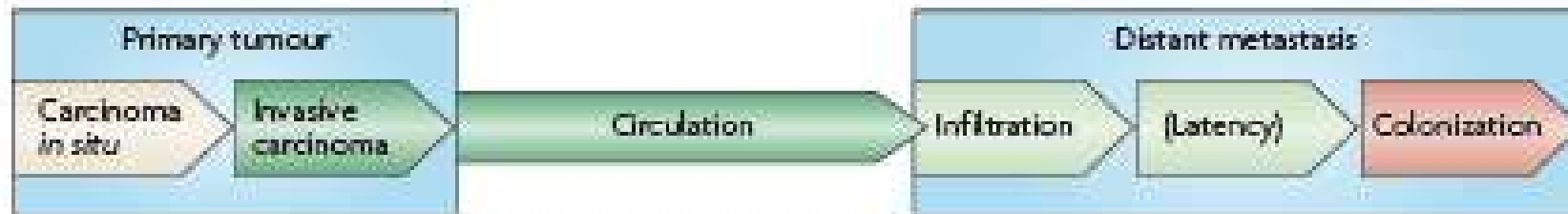


Invasion and metastasis

József Tímár

Cancer Progression



Tumour initiation functions: growth, survival, progenitor-like state and genomic instability

Oncogenes: *ERBB2*, *CTNNB1* (β -catenin), *KRAS*, *PI3K*, *EGFR*, *MYC*

Tumour suppressors: *APC*, *TP53*, *PTEN*, *BRCA1*, *BRCA2*

Metastasis initiation functions: invasion, angiogenesis, marrow mobilization and circulation

Gain of *TWIST1*, *SNAI1*, *SNAI2*, *MET*, *IC1*,

Loss of *KISS1*, *miR-126*, *miR-335*, *DARC*, *GPR56*

Metastasis progression functions: extravasation, survival and reinitiation

PTGS2, *EGF*, *MMP1*, *LOX*, *ANGPTL4*, *CCL5* targets

Metastasis virulence functions: organ-specific colonization

PTHrP, *IL11*, *CSF2RB* (GM-CSF), *IL6*, *TNF α*

Origin of metastatic cancer cell

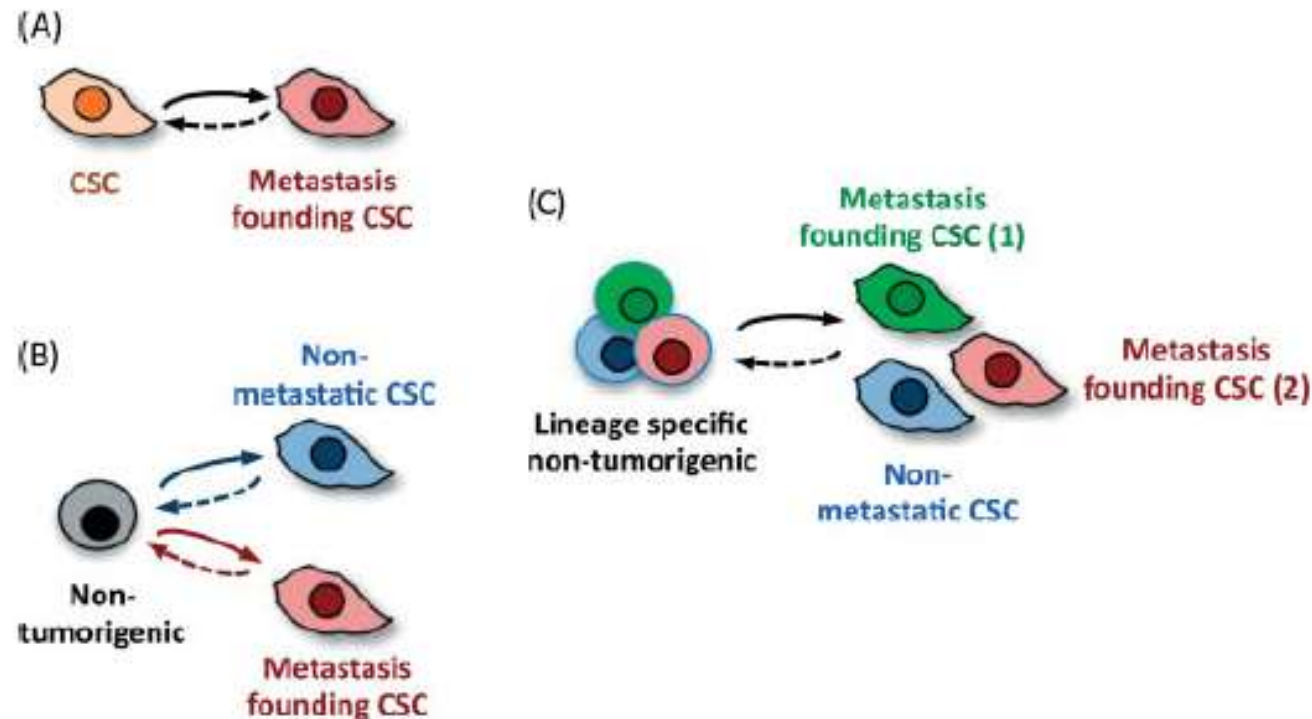


Figure 2. Simplified models for the origins of metastasis-founding cells. (A) A cancer stem cell (CSC) possesses the ability to propagate a tumour yet requires additional genetic changes (solid arrows) to metastasize. These alterations can also be epigenetic and hence reversible (dotted arrows). (B) Different CSC subpopulations arise due to distinct sets of oncogenic changes in non-tumorigenic cells. These different CSCs may all propagate a tumour but the subpopulations will vary in their metastatic competence, depending on the initial pathways that lead to their emergence. (C) Further heterogeneity may be observed within metastasis-initiating CSCs (subpopulation 1 versus 2). Differences in phenotypes could include organ tropism or differential response to therapies in specific tissues. Moreover, the plasticity of metastasis-founding CSCs could be predicated by the lineage of the premalignant cell of origin.

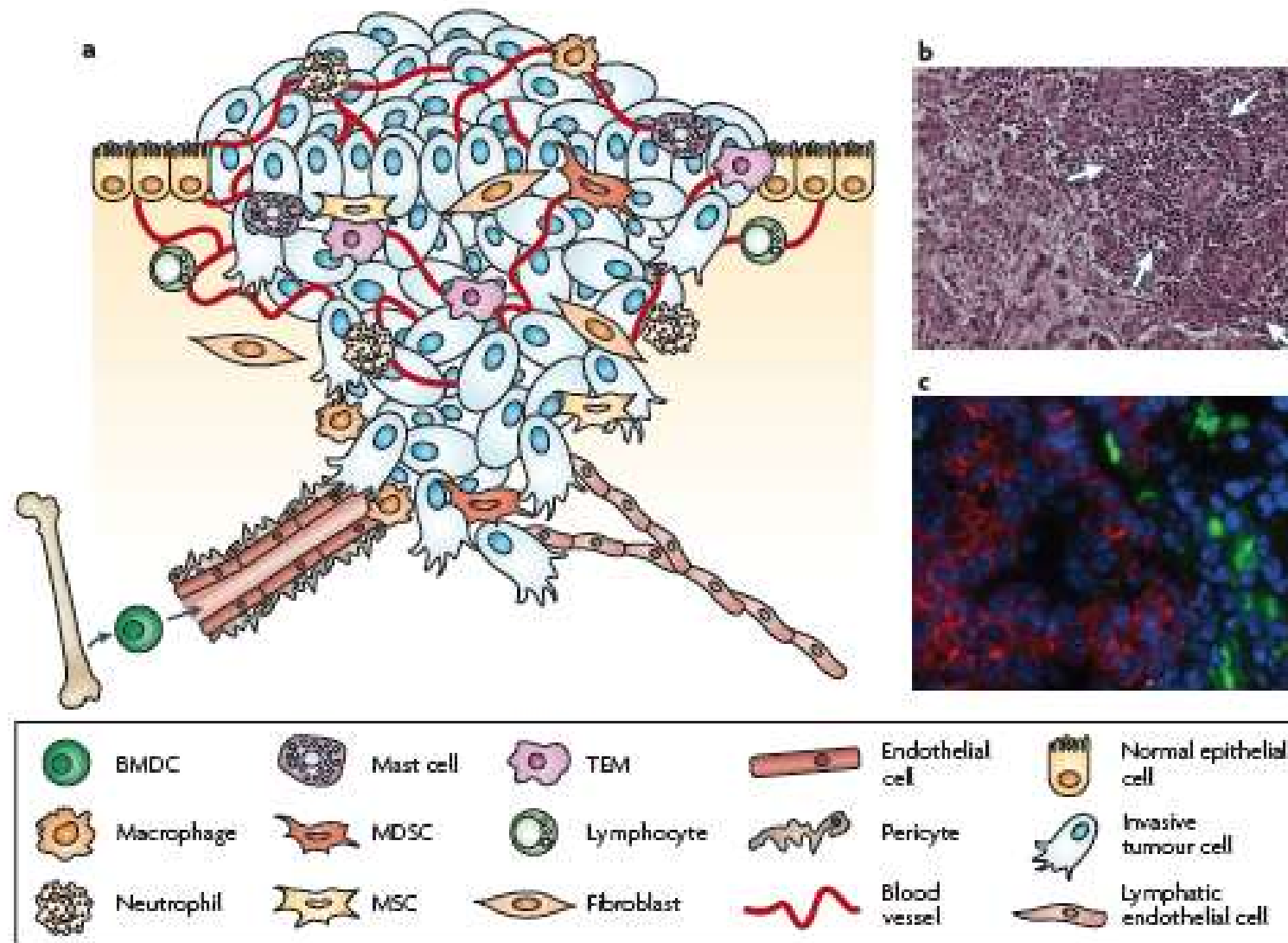
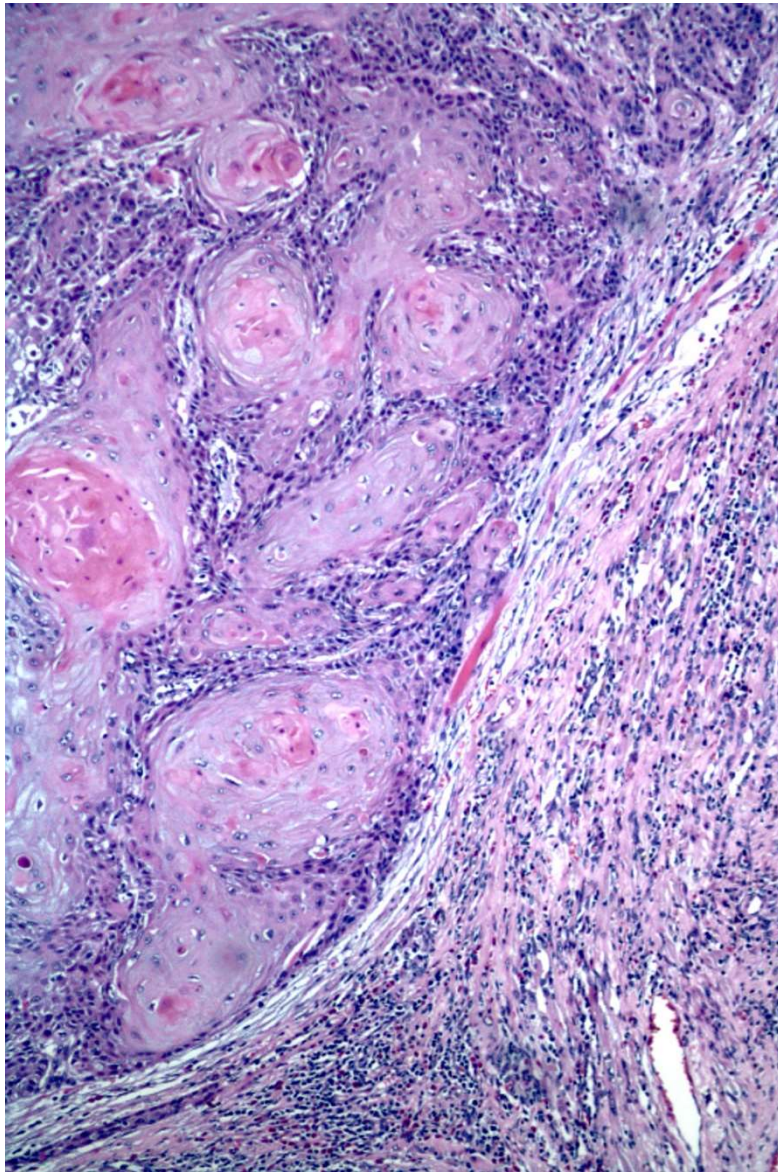
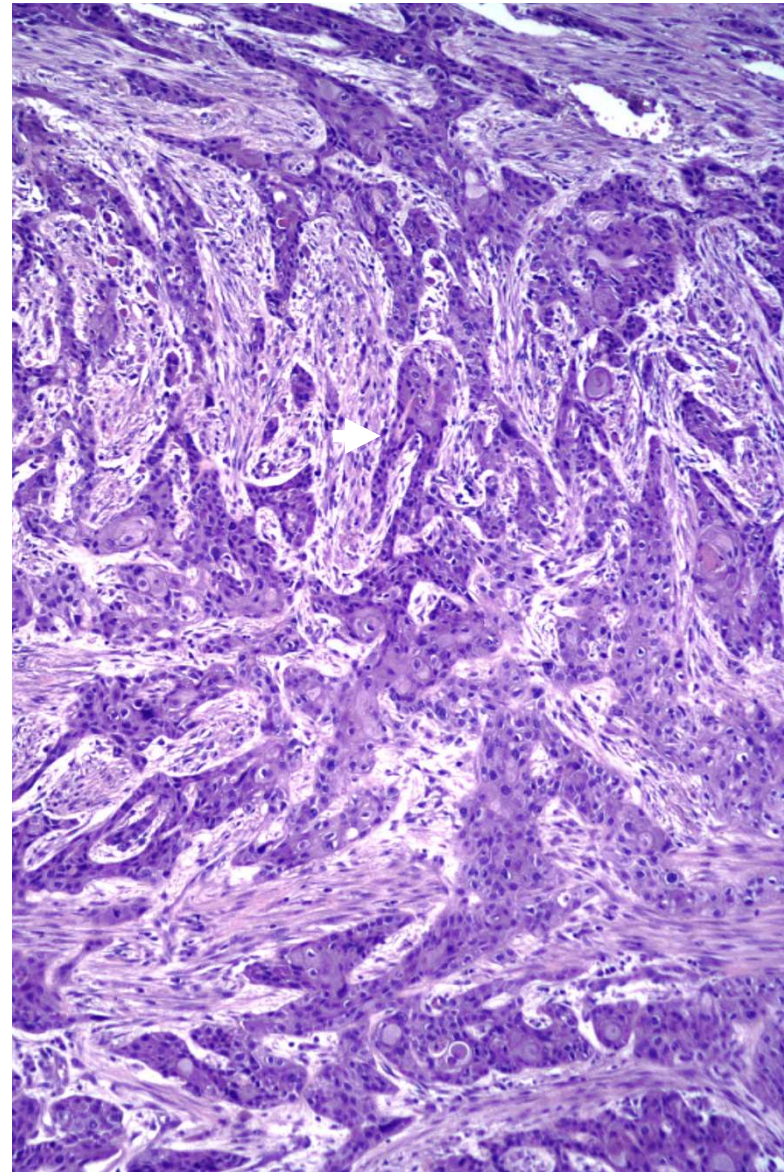


Figure 1 | The primary tumour microenvironment. a | Cancer cells in primary tumours are surrounded by a complex microenvironment comprising numerous cells including endothelial cells of the blood and lymphatic circulation, stromal fibroblasts and a variety of bone marrow-derived cells (BMDCs) including macrophages, myeloid-derived suppressor cells (MDSCs), TIE2-expressing monocytes (TEMs) and mesenchymal stem cells (MSCs). b | Invasive human breast cancer stained with haematoxylin and eosin, in which a prominent infiltration of leukocytes (indicated by white arrows) is evident at the invasive margin. c | Macrophages at the invasive edge of pancreatic islet cancers express cathepsin B (green), which is associated with loss of epithelial cadherin (red) on the neighbouring cancer cells. Cell nuclei are visualized by DAPI (blue). Part c reproduced, with permission, from REF. 151. © (2006) Cold Spring Harbor Laboratory Press.

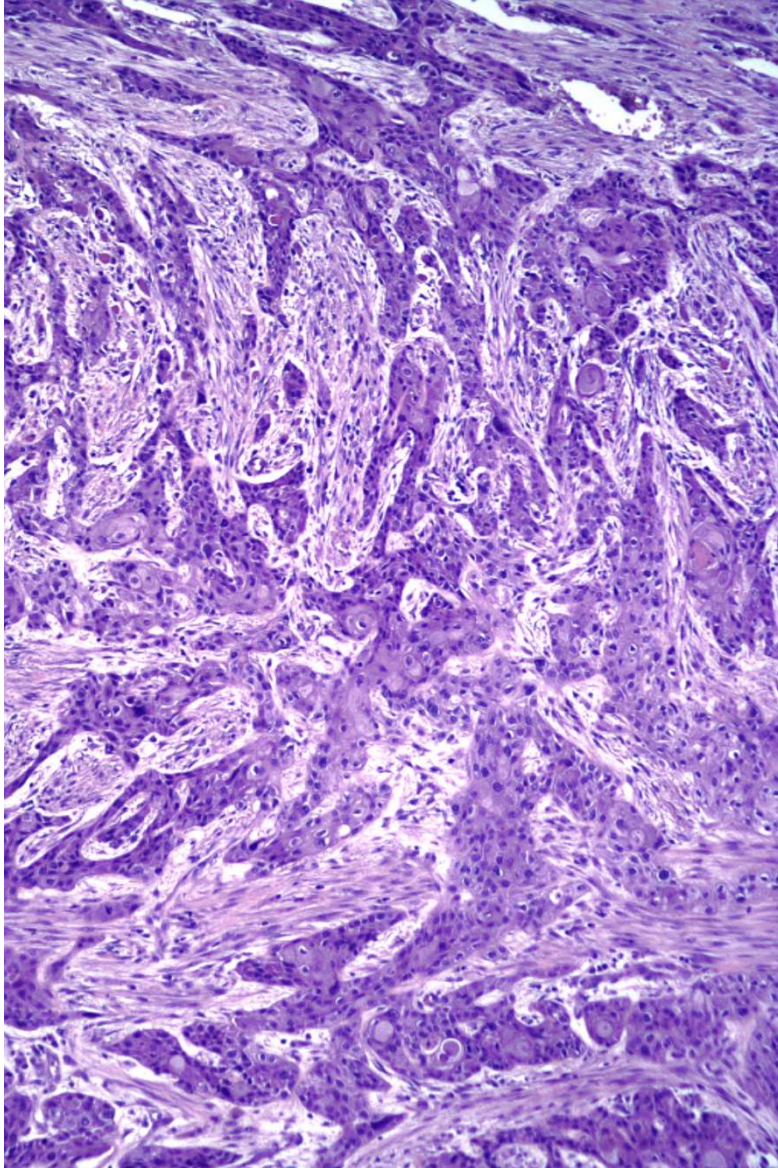
Compressive growth



Invasive growth



Local Invasion



- **Detachment from the tumor nest**
(decrease in cell adhesion)
- **Recognition of ECM**
(integrin expression)
- **Defradation of basement membrane
And interstitial stroma**
(metalloprotease, cathepsin, uPA)
- **Migration in the degraded matrix:**

toward capillaries
toward lymph vessels
toward nerves

- **Intraluminalis dissemination !!!!!!!!!!!**
(Pleura, peritoneum etc.)

