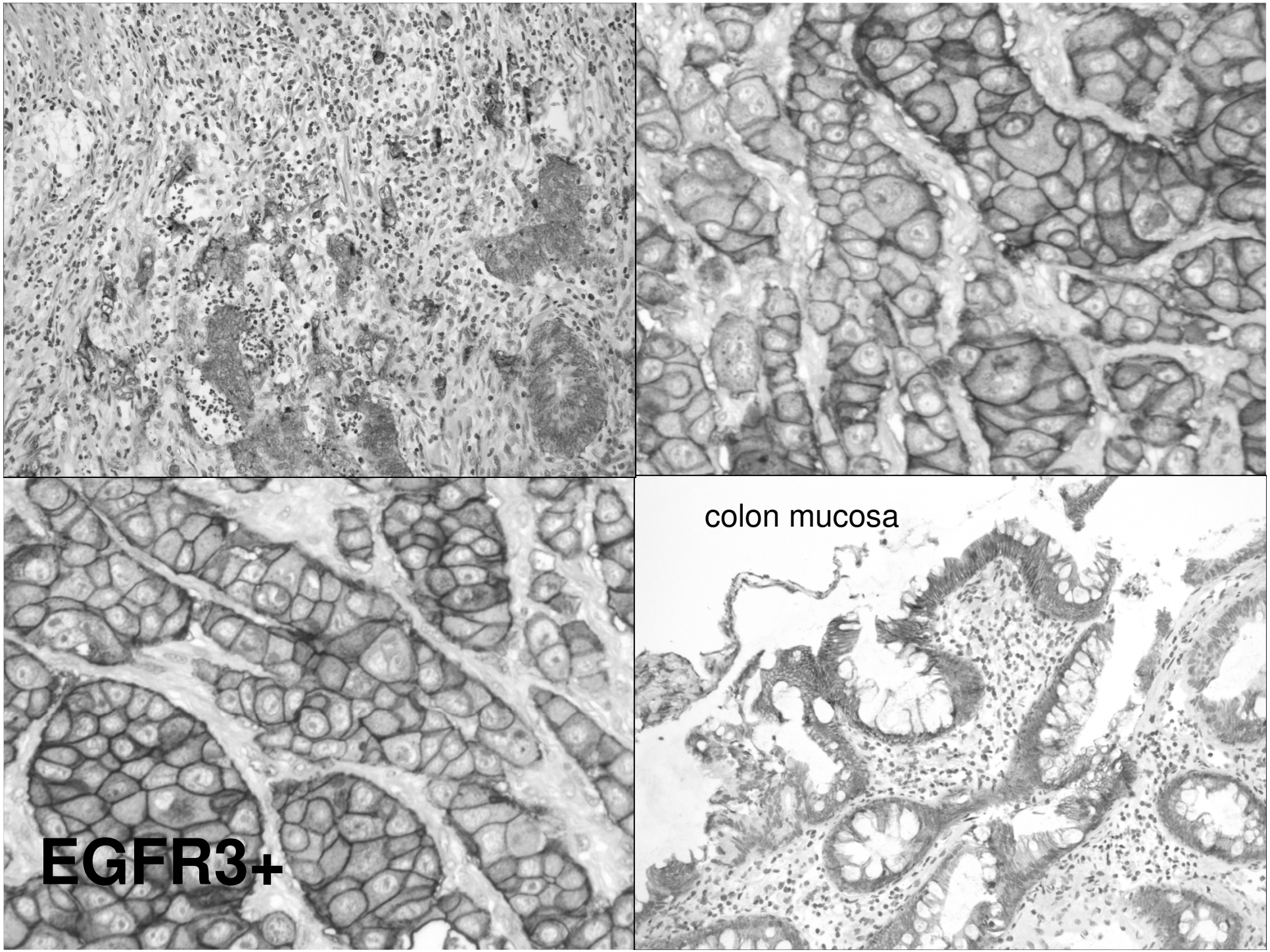
3+ CB11…immunhistochemistry



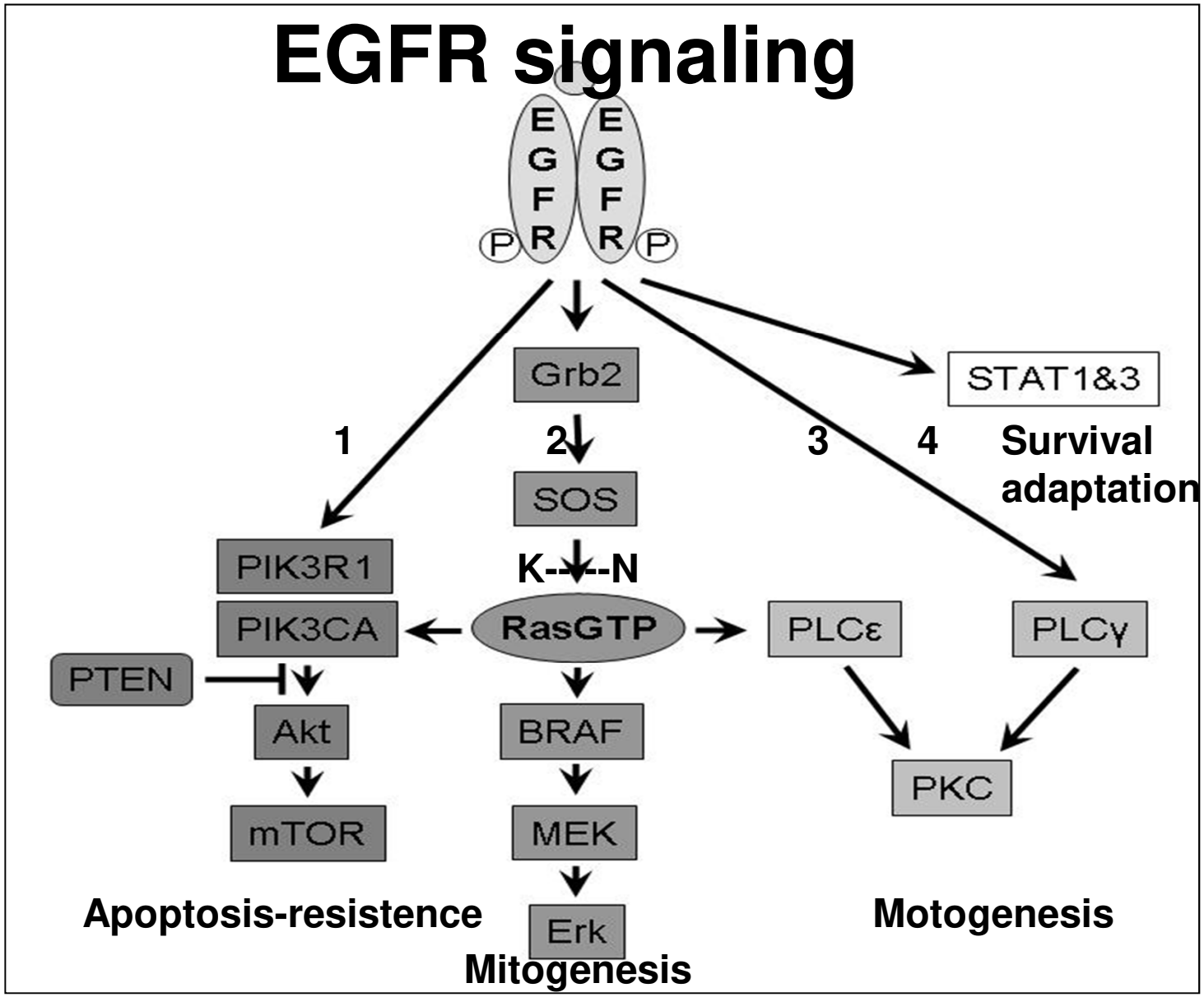
EGFR genetics in cancer

EGFR	mutation	amplification
▪ GBM	vIII/EC-del	+
▪ AC-lung	TKex19-21	+
▪ Head,neck	EC-del	+
▪ Colonic	EC-del	+
▪ melanoma	EC-del	+(NM)


EGFR(1)
Extracellular
Egf
Egf
Egf
Egf
Egf Receptor
Egf Receptor
DG
PIP2
H2O
Plcγ
Pi3k
PI3K
p85: p110
Jak1
IP3
Plcγ
Jak1
Pkc
Pkc
Ca++
Ca++
Ca++
Ca++
Stat1
Stat3
Stat3
Stat3
Stat1
Stat3
Gap
SOS-1
Grb2
Grb2
Shc
Grb2
Gap
Sos-1
Sos-1
GTP
GDP
P
Ras
GDP
P
Ras
GTP
Gap
AKT/mTOR
S6K, GSK
Raf1
Mekk1
Mek
Jnkk1
Erk
Jnk1
Erk
Elk-1
Elk-1
c-Fos
EBS
SRE
SIE
TRE
AP1
Elk-1
Srf
Srf
Stats
Stats
SIF
c-Jun
c-Fos
c-Jun
Srf
Ck-II
Ck-II
P
c-Jun
Nucleus
Cytoplasm
BIOCARTA

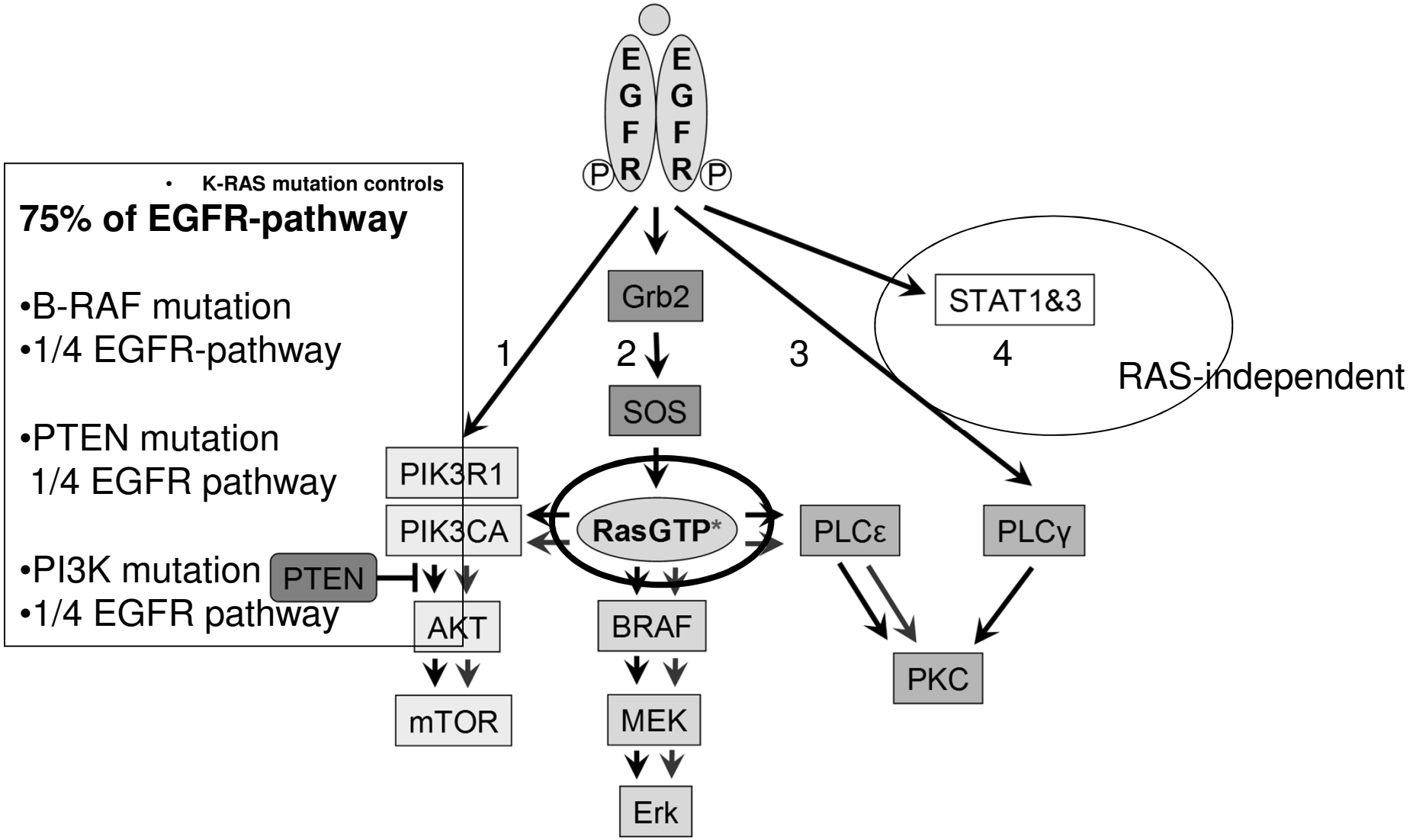

colon mucosa
EGFR3+

EGFR signaling



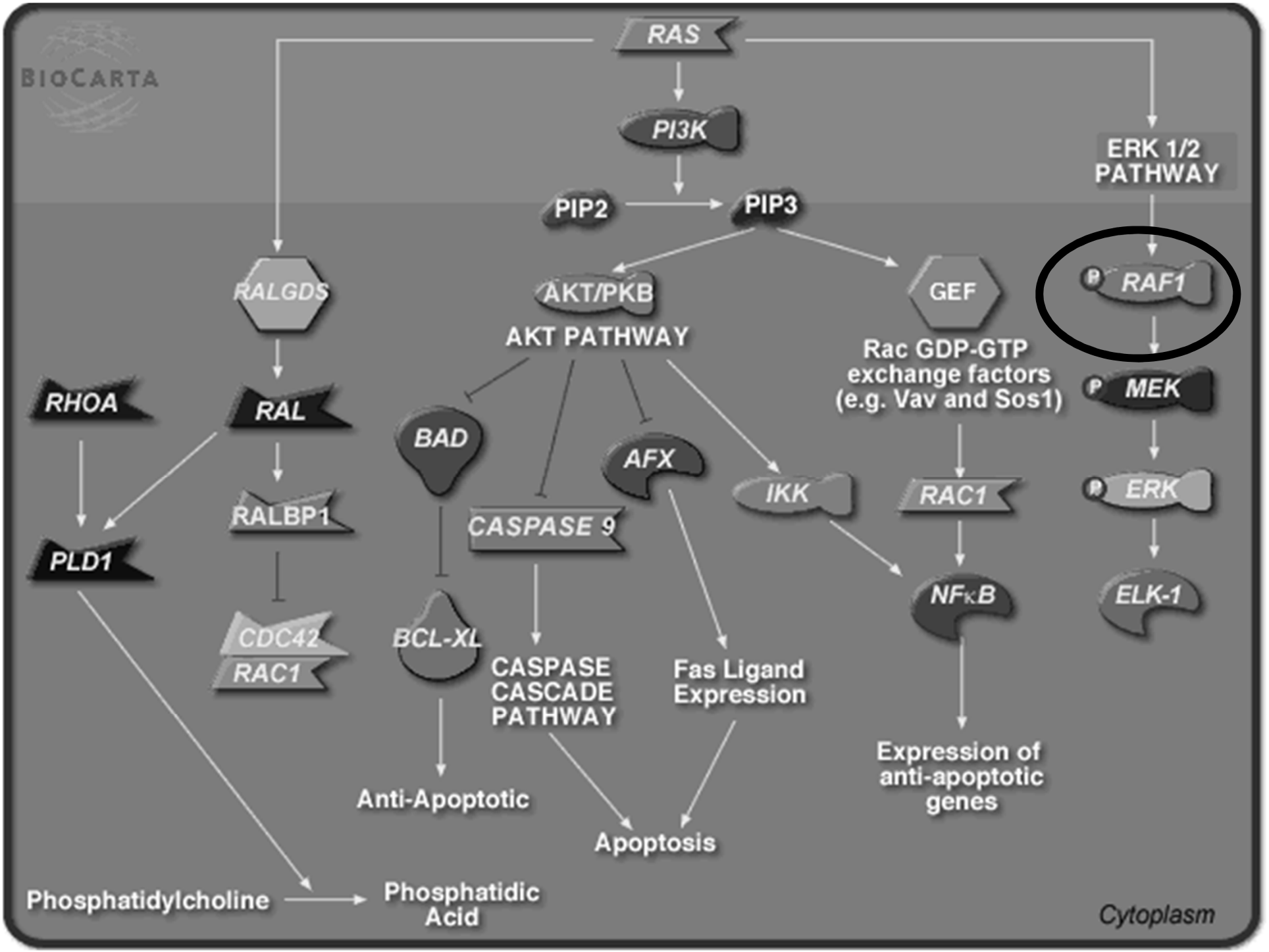
RAS and B-RAF mutations in human cancer

Cancer type	K-RAS	H-RAS	N-RAS	B-RAF
Pancreatic	60 (70-80)*	0	2	3
Colorectal	32 (45-50)*	0	3	14 (10-15)*
Bile duct	33	0	1	14
NSCLC (adenocarcinoma)	19 (35)*	1	1	2
Ovarian	17	0	4	15
Endometrial	15	1	0	1
Cervical	9	9	1	0
IIepatocellular	8	0	10	3
Myeloid leukemia	5	0	14	1
Thyroid	4	5	7	27
Breast	4	0	0	2
Urinary bladder	4	11	3	0
Malignant melanoma	2	6	18	43 (70)*
Renal cell	1	0	0	0


• K-RAS mutation controls
75% of EGFR-pathway
•B-RAF mutation
•1/4 EGFR-pathway
•PTEN mutation
1/4 EGFR pathway
•PI3K mutation
•1/4 EGFR pathway
EGFR
EGFR
P
P
Grb2
STAT1&3
1
2
3
4
RAS-independent
SOS
PIK3R1
PIK3CA
RasGTP*
PLCε
PLCγ
PTEN
AKT
BRAF
PKC
mTOR
MEK
Erk

EGFR-RAS paradoxon

- **wtEGFR----------wtRAS (EGFR ampl)**
  CRC, NSCLC, HNSC
- **wtEGFR---------mutánsRAS (EGFR amp)**
  CRC, NSCLC, pancreatic cancer
- **mutánsEGFR---------wtRAS (EGFR ampl)**
  NSCLC
- Mutant EGFR----mutant RAS…extreme rare


