Positive and Negative Regulators of Metastasis

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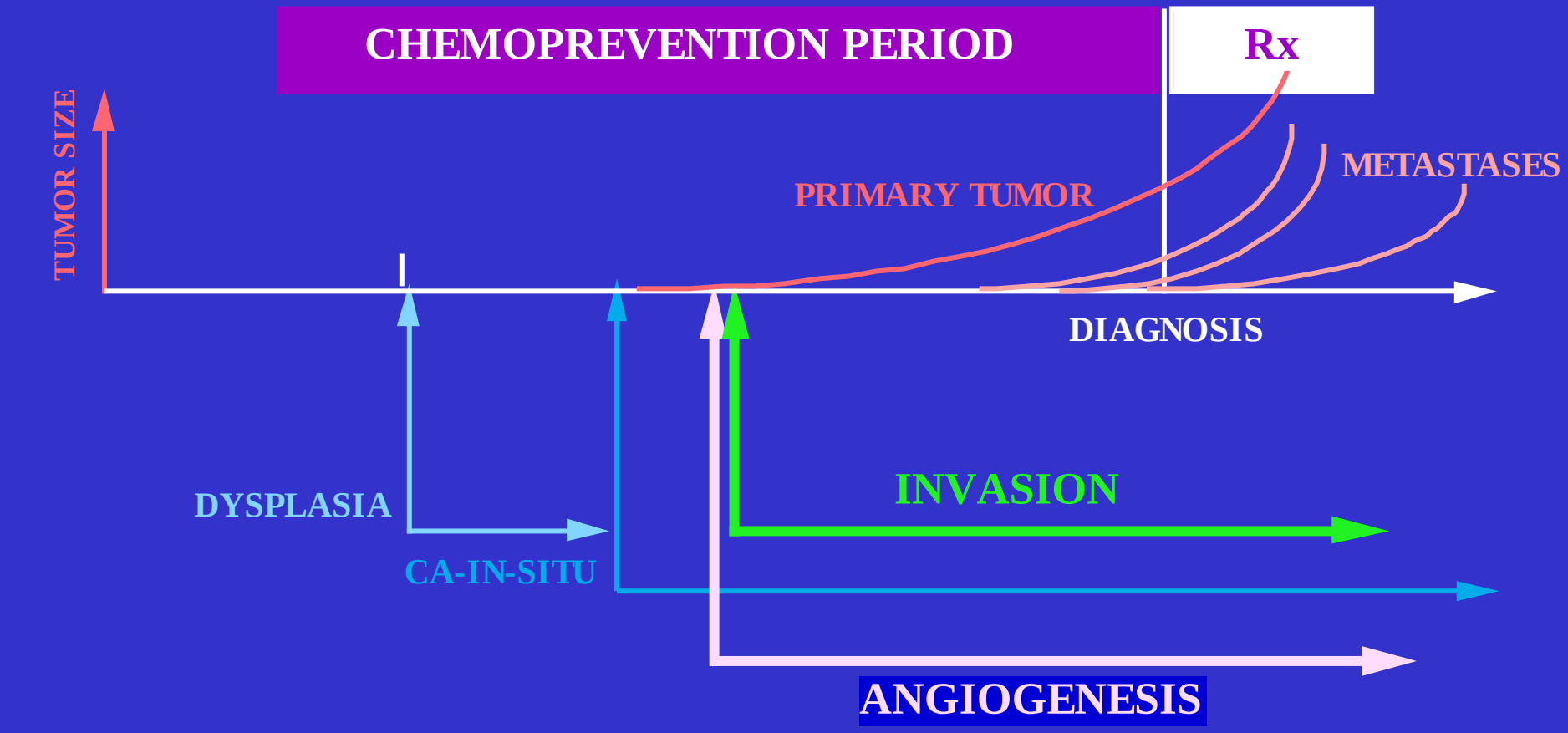
TIME SCALE OF TUMOR PROGRESSION



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QuickDraw decompressor
are needed to see this picture.

The Metastatic Cascade

When does metastasis begin?

Commitment to the metastatic phenotype:

- How early does it occur?
- Can it be reversed?

Progenitor lesions:

- What are the key progenitor lesions?
- What is the efficiency of transition to invasion?
- Are all metastasis precursors clonal?

What is the role of the host?

- Under what conditions does the host drive or suppress the process?
- Does the transition from pre-invasive to invasive lesions require host participation?
- If so what are the molecular and cellular players that are functionally important?
- The circuitry of the tumor host communication may be the key to prevention of invasion.

Physiologic basis of metastasis

- Is metastasis a normal physiologic program which is disregulated or inappropriately activated?
- Does a physiologic motility and invasion program exist for development, angiogenesis morphogenesis and wound healing?
- Is metastasis colony formation a natural ongoing process conducted by stem cells?

What is the driving force?

- Is the metastatic phenotype pre determined within the primary tumor? Within the host microenvironment?
- Are malignant cells a product of adaptation and selection?
- What is the selection factor? If malignant cells are survival of the fittest, then what is the fitness test?
- Is cell survival in a foreign (non home) tissue the ultimate selection factor?

Metastasis Pre-1900

Hippocrates (460–375 B.C.)
Galen (131–201 A.D.)

Pre-1700: The Greek physician Hippocrates coined "carcinoma" from *karkinos*, the word for crab.

Pre-cellular theory of invasion and metastasis: recognition of malignant tumors and localized versus metastatic disease

LeDran 1757: Noted that malignant tumors begin as localized disease, then spread to regional lymph nodes and then enter the circulation to subsequently appear in the lung

Bichat 1801: Tumors contain both parenchyma and stroma

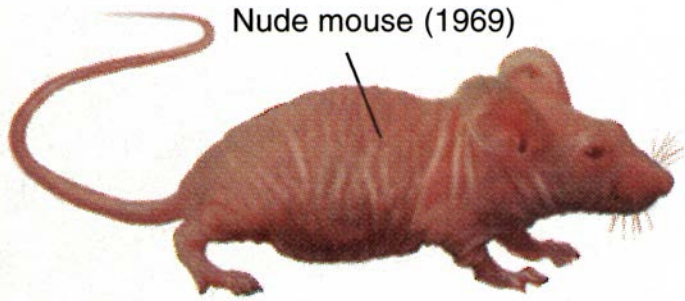
Recamier 1829 : Used the term "Metastases"

Validation of the cellular theory of cancer metastasis

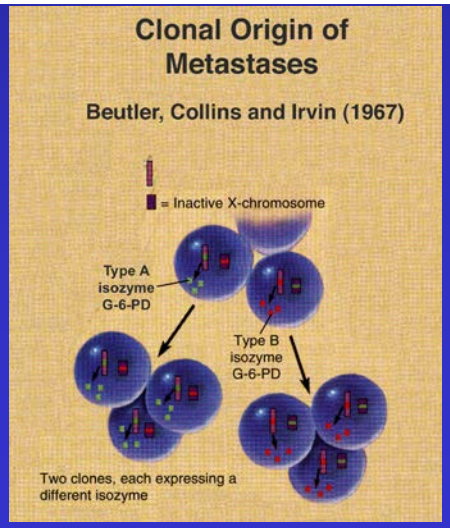
Takahashi: (1915)

1900–1949: Takahashi found that the cells of various mouse carcinomas and sarcomas produce reproducible patterns of metastases when injected into other mice.