Basic characteristics of invasive cancer cells

Altered matrix interaction **adhesion**

Integrins
CD44, HSPG
cell adhesion molecules

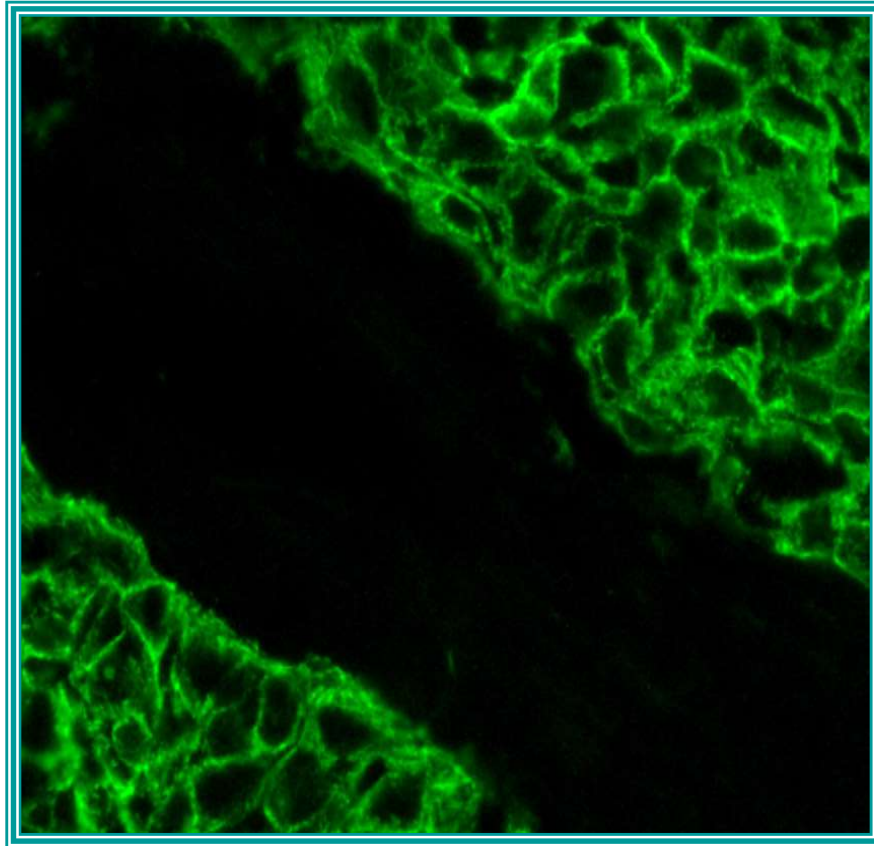
Degradation

metalloproteases (Zn)
cathepsins
plasminogen activators
heparanase

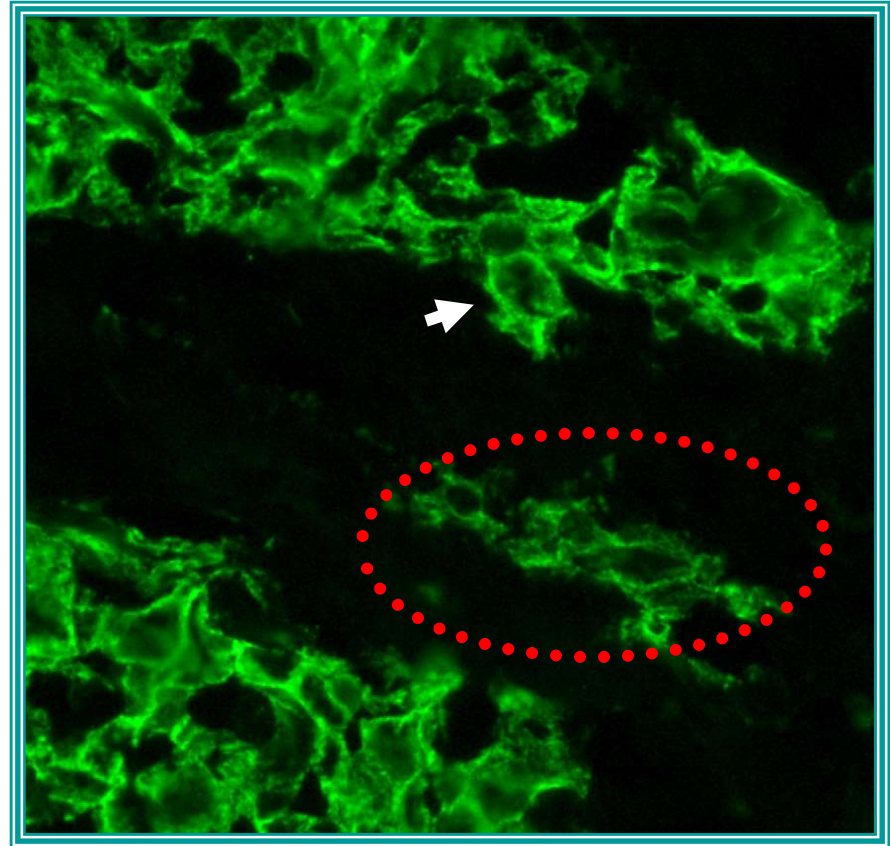
MIGRATION

adhesion/detachment
motogenic signaling
cytoskeletal rearrangement

Non-invasive cancer



Invasive cancer



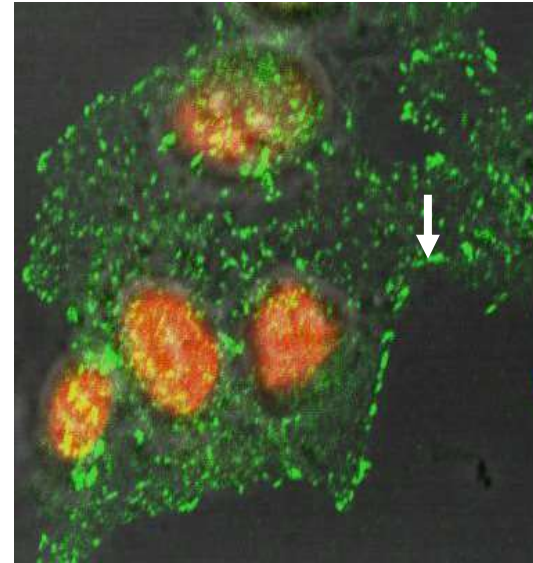
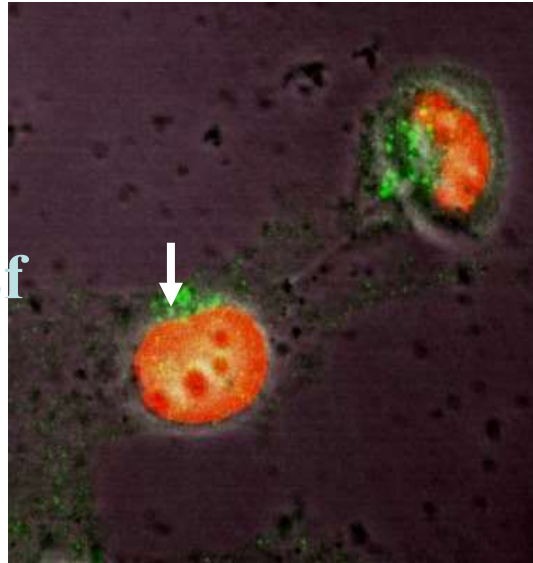
CD44v3 matrix receptor

α IIb

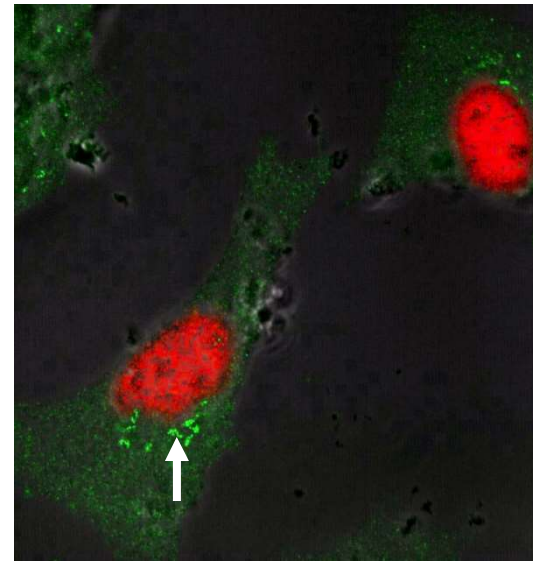
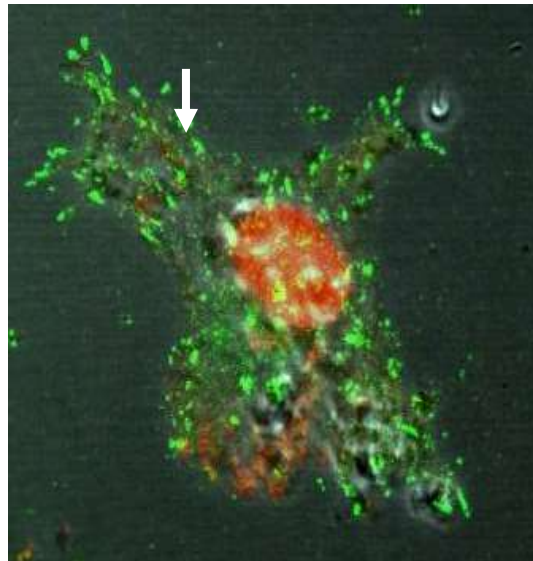
integrin

α v

Mock transf

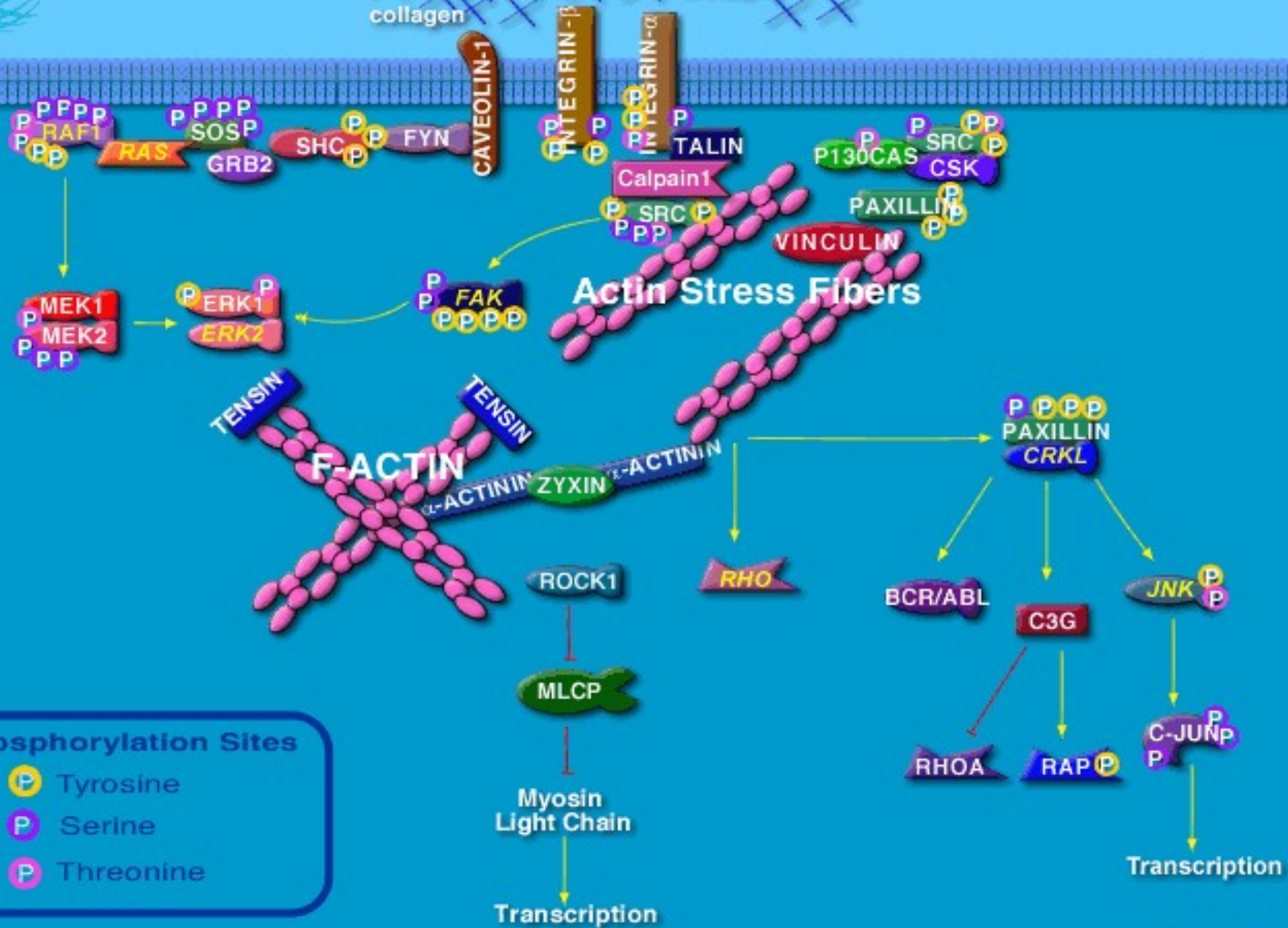


1990High



INTEGRIN SIGNALING

Extracellular

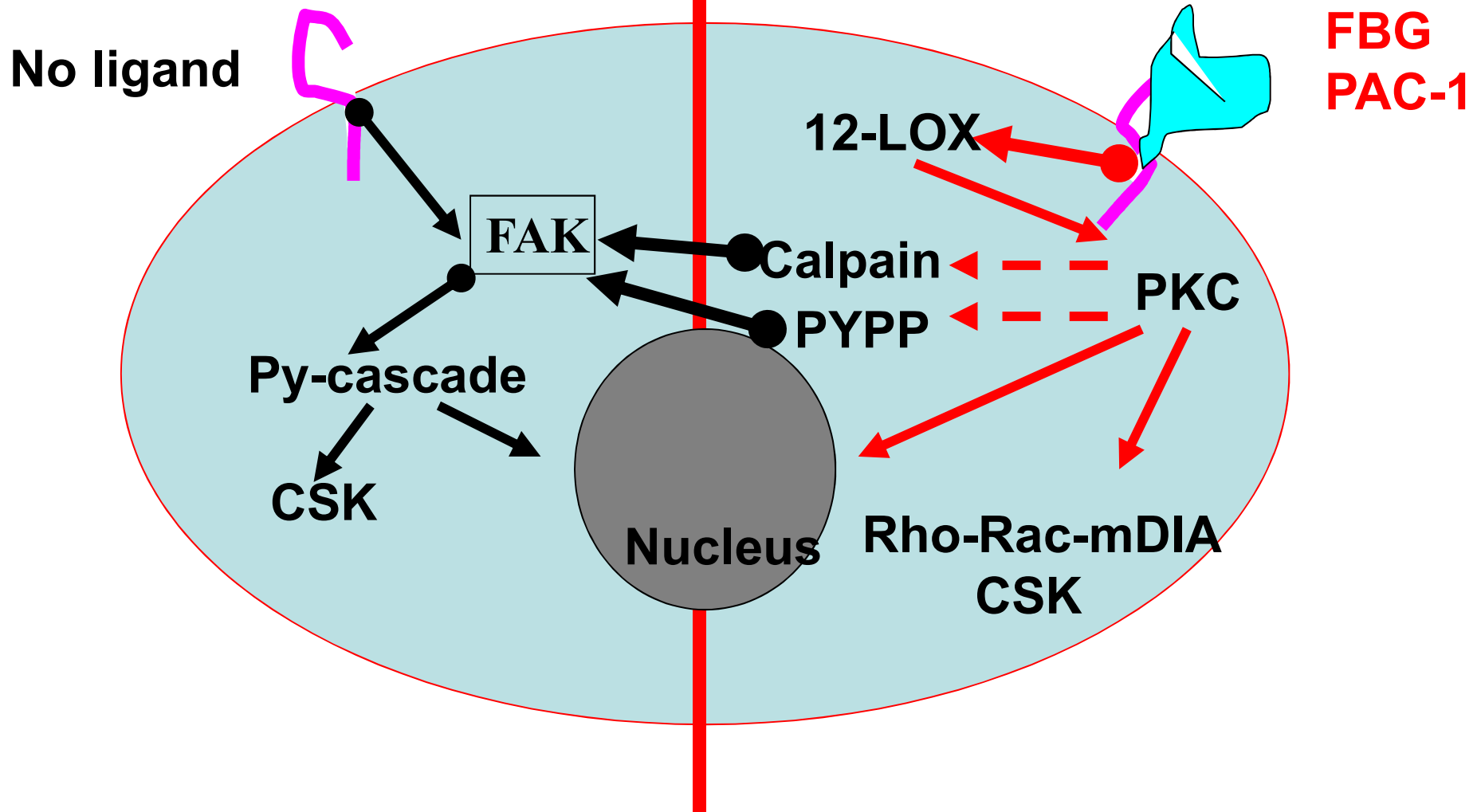


Cytoplasm

Integrin pathology

- ☐ α IIb és/vagy β 3 mutáció: thrombasthenia/vérzékenység
- ☐ β 2 integrin mutáció/hiány: leukocita adhézions betegség (baktériális fertőzések)
- ☐ β 4 mutáció: α 6 β 4 funkciózavar : epidermolysis bullosa
- ☐ α v β 3(/ α v β 5): (retinopátia) neoangiogenezis
- » osteoporosis
- » HIV fertőzés
- ☐ α v β 3: tumor-indukált neoangiogenezis
- ☐ α 5 β 1 elvesztés: carcinogenezis
- Illegitim integrin expresszió: tumor progresszió
- Integrin mutáció/konstitutív „ON”: tumor progresszió
- Integrin splice variáns predominancia: tumor progresszió

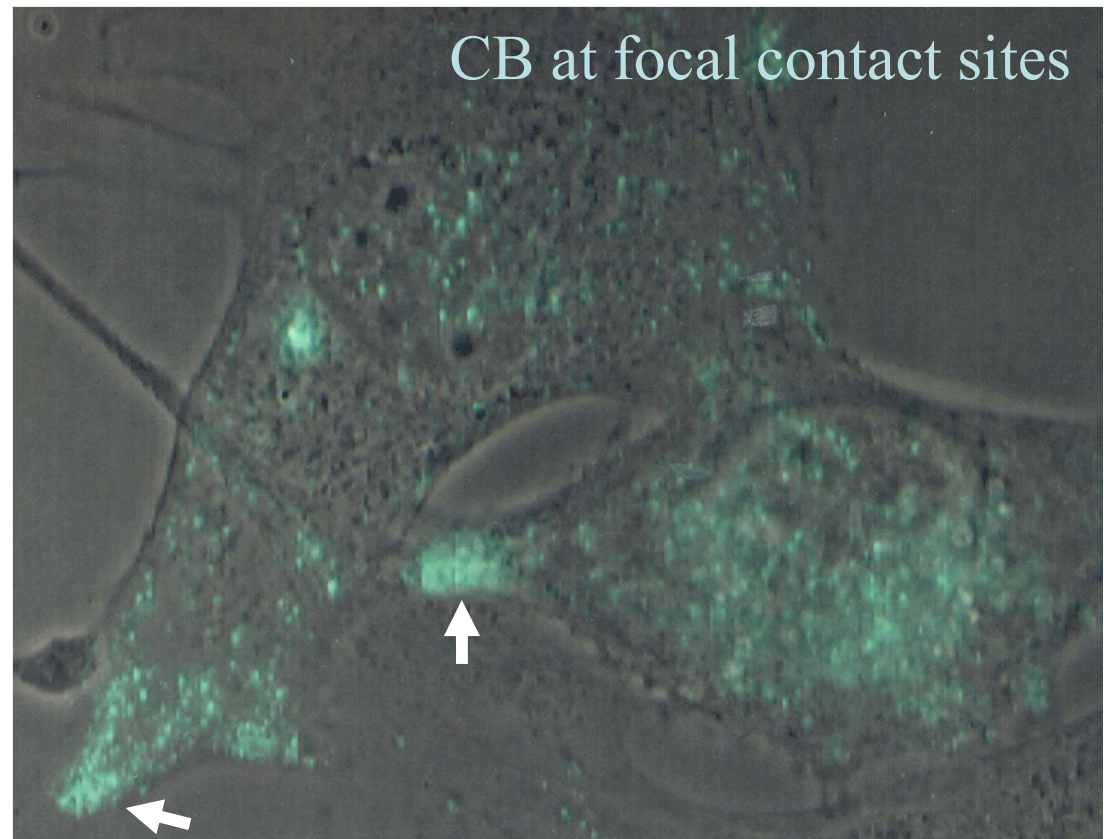
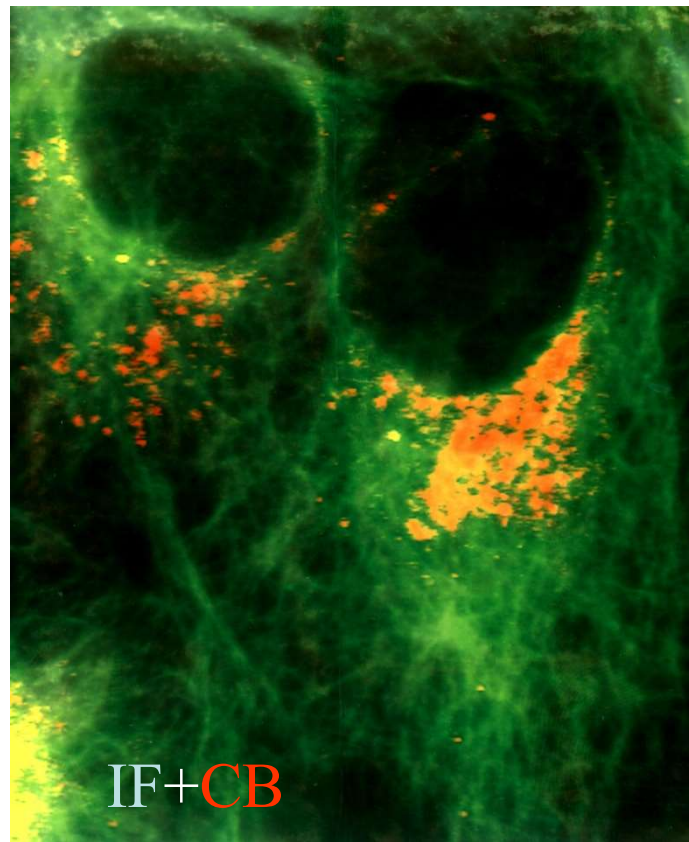
Signaling of the ectopic $\alpha\text{IIb}\beta\text{3}$ integrin



Proteases

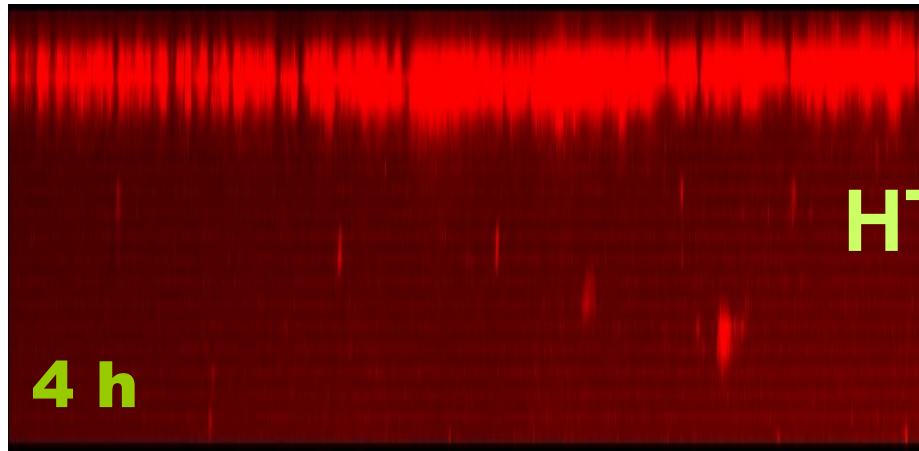
- **MMP (matrix metalloproteases, 30) / TIMP**
- **cathepsins (cysteine proteases) / stefins**
- **Serine proteases (plasminogen activators)**
- **PAI**
- **Protease ---- protease inhibitor**

Dynamics of Cathepsin B secretion in cancer cells

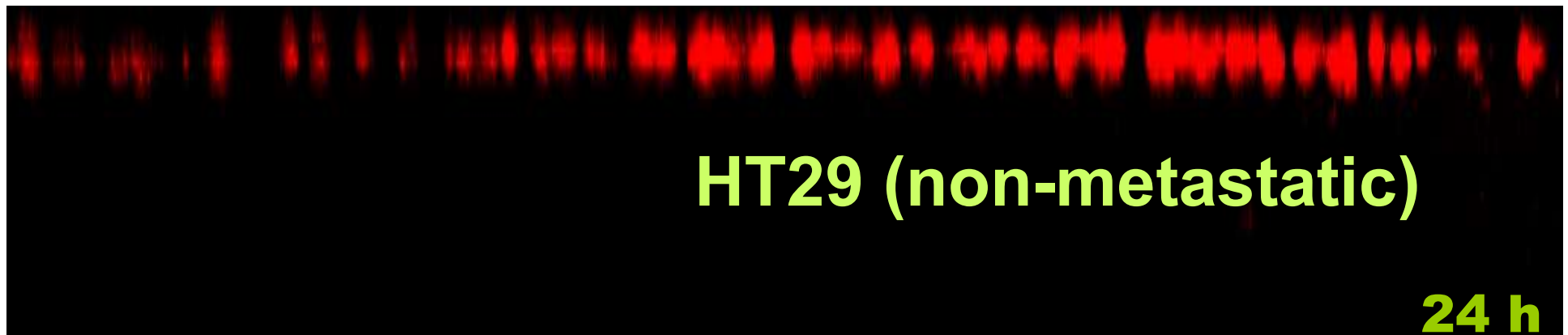
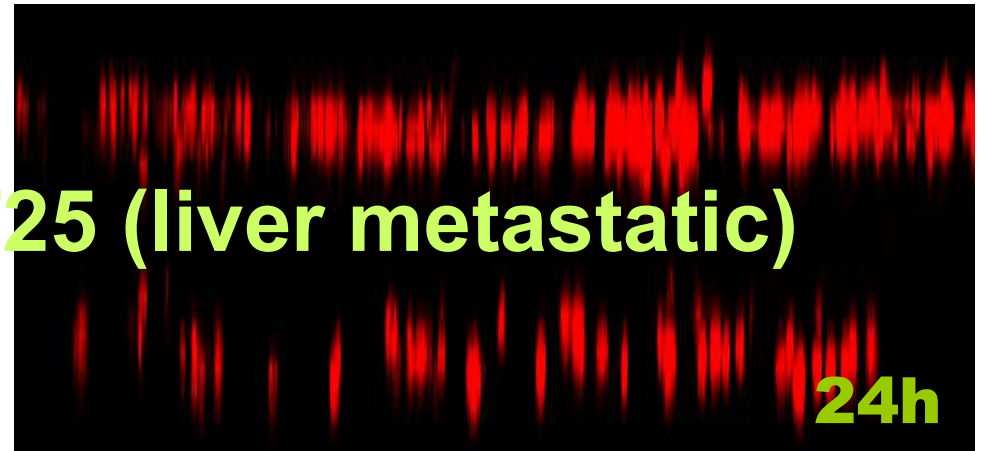