BIOCARTA
RAS
PI3K
PIP2
PIP3
ERK 1/2 PATHWAY
RALGDS
AKT/PKB
AKT PATHWAY
GEF
Rac GDP-GTP exchange factors (e.g. Vav and Sos1)
P RAF1
P MEK
P ERK
RHOA
RAL
BAD
AFX
IKK
RAC1
RALBP1
CASPASE 9
PLD1
NFκB
ELK-1
CDC42
RAC1
BCL-XL
CASPASE CASCADE PATHWAY
Fas Ligand Expression
Anti-Apoptotic
Apoptosis
Expression of anti-apoptotic genes
Phosphatidylcholine
Phosphatidic Acid
Cytoplasm

B-RAF acivating mutations (V600E)

- Cutan melanoma 70%
- Thyroid cancer (pap) 50%
- Ovarian cc (low grade, serosus) 30%
- Colorectal cancer 5-10%

NO activating mutation

- Hepatocellular carcinoma
- Gastric cancer
- Endometrial cancer

RAS- BRAF paradoxon

- K-RAS- B-RAF mutations exclude each other

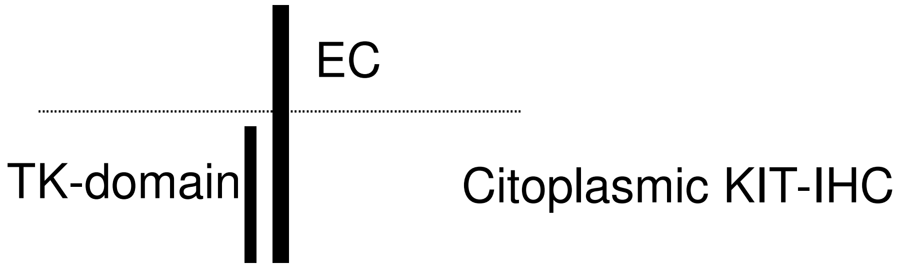
  (NSCLC, CRC)
- N-RAS- B-RAF mutations exclude each others (melanoma)

Other tyrosine kinases

KIT	**mutation**	**amplification**
▪ **GIST**	**jm, TK**	**-**
▪ **nonUV melanoma**	**TK**	**-**
PDGFR		
▪ **GIST**	**EC-del, TK**	**-**
▪ **DFSP**	**fusion gene (coll)**	**-**
ABL		
▪ **CML**	**fusion gene (BCR)**	**-**

C-KIT mutations in GIST

- Exon 11 (juxtamembrán): EC domain deletion

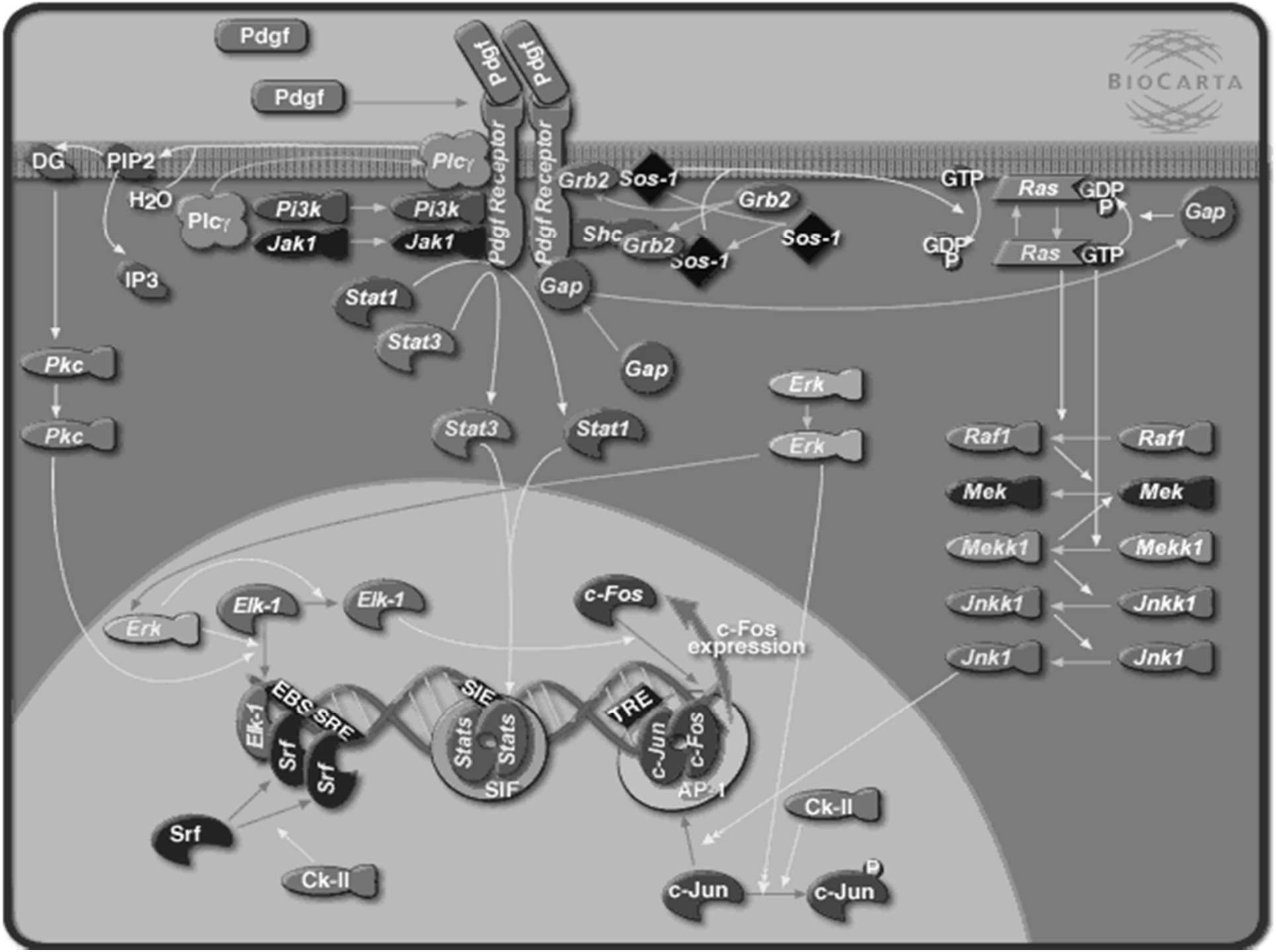


Cod557,558: BAD PROGNOSIS!!

•Exon 9, 13,17 mutations are rare (9-EC, 13,17 TK)
•**Ex 17-mut: GLEEVEC RES**

•Classic morphology:::::::::::::::::::::::::::::::::

NO GENE-AMPLIFICATION (FISH nem szükséges)
Tabone et al BBA 1741:165,2005


BIOCARTA
Pdgf
Pdgf
Pdgf
Pdgf
Pdgf Receptor
Pdgf Receptor
DG
PIP2
H2O
Plcγ
Plcγ
IP3
Pi3k
Pi3k
Jak1
Jak1
Grb2
Sos-1
Grb2
Sos-1
Shc
Grb2
Sos-1
GTP
GDP
P
Ras
GDP
P
Ras
GTP
Gap
Gap
Gap
Stat1
Stat3
Stat3
Stat1
Pkc
Pkc
Erk
Erk
Raf1
Raf1
Mek
Mek
Mekk1
Mekk1
Jnkk1
Jnkk1
Jnk1
Jnk1
Erk
Elk-1
Elk-1
c-Fos
c-Fos
expression
EBS
SRE
Elk-1
Srf
Srf
SIE
Stats
Stats
SIF
TRE
c-Jun
c-Fos
AP-1
Srf
Ck-II
Ck-II
c-Jun
c-Jun
P

A PDGF receptor mutations in GIST

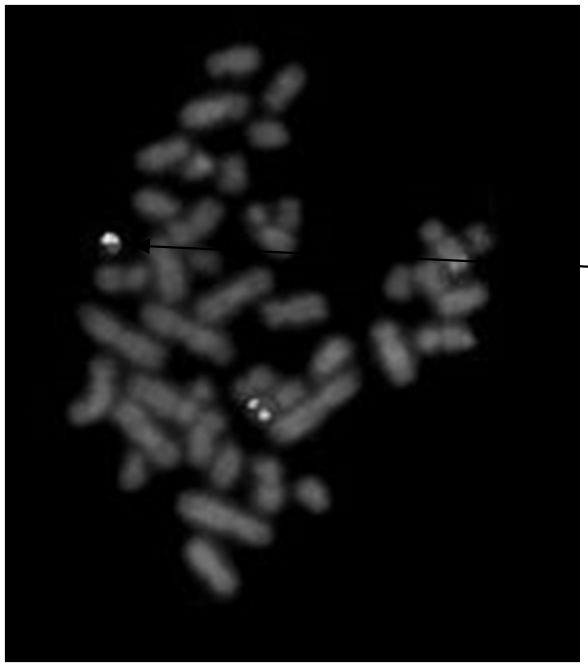
- Exon 12 (deeletion or missense) EC domain del
- **Exon 18 (del or missense) GLEEVEC-RES**
- Exon 14 point mutation EC domain

- No parallel c-kit mutation

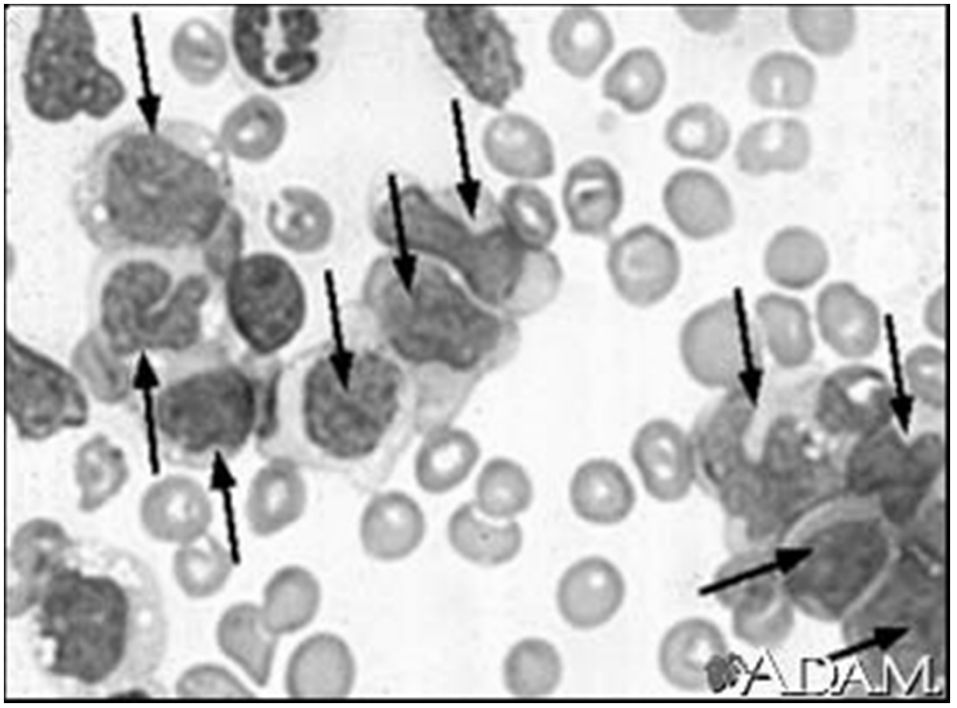
- Morfphology: pleiomorphic, giant cell
- Localisation: stomach (ex14/cod2125) epitheloid
- PDGFR: citoplasmic (dot)

Lasota et al. Lab Invest 86:94,2006 Martin et al. JCO 23:6190,2005

Breakpoint Cluster Region (BCR)

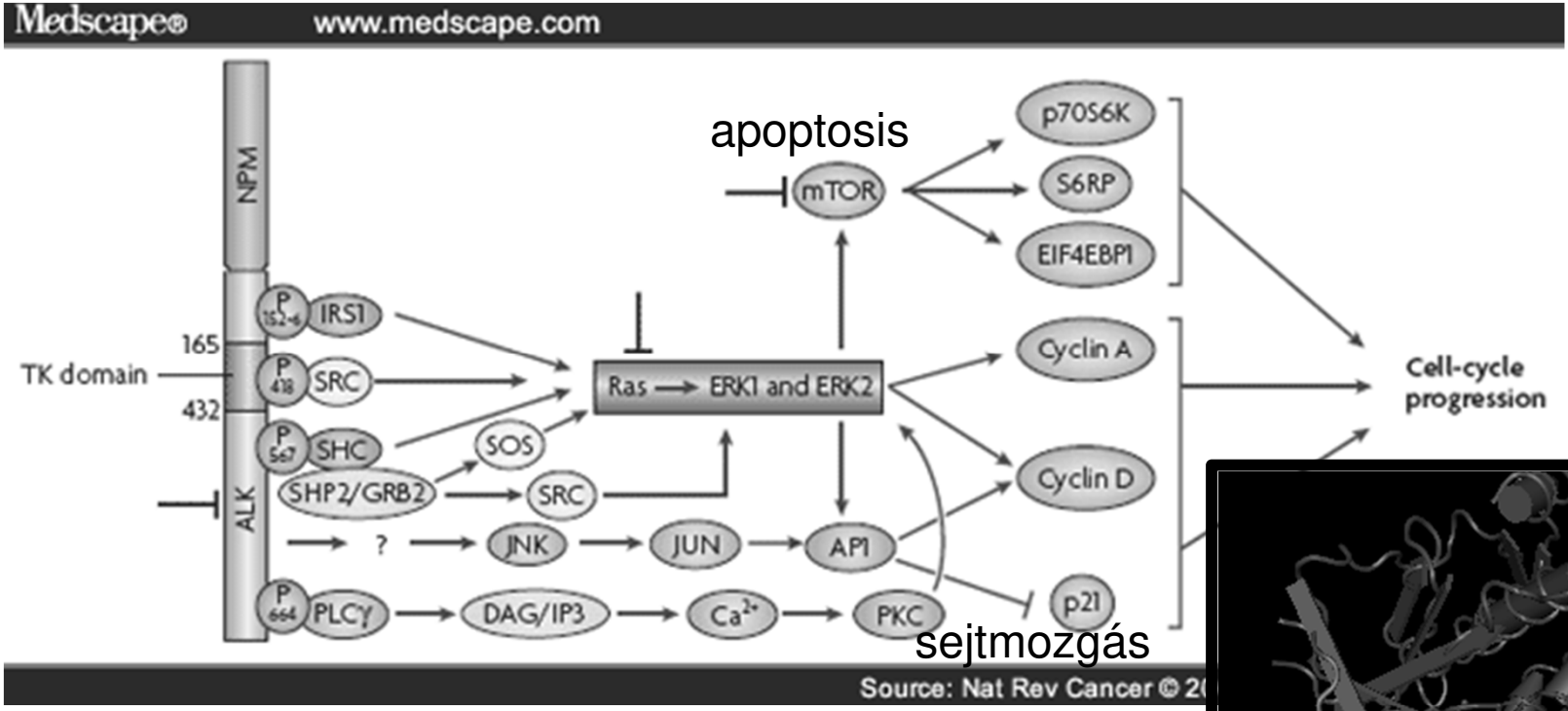


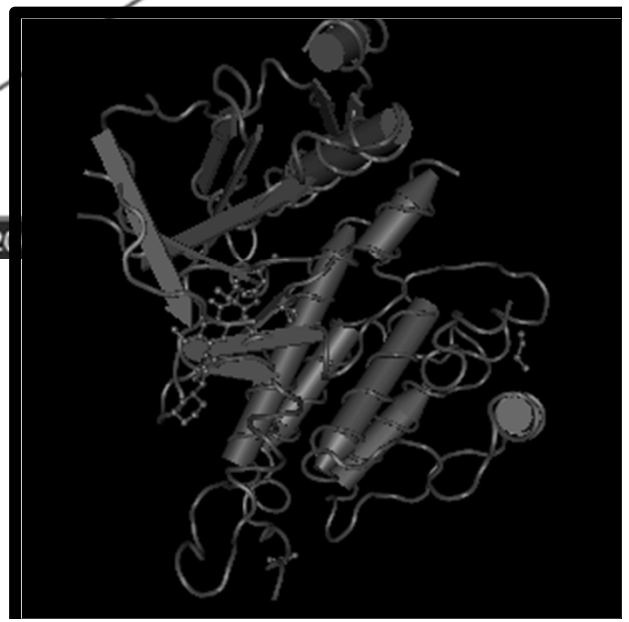
Chr22-BCR (S/TK)
Chr9-ABL oncogene (TK)
t22-9: BCR-ABL (p210)



Chronic Myeloid Leukemia

Anaplastic Lymphoma Kinase structure and role



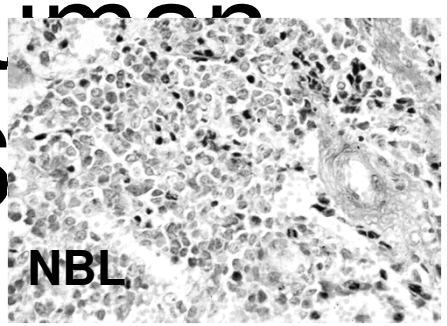


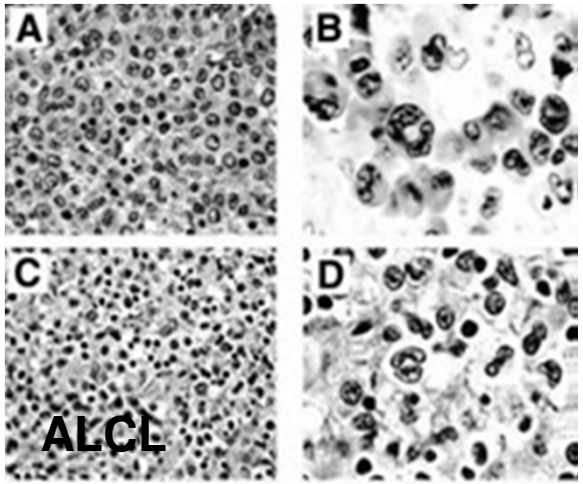
Insulin-receptor family – tirosine kinase member
Ligand: **Midkine/pleiotropin**