Tyzzer 1913: Experimental Metastasis



1950–1969: Rygaard and Povlsen showed in 1969 that human tumors can grow in nude mice, which lack a thymus and are T cell deficient. This experimental animal model of human cancer continues to be refined and used today.



Ziedman and Fidler 1970-80: Intravenous metastasis models

Paget
(1889)

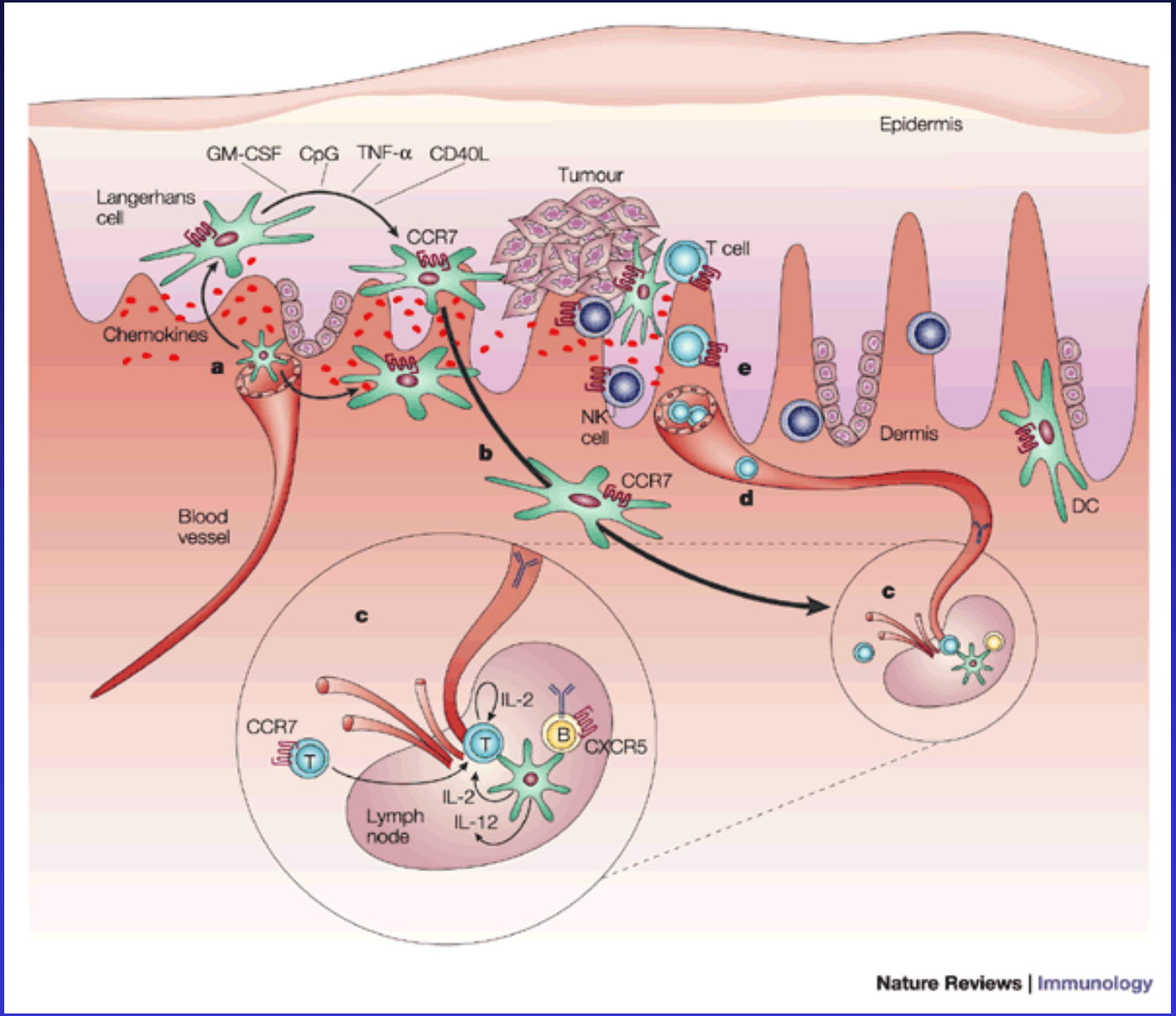
1700–1899: Paget proposed that metastases form specifically in organs that are "soil" to a metastatic cell's "seed."

The organ pattern of metastasis is characteristic of the tumor type and tissue of origin. 50-70% of the metastatic pattern can be predicted by the venous drainage blood flow. The remaining 30-50 % may be caused by specific molecular homing mechanisms.

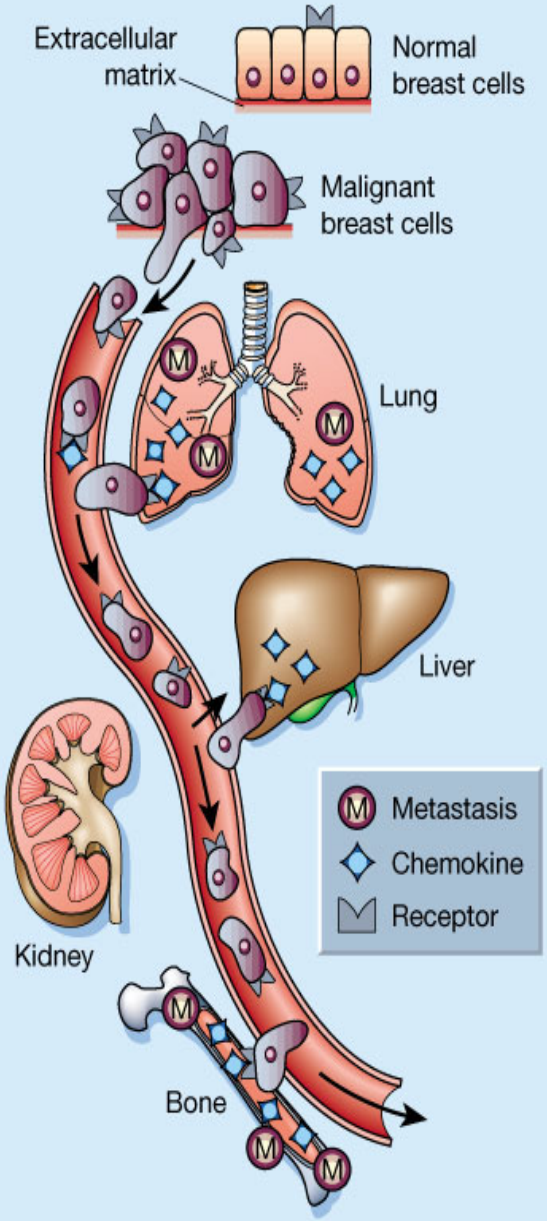
Potential molecular mechanisms:

a) Preferential adhesion in the vessels of the target organ
b) Selective extravasation
c) Organ attractants
d) Organ specific survival and growth

Chemokines regulate leukocyte recirculation and trafficking to sites of inflammation and infection



Premise: Metastasis homing is dictated by relative abundance of chemokines and cognate receptors on the tumor cell.

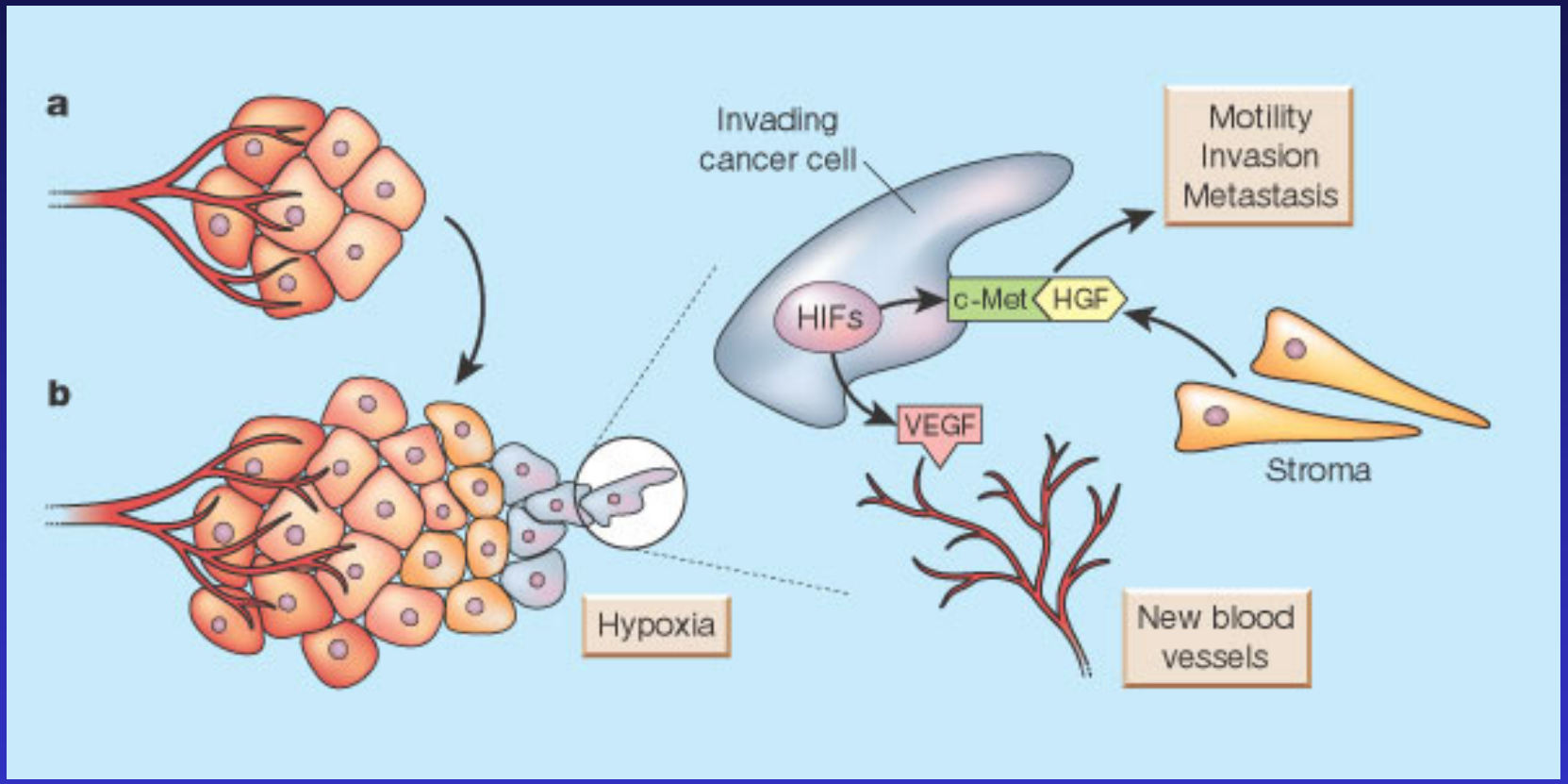


Why do the tumor cells express the chemokine receptors in the primary tumor prior to dissemination?

Therapeutic utility is limited because dissemination has already occurred at the time of diagnosis