Honn KV, Tímár J et al. Exp Cell Res, 1994

Microinvasive potential of human colon cancer cell lines

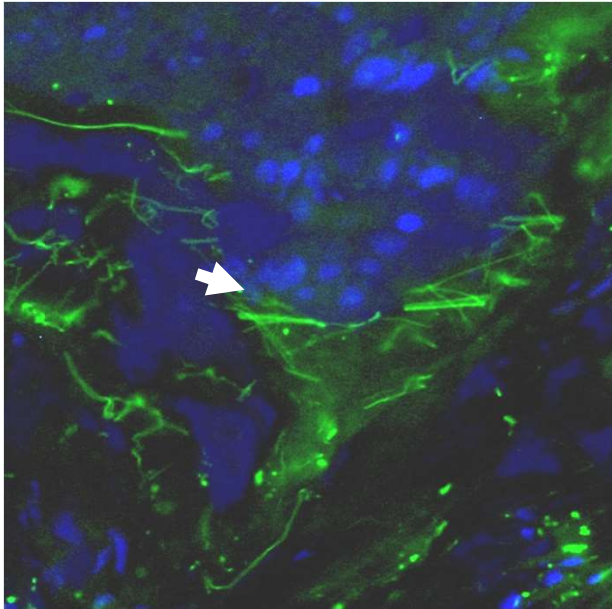


HT25 (liver metastatic)

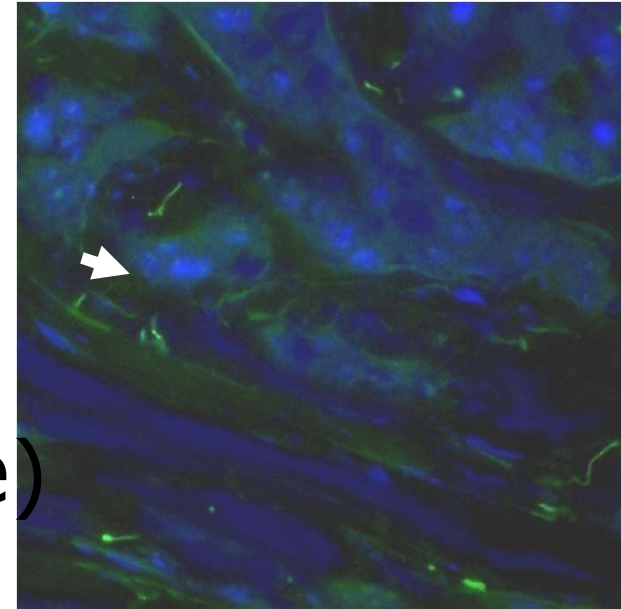


HT29 (non-metastatic)

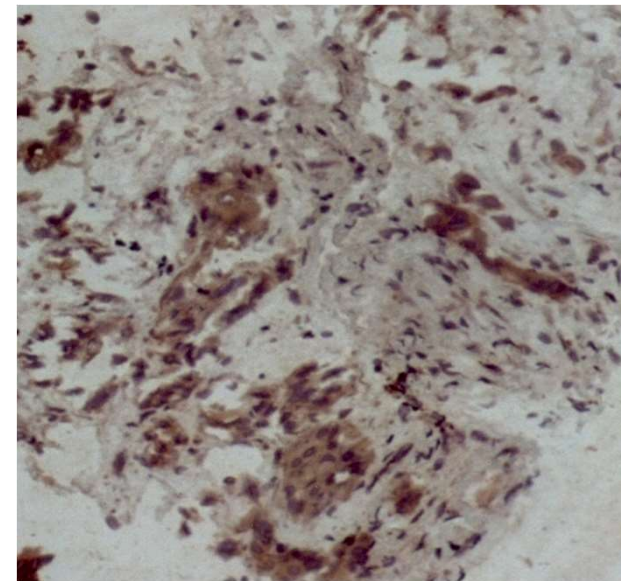
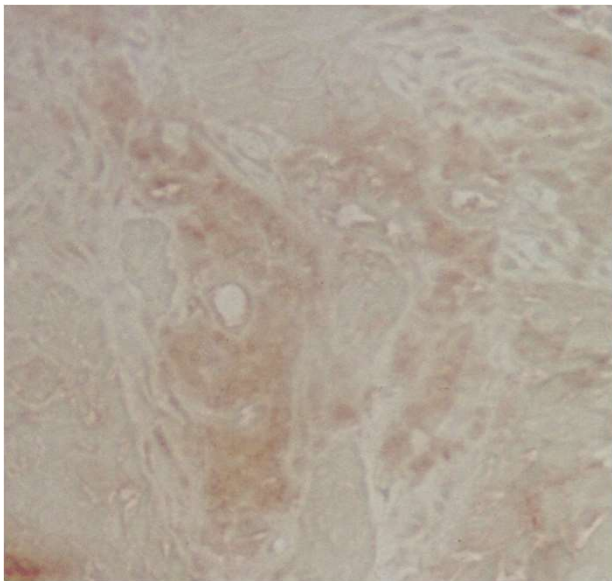
Non-invasive tumor



Invasive cancer

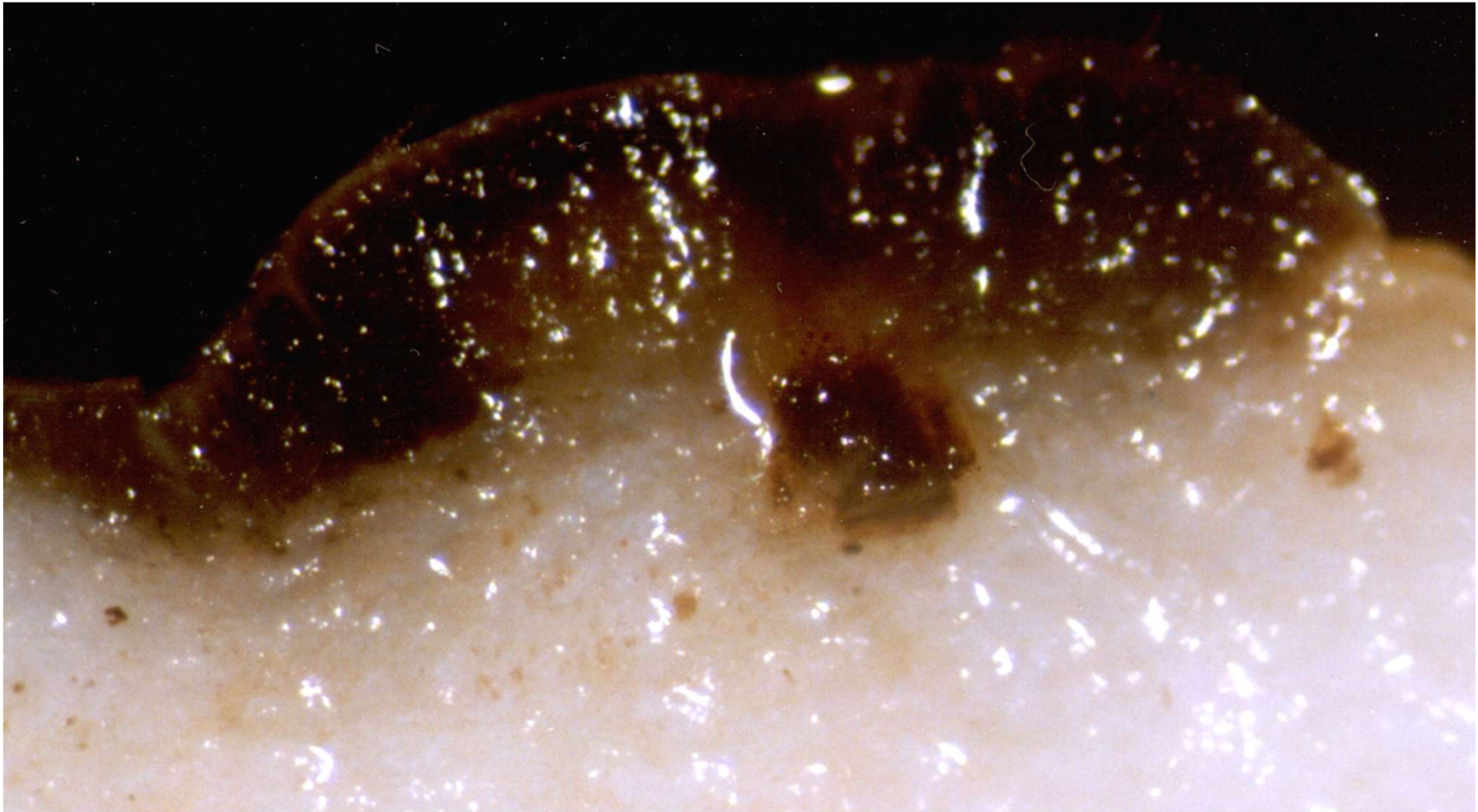


Elastin
(elastase)

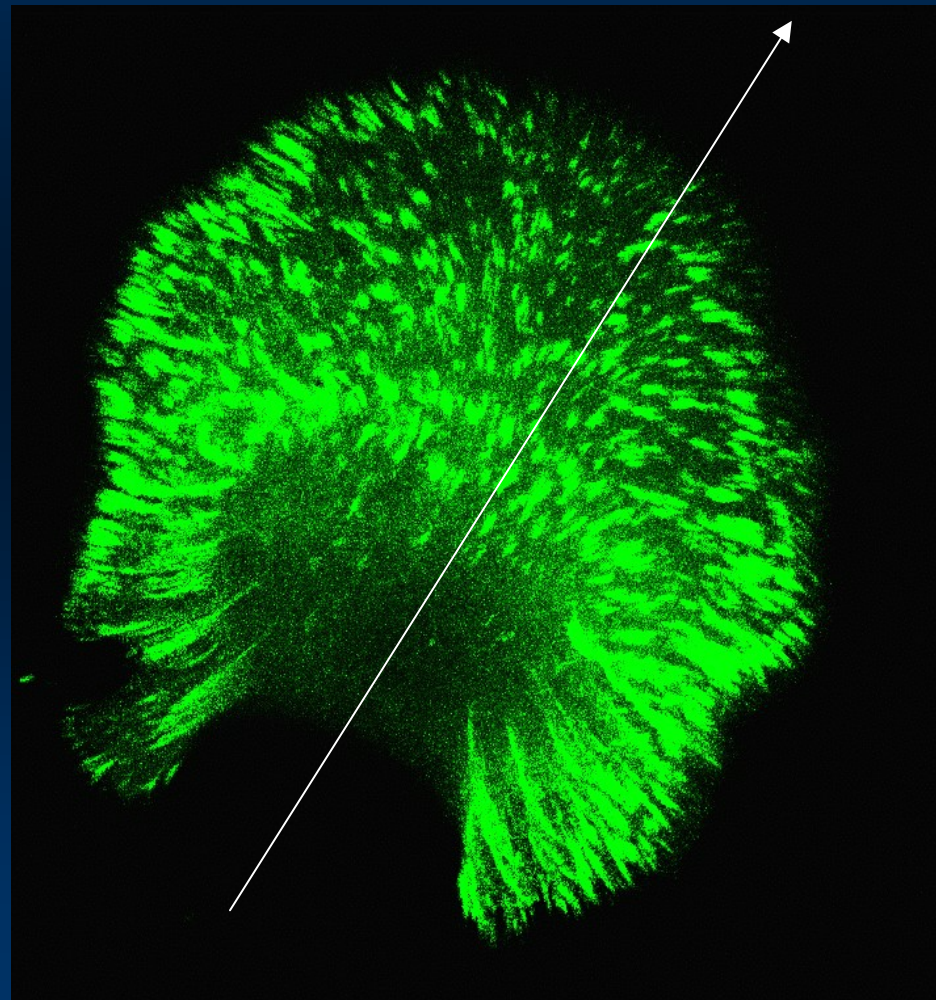


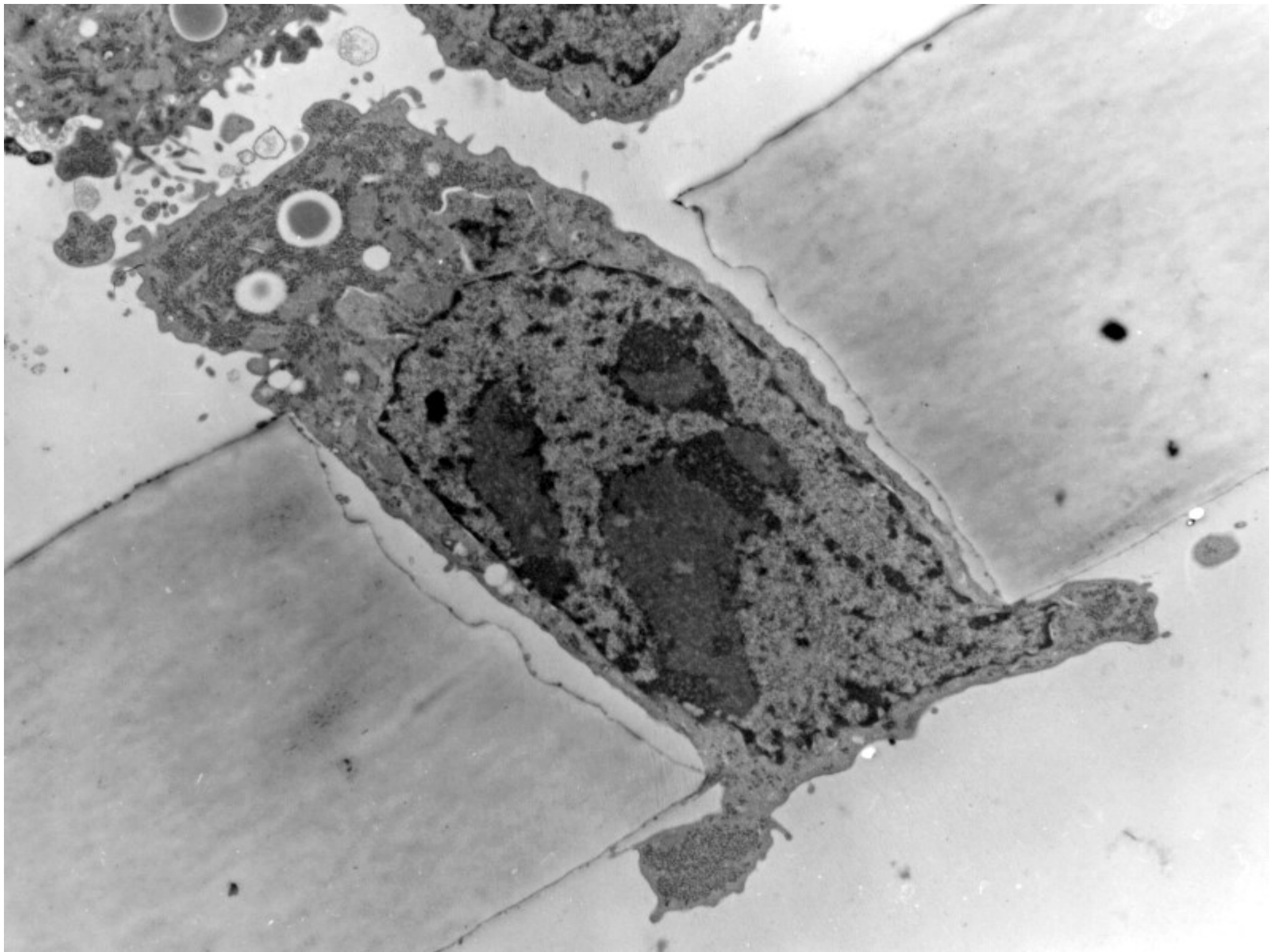
MMP-2

Local invasion (melanóma)



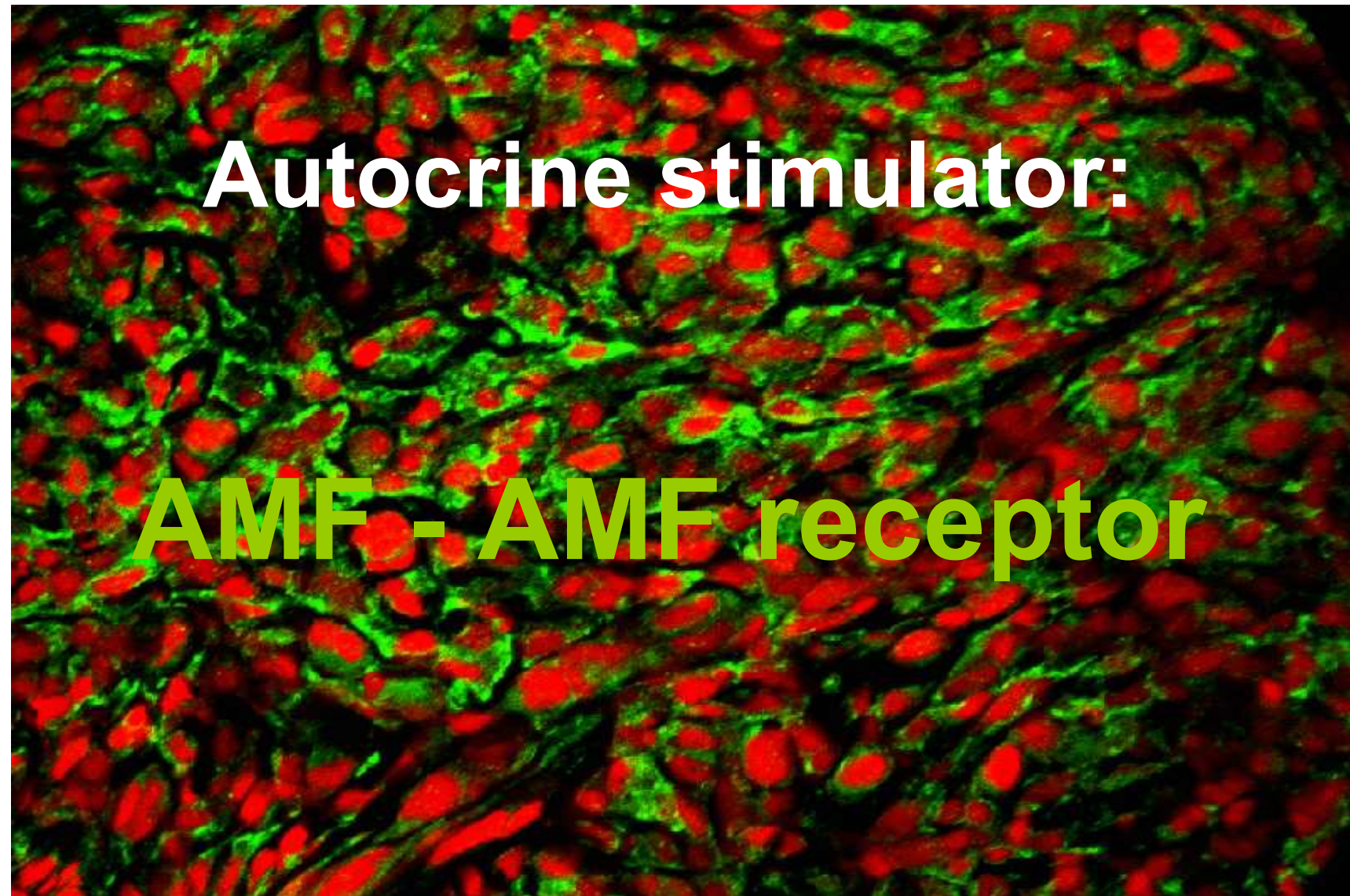
Invasion: adhesion + motility





Motility cytokines and their receptors

Citokine	effect	Receptor
AMF	Motility	gp78/p-hexose-
isomerase		
ATX	Motility	?
MSF	Motility	?
MIF	Motility	?
MCP	Motility	?
Inzulin	Prolifertion	IR
EGF	Proliferation	EGF-R, c-erb
TNF α	both (motility + proliferation)	TNF-R55, TNF-R75
TGF β	double	TGF β -R I., II.
HGF/SF	double	c-met
bFGF	double	TGF-R1-R4
GM-CSF	double	P80, p120
IL-6	double	IL-6R, gp130
PDGF	double	R α p80 , R β p170



Autocrine stimulator:

AMF - AMF receptor

Metastasis: heterotypic cellular interactions

Cancer cell – mesenchymal interactions (fibroblast..., smooth muscle etc)

Cancer cell – endothelial cell interactions (hematogenous/limphogenous)

Cancer cell - immune effector interactions (local + circulation, distant)

Cancer cell – platelet interactions (macro/microaggregation)