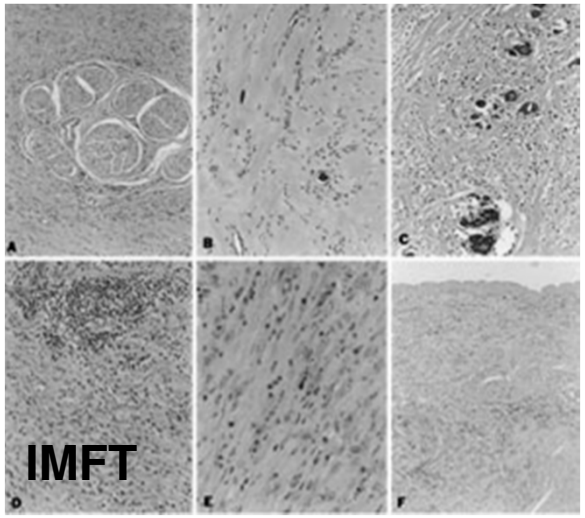
Brain development, regulating nejuronal development

ALK translocations in human cancer: ALKOMAS

	génhiba	daganat
ALK	TK mutáció/ampl	NBL
ALK	ALK-NPH 2/5	ALCL
ALK	ALK-ALO17	ALCL
ALK	ALK-TFG	ALCL
ALK	ALK-SQSTM1	ALCL
ALK	ALK-MSN moesin	ALCL
ALK	ALK-MYH9 myosin	ALCL
ALK	ALK-HMGIC	IMFT
ALK	ALK-RANBP2	IMFT
ALK	ALK-CARC	IMFT
ALK	ALK-ATIC 2/2 inv	ALCL, IMFT
ALK	ALK-CLTC 2/17 clathrin	ALCL, IMFT
ALK	ALK-SEC31A	ALCL, IMFT
ALK	ALK-TPM3 2/1 troponin	ALCL, IMFT, histioc
ALK	ALK-TPM4 2/19	ALCL, MFT, oeCC



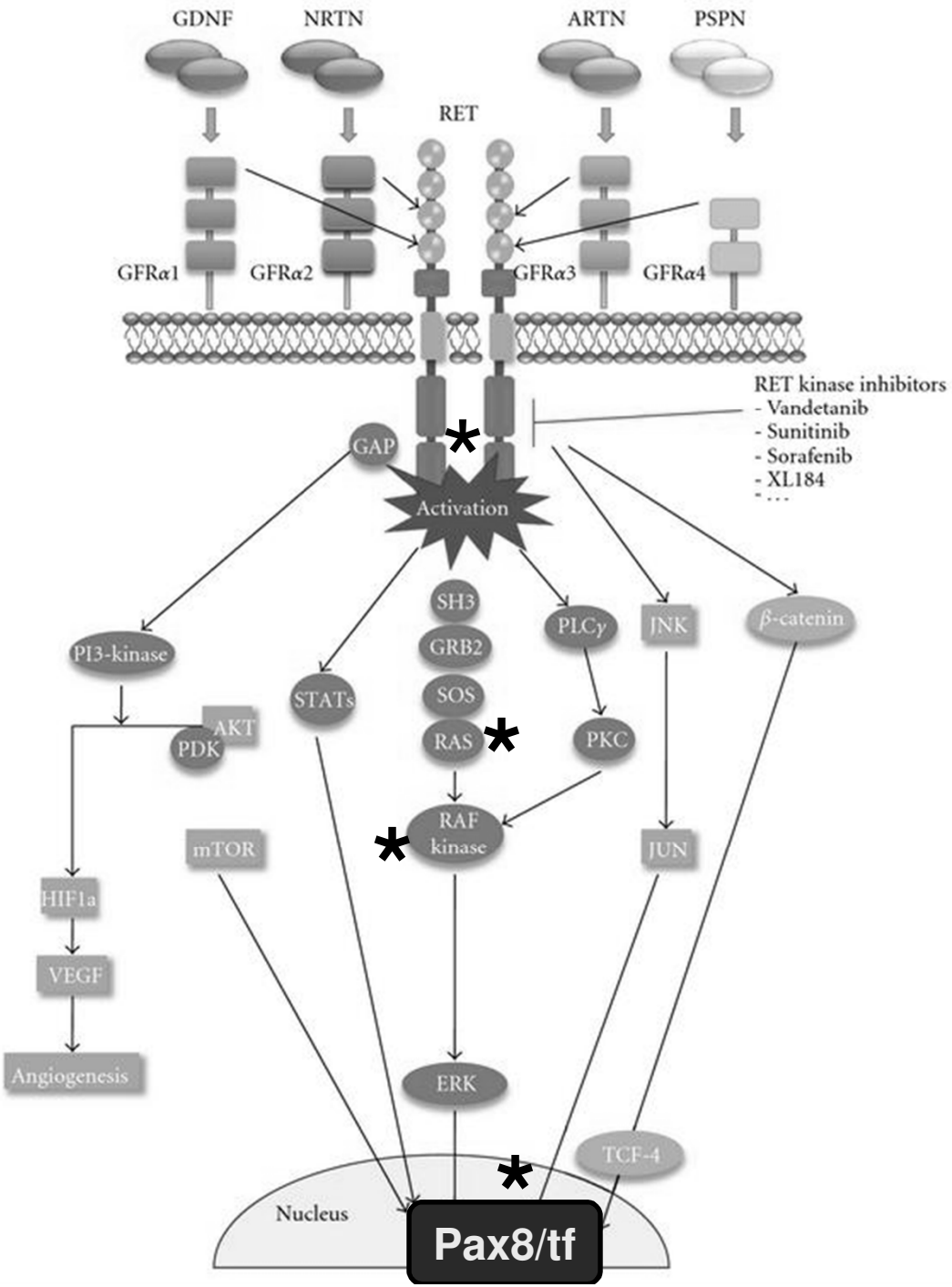




ALK-translocations and lung cancer

	génhiba	következmény
ALK	Mutáció/amp/LOH	Y1604+ALK
ALK	**EML4-ALK inv(2)(p21p23)**	**ALK+IHC Konst akt**
ALK	EML4-ALK	CRC,BRC
ALK	TGF-ALK 2/3tr	ALK+, konst akt
ALK	KIF5B-ALK 2/10tr	ALK+, konst akt

- **ALK gene defect: EGFRwt, KRASwt**
- **ALK gene defect: adenocarcinoma (signet ring)**
- **ALK gene defect: non smokiing, young male**

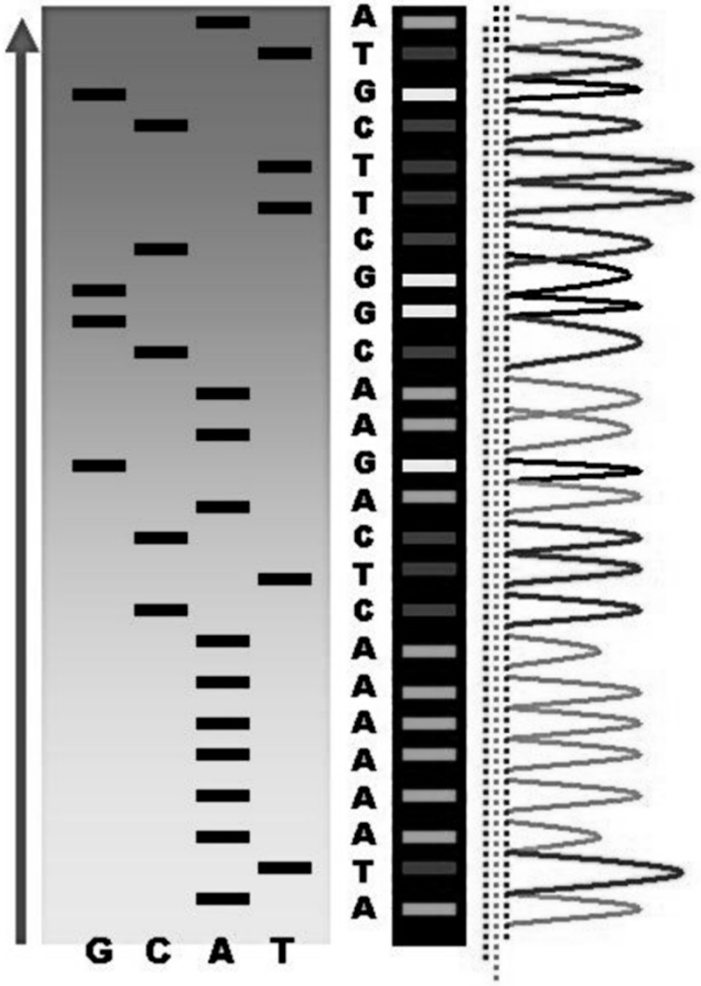

GDNF
NRTN
ARTN
PSPN
RET
GFRα1
GFRα2
GFRα3
GFRα4
RET kinase inhibitors
- Vandetanib
- Sunitinib
- Sorafenib
- XL184
- ...
GAP
Activation
SH3
GRB2
SOS
RAS
RAF kinase
PI3-kinase
AKT
PDK
STATs
PLCγ
PKC
JNK
β-catenin
mTOR
JUN
HIF1a
VEGF
Angiogenesis
ERK
TCF-4
Nucleus
Pax8/tf

Molecular classification of thyroid cancer

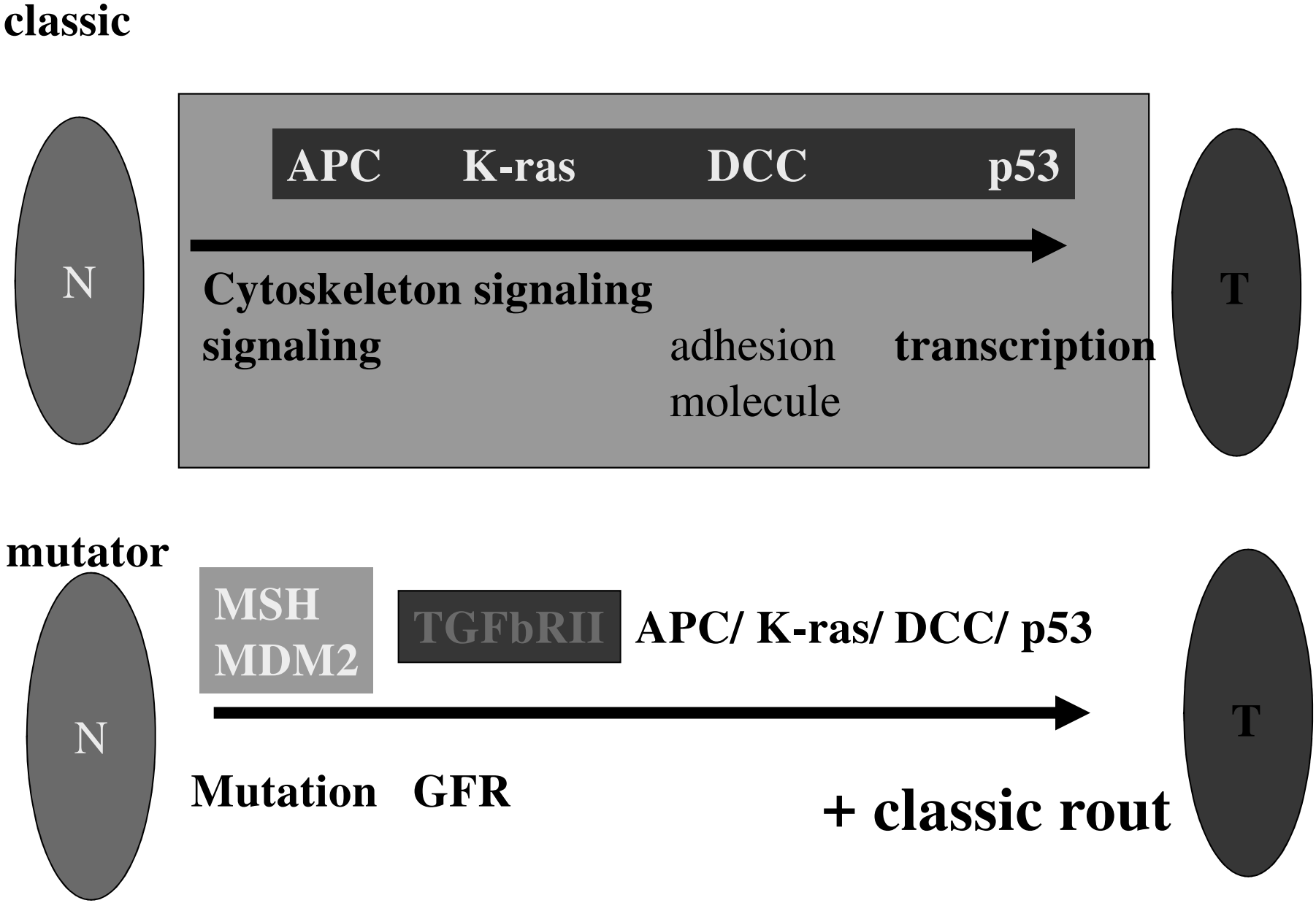
pathway	Gene defect	histology	%
Membrane receptor	**RET-TK mutation**	**medullary**	**100**
	RET-TK-fusion gene	**papillary**	**15**
MAPK signaling	RAS mutation-**c61**	follicular	40
	RAS mutation-**c61**	papillary	15
	B-RAF mutation	**papillary**	**45**
	PAX8/PPARg fusion	**follicular**	**30**

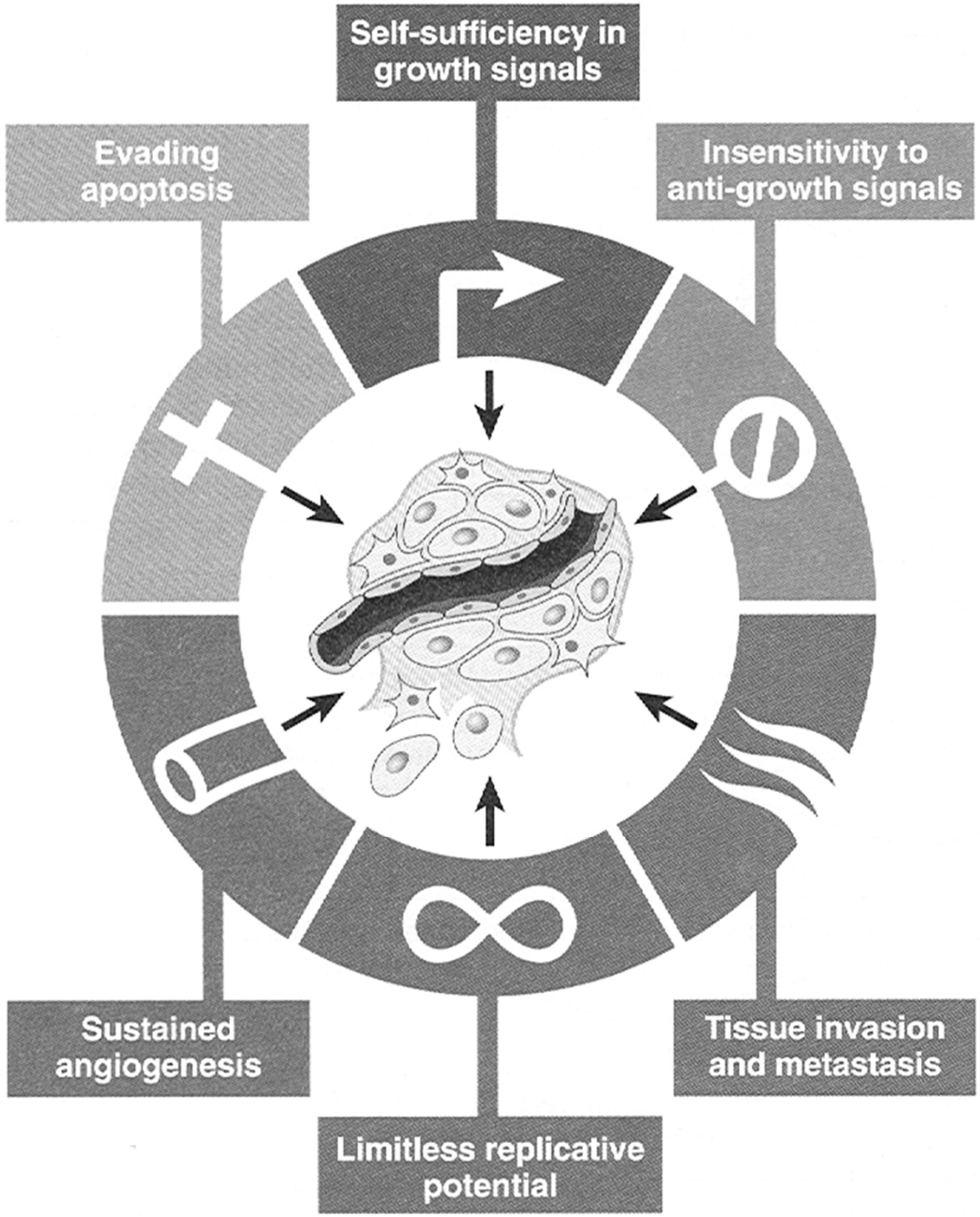
Next Generation Sequencing. Precision medicine

Myseq: 1000-USD genome



Carcinogenesis of colon cancer (Weinberg)




Self-sufficiency in growth signals
Evading apoptosis
Insensitivity to anti-growth signals
Sustained angiogenesis
Tissue invasion and metastasis
Limitless replicative potential