Tumor necrosis is a bad prognostic indicator

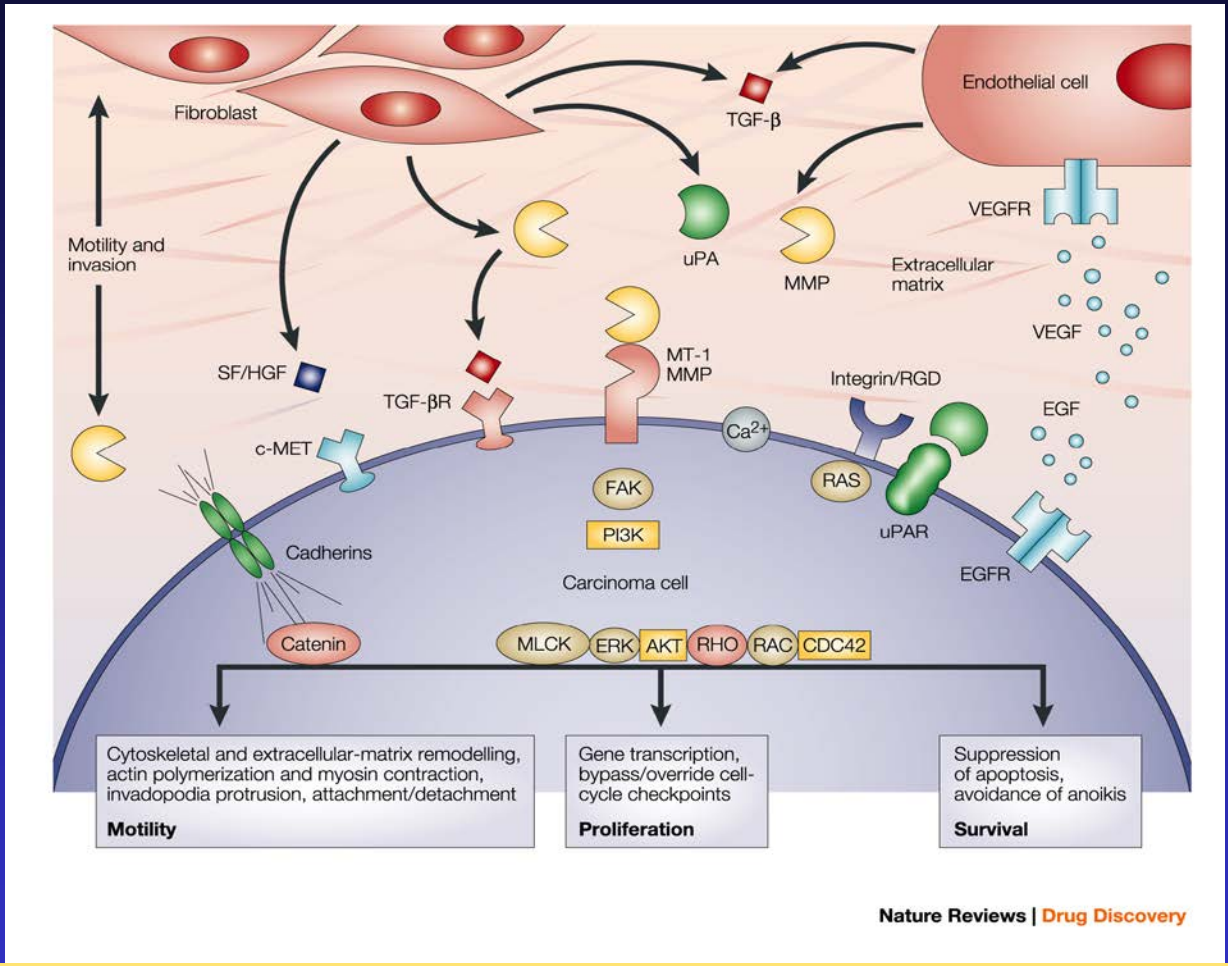
Hypoxia induces angiogenesis and promotes invasion



Outgrowth of vascular supply

Selection of aggressive cells

Molecular Ecology of the Tumor-Host Microenvironment



Translational Applications

- Extracellular signalling networks are the therapeutic target
- Pro survival pathways may be a key selection factor within a given cellular context

Pathogenic defects in signal pathways extend into the tumor host interface.

- Cross regulation, and exchange, of enzymes, substrates, cytokines and mitogens stimulates motility, invasion, survival and growth of tumor and host populations
- Invadopodia are localized regulated at the leading edge of the invading cancer cell

Key Theme

As with the other stages of carcinogenesis, metastasis is genetically controlled with the involvement of both enhancing and suppressing modifiers.

Metastasis Promoting Genes - I

Gene	Tissue Site	Function
ARM-1	Lymphoma	Promotes adhesion of tumor cells to the endothelium
ATX	Breast, Liver, Lung, Melanoma, Teratocarcinoma	cytoskeletal reorganization and motility; G-protein coupled receptor activation
CD44	Multiple sites	cell-cell interactions; activates HGF/c-Met pathway
Cox2	Breast, Colorectal, Gastric	Prostaglandin synthase; induces VEGF
Cyr61	Breast	Mediates adhesion; Erb-B2/3/4 pathway
Ezrin	Liver, Ovary, Pancreas, Prostate, Uterus	Membrane-cytoskeletal linker; RHO and RAC interactions
HMG-I(Y)	Breast, Cervical, Colorectal, Prostate, Skin, Thyroid, Uterus	Regulated by EGF and MMP-9
Laminin-5	Multiple sites	EGF and TGF-α induce expression of laminin subunits; cell adhesion, motility
c-Met	Multiple sites	Activated by HGF; Modulates Ras and PI3 kinase

Metastasis Promoting Genes - II

Gene	Tissue Site	Function
MTA1	Breast, Cervix, Melanoma, Ovary	Neucleosome remodeling; histone deacetylase complex
Oncostatin M	Lung	Activates PKA-dependent pathway
PP2A	Not determined	Activated by p38/MAPK; inhibits MEK1, MEK2, and MMP-1
RAGE	Gastric, Lung, Pancreatic, Renal	transmembrane receptor; activates p21, MAPKs, NF-6B, cdc42/rac
S100A4	Breast, Colorectal, Prostate	Calcium-binding protein; activates c-erbB-2
S100A9	Colon, Gastric, Skin	Calcium-binding protein; Modulates Mac-1 integrin receptor through G-protein
Semaphorins	Gastric, Leukemia, Lung, Skin	cell-cell interactions; Receptor crosstalk with c-Met binding semaphorin receptor, plexin
Thymosin-β15	Prostate	actin binding; motility
Wnt-5a	Breast, Colon, Lung, Melanoma, Pancreas, Prostate	PKC activation with associated changes in cytoskeleton, cell adhesion, and motility

Cellular Phenotypes Modulated by IGF1

- Growth
- Apoptosis
- Invasion
- Metastasis
- Angiogenesis
- Response to chemotherapy

IGF-1/IGF-R as Positive Regulators of Metastasis

- Mutant IGF-R(soluble receptor) blocks metastasis but not tumor growth of breast cancer cells(Dunn/Barrett).
- Serum IGF-1 levels influence metastasis of colon cells(Wu/LeRoith).
- IGF-R overexpression accelerates metastatic progression in RIP-Tag mice(Lopez/Hanahan).