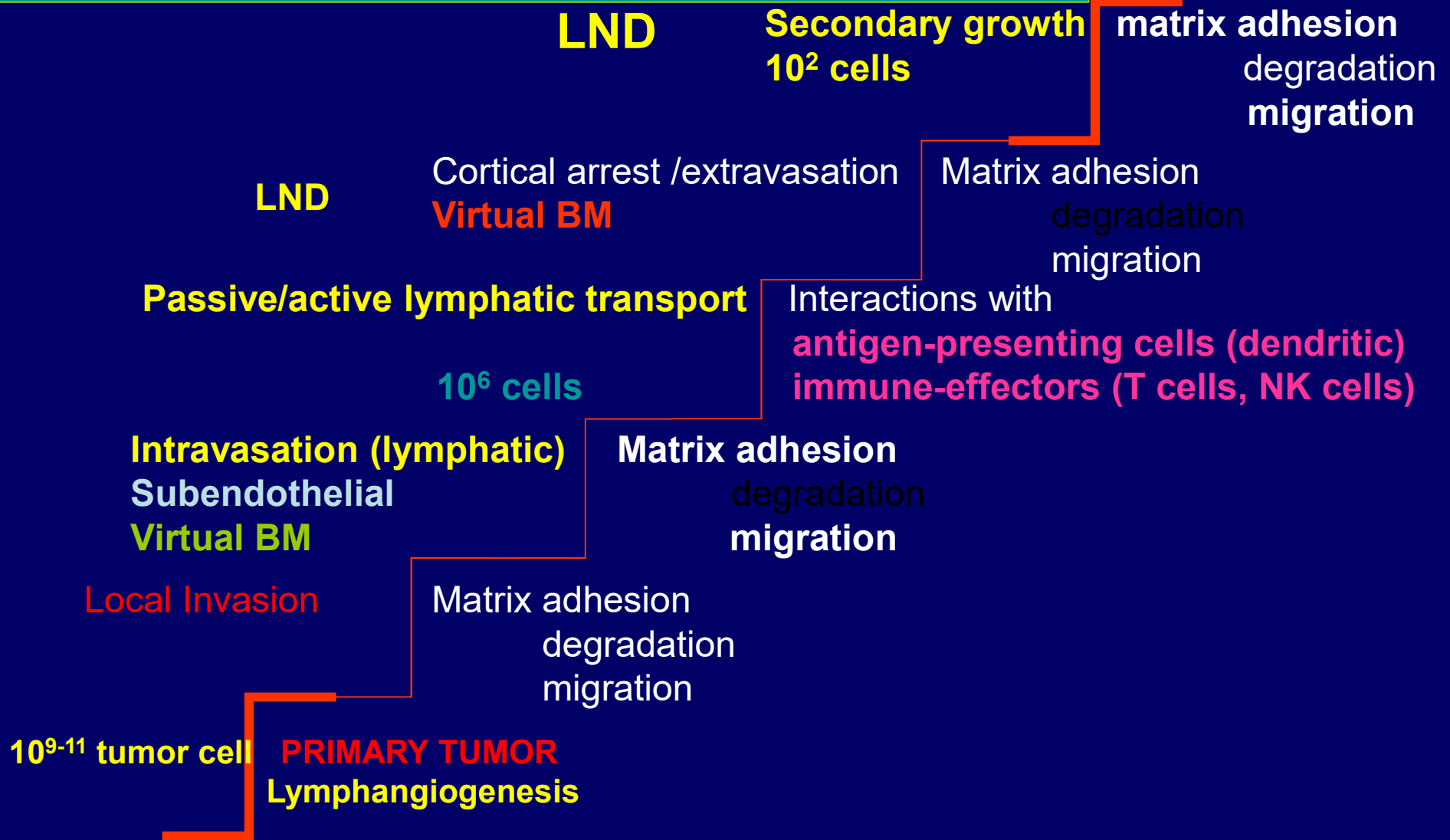
Characteristics of metastatization

Survival potential is more important than proliferative one

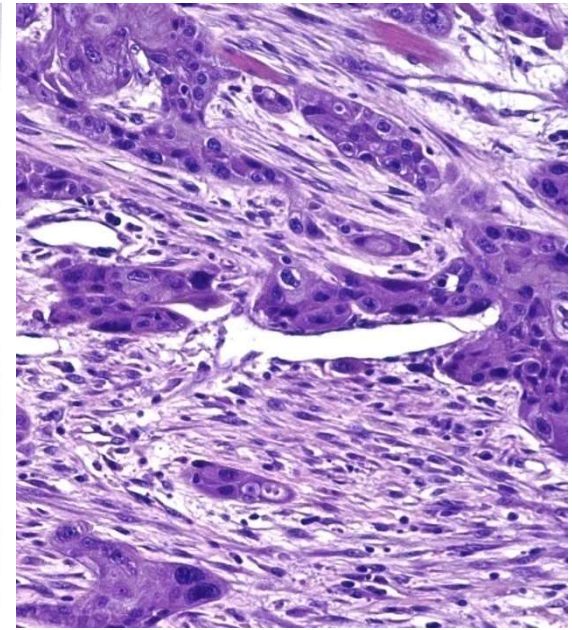
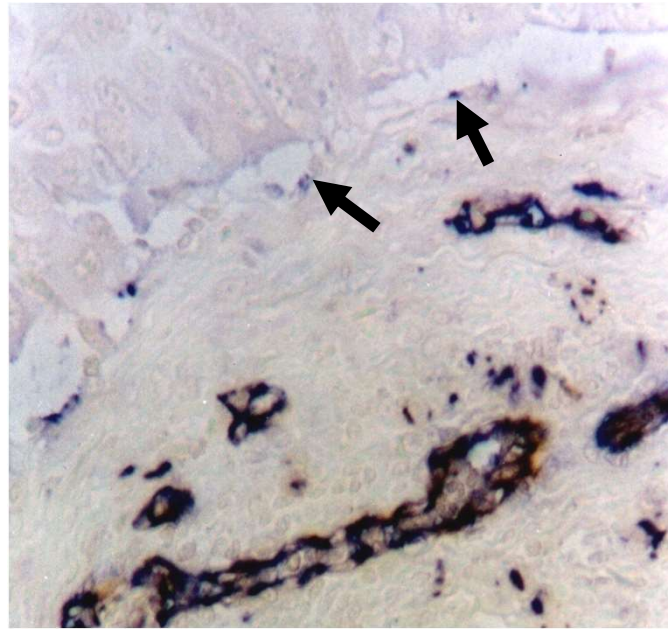
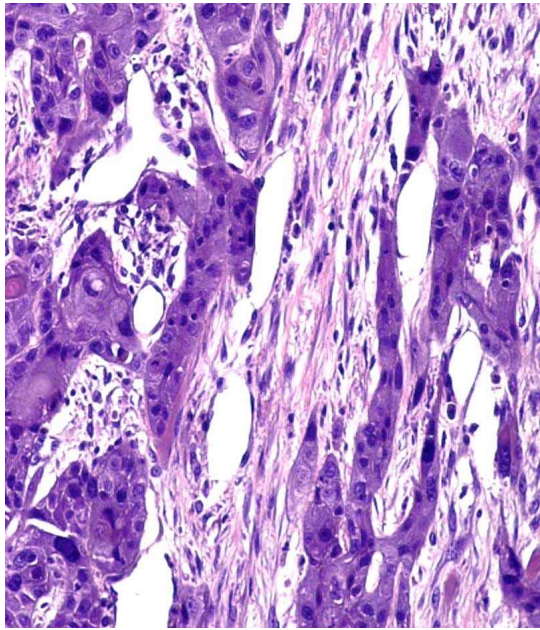
Organ selectivity

Genetic heterogeneity

Lymphatic metastatic cascade (immune selection)



Lymphatic vessels in laryngeal cancer

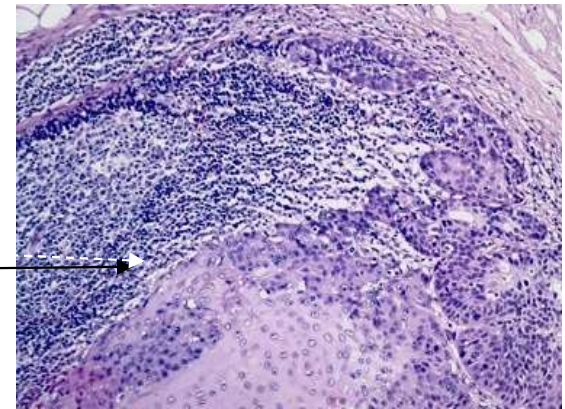


CD34, FVIII (blood vessel)

Ly vessel: CD31
LYVE (HA rec)
podoplanin



Primary tumor



regional lymph node

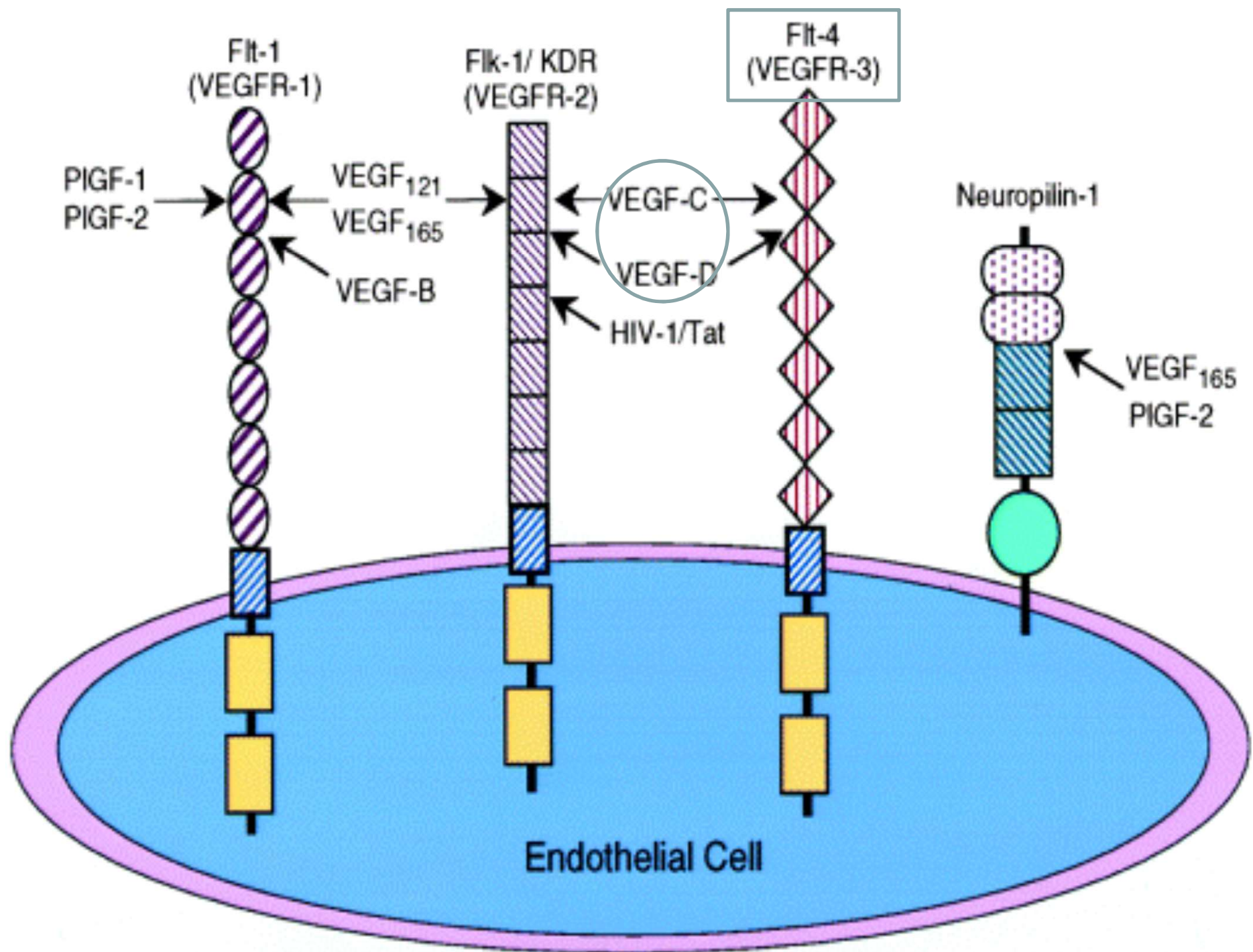
Lymphatic capillary

- No continuous basement membrane
- No pericytes

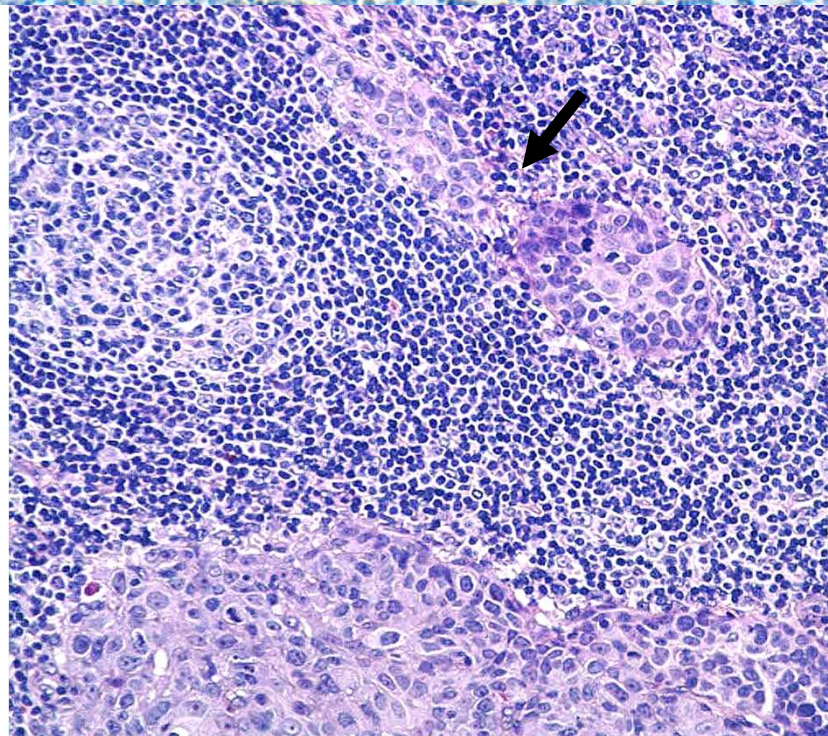
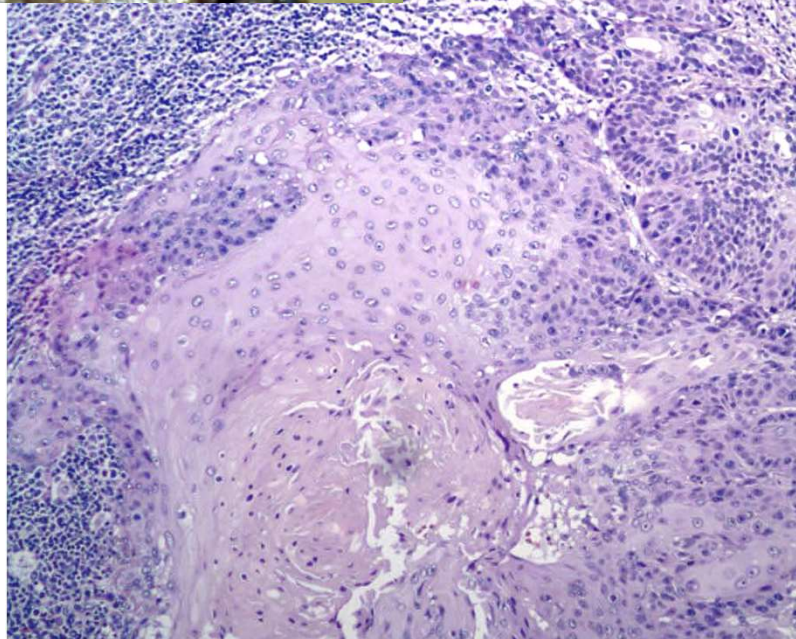
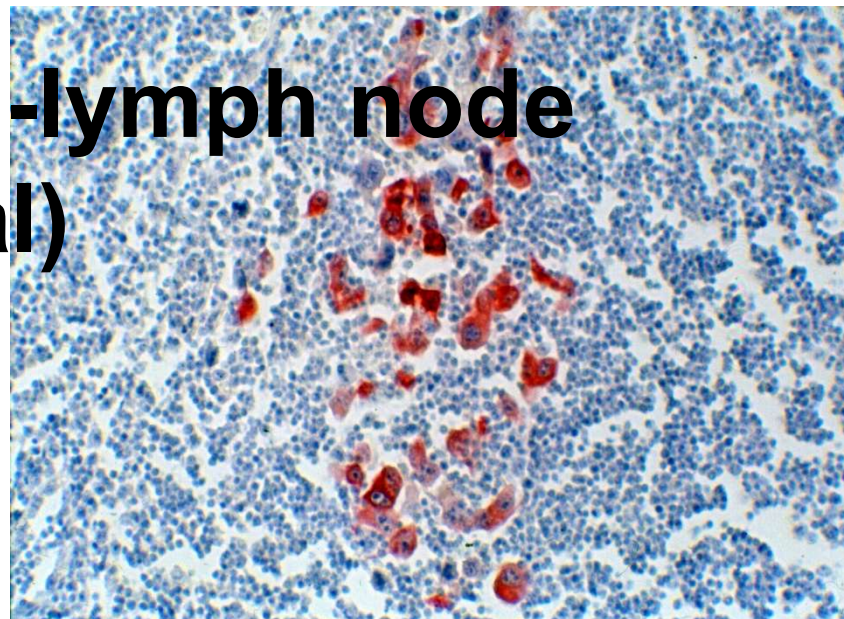
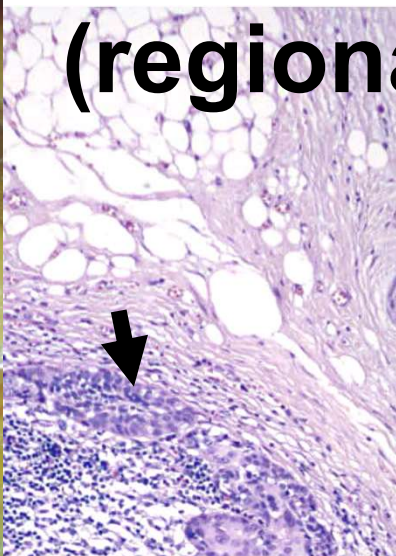
- Easy trans migratory potential

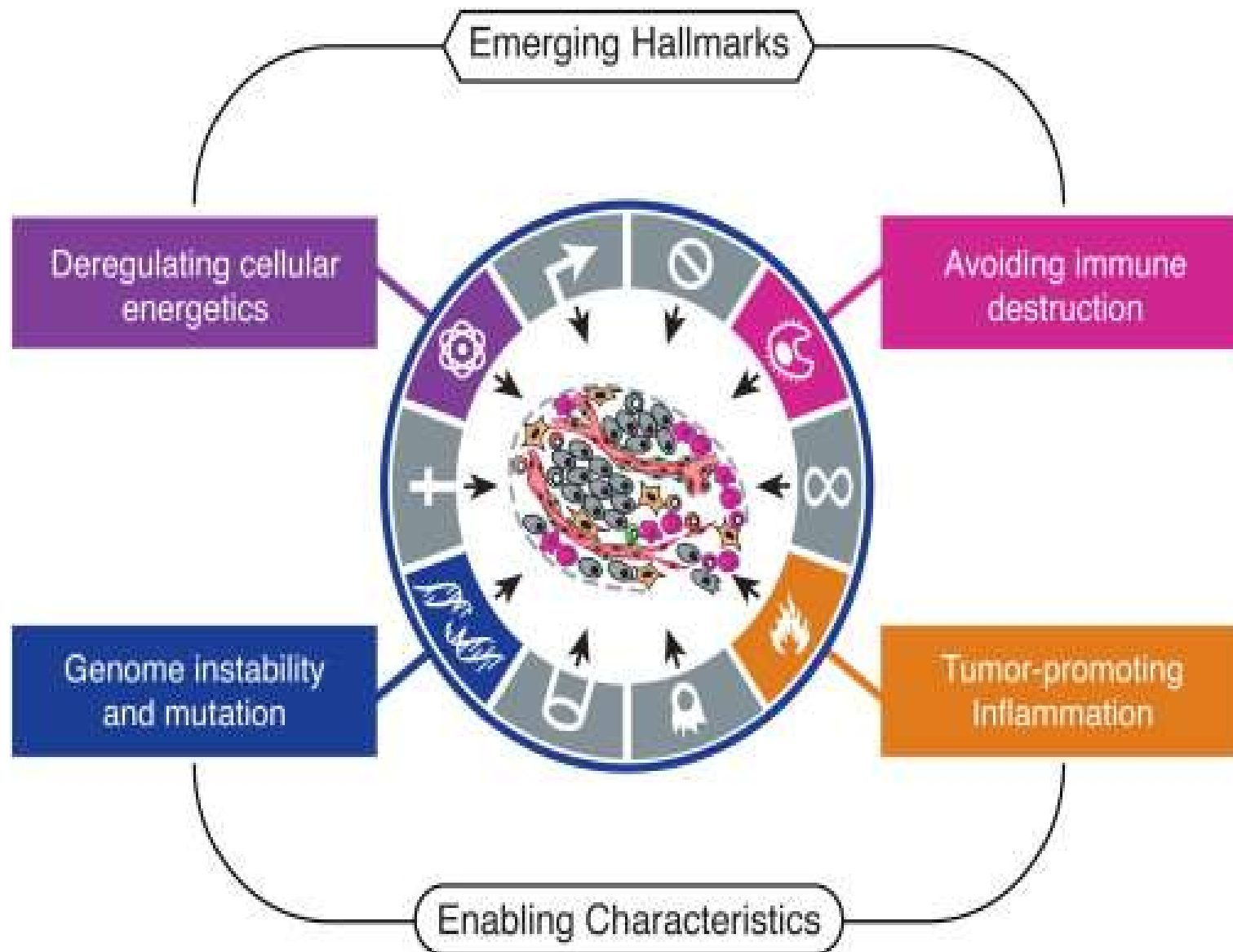
- **Marks:**

- HA receptor (lyve1)
- Desmoplakin
- Podoplanin (mucin)
- VEGFR3
- PALE



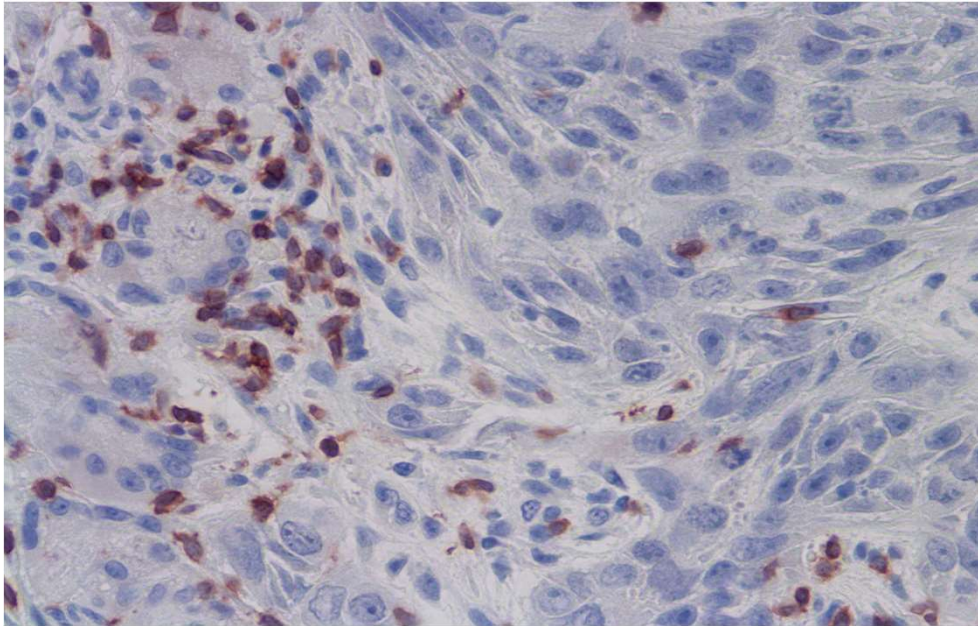
Sentinel-lymph node (regional)





Figure?3 Emerging Hallmarks and Enabling Characteristics An increasing body of research suggests that two additional hallmarks of cancer are involved in the pathogenesis of some and perhaps all cancers. One involves the capability to modify, or ...