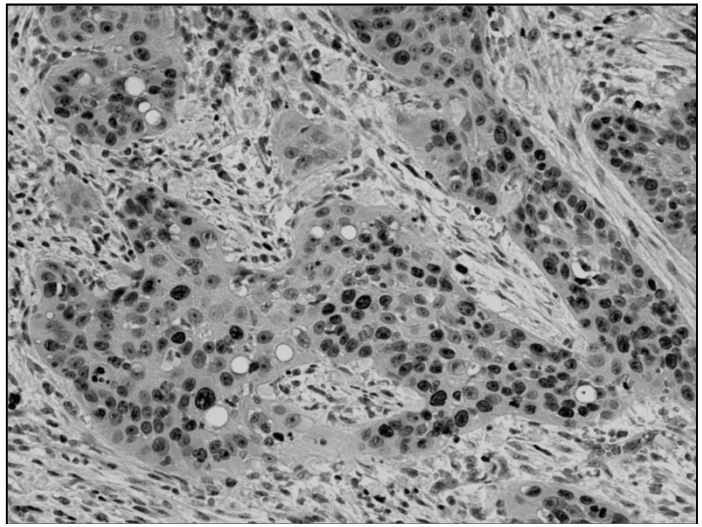
Accumulation of cancer tissue

- Labeling index (LI):

  Ts/Tc (S-phase,cell cycle time)

Ki67 (cycle marker), mitotic index (M phase)

- Growth fraction: proliferating/ P+steady)
- Rate of cell loss: 1-estimatedDT/measuredDT

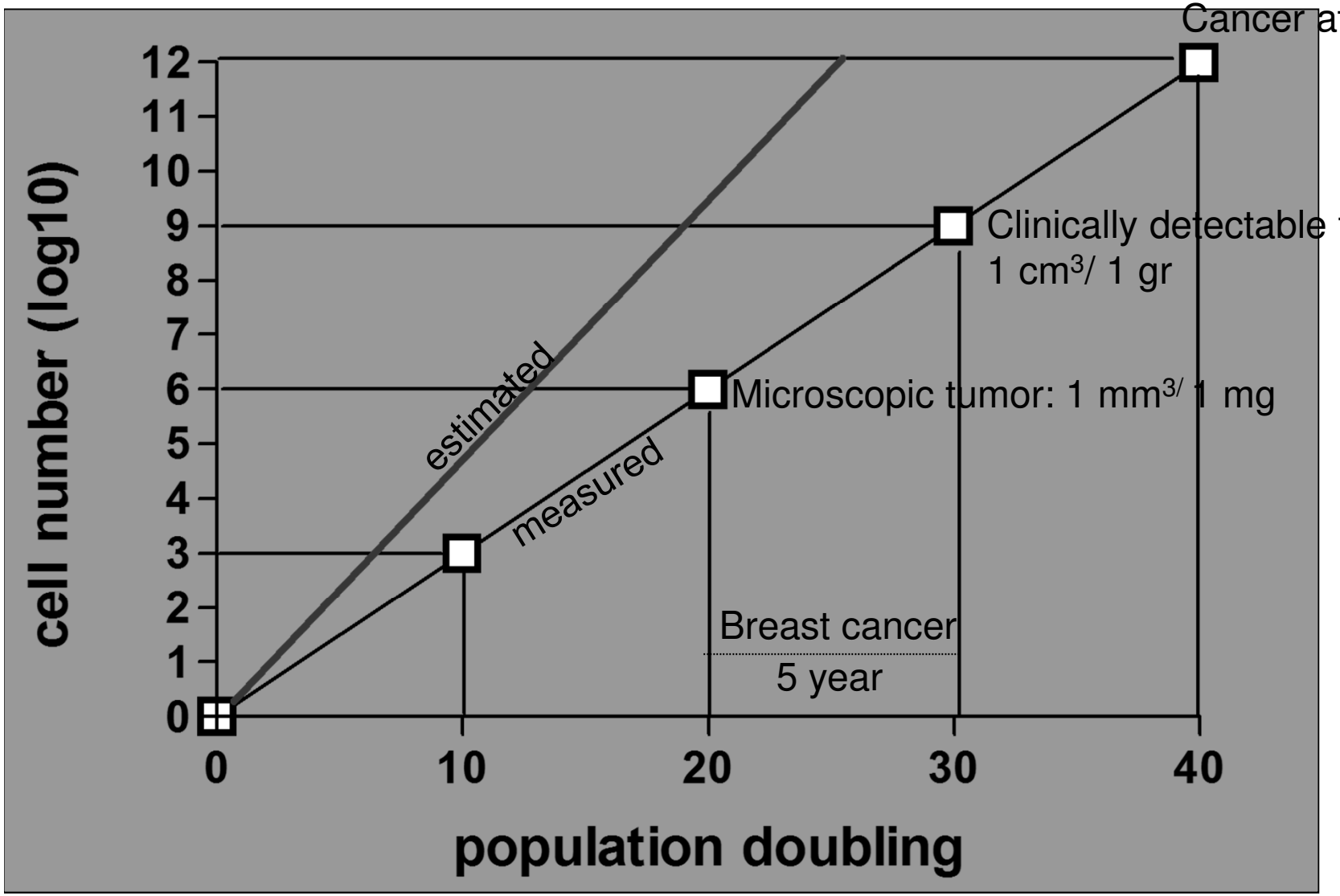


Cell kinatic characteristics of various cancers

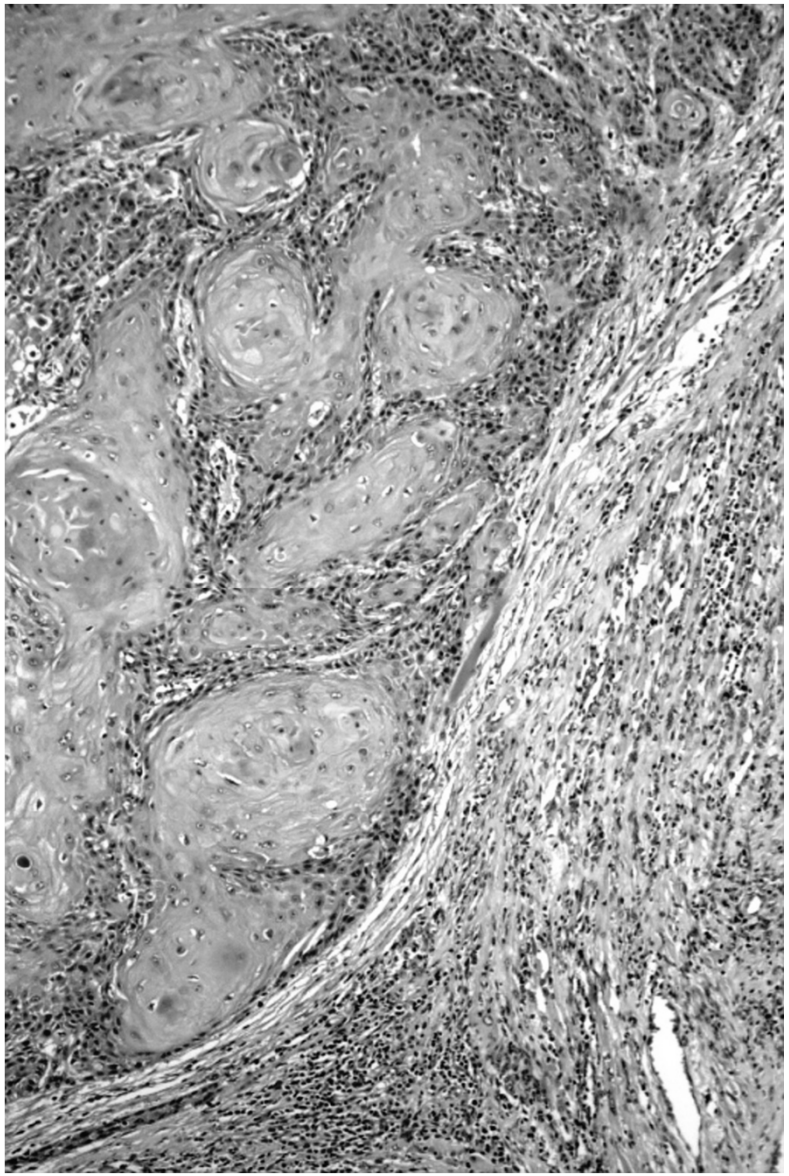
	DT (weeks)	S-phase (hr)	Cycle (hr)	LI (%)
Breast cancer	14	21	60	2
Colon cancer	90	17	72	3
Lung cancer	11-21	20	108	8
NHL	4	12	48	30
AML		18	60	8

Mitotic rate
MI/apoptosis rate

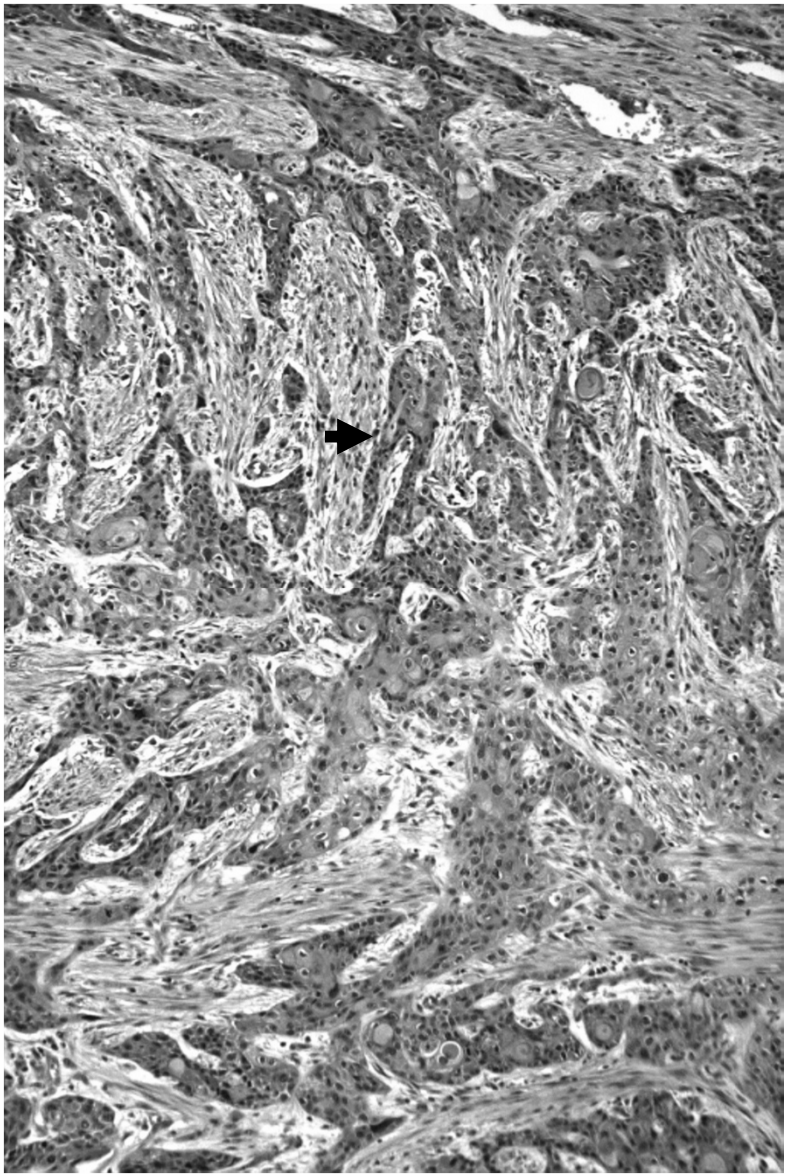
Growth dynamics of cancer



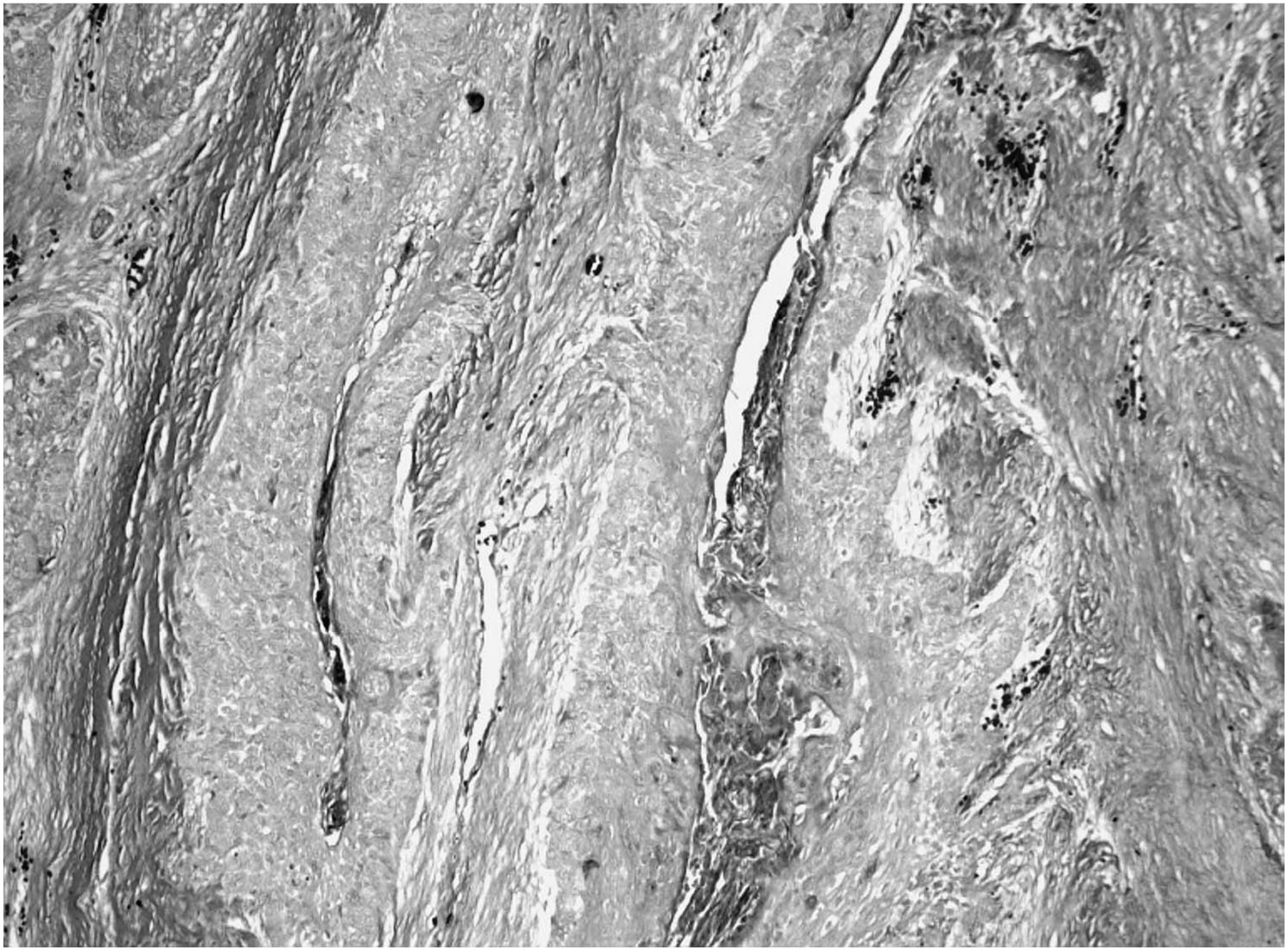
Compressive growth



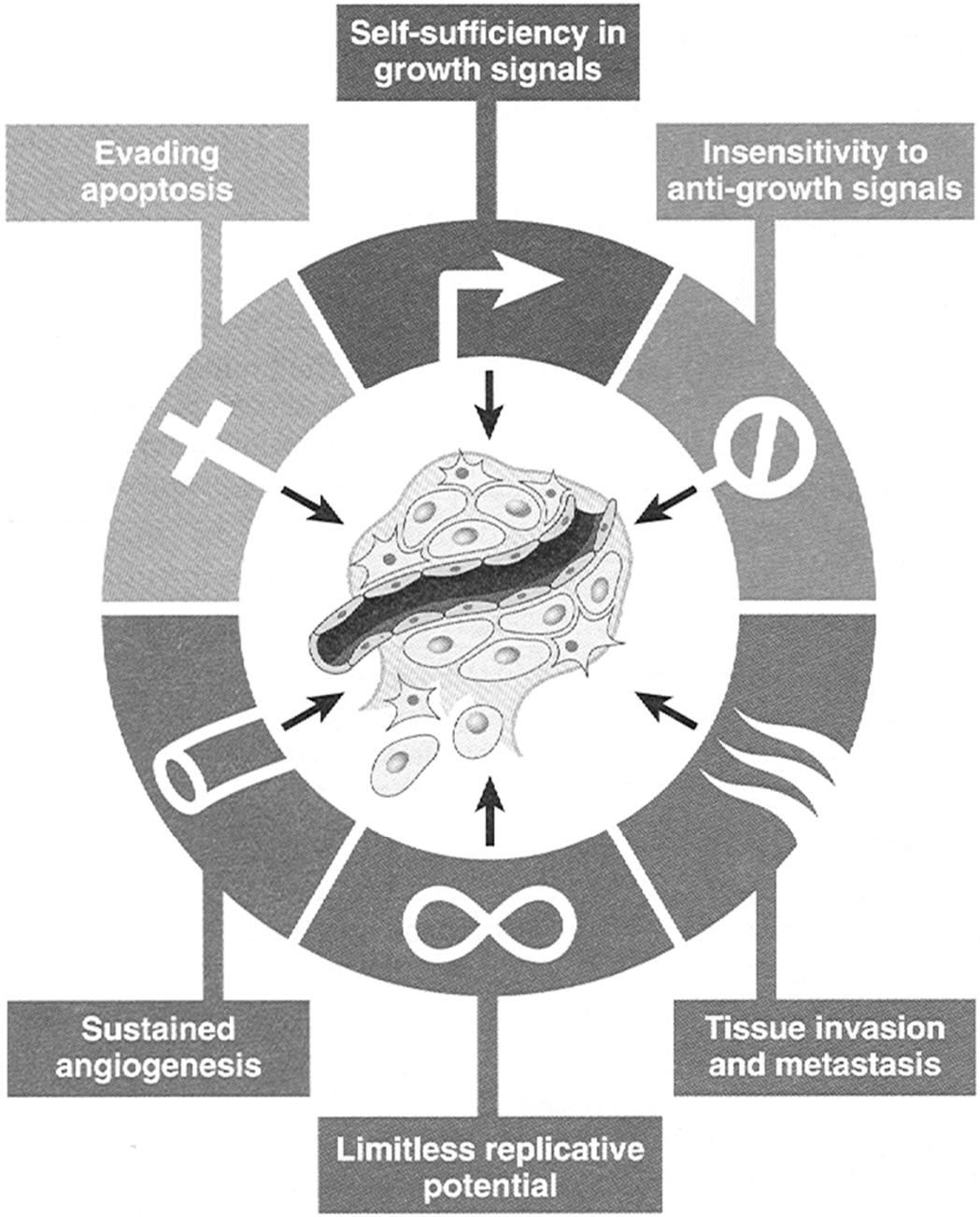
Invasive growth



Tumor stroma (non-transformed, abnormal)