Evidence for Genetic Influences on Metastatic Potential

- Metastasis formation(independent of tumor initiation and growth) in mice is dependent on the genetic background of the mouse and map to multiple loci (Kent Hunter, NCI)
- Hybrids between metastatic cells and non metastatic cells are suppressed for metastasis independent of tumor forming ability
- Specific genes can control metastasis independent of tumorigenesis

Metastasis Suppressor Genes - I

Gene	Tissue Site	Function
Annexin7	Prostate	calcium-dependent GTPase; substrate for PKC and other kinases associated with proliferation
BRMS1	Breast, Melanoma	gap-junctional communication
CC3	Colon, Lung	serine/threonine kinase
CEACAM1-4S	Breast, Colon	Bax pathway
CRSP3	Melanoma	transcriptional co-activator
DAP-kinase	Multiple sites	calcium/calmodulin-dependent serine/threonine kinase; pro-apoptotic pathway
E-cadherin	Multiple sites	Wnt signaling; cytoskeleton; cell-cell adhesion
HEPSIN	Ovarian, Prostate, Renal	transmembrane serine protease
HPI$^{HS\alpha}$	Breast	non-histone heterochromatin-associated protein
KAI-1	Breast, Prostate	Transmembrane tetraspondin; role in adhesion, motility, growth regulation, and differentiation; integrin interaction
KiSS1	Breast, Melanoma	Modulates Rho, Rac, and MAPK signaling
Maspin	Breast, Colon, Oral Squamous Cell, Prostate	Serine protease inhibitor; binds collagen and can modulate integrins
Melastatin	Melanoma	Calcium channel protein

Metastasis Suppressor Genes - II

Gene	Tissue Site	Function
MKK4	Ovary, Prostate	MAPK; phosphorylates and activates p38 and JNK kinases
NESH	Lung, Prostate	src homology 3 adapter protein; down regulates p21 pathway
NM23-H1	Breast, Colon, Melanoma, Oral Squamous Cell	histidine kinase; phosphorylates KSR, which might reduce ERK 1/2 activation
PTEN	Multiple sites	phosphatase; growth regulation, cell motility
RhoGD12	Bladder	Inhibits GTP binding; regulates RHO and RAC
SFRP1	Breast, Colorectal	Modulates Wnt signaling pathway
SHPS-1	Breast, Leukemia	glycoprotein; may regulate RAS-MAPK signaling; suppresses anchorage independent growth
Syk	Breast, Colon, Pancreas, Skin	Tyrosine kinase; inhibits PI3 kinase; necessary for MAPK activation
TSP-1	Multiple sites	inhibits endothelial cell proliferation and migration; c-Myc expression inhibits TSP-1
tropomyosins	Breast	interacts with e-cadherin/catenin complex
VDUP1	Melanoma	Thioredoxin inhibitor; upregulates *KiSS1; interacts with CRSPs*

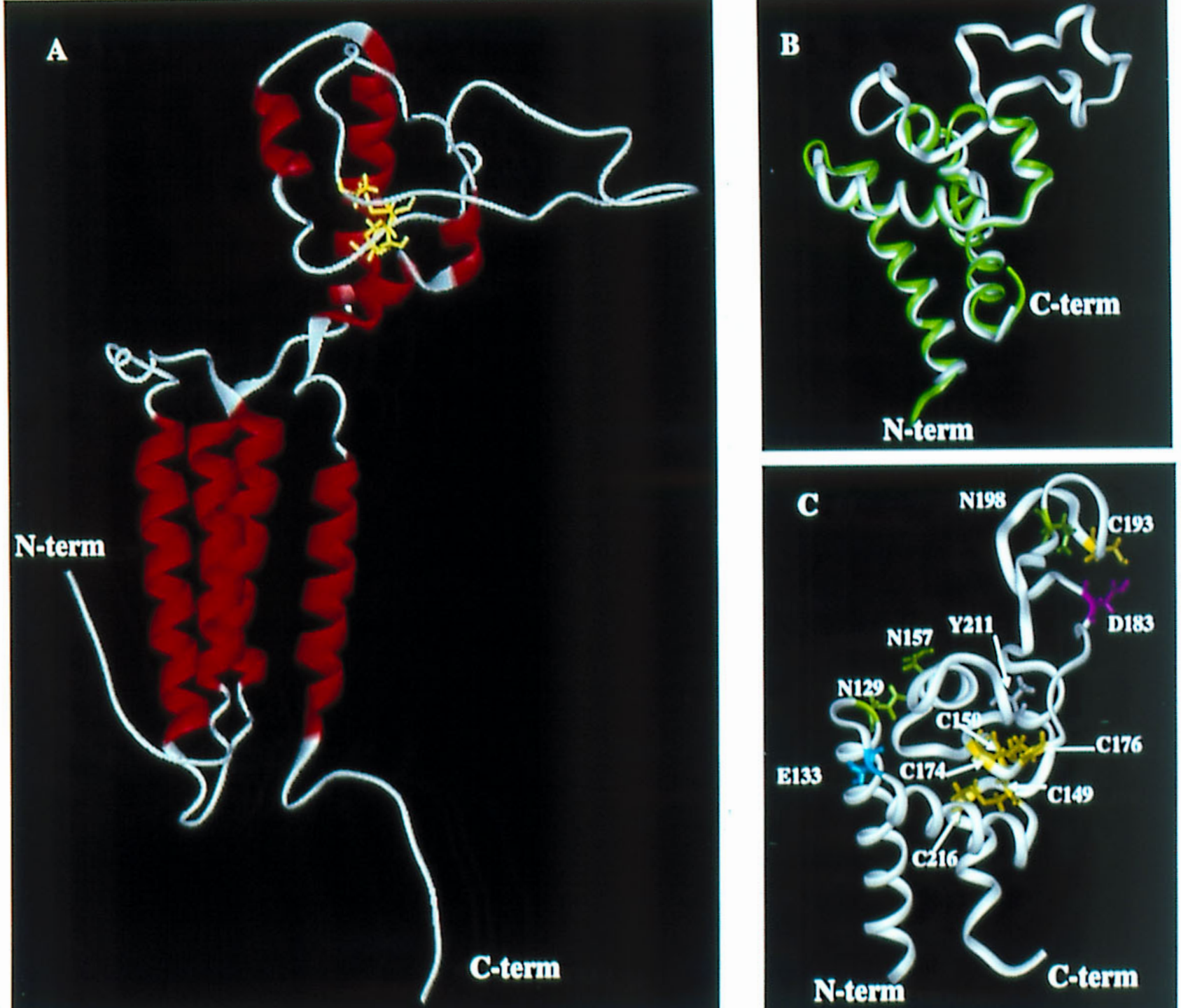
Lessons Learned from Studies of Genes Involved in Metastasis

- Both positive and negative regulation of metastasis are involved in cancers.
- Metastasis suppressor genes can be lost early in the development of cancers.
- Multiple mechanisms are involved in metastasis.
- Interactions and possible pathways of proteins involved in metastasis are observed.
- Negative regulators of metastasis often exhibit epigenetic silencing rather than mutations in cancers.
- Negative regulators of metastasis exhibit plasitcity of expression and function .