Tumor infiltrating (lymphoid) cells: TIL

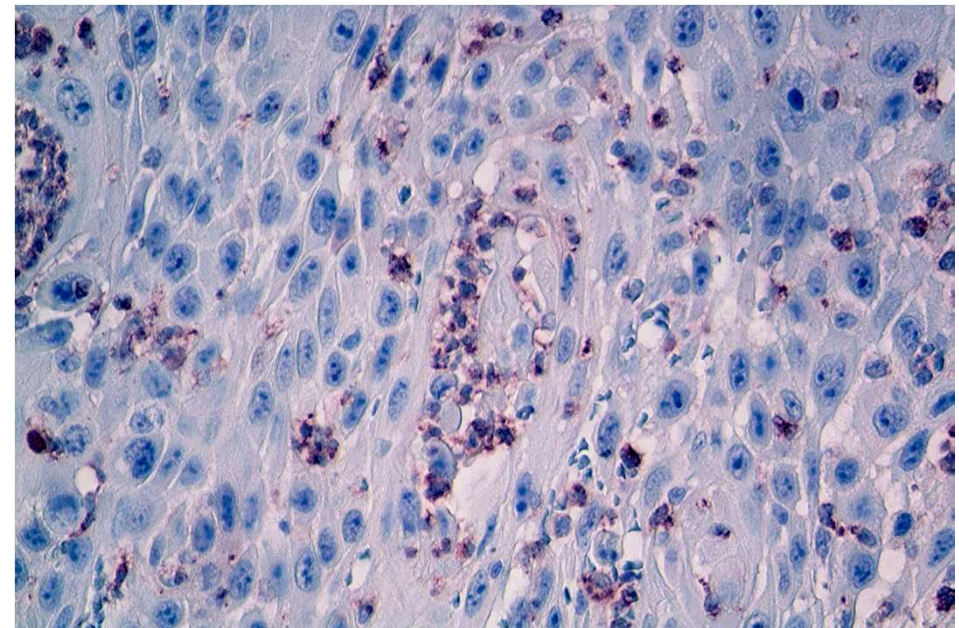


T cells

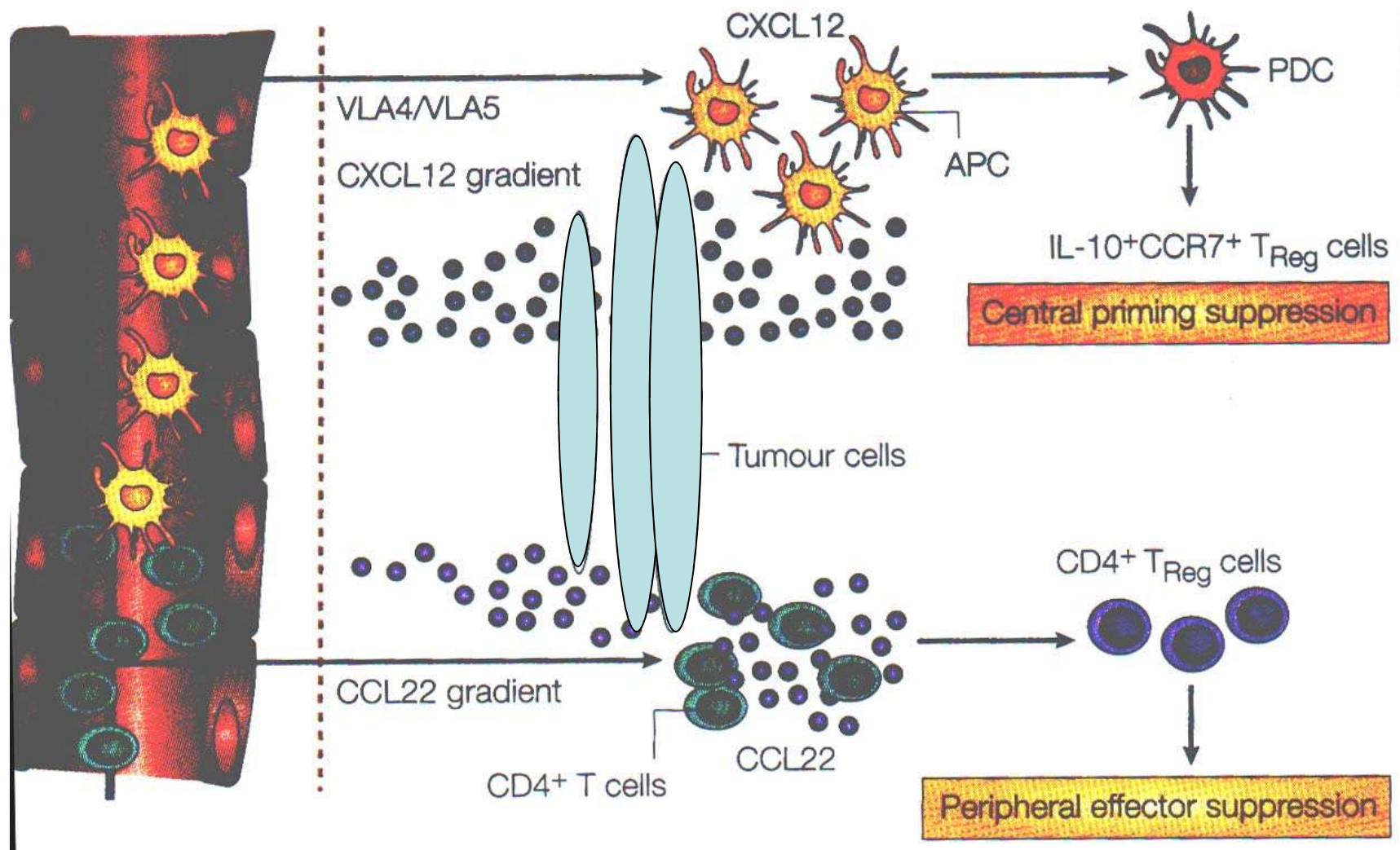
B cells

Macrophages

neutrophils

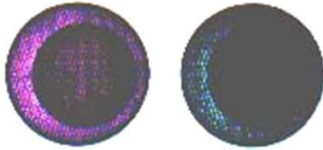


Immunosuppression by CANCER



Immunosuppression by CANCER

Effector T cells

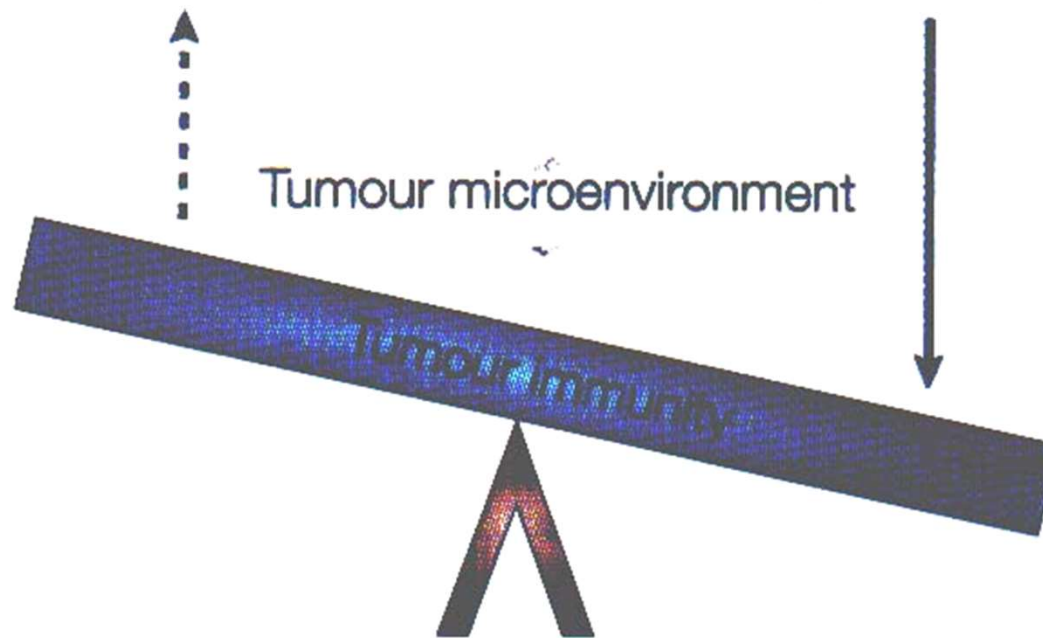


TAA-specific effector CTL
TAA-specific effector CD4⁺ T cells

Regulatory T cells



IL-10⁺ suppressive T cells
CD4⁺CD25⁺ T_{Regs}



Haematogenous metastatization

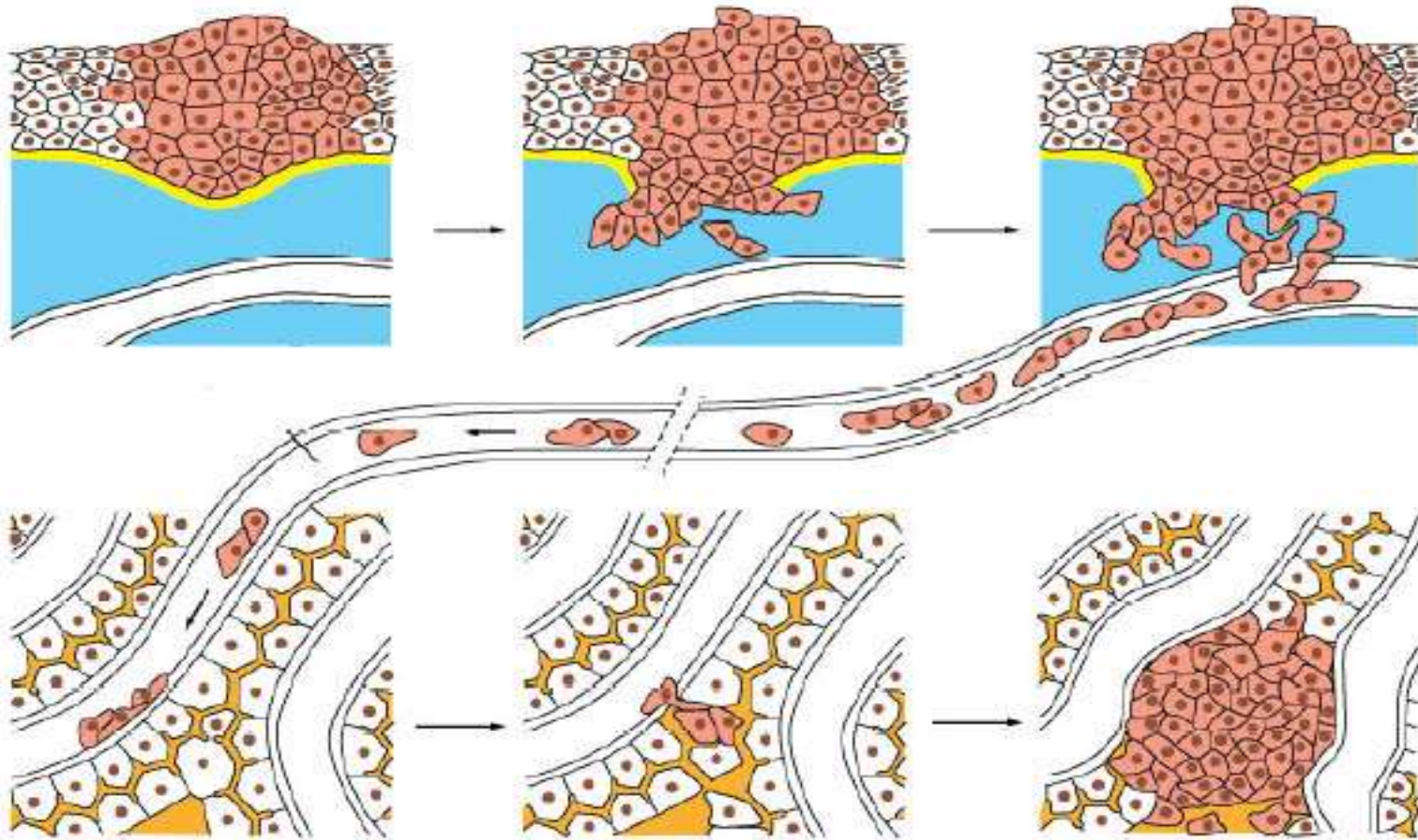
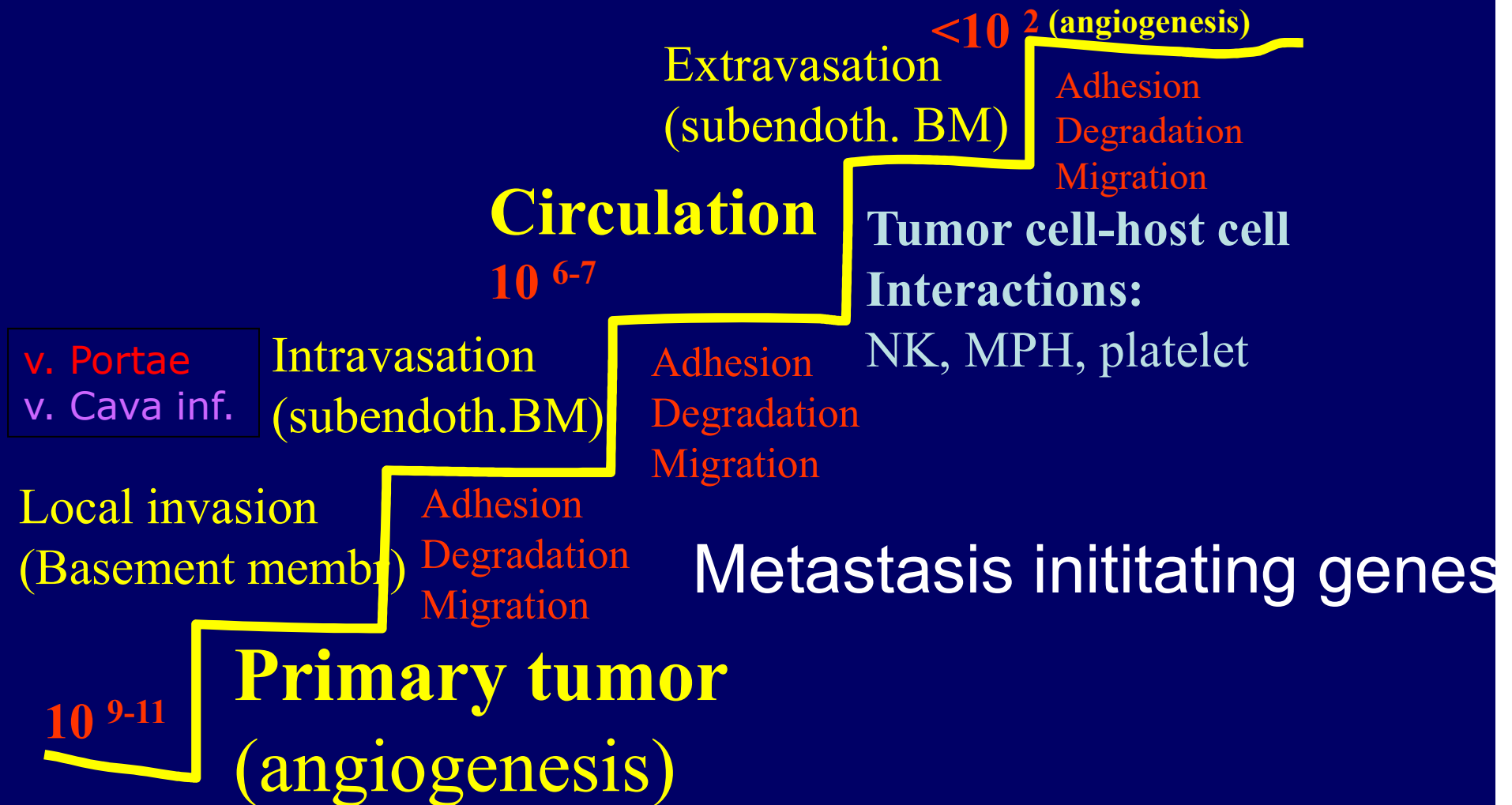


Figure 23-15. Molecular Biology of the Cell, 4th Edition.

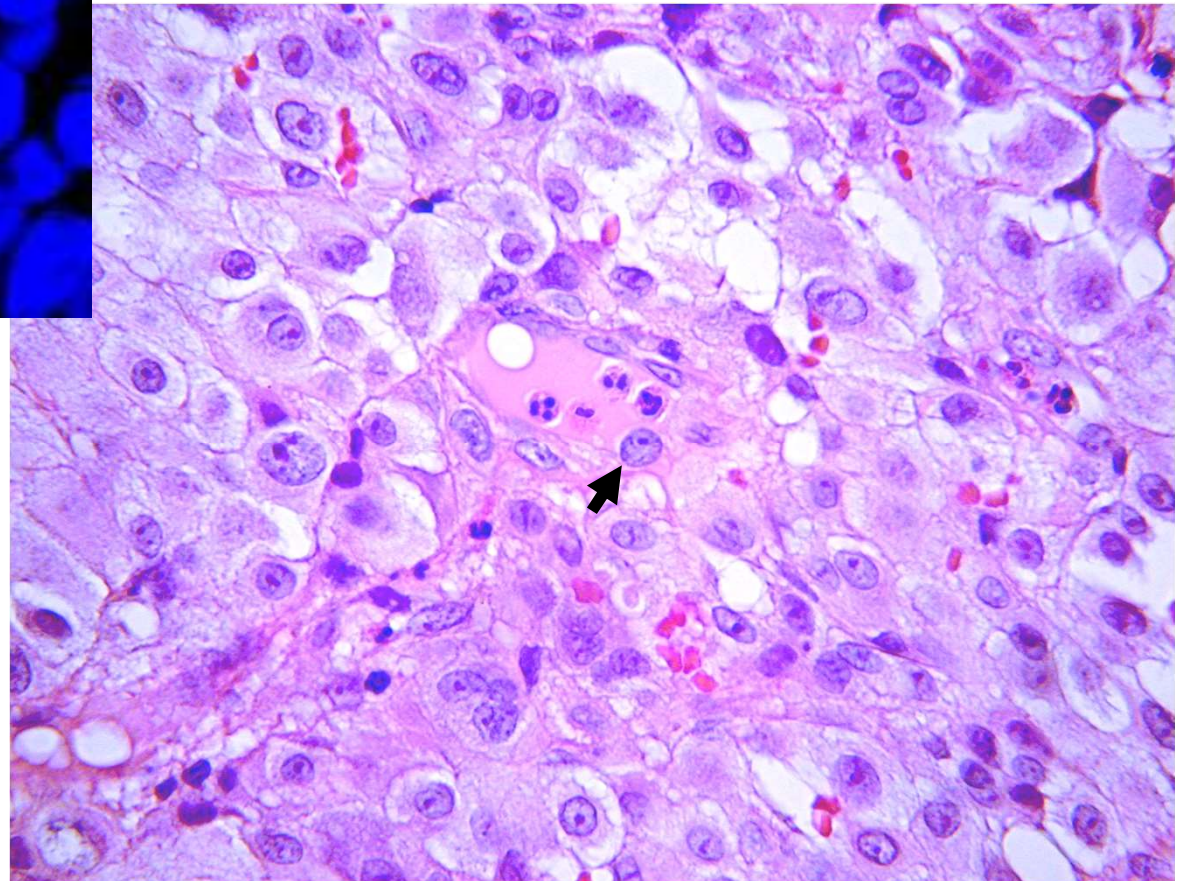
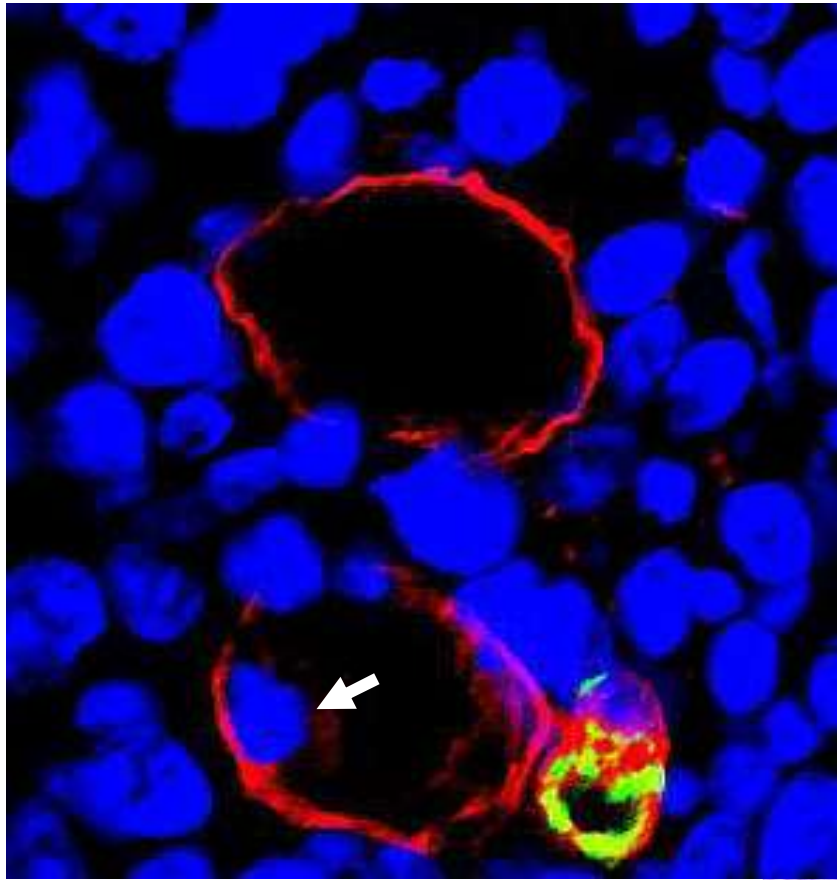
Hematogenic metastatic cascade (organ selectivity)