Connective tissue (fibroblasts)- Mallory trikróm festés


Self-sufficiency in growth signals
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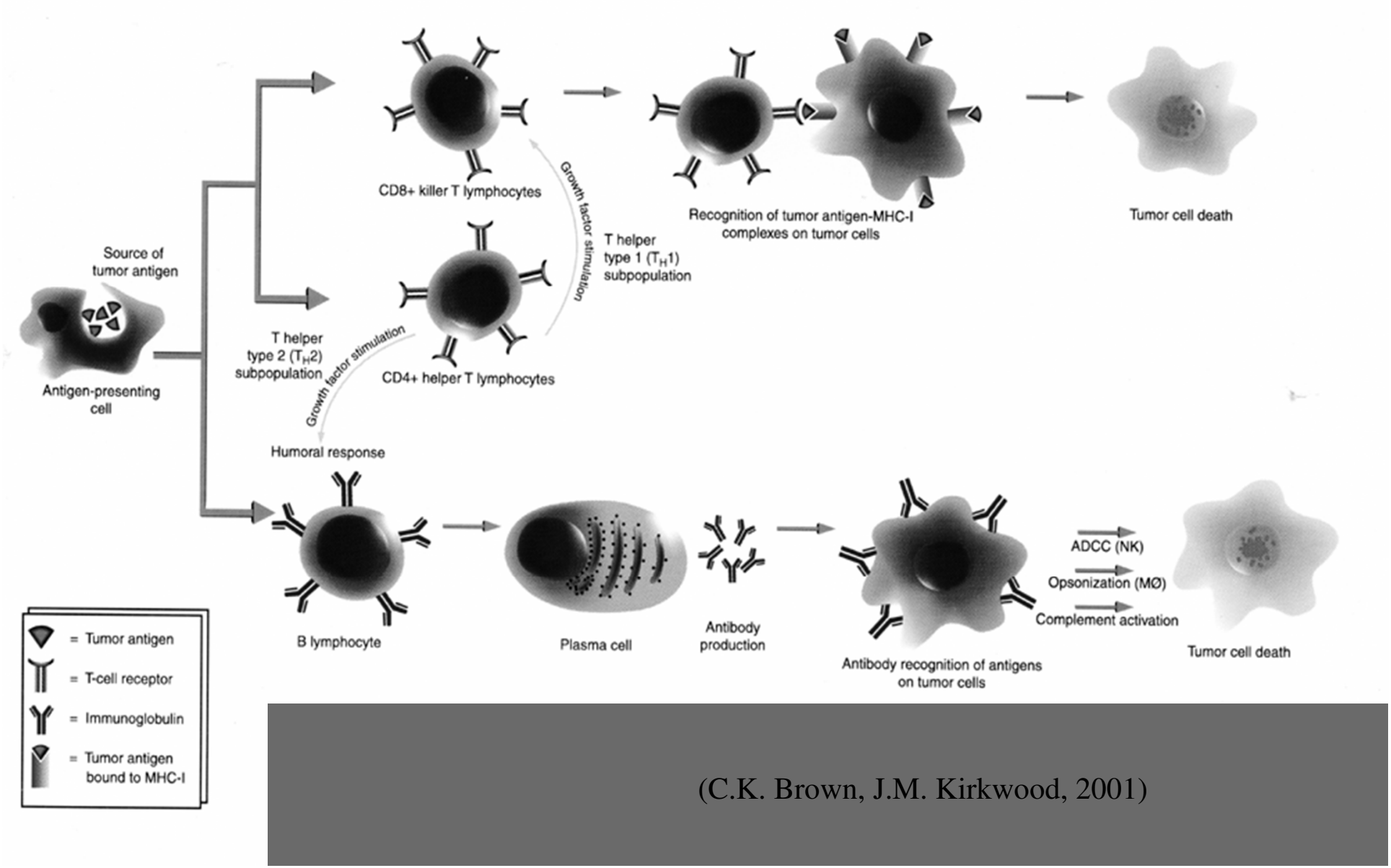
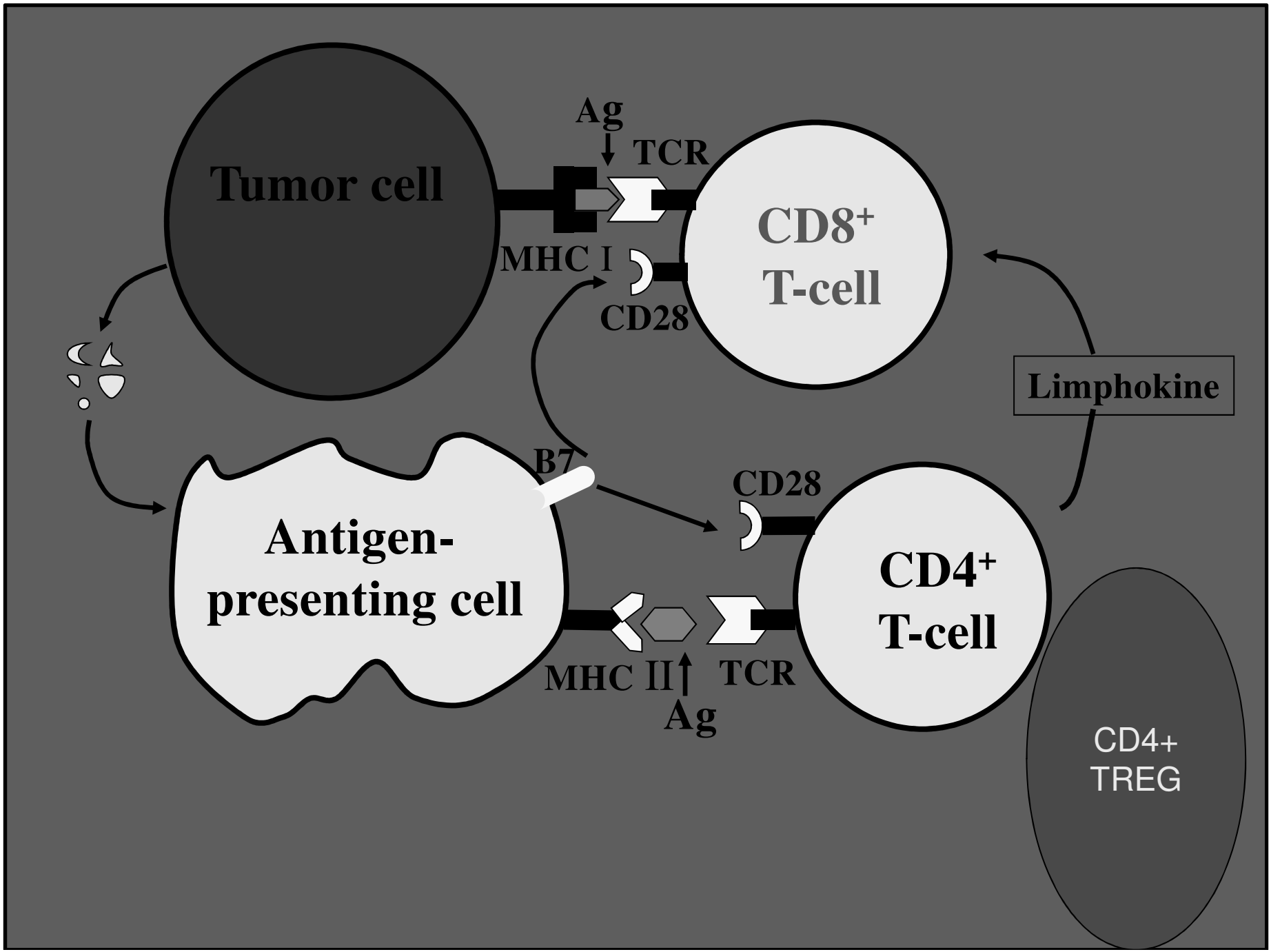
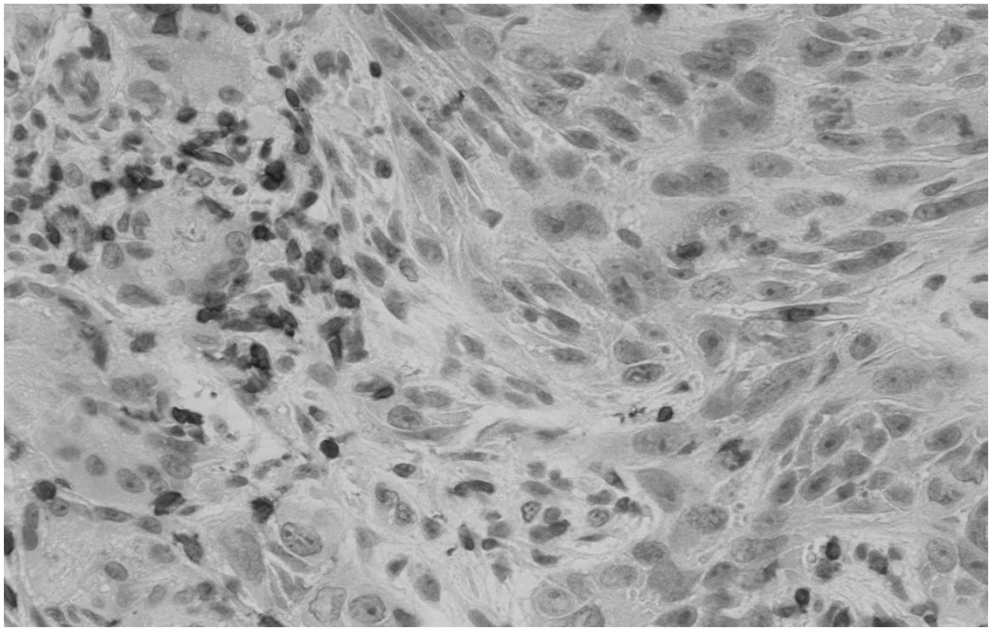
Antitumor immune response



(C.K. Brown, J.M. Kirkwood, 2001)


Tumor cell
Ag
TCR
MHC I
CD28
CD8+
T-cell
Limphokine
B7
CD28
Antigen-
presenting cell
CD4+
T-cell
MHC II
TCR
Ag
CD4+
TREG