Prostatic Metastasis Suppressor Gene

KAI-1

Kang-ai ---- Anti Cancer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N-term
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E133
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C216
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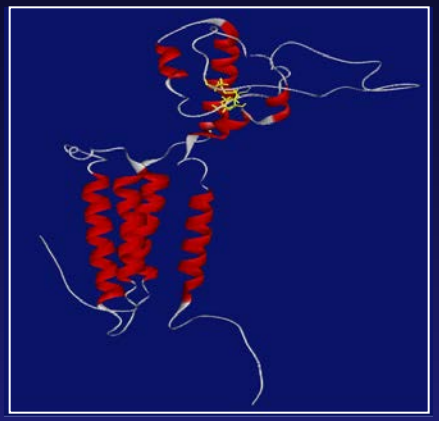


KAI1 / CD 82

Names : KAI1 / CD82, (C33, R2, IA4)

Gender : Transmembrane Glycoprotein

Ligands ? **Signal Pathways : ?**

Biological Function :

- **motility**
- **invasiveness**
- **cell-cell interactions**

Particularity

- **Member of the tetraspanin or transmembrane 4 superfamily (TM4SF)**
- **Contains an internalization sequence at its C-terminus (YSKV)**

***Current Address* :**

Cell membrane (lymphocytes, epithelial cells)

Lysosomes

Vesicles

KAI1 / CD 82 and Cancer

Correlations

High level of KAI1/CD82 is a good prognosis factor or associated with low grade histology :

- prostate
- pancreas
- colon
- lung carcinoma

KAI1/CD82 expression is inversely related to the metastatic potential :

- prostate
- lung carcinoma
- colon
- hepatoma
- breast
- lung (non-small-cell carcinoma)
- bladder cancer
- *ovary*
- *melanoma*

Experimental Data

Transfection of tetraspanin reduces metastatic potential

melanoma	B16
	MDA-MB-435 *
prostate	AT6.1, AT6.3
breast	MDA-MB-231

(from Boucheix & Rubinstein , 2001

Loss of KAI-1 Expression in Prostate Cancer

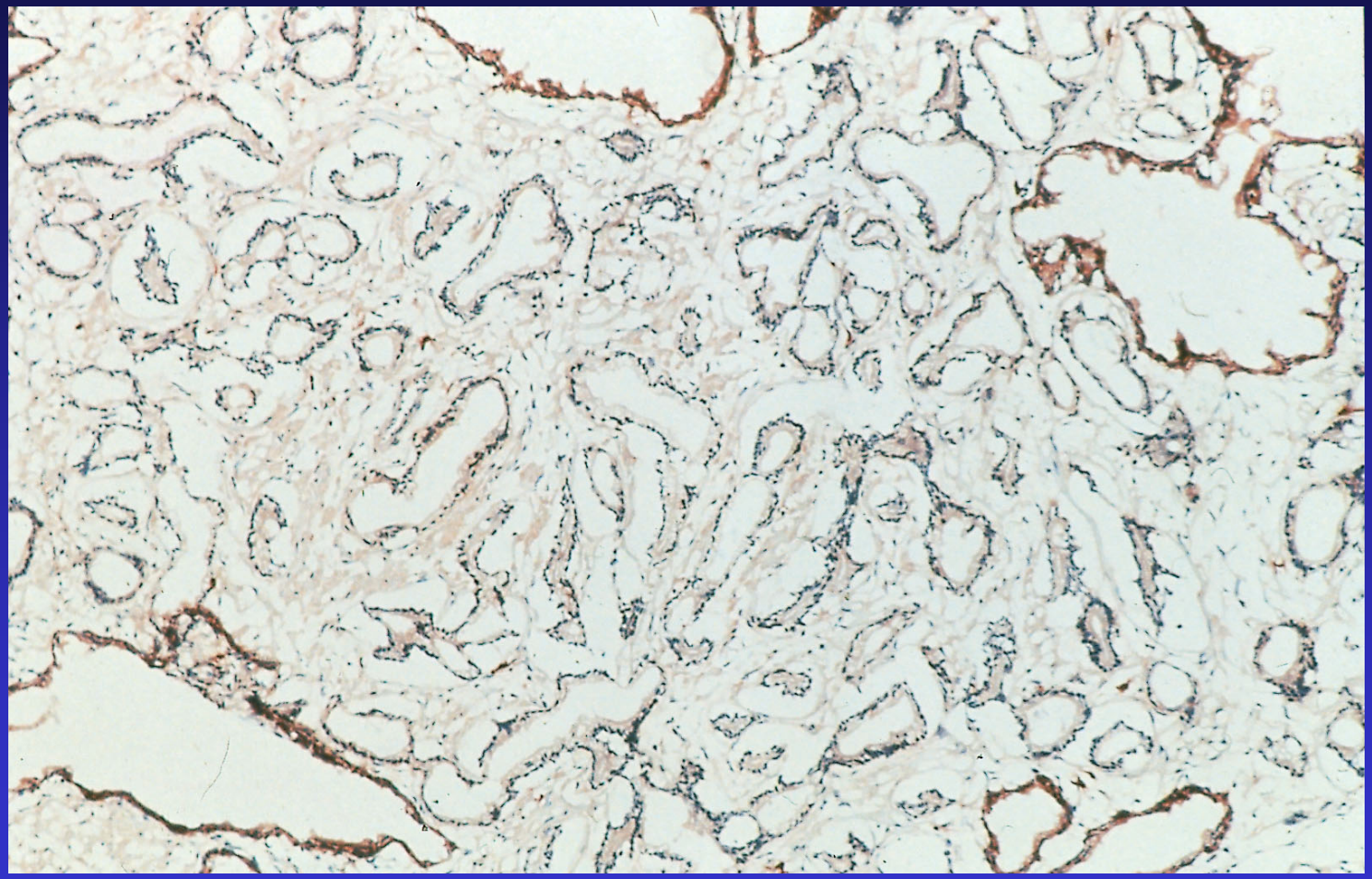
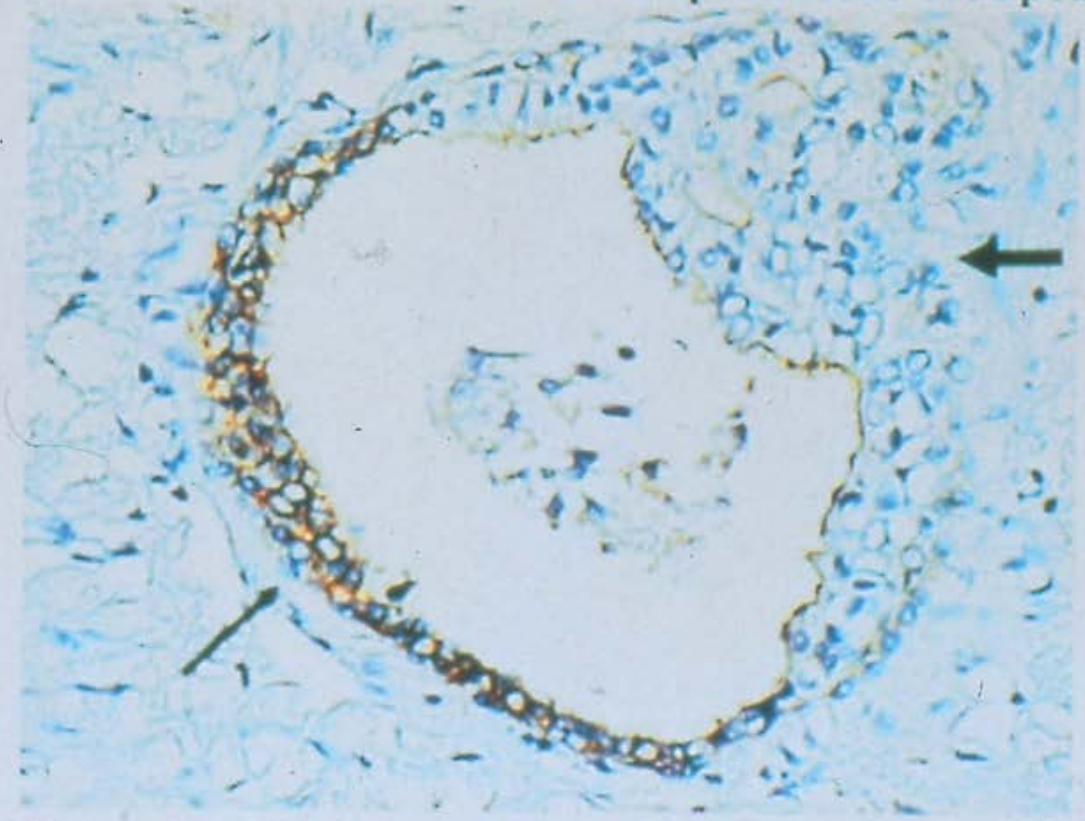