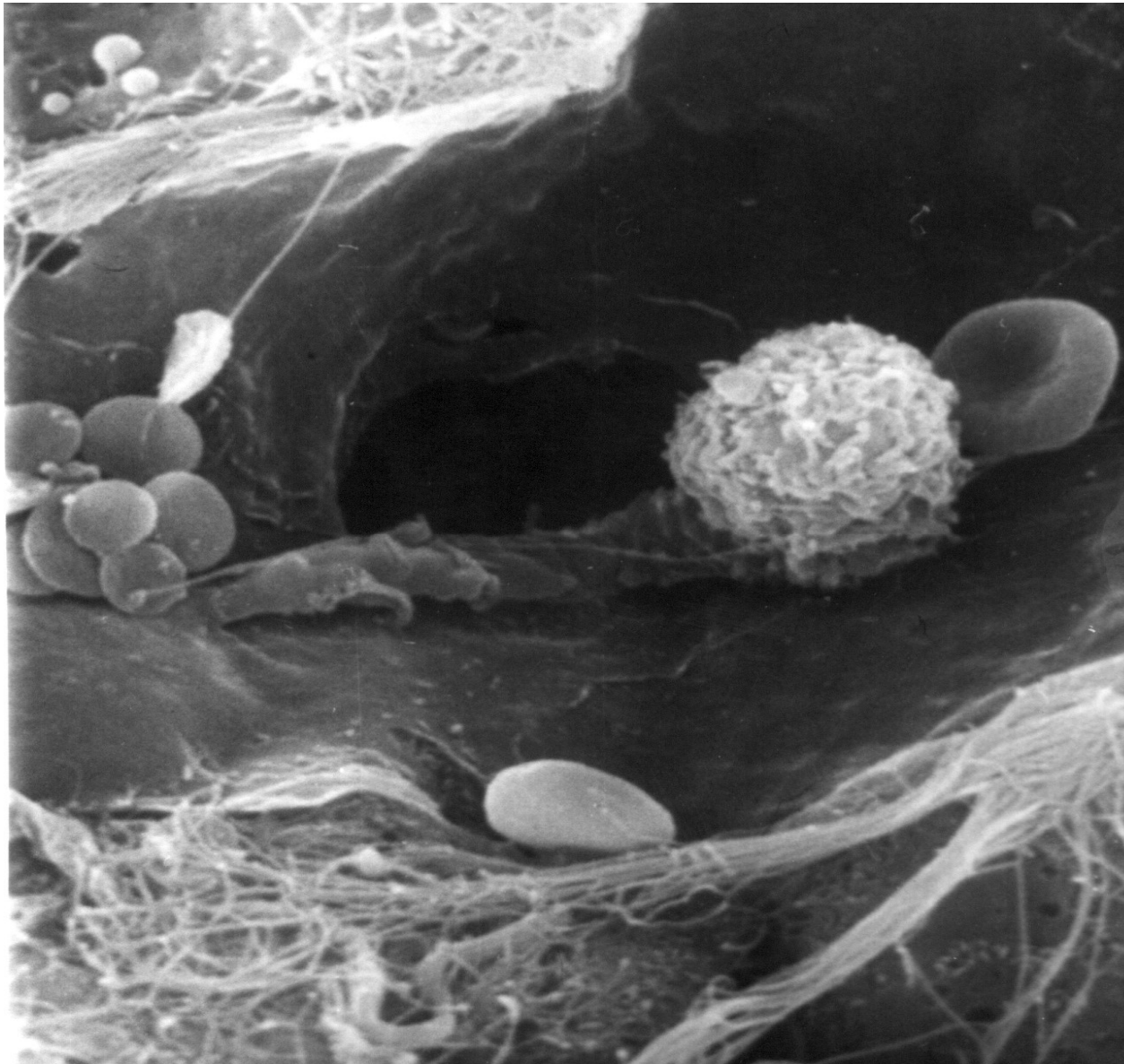
Metastasis maintenance
genes

(LUNG) **Liver metastasis**



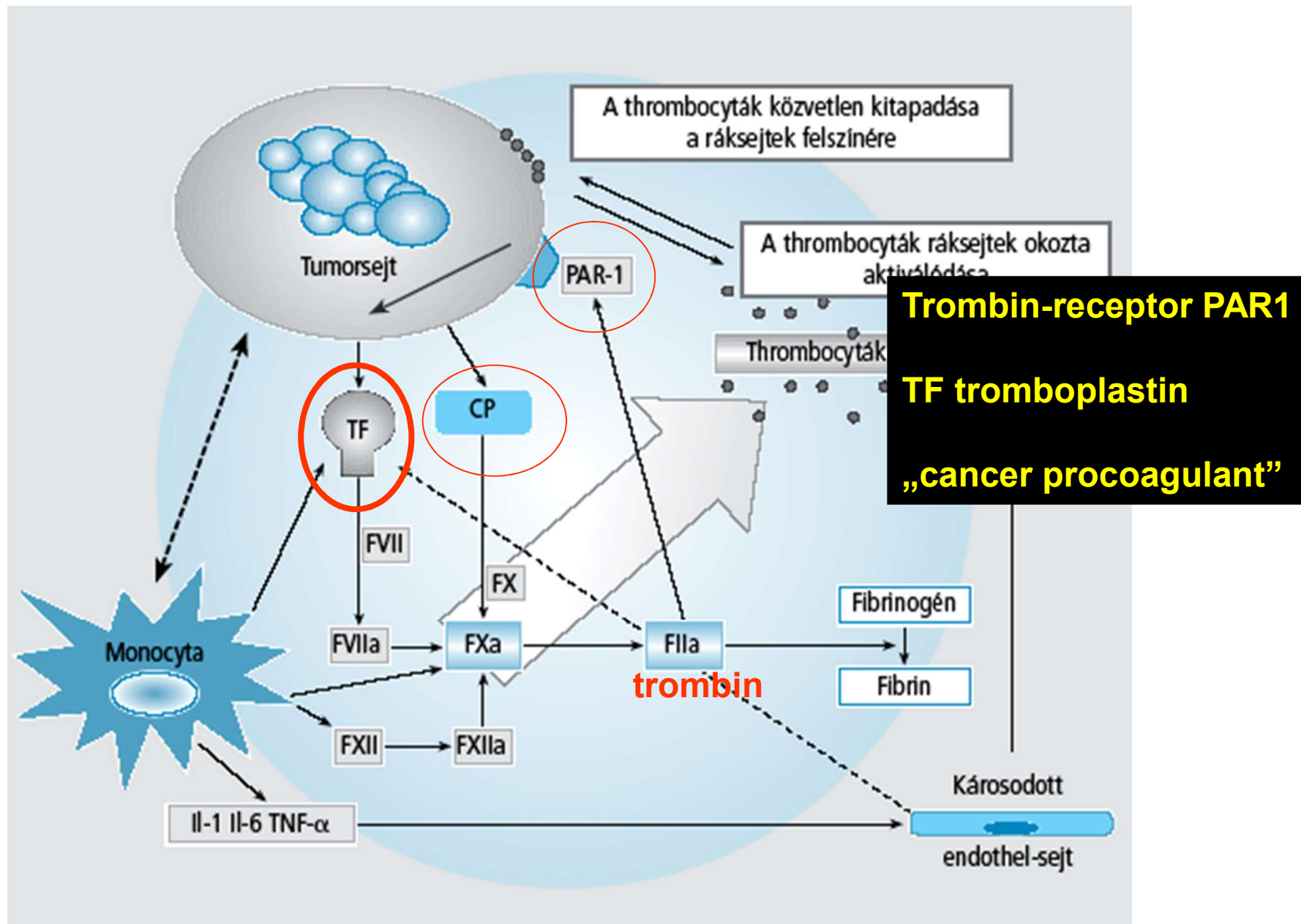
intravasation





Circulation - dissemination

Procoagulants in cancer cells

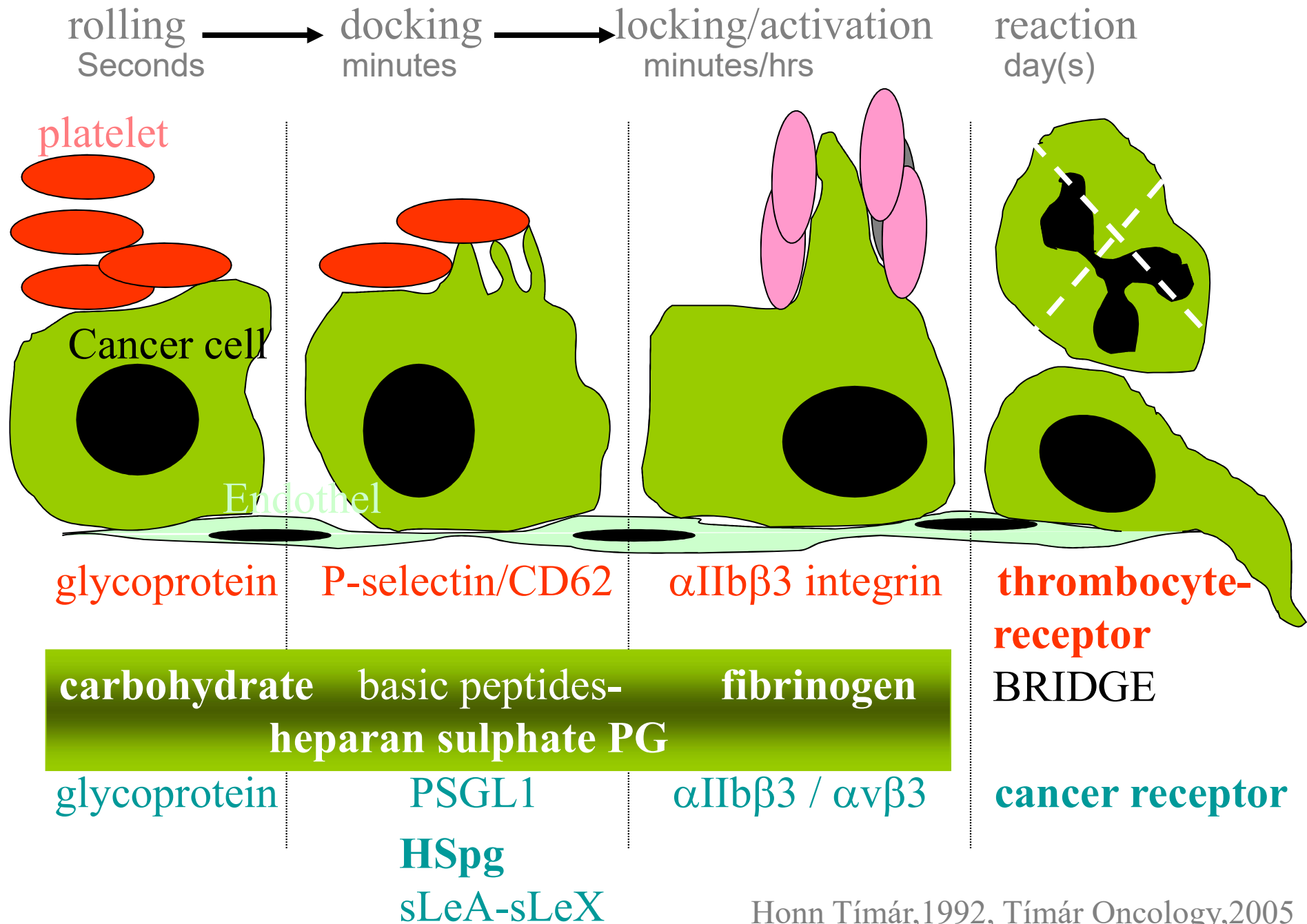


Invazive cancer cell

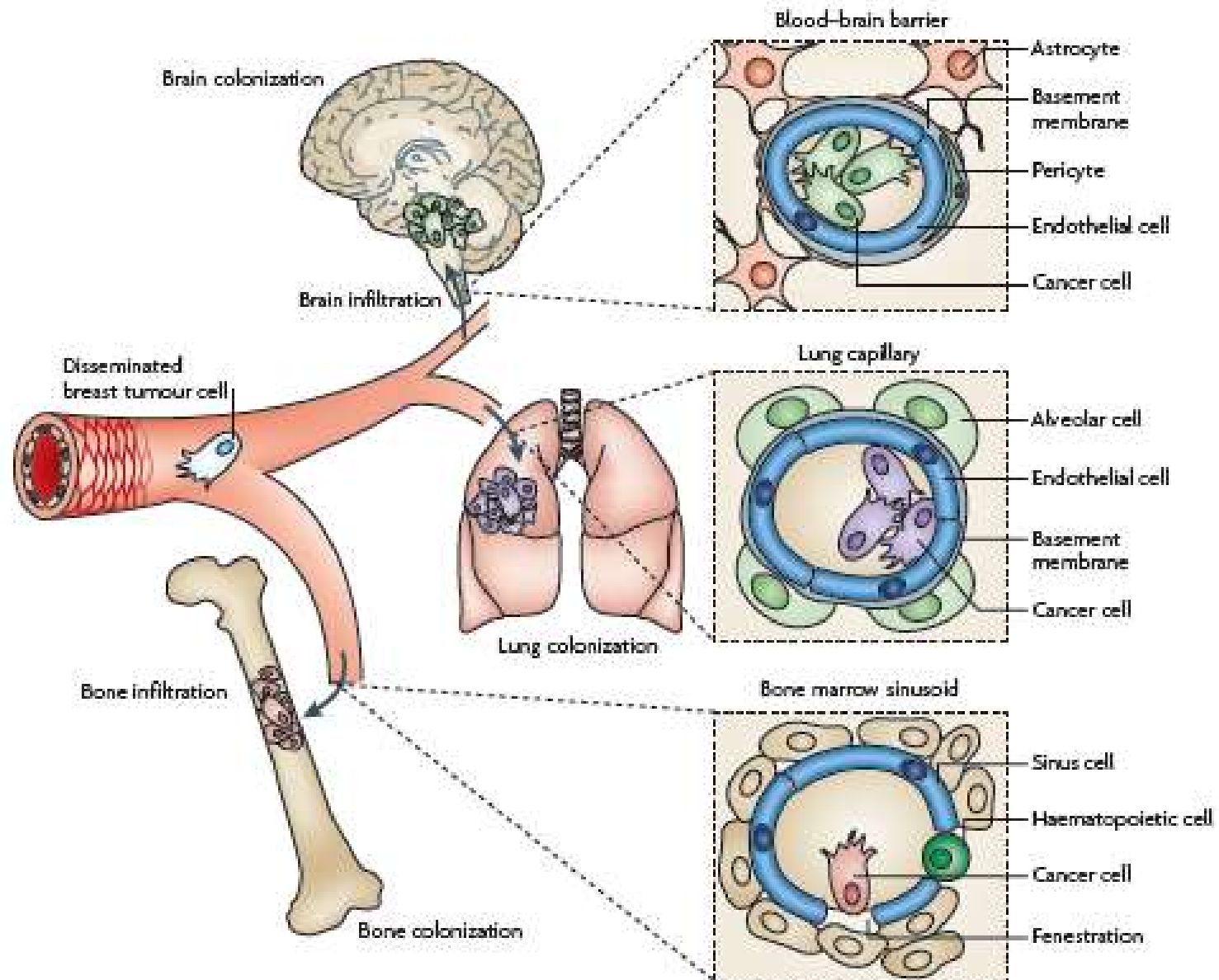
- **Matrix metalloprotease production /TIMP loss**
- **Cisteine protease: Cathepsin production /
steffin-loss**
- **Serine protease: uPA production / PAI loss**
- **Plazmine-production..... (fibrinolysis)**

Thrombocyte genes in cancer

- CD41 (GPIIb, integrin α IIb)
- Thr. 12-lipoxygenase
- PECAM/CD31 (thrombocyte adhéziós molekule)
- Thrombin-receptor (PAR1-4)



Extravasation: organ specificity



Organ metastatic niche

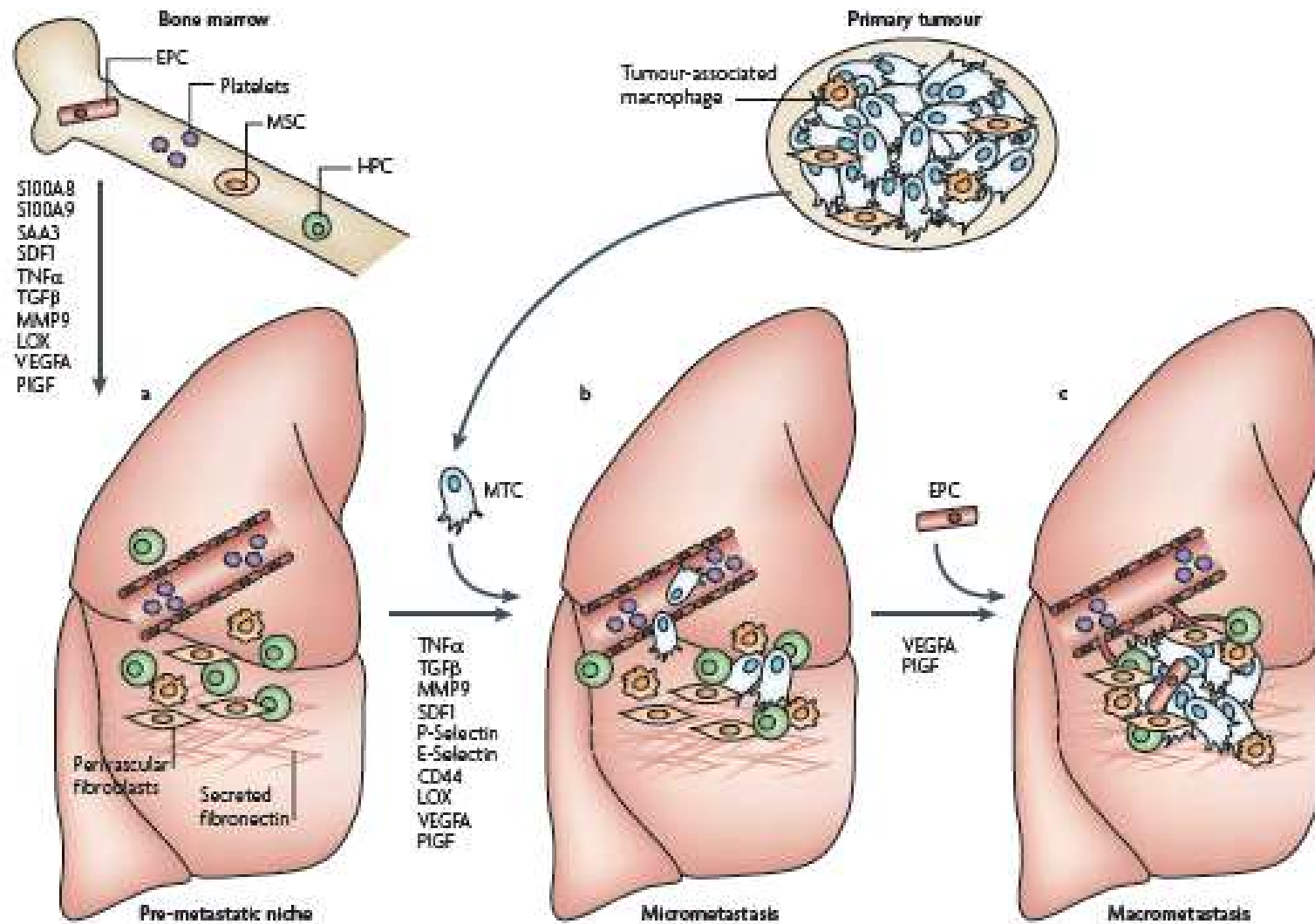
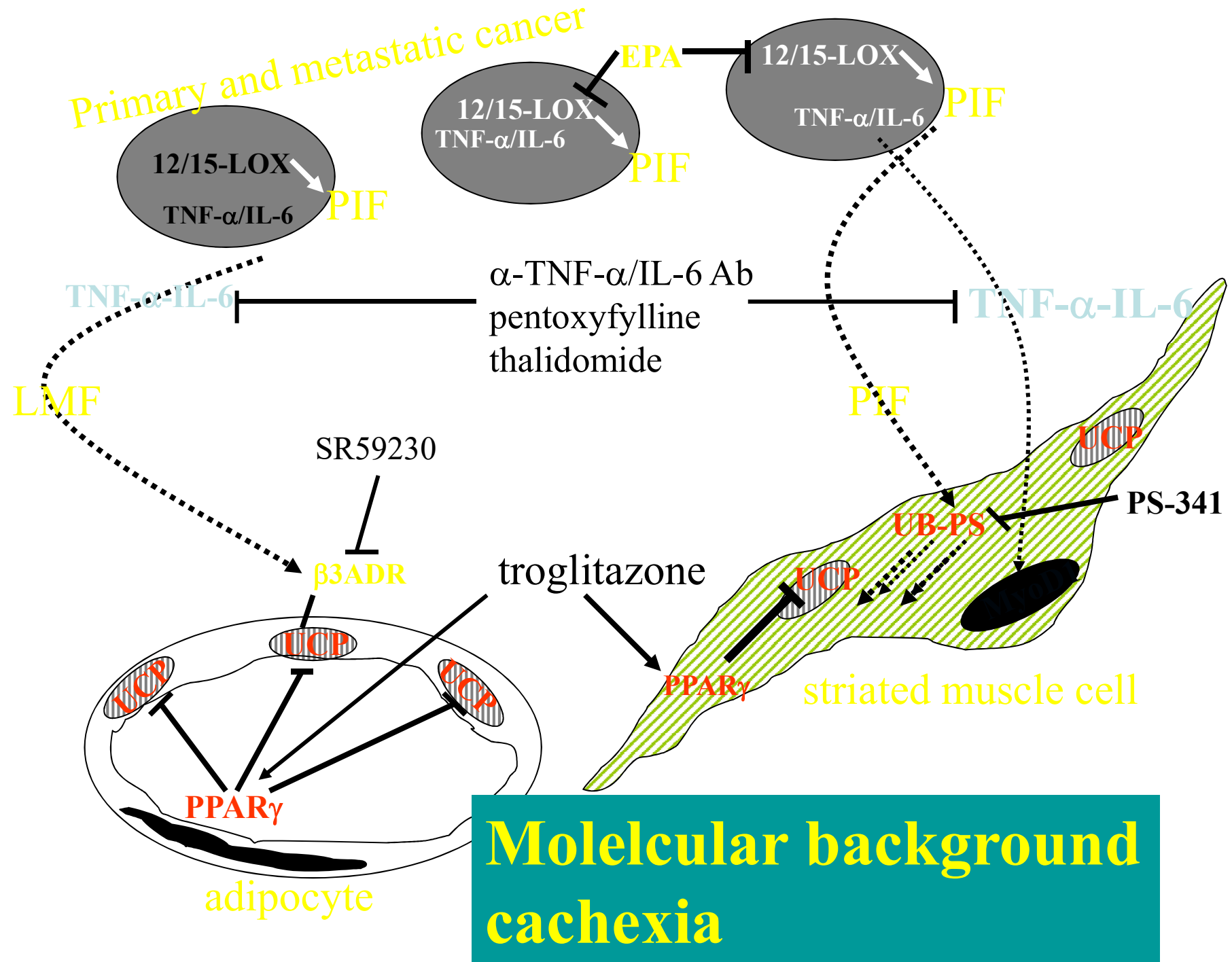


Figure 1 | A model of the evolution of a metastatic niche. This figure depicts the pre-metastatic, micrometastatic to macrometastatic transition.

metalloproteinase 9 (MMP9) and TGF β ^{14/14}. Activated fibroblasts, possibly derived from mesenchymal stem cells (MSCs), secrete fibronectin, an impor-



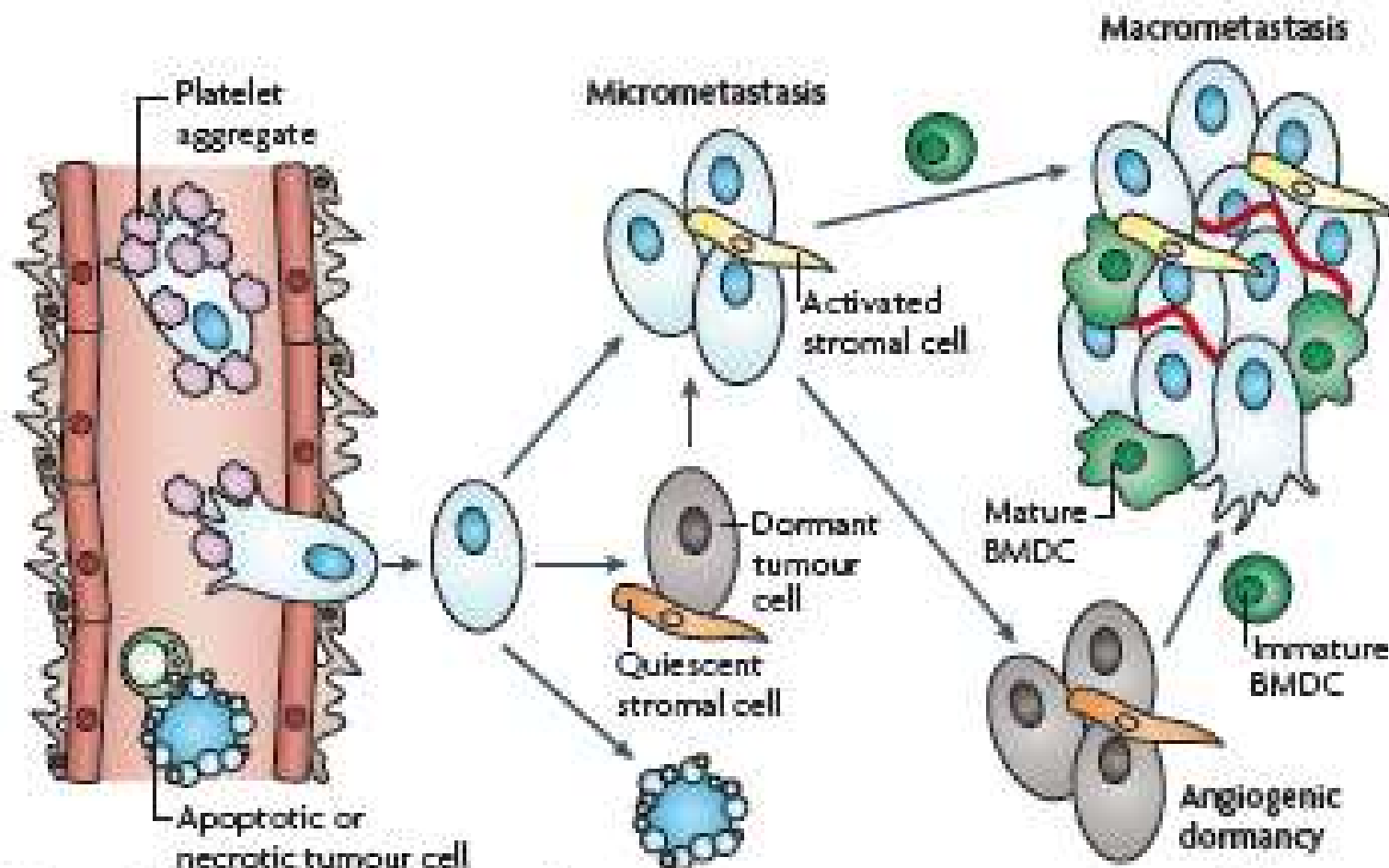


Figure 3 | The fate of tumour cells in the metastatic microenvironment. Following cancer cell intravasation, a series of rate-limiting steps affect the ability of these cells to establish secondary tumours in the metastatic site. At each step, the tumour cells can meet several different fates (death, dormancy or survival), which can be modulated by microenvironmental factors, including shielding by platelet aggregates in the circulation, the activation of resident stromal cells, and the recruitment and differentiation of bone marrow-derived cells (BMDCs).