Tumor infiltrating (lymphoid)cells: TIL

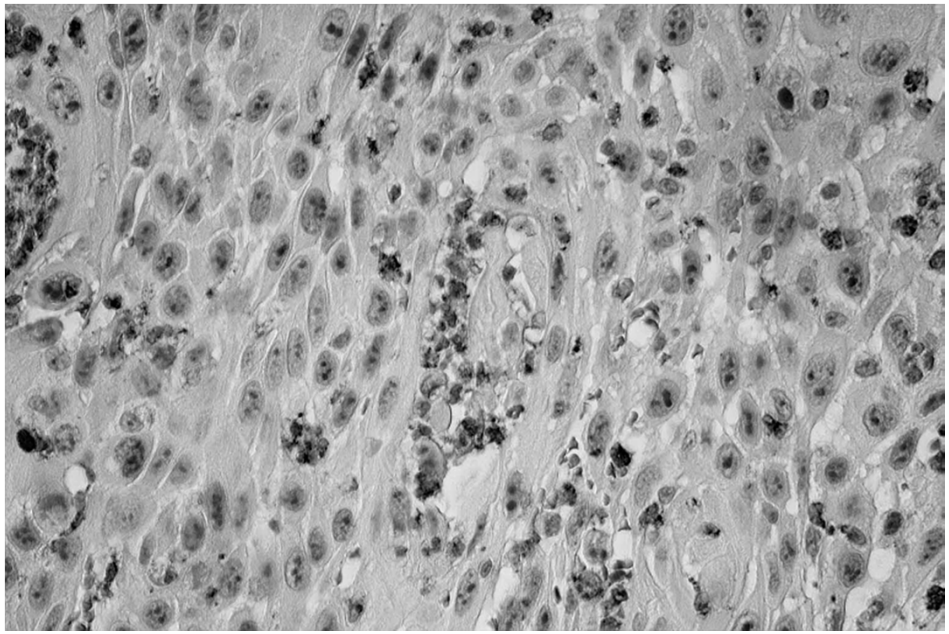


T cells

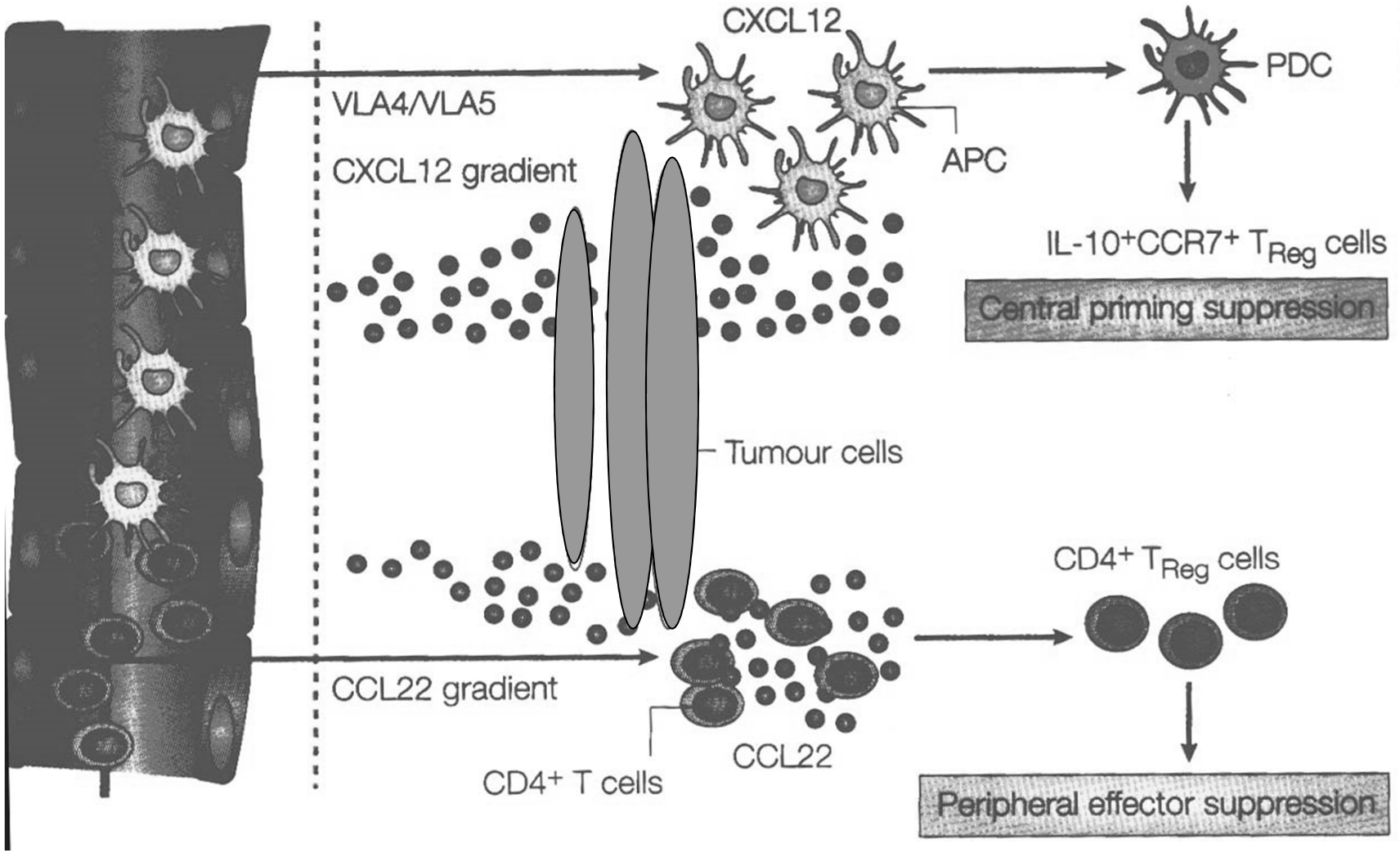
B cells
macrophages

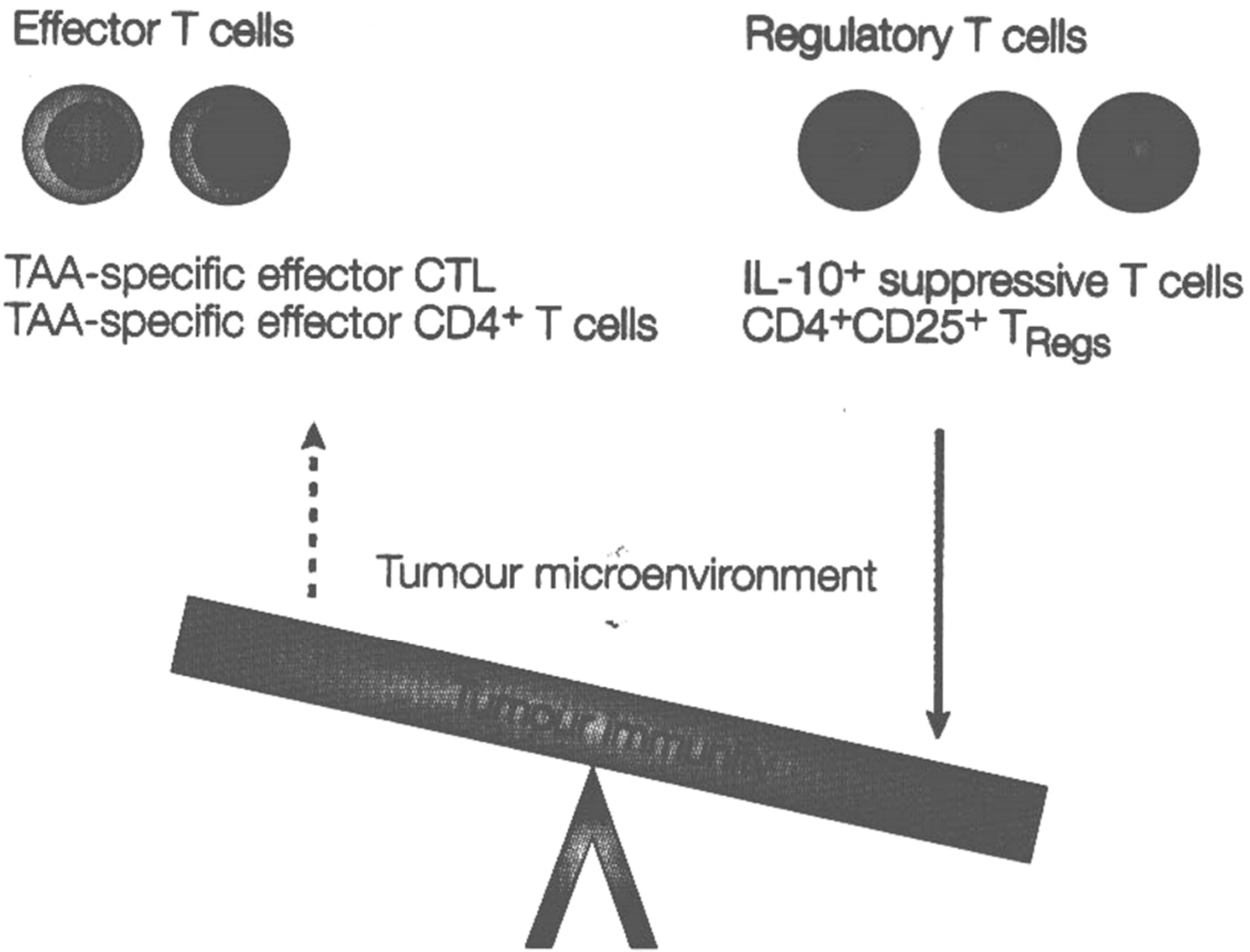
Dendritic cells

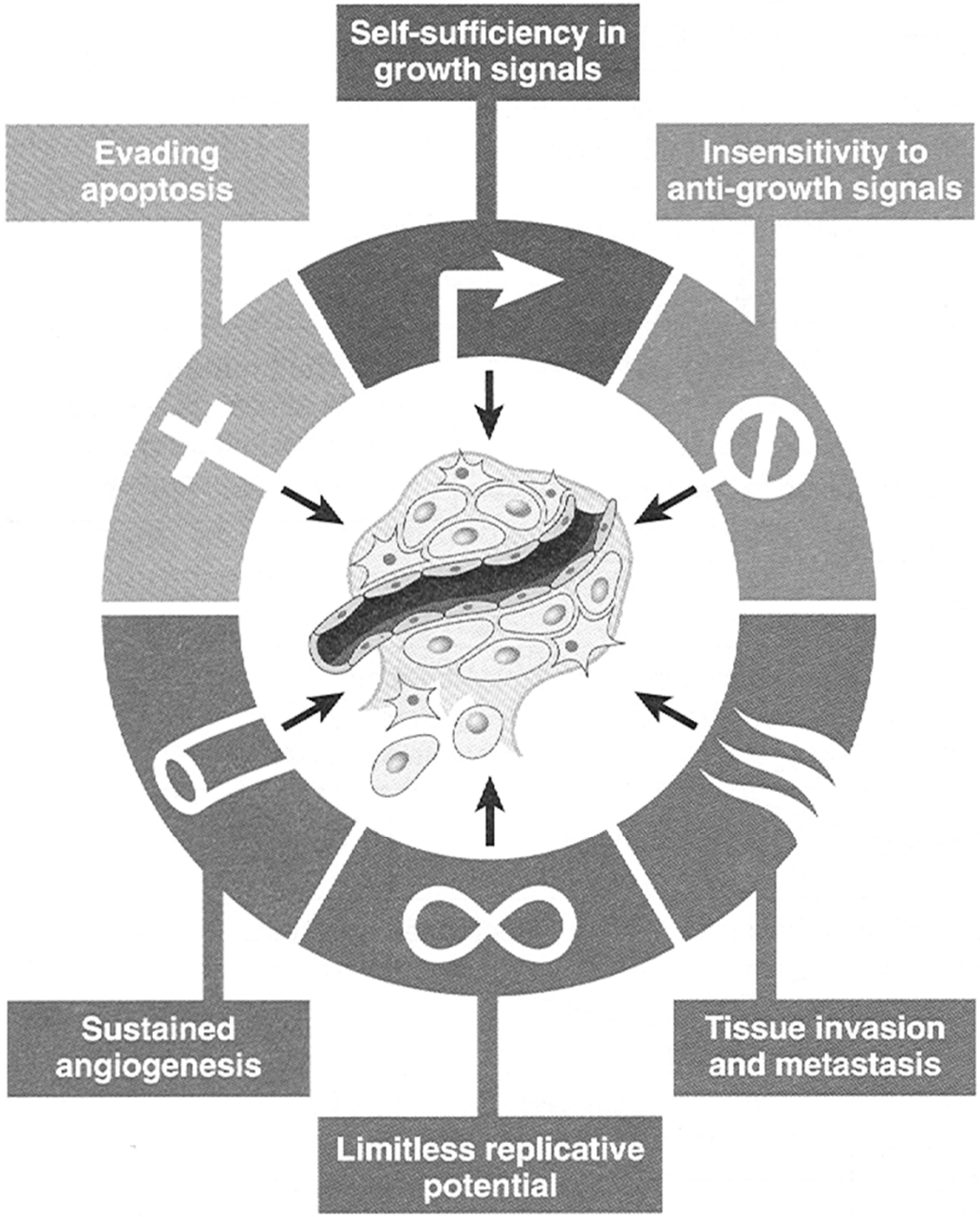
PMN



Immunosuppression in cancer




Effector T cells
Regulatory T cells
TAA-specific effector CTL
TAA-specific effector CD4+ T cells
IL-10+ suppressive T cells
CD4+CD25+ TRegs
Tumour microenvironment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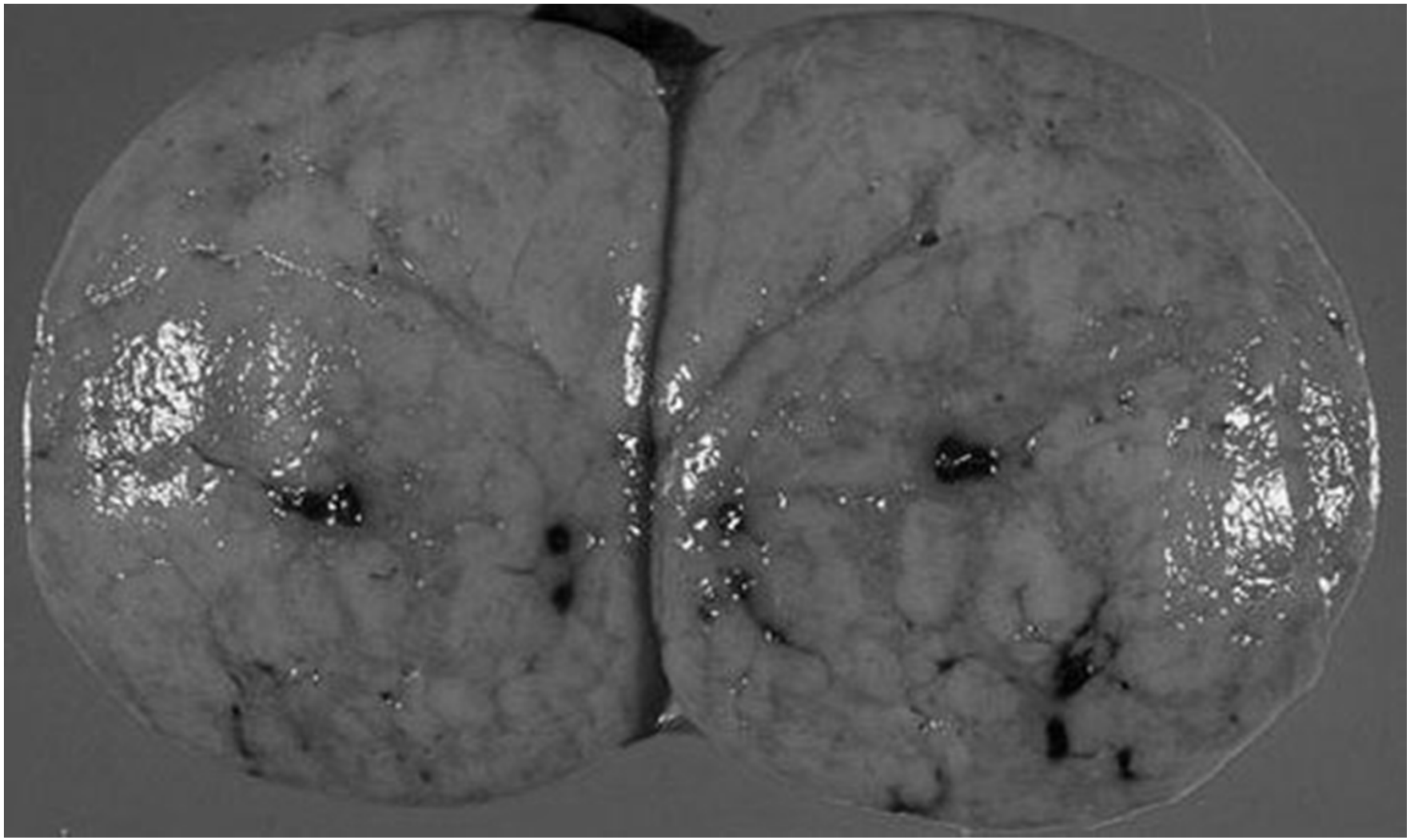
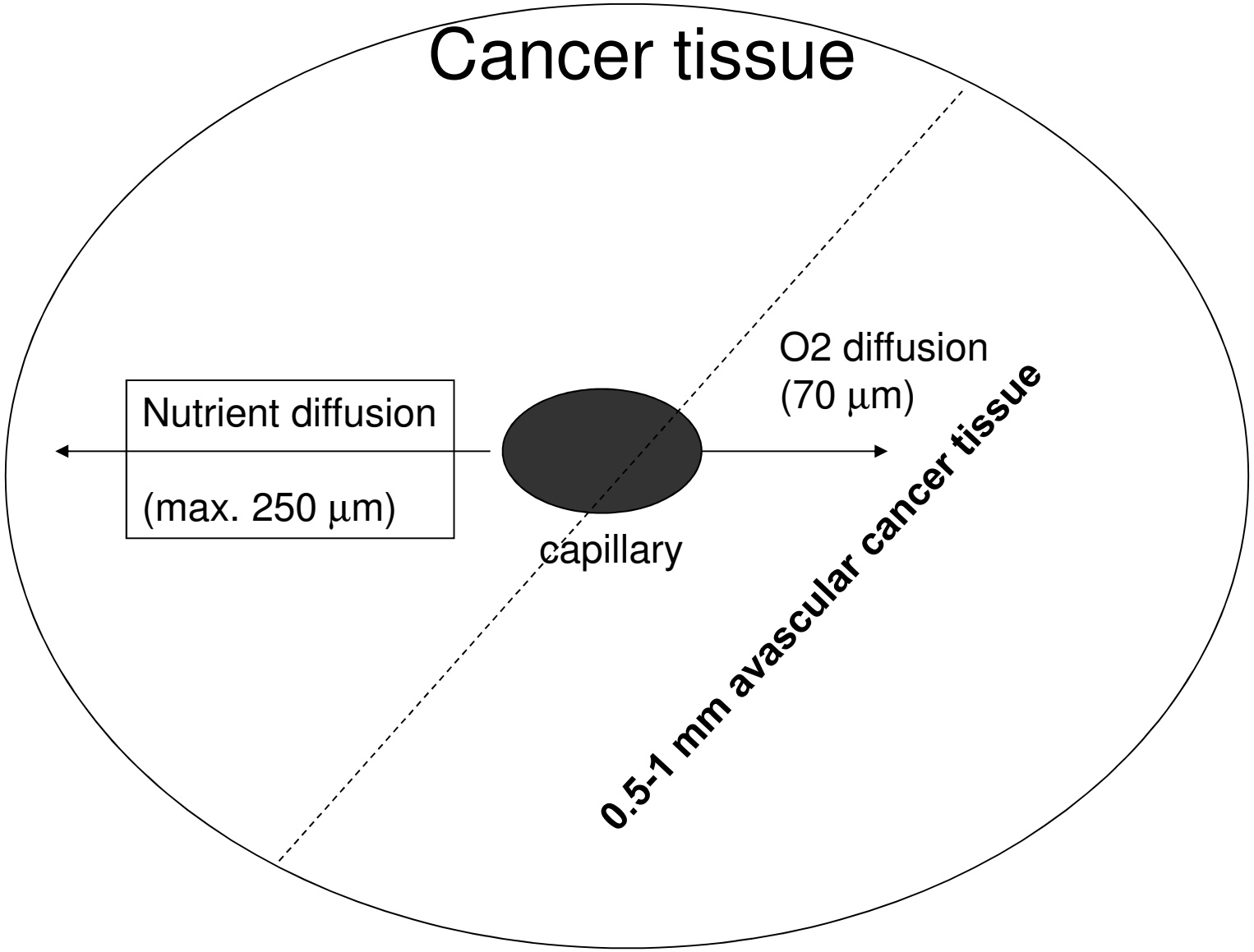
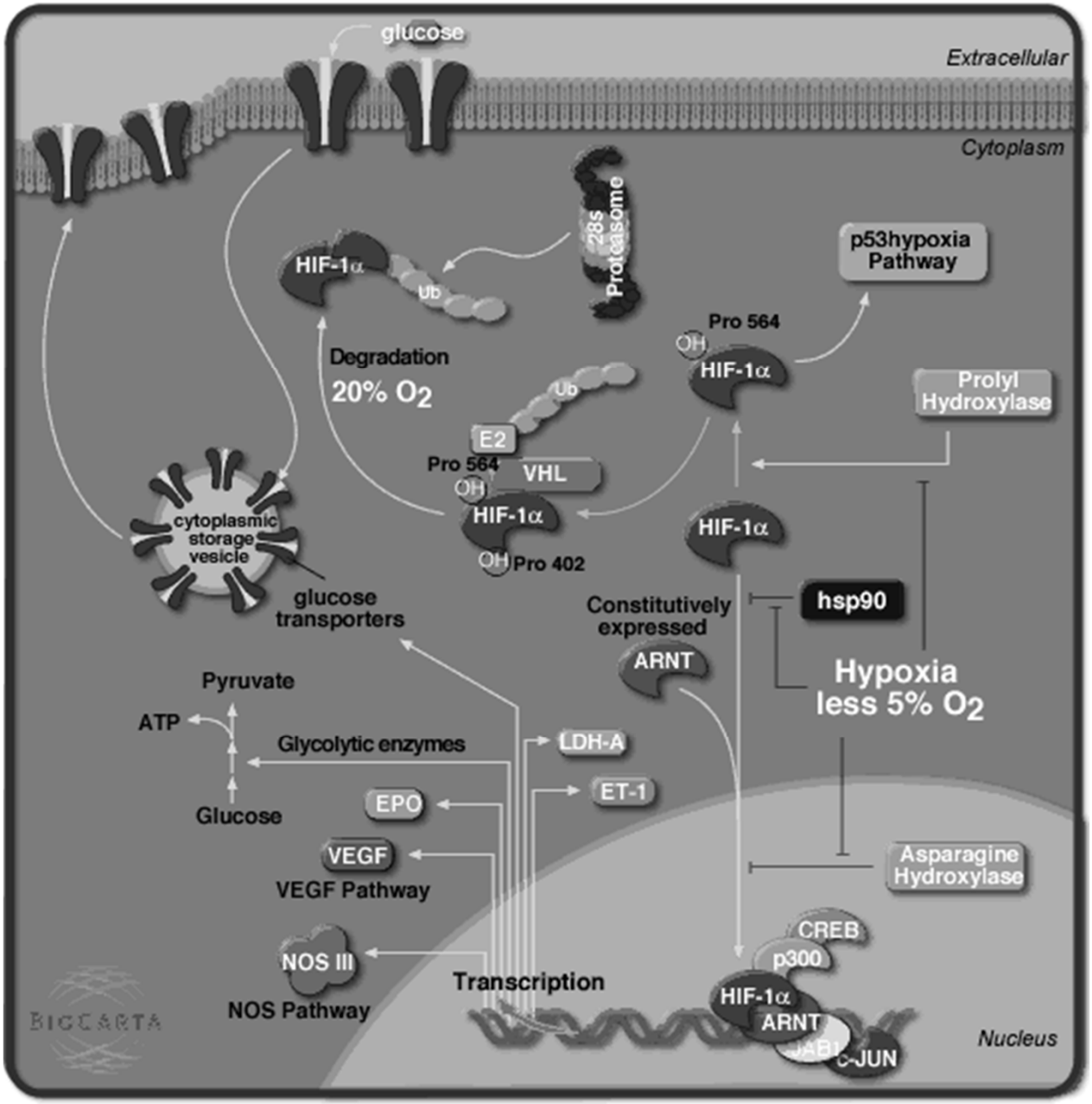


Pediatric kidney cancer (nephroblastoma)


Cancer tissue
Nutrient diffusion
(max. 250 μm)
O2 diffusion
(70 μm)
capillary
0.5-1 mm avascular cancer tissue