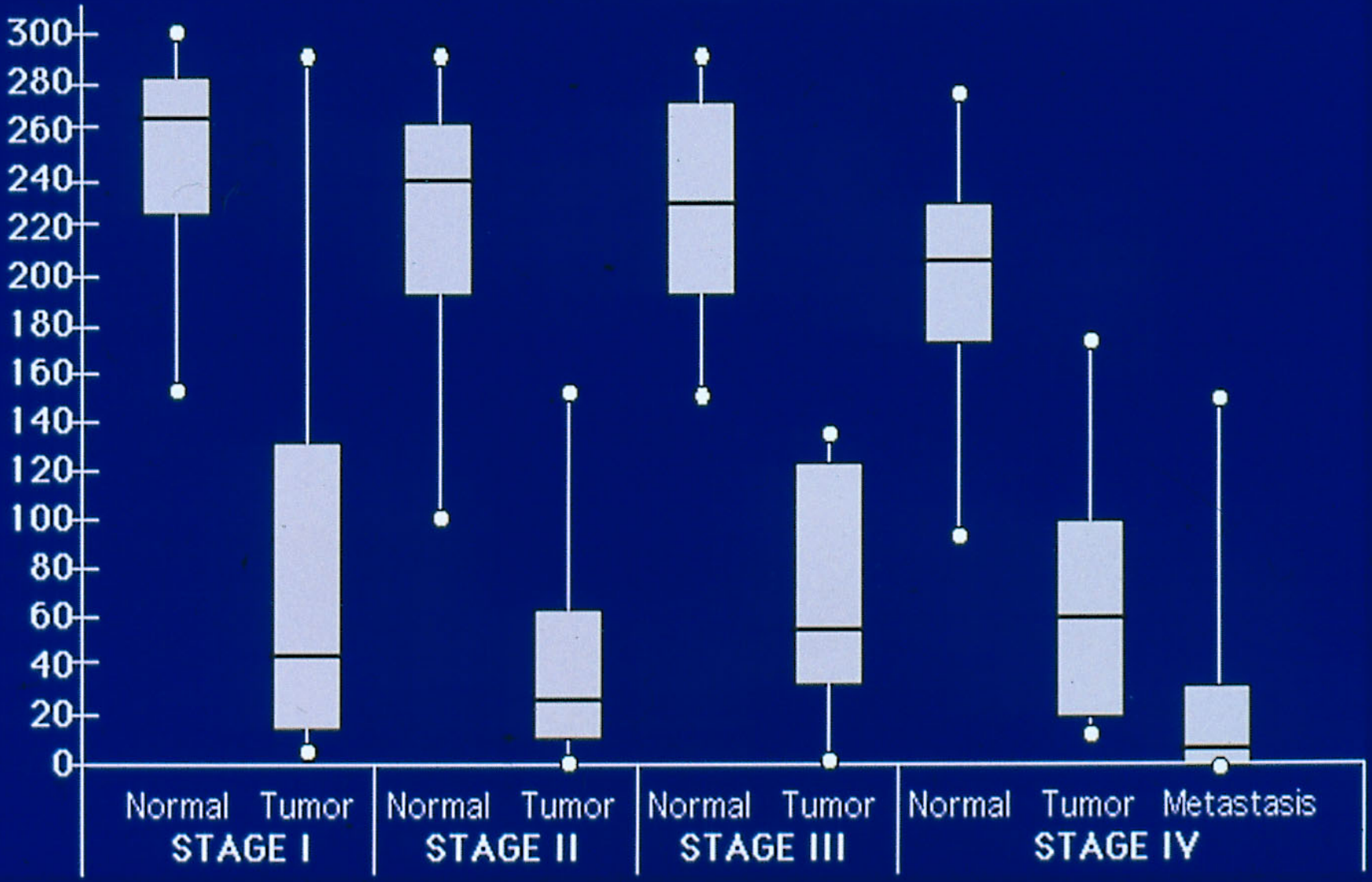
Figure 6. Immunohistochemistry of KAI1 in Prostatic Intraepithelial Neoplasia (PIN)



normal appearing epithelial cells, KAI1 +

high-grade PIN, KAI1 -

Normal Colon and Tumor KAI1 Scores by Stage



KAI-1 Functions

+ (Promotes)	- (Inhibits)
Cell Aggregability Cell Adhesion	Invasion Motility Metastasis

The Key Question :

How does KAI1 exert it's effect on the ability of cancer cells to invade ?