Time-frame of metastatic progression

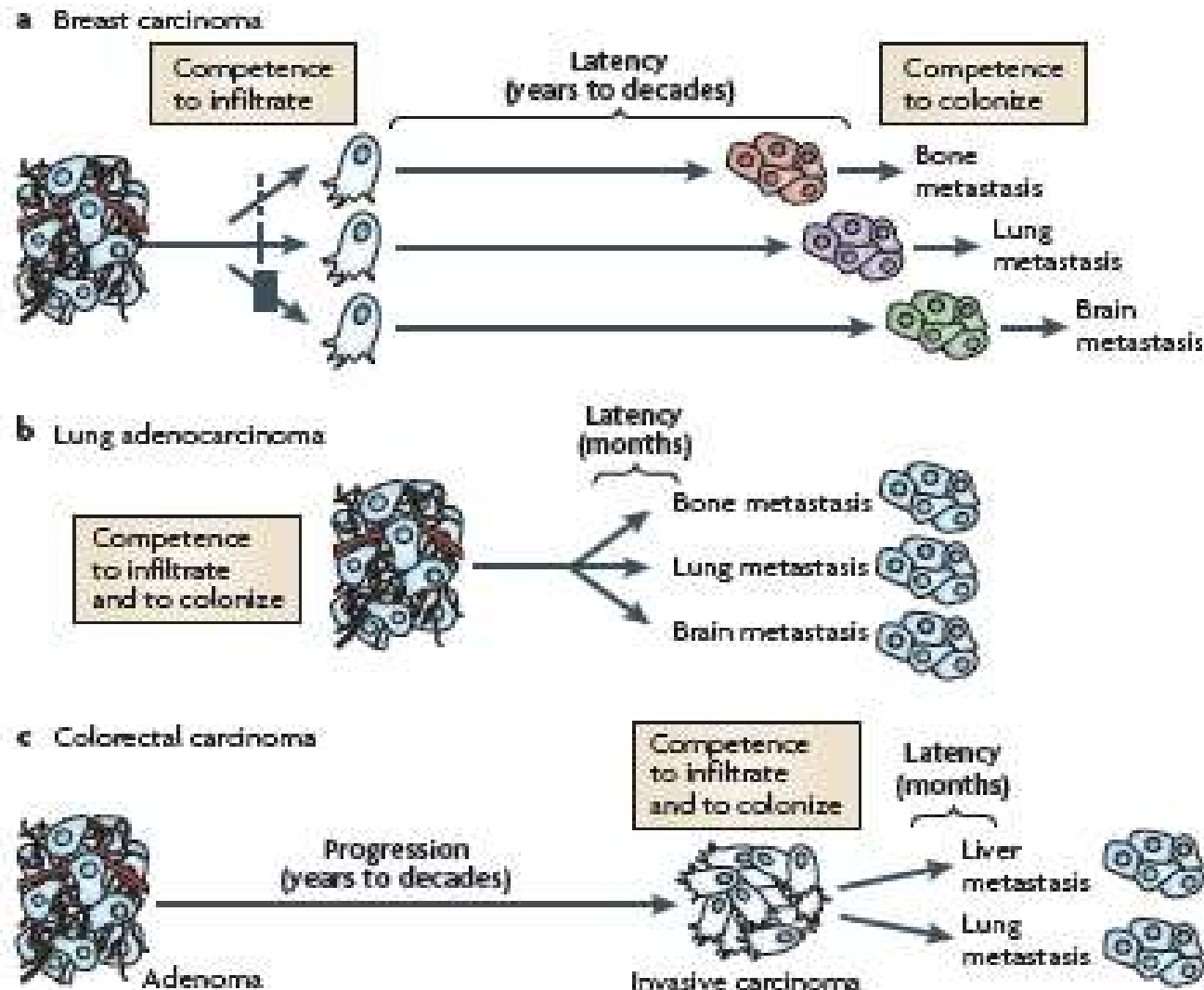
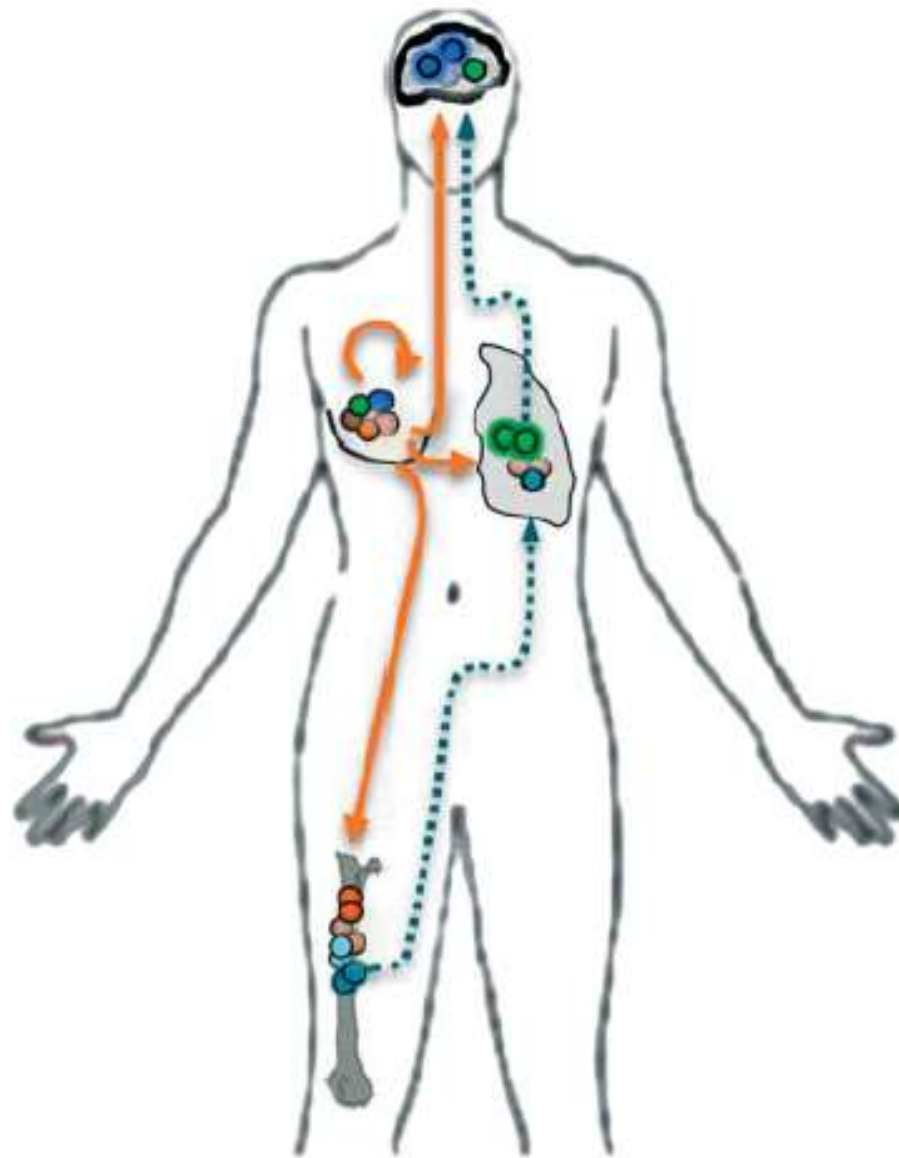
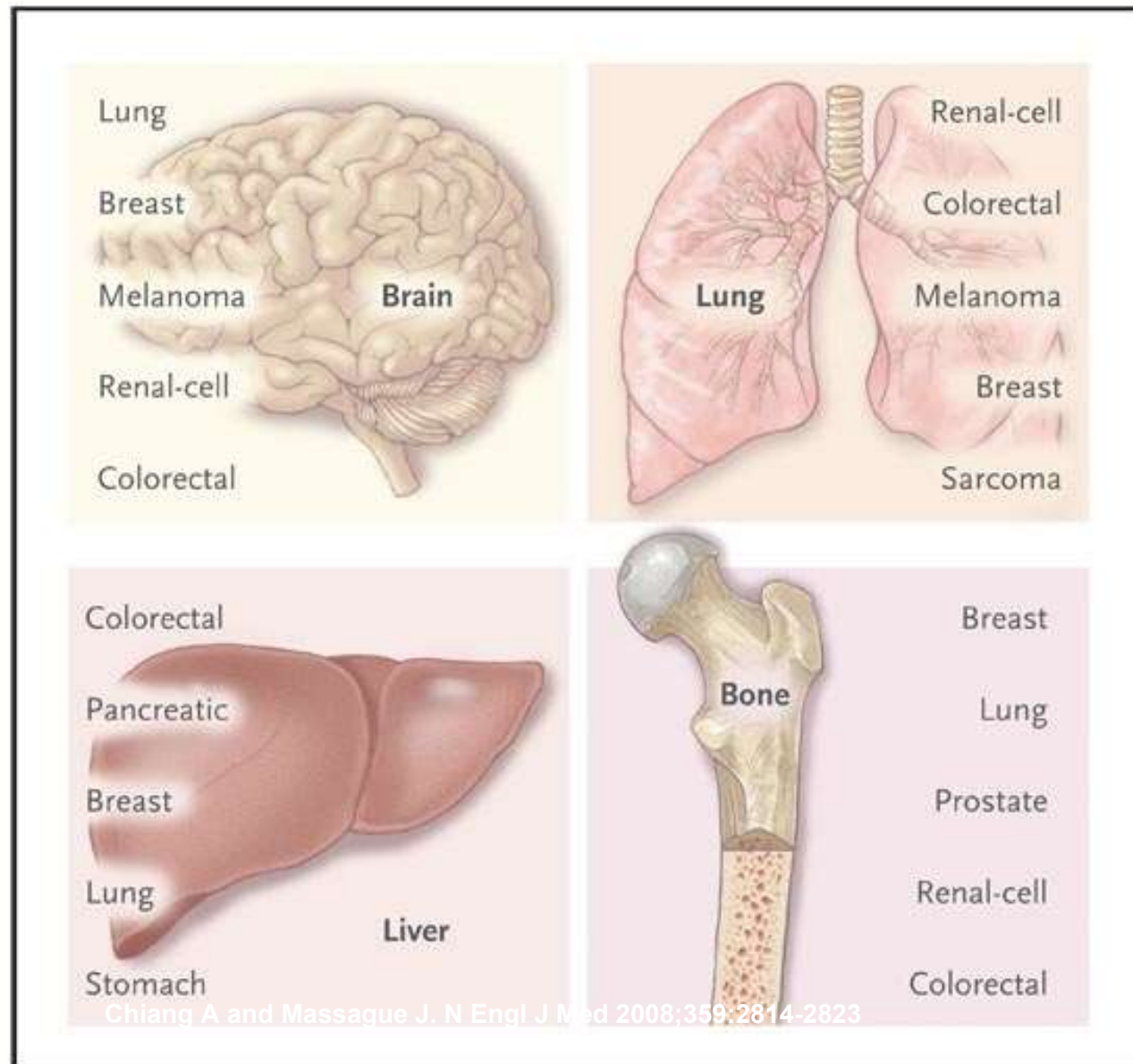
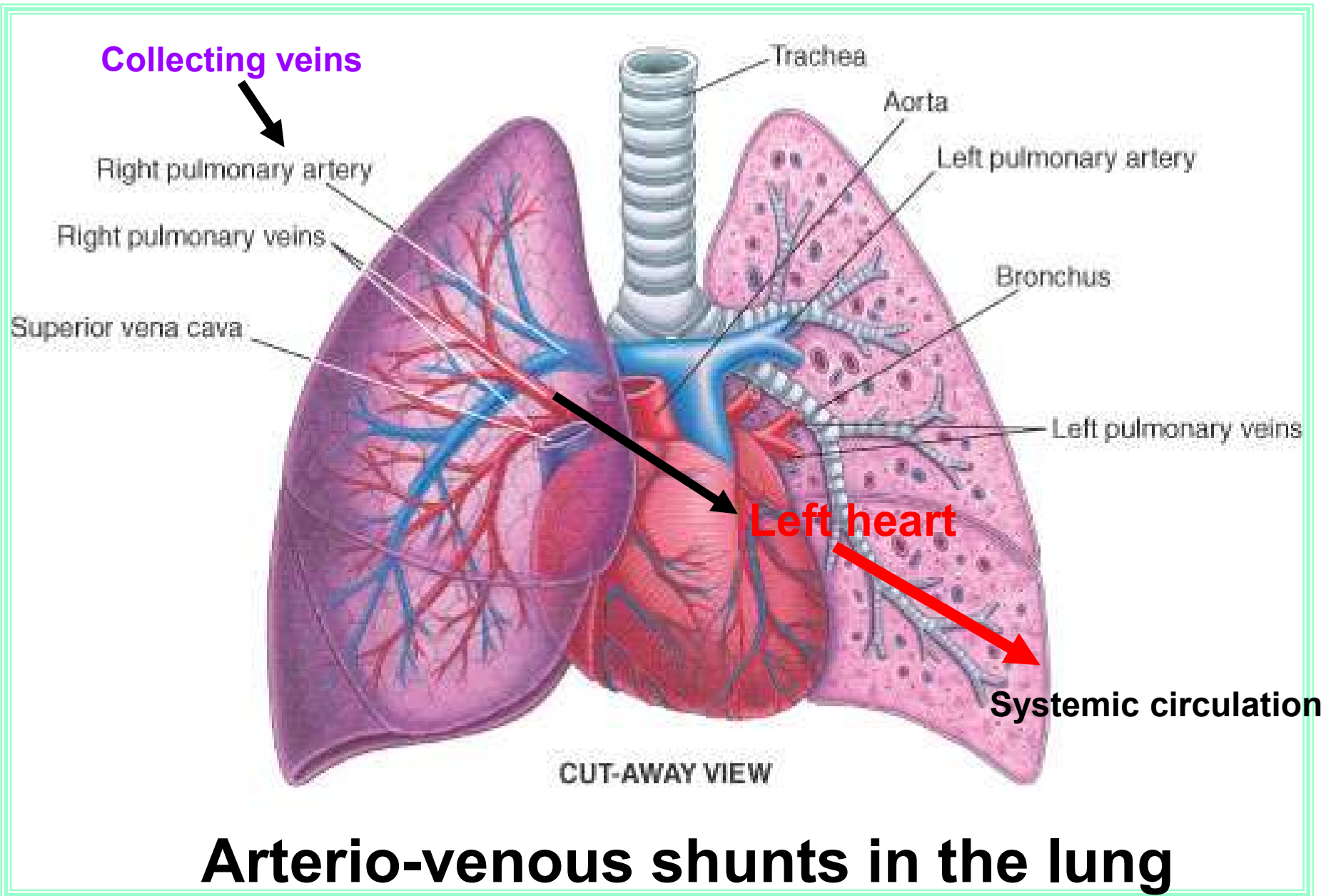


Figure 4 | The temporal course of metastasis. A model depicting the mode of



Organ specificity of metastatic progression





Lung metastatisation

- **All cancer types**

Superior/inferior v. cava.....

(**Lymphatic dissemination d. thoracalis**
v. cava superior.....)

Lung meta- breast cancer genes

- Desmocollin2 (cell adhesion)
- UDP glikoziltraszferáz8 (glikoszfingolipid szintézis)
- Integrin $\beta 8$ (matrix adhesion)
- Fermitin-1 (cell adhesion)
- Landemaine et al. CR, 2008
- Epiregulin (EGFR ligand)
- COX2
- MMP1/2 matrix metalloproteases
- ANGPTL4 angiogenic cytokine
- Massague et al. NEJM 2008



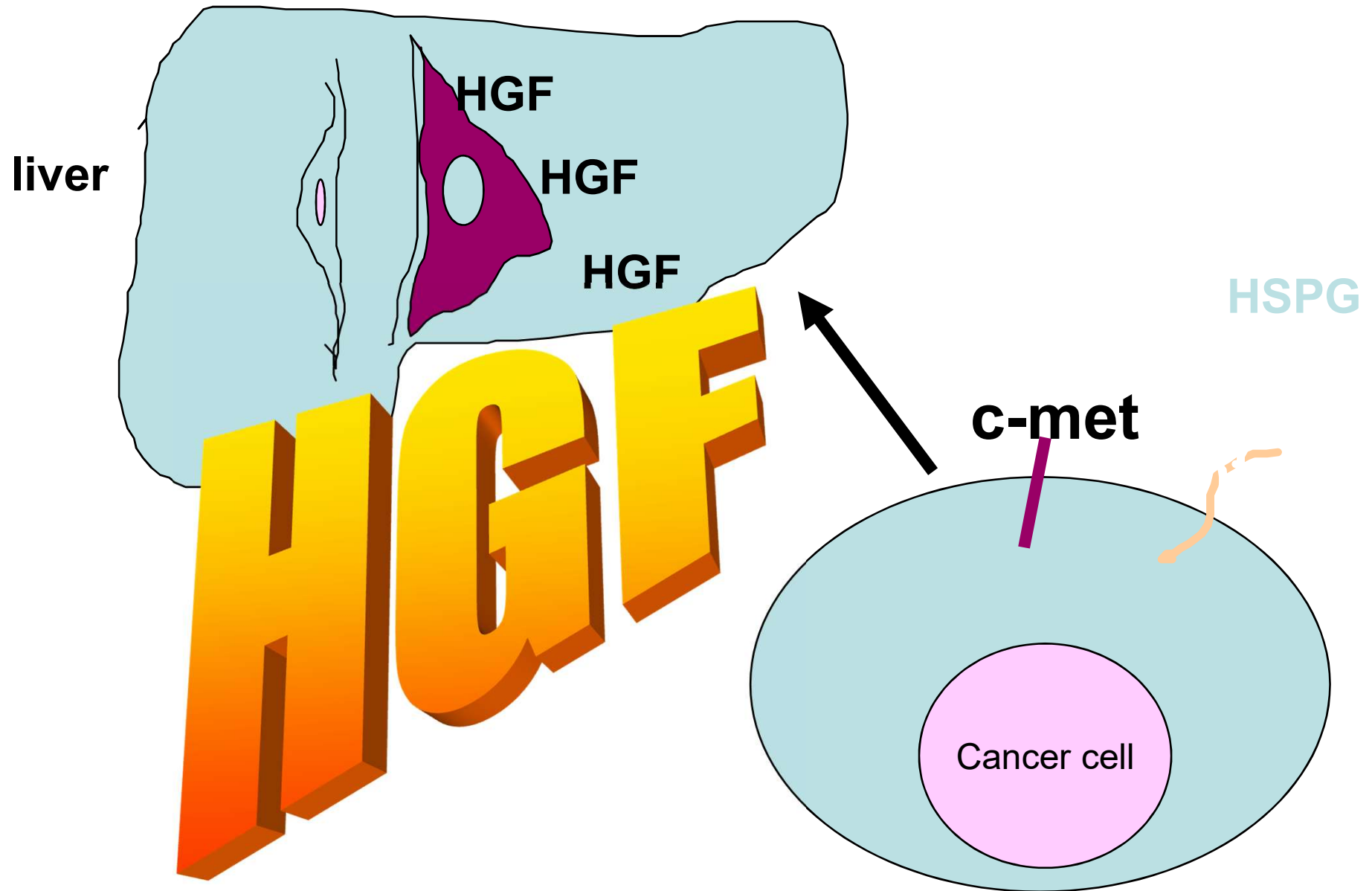
Liver metastatisation

- Colorectal cancers (lower 3rd rectal: NOT)
- Gastric cancers (portal vein)
- Pancreatic cancers (splenic vein/portal vein)