Hypoxic signaling (cancer)



Oncogenic signaling and HIF activation

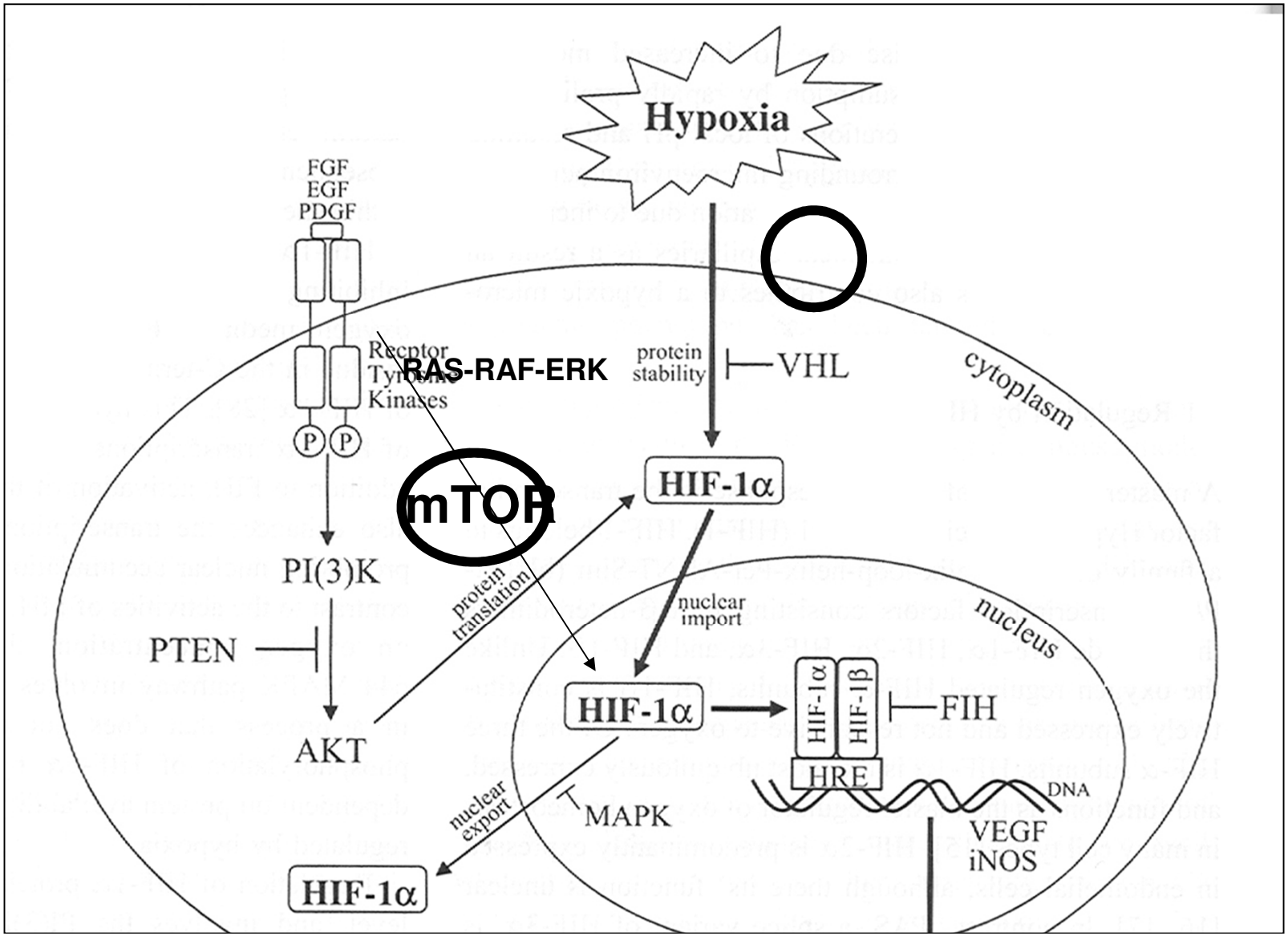
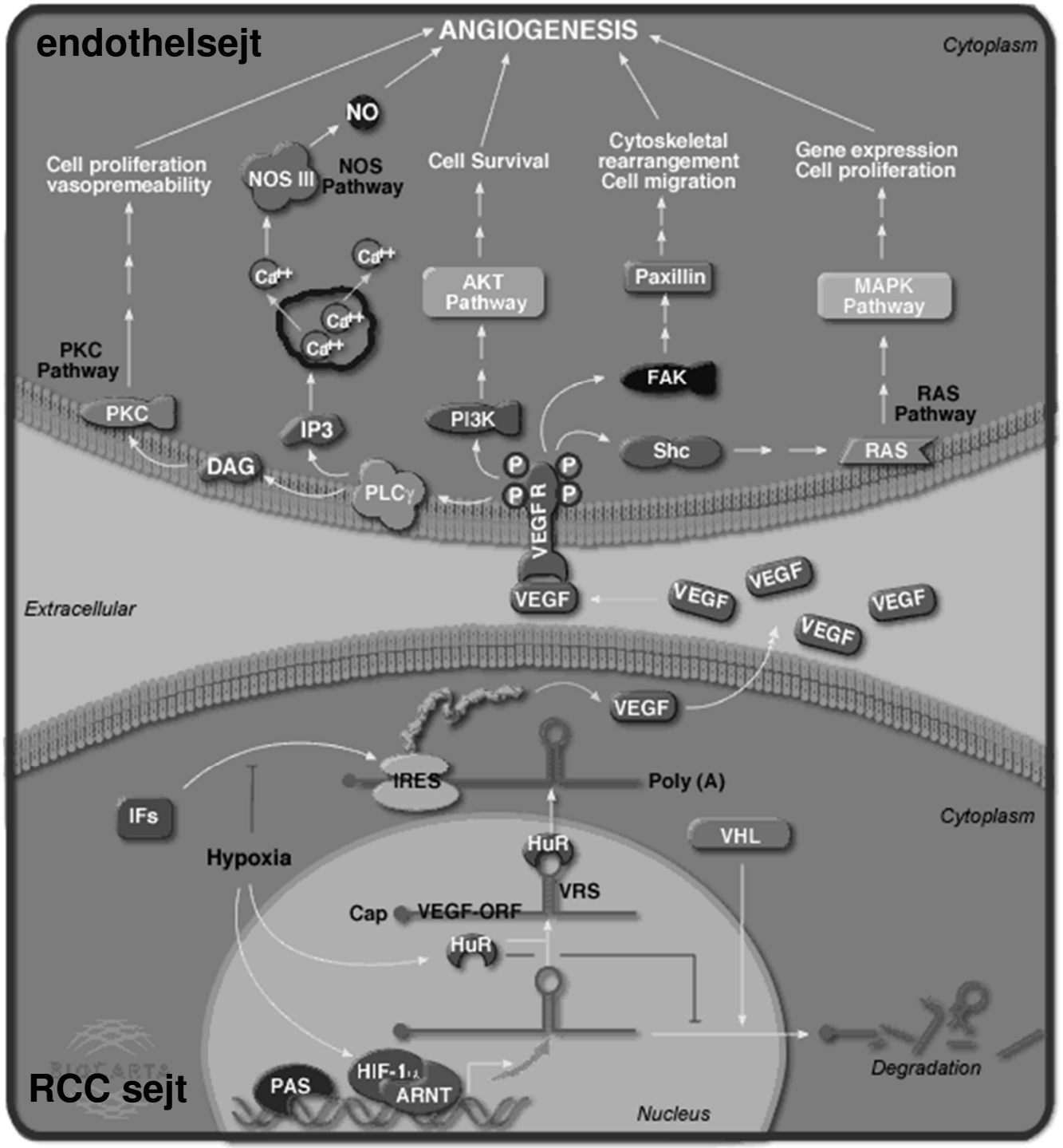


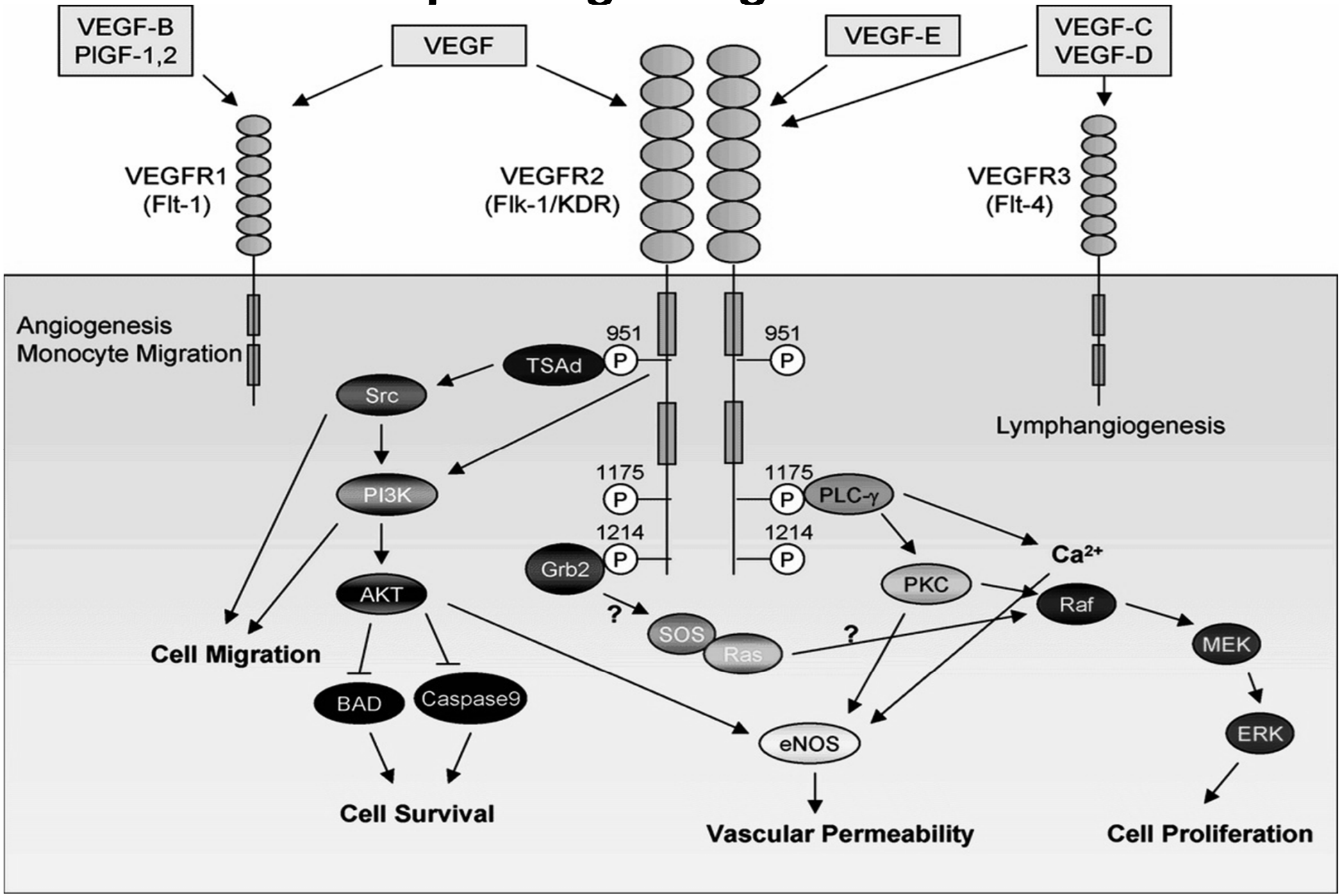
Table 1. Physiological pro-angiogenic factors

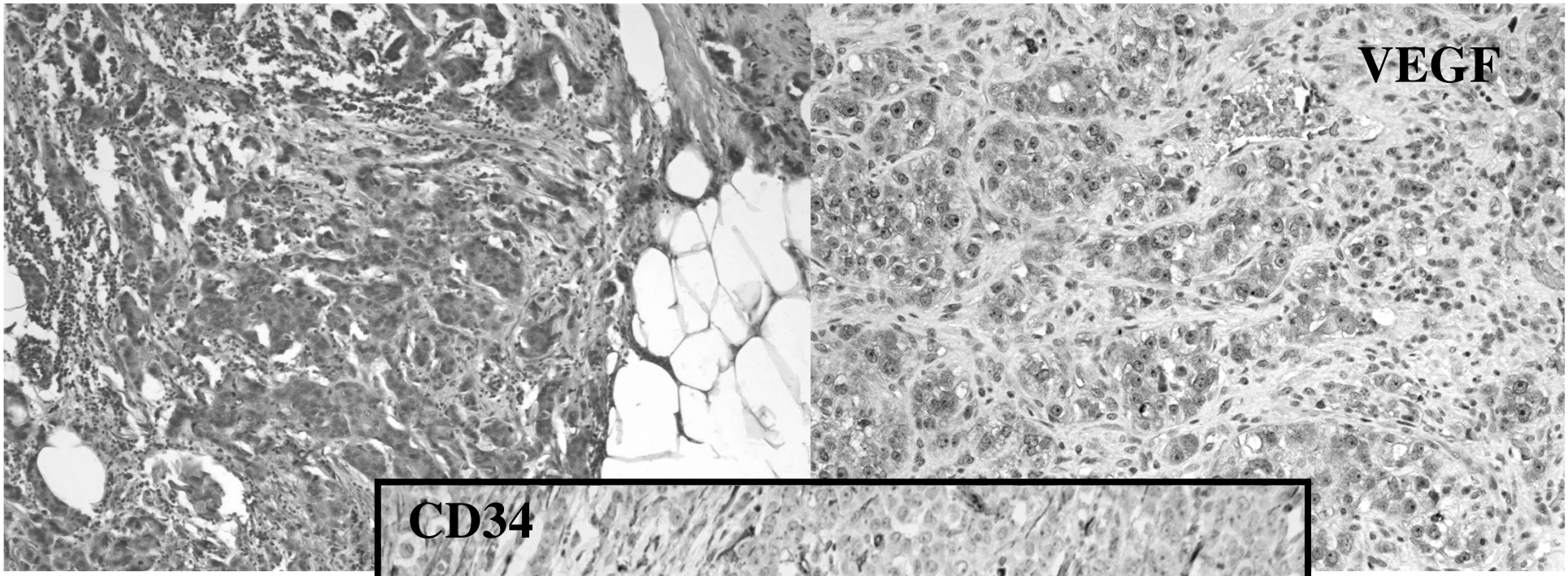
factor	receptor
Growth factors	
VEGF165	FLT1
VEGF121	FLK1/KDR
VEGF189	
VEGF206	
PLGF-152/131	Neuropilin
Ang-1	TIE2
FGF1-9	FGFR
HGF	c-met
IGF-1,2	IGFR
PDGF	PDGFR
EGF	EGF
G-CSF	GCSFR
PD-ECGF (thymidine phosphorylase)	
Cytokines	
TNFα	TNFR1
IL-1b	IL-1R
IL-6	IL-6R
IL-8	IL-8R
Chemokine	
PBSF/SDF1	CXCR4
Hormones	
Estrogen	ER-β
androgen	AR
leptin	OB-Rb
Bioactive lipids	
PAF	PAFR
PGE1,2	PGR
TXA2	TXR
12-HETE	HETE-R (?)
Matrix proteins	
thrombin	THRR
Fibrin	αvβ3, V-cadherin
CYR61, CTGF	αvβ3

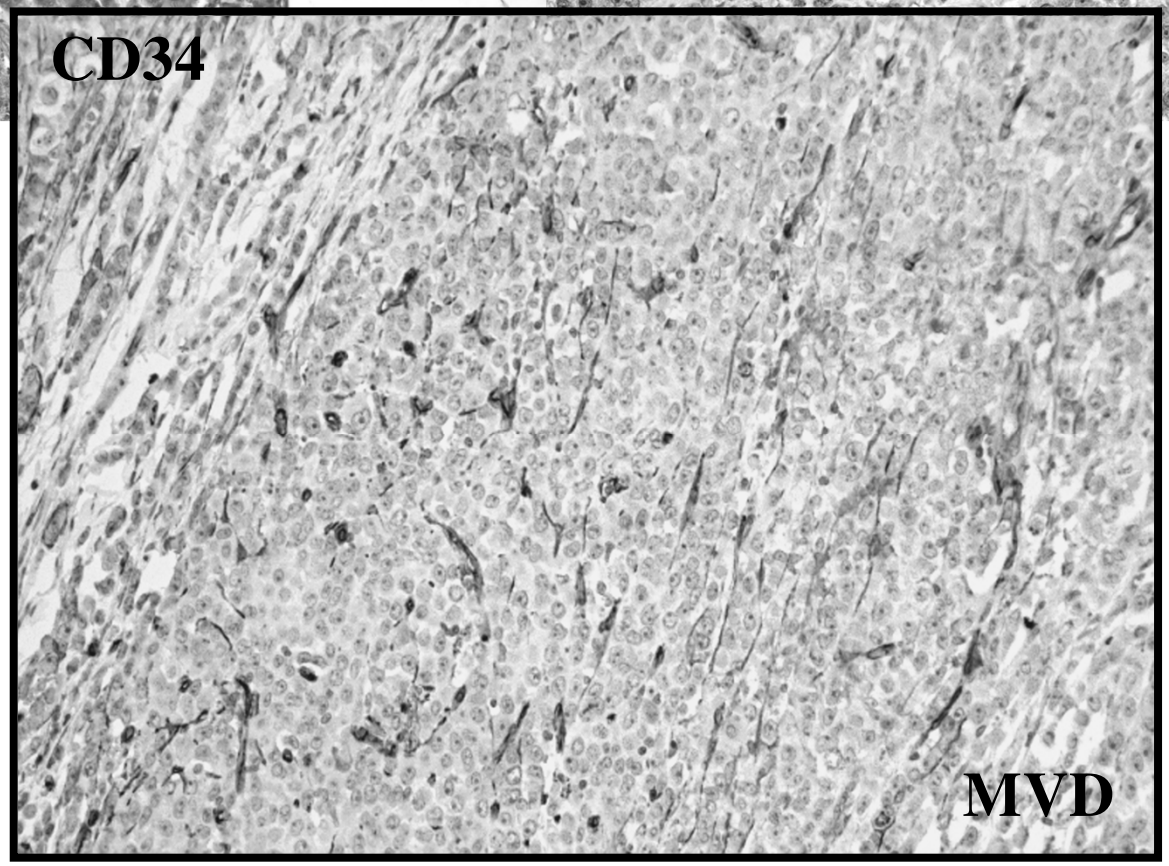
Footnotes: EGF: epidermal growth factor, FGF: fibroblast growth factor, G-CSF: granulocyte-colony-stimulating factor, HGF: hepatocyte growth factor, IGF: insulin-like growth factor, PAF: platelet-activating factor, PDGF: platelet-derived growth factor, PD-ECGF: platelet derived endothelial growth factor, PGE1,2: prostaglandin E1,2, PLGF: placental growth factor, TXA2: thromboxaneA2, VEGF: vascular endothhial growth factor


endothelsejt
ANGIOGENESIS
Cytoplasm
NO
Cell proliferation
vasopremeability
NOS III
NOS
Pathway
Cell Survival
Cytoskeletal
rearrangement
Cell migration
Gene expression
Cell proliferation
Ca++
Ca++
Ca++
Ca++
AKT
Pathway
Paxillin
MAPK
Pathway
PKC
Pathway
FAK
RAS
Pathway
PKC
IP3
PI3K
Shc
RAS
DAG
PLCγ
P
P
P
P
VEGF R
VEGF
Extracellular
VEGF
VEGF
VEGF
VEGF
VEGF
IRES
Poly (A)
IFs
Hypoxia
Cytoplasm
VHL
HuR
VRS
Cap
VEGF-ORF
HuR
HIF-1α
ARNT
PAS
Degradation
Nucleus
RCC sejt