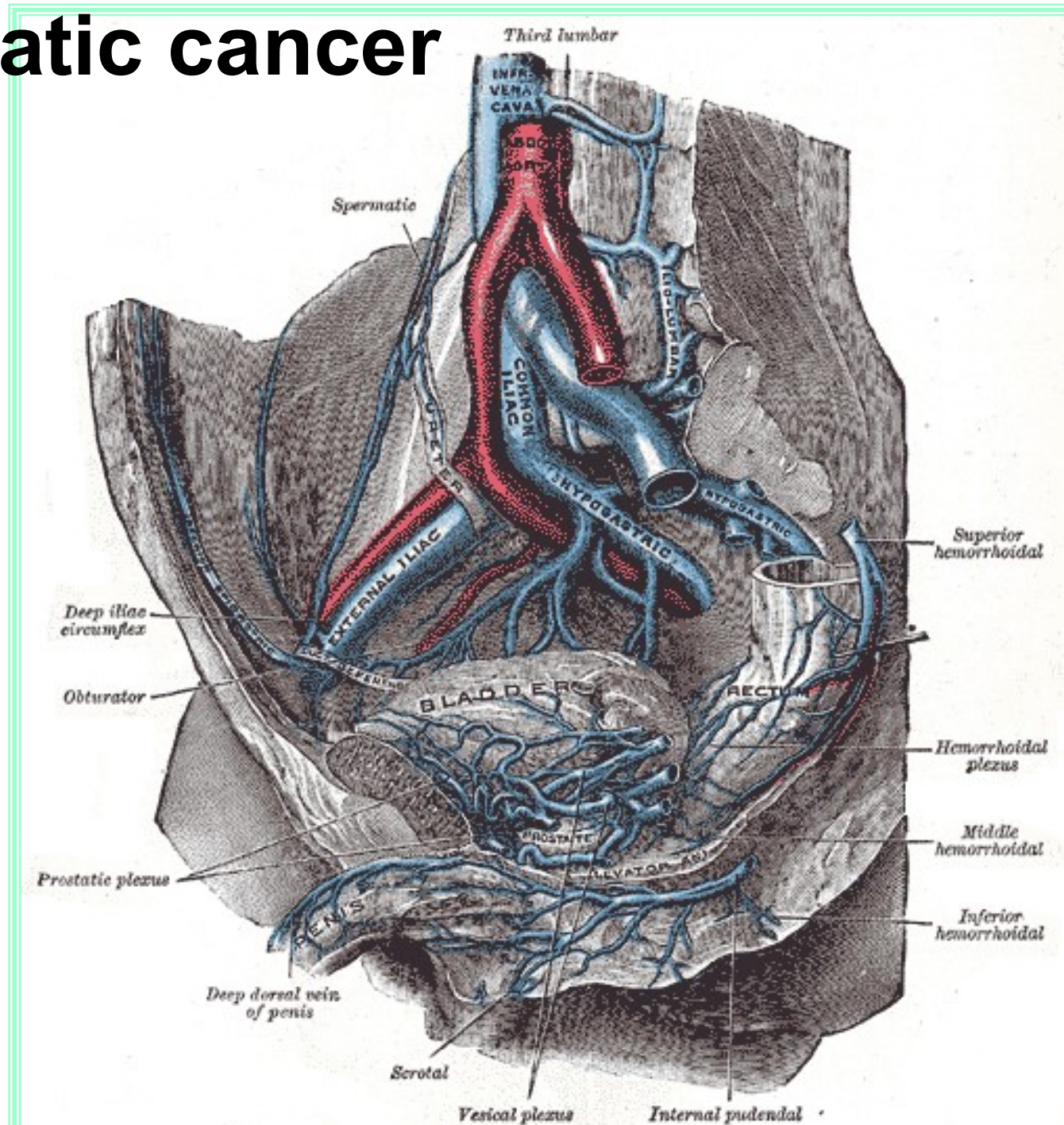
(**v. portae**)

- Malignant melanoma / uveal melanoma
- Any cancer type.....**arterial dissemination**

Paracrine regulator of cell motility:



Prostatic cancer



- Periprostatic veins
- Batson veins
- v. vertebrae

Lokális invázió
(BM)

10^{9-11}

Prostate cancer
(angiogenesis)

Intravazáció
(subendoth. BM)

Adhézió
Degradáció
Migráció

Keringés
 10^{6-7}

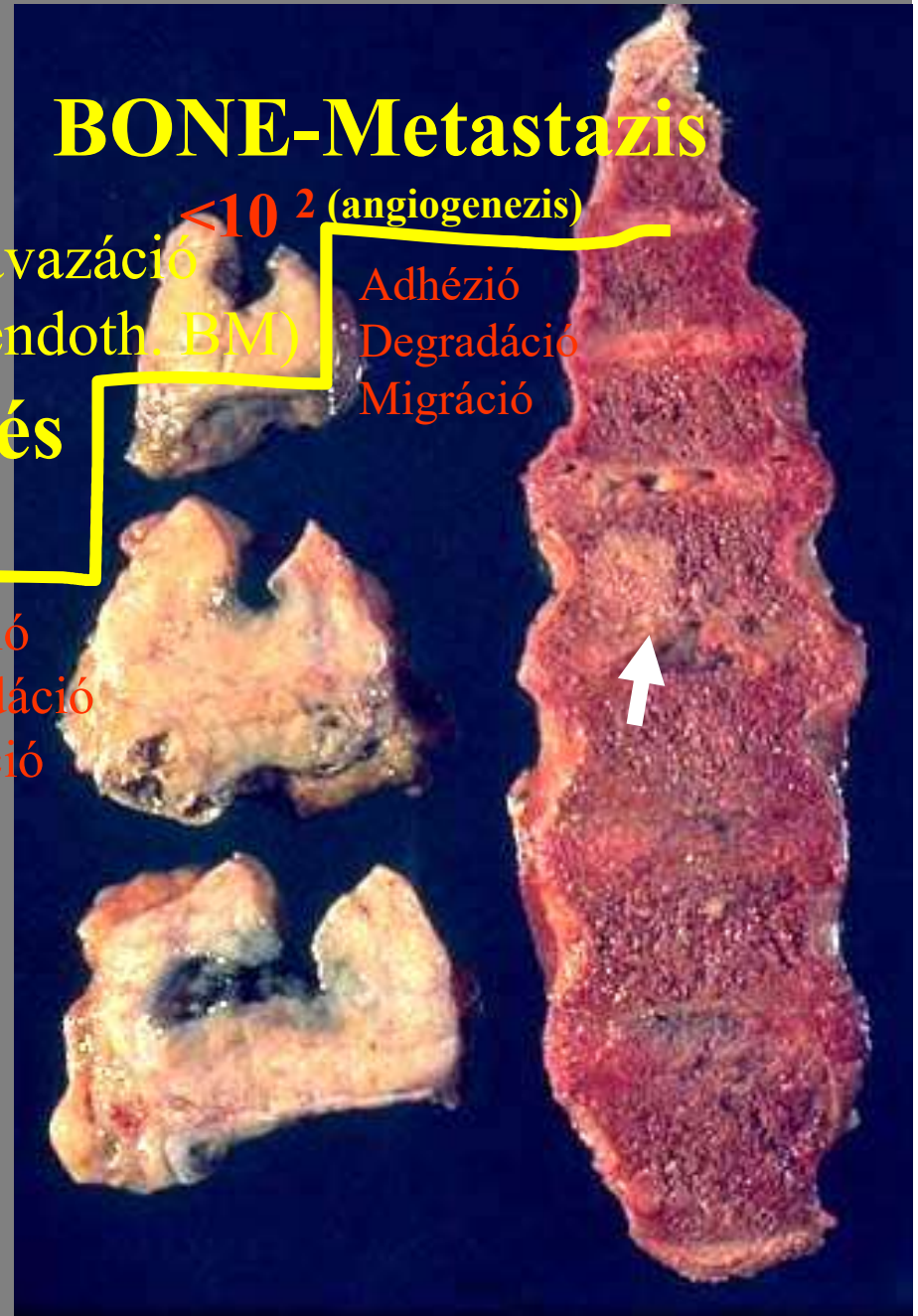
Extravazáció
(subendoth. BM)

Adhézió
Degradáció
Migráció

BONE-Metastazis

$<10^2$ (angiogenesis)

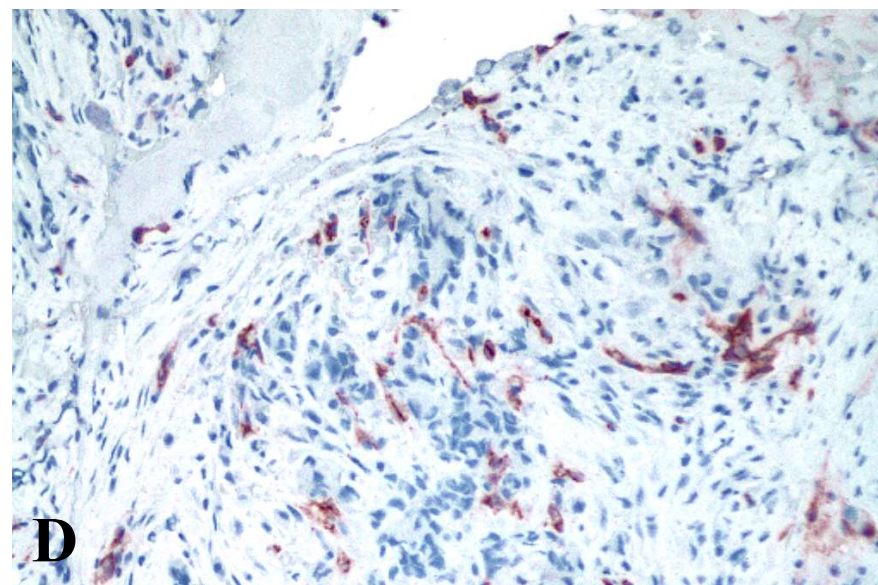
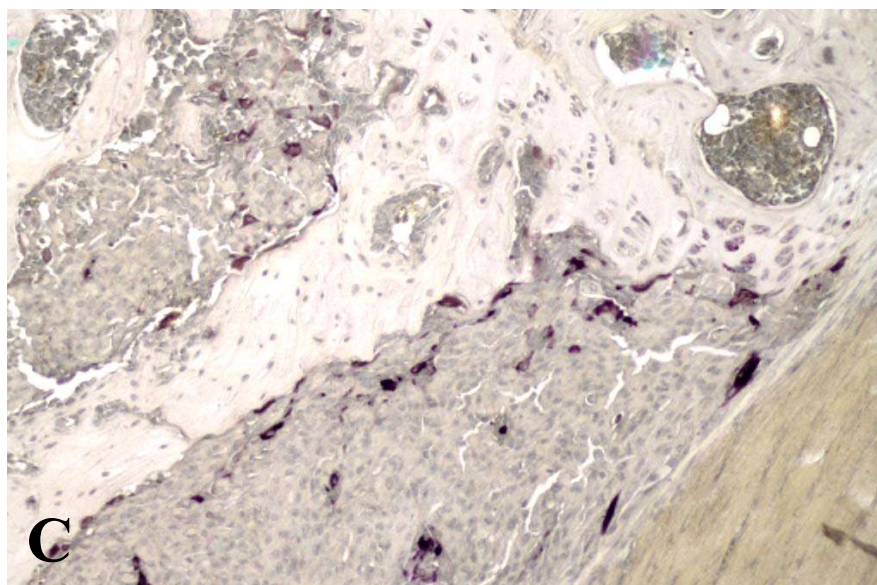
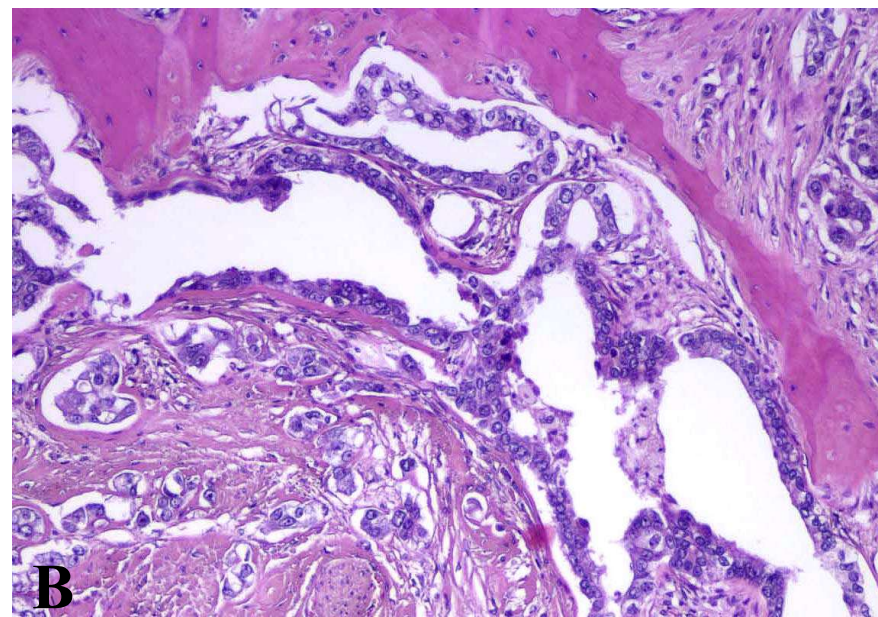
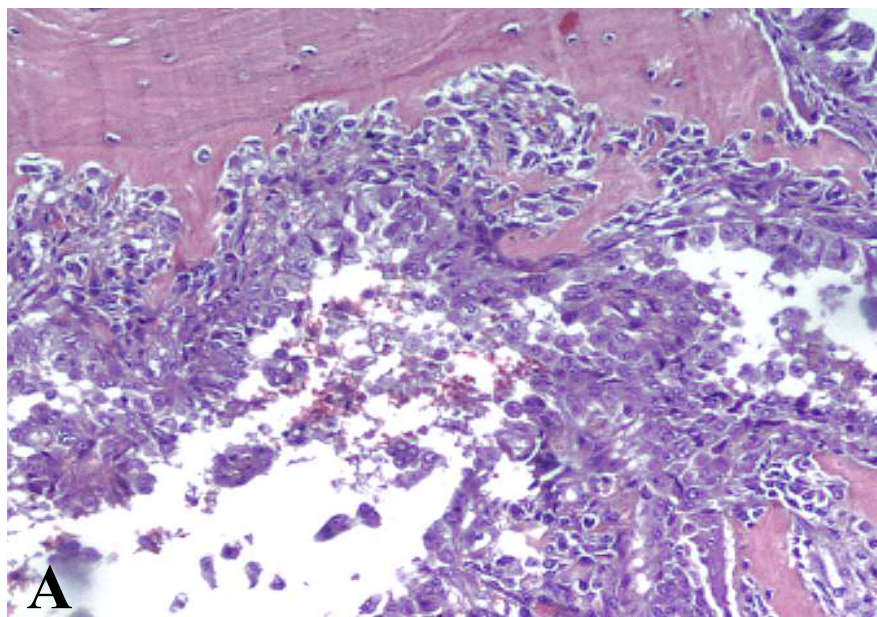
Adhézió
Degradáció
Migráció



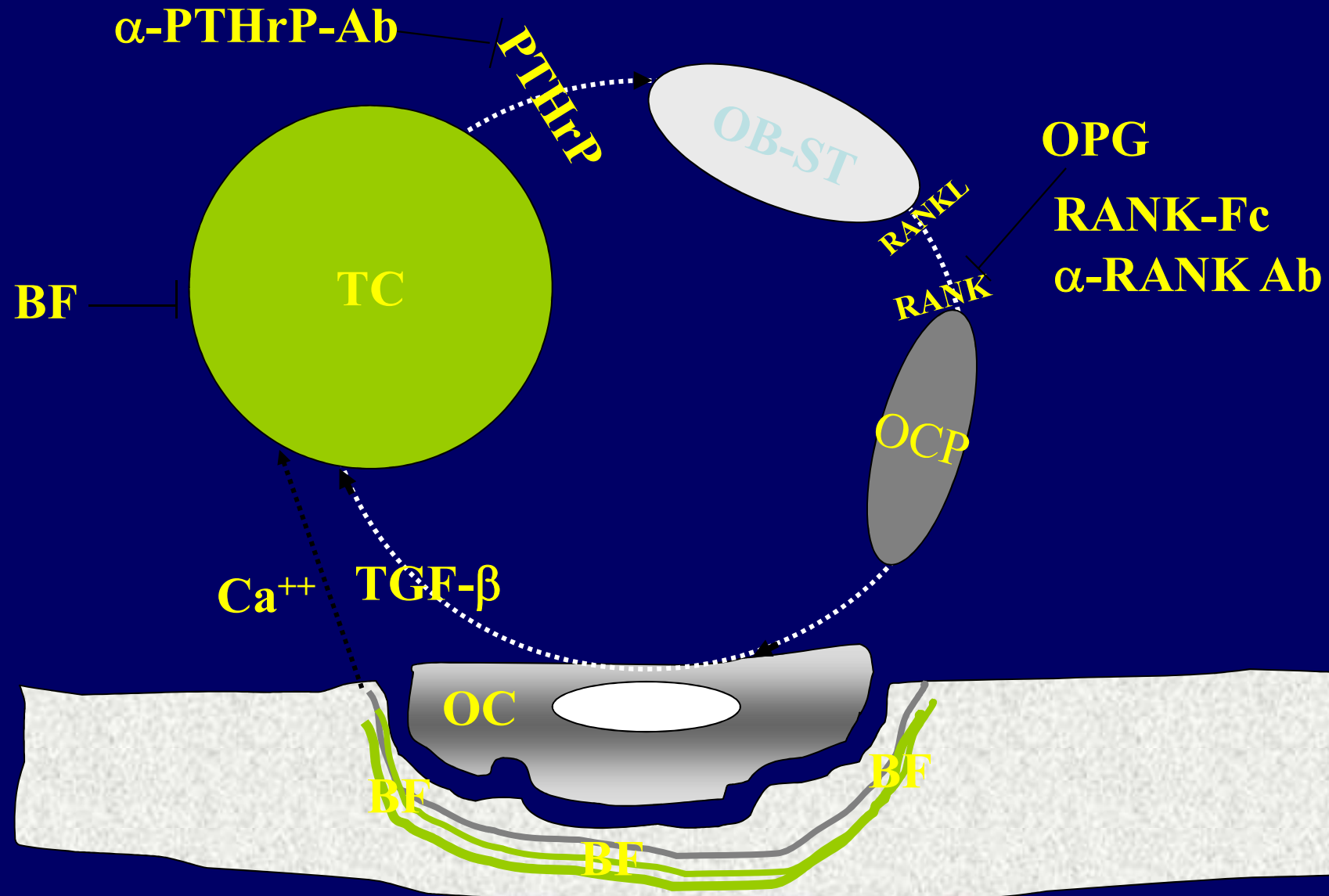
Bone metastatic cancers

- Prostate cancer
- Breast cancer **common:**
- Lung cancer **bone matrix protein expression**
- Renal cell cancer
- Thyroid cancer

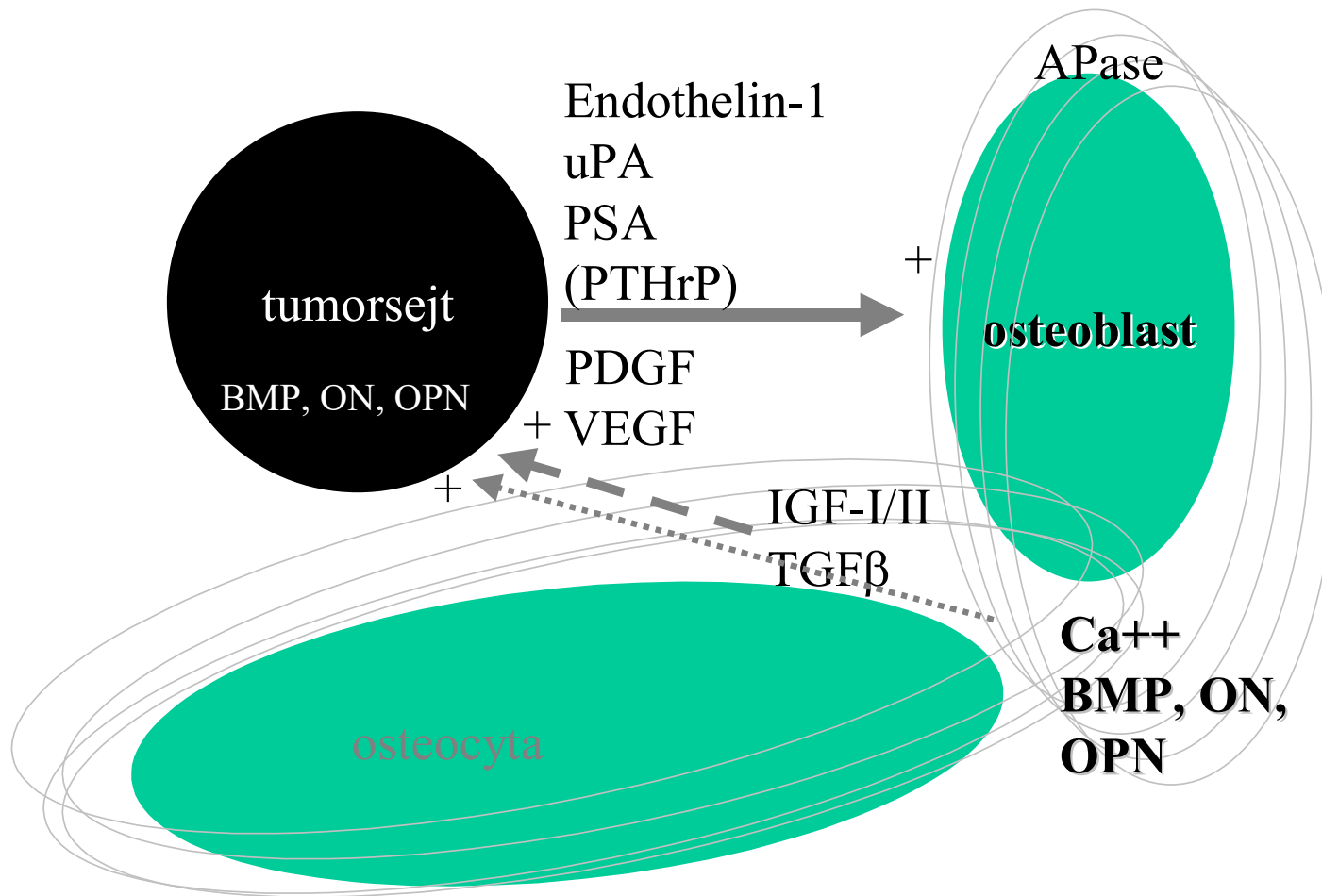
Arterial dissemination (except: prostate cancer)



Molecular targets in bone metastasis



Osteoplasticus csontáttétképzés



Brain metastasis

- Breast cancer
- Lung cancer
- Renal cell cancer
- Malignant melanoma
- Common: from systemic arterial circulation

Brain metastatic breast cancer genes

- COX2
- Hb-EGF EGFR ligand.....
- ST6GALNAC5 szialil-transzferáz
- MMP1 metalloprotease
- ANGPTL4 angiogenezis
- Bos et al. Nature, 2009

Meta-genes

- CD44v6/v3
- NEDD9: FAK regulator.....
- **EMT regulators:**epithelial-mesenchymal transition
- **TWIST** tf (bHLH)
- E47/E2A (bHLH)
- SIP1, Slug, Snail (Zn finger type)
- FOXC2
- goosecoid

Meta-szuppresszor genes

- BRMS1 (chromatin remodeling), gap-junction comm
- CRSP3 tf
- KAI1/CD82 sejtadhéziós molekula (EGF antagonista)
- KISS1(metastin): a GPCR45 ligandja (DRIP130 tf)
- E-cadherin (CDH1): CAM
- RhoGDI-2 : Rho inhibitor
- MAPK inhibitors: NM23(NDPK), VDUP1, MKK4
- TIMPs (MMP inhibitorok)

Genetic models for metastatization