VEGFR2 receptor signaling in endothelial cells

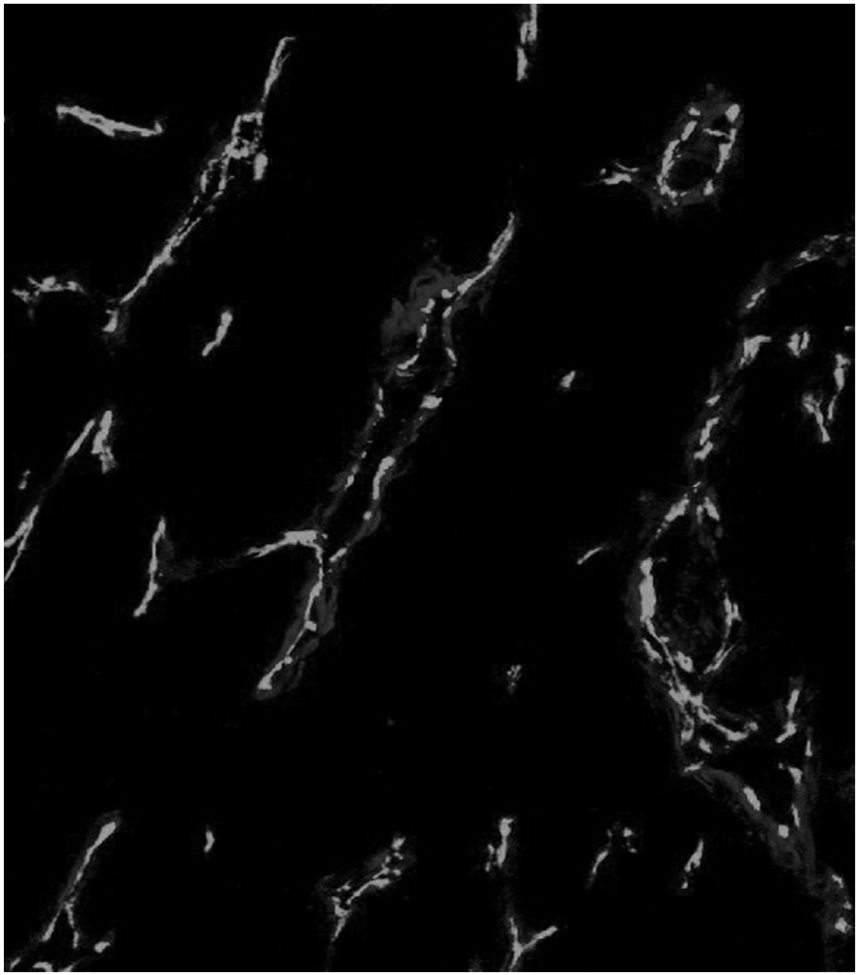






Breast cancer
(DIC)

Cancer vessels

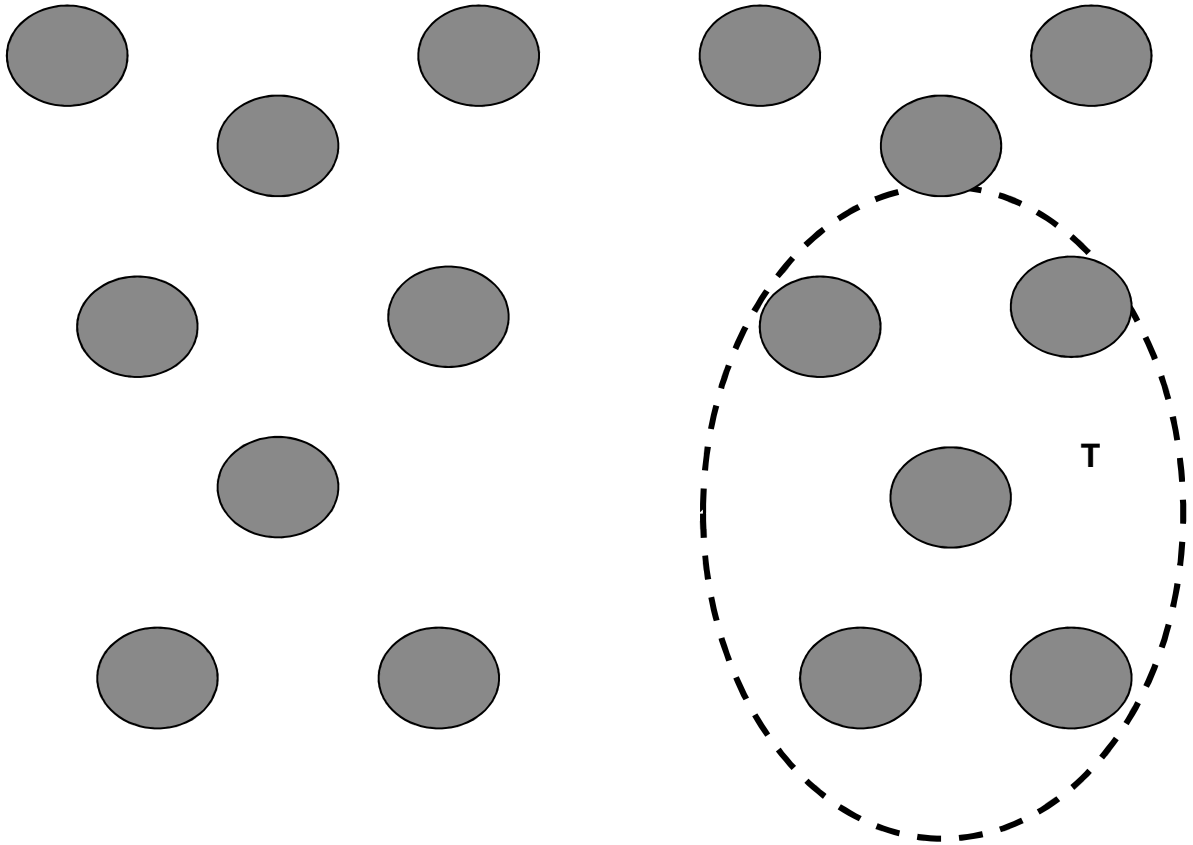


Endothel----pericyta

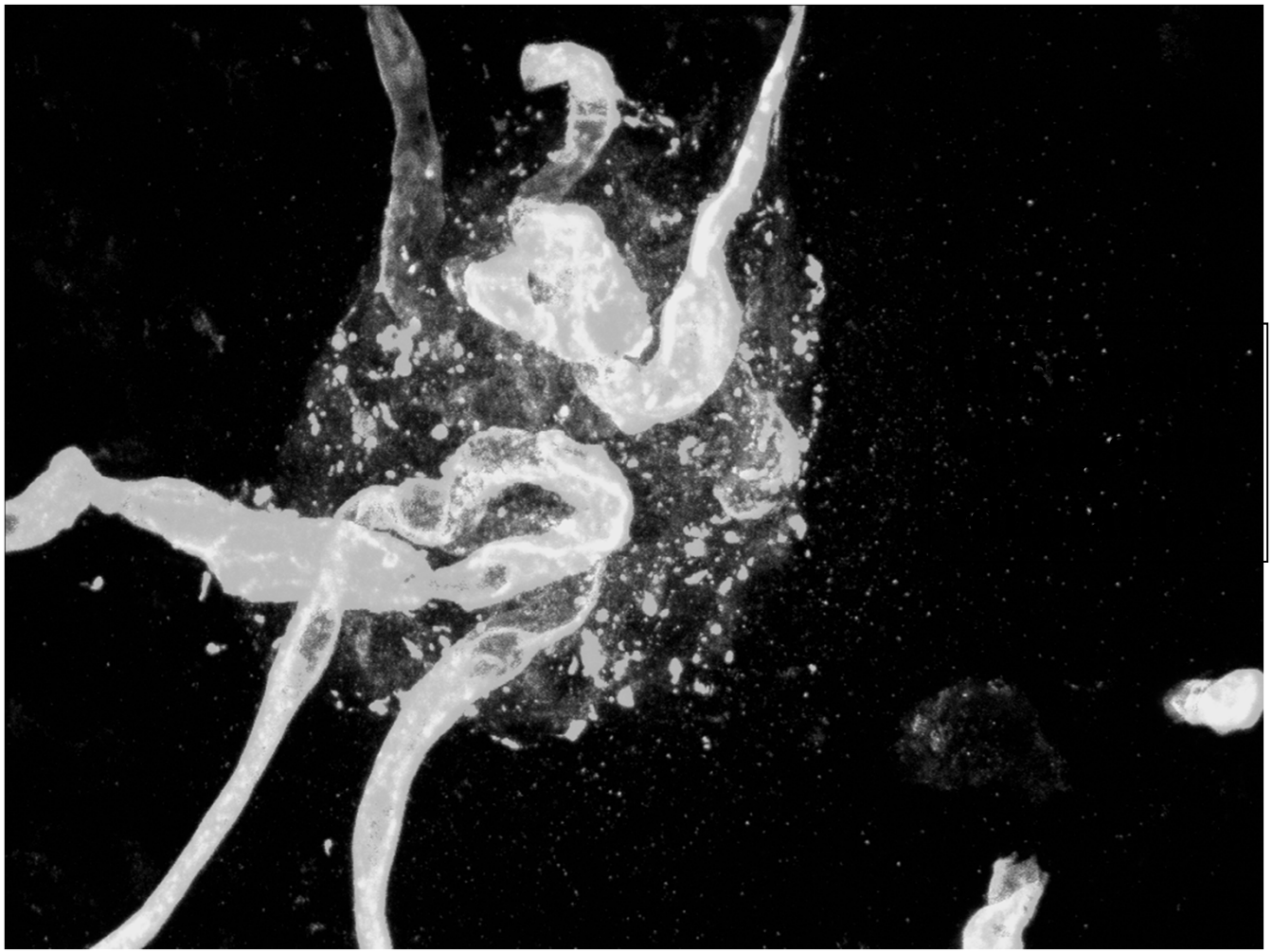
Vascularization of cancer

- Neoangiogenesis
- Postnatal vasculogenesis
- Vessel incorporation (cooption)
- Glomeruloid „angiogenesis”
- Vasculogenic mimicry

Vessel incorporation

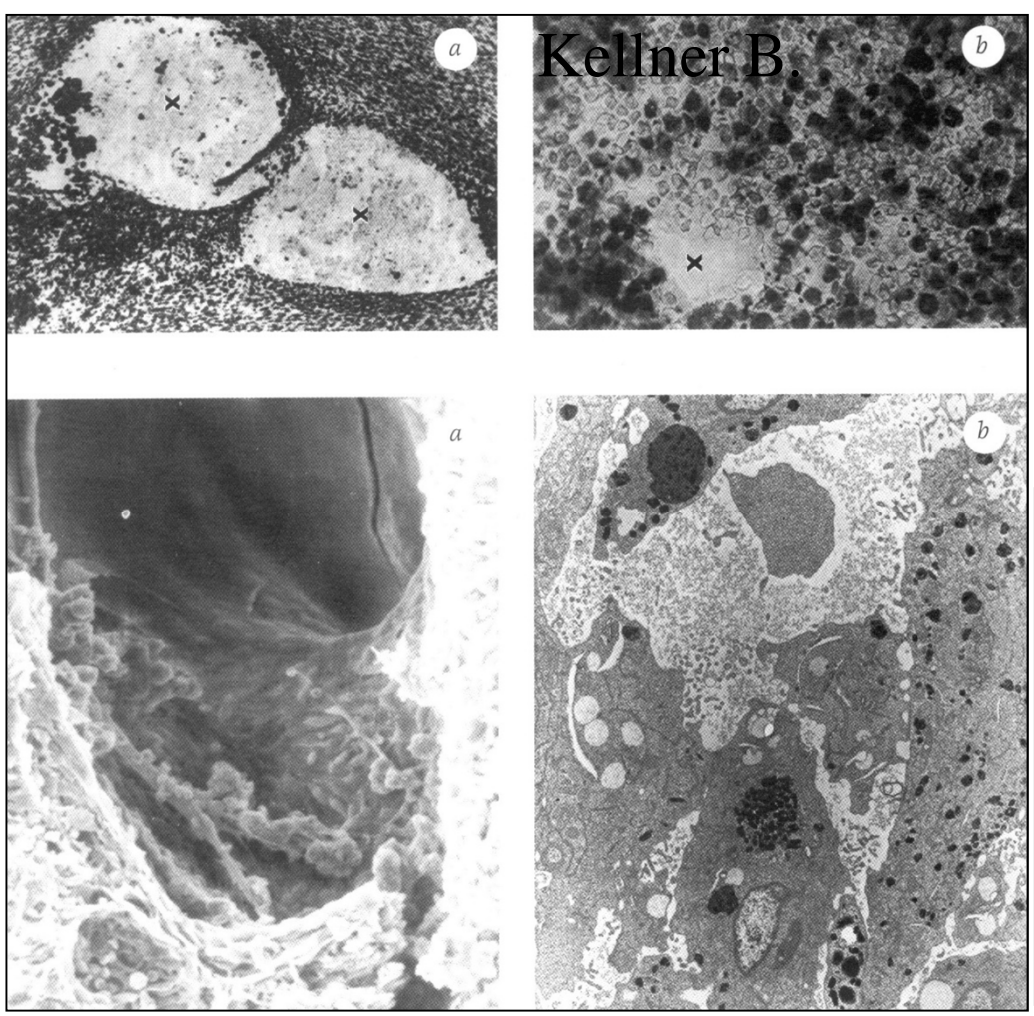


Glomeruloid vessels in brain micrometastases

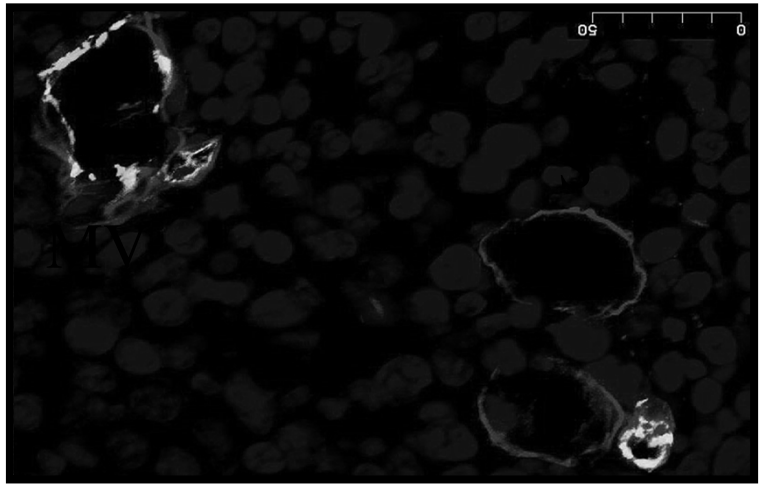


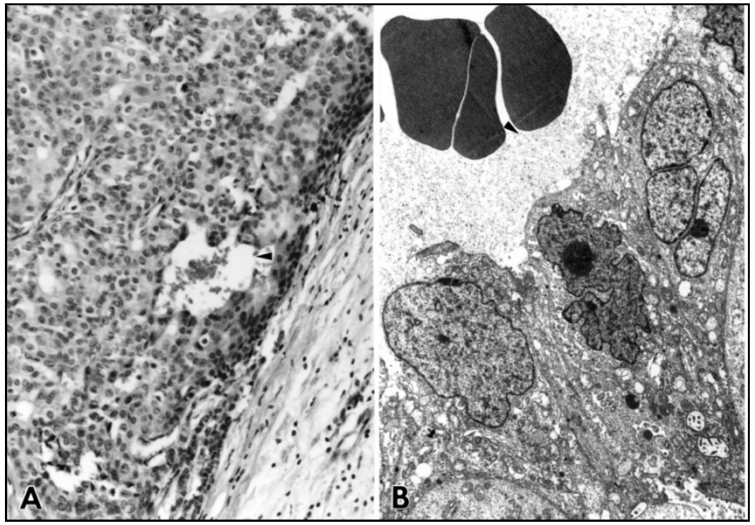
Döme et al Neuropath Exp Neurol,2003

Tumoral Sinuses in Melanoma and Breast Cancer



Kellner B.





Tímár et al.POR,2000