KAI-1 as a Molecular Facilitator

Association of KAI1 / CD82 with other cell surface molecules

TM4SF members } **the tetraspanin web**

β integrins

α3β1, α6β1, α4β1, α1β2

Molecules of the immune system

HLA-DR, MHC class I, CD4, CD8, CD19, CD46,

Others

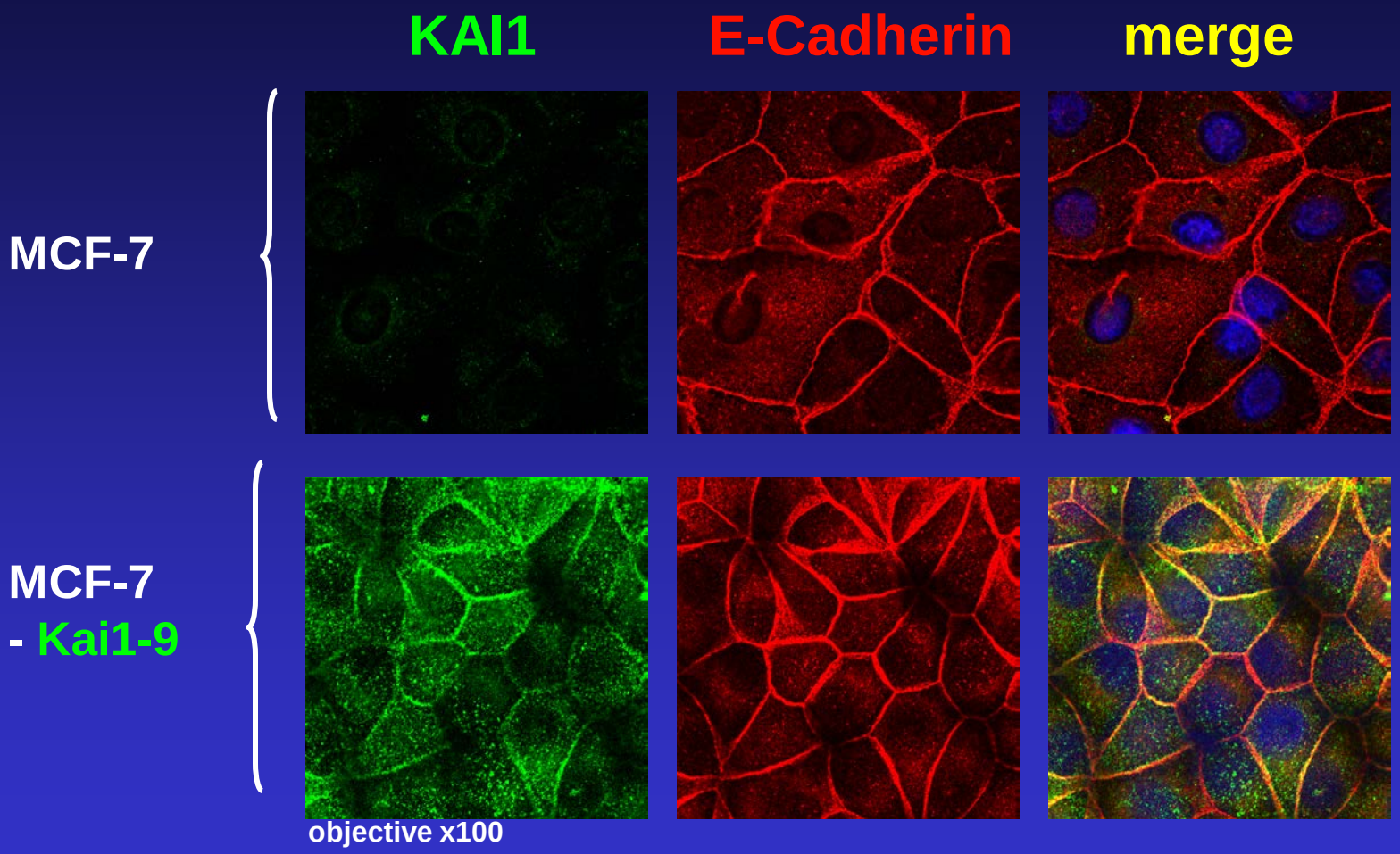
CD9P-1, ProHB-EGF, γ-glutamyl transpeptidase,

EGF-R

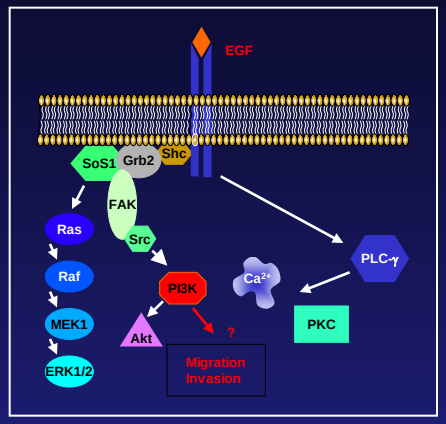
DARC

E-Cadherin

Confocal microscopy



EGF-R



EGF-R pathway

Names : EGF-R (erb-1)

***Gender* :** Receptor Tyrosine Kinase (RTK)

Ligands: EGF
TGFβ

Signal Pathways : MAPK
PI3K

Biological Function :

morphogenesis
growth regulation

Oncogenic effects :

(over-expression EGFR)
initiation of DNA synthesis
enhanced cell growth
invasion
metastasis

Current Address :

Cell membrane (membrane microdomains)

Vesicles

Nucleus (?)

Why KAI-1 and EGF-R pathways ?

Attenuation of EGF receptor signaling by a metastasis suppressor, the tetraspanin CD82/KAI1

E. Odintsova et al., 2000

Facilitation of ligand-induced endocytosis of the EGF-R and its subsequent desensitization by CD82/KAi1

⇒ **Opposite effects of KAI1 and EGF-R pathway**

Selective enrichment of Tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B- lymphocytes

J.M. Escola et al., 1998

⇒ **“Co-localisation” in endosomes of KAI1 and EGF-R**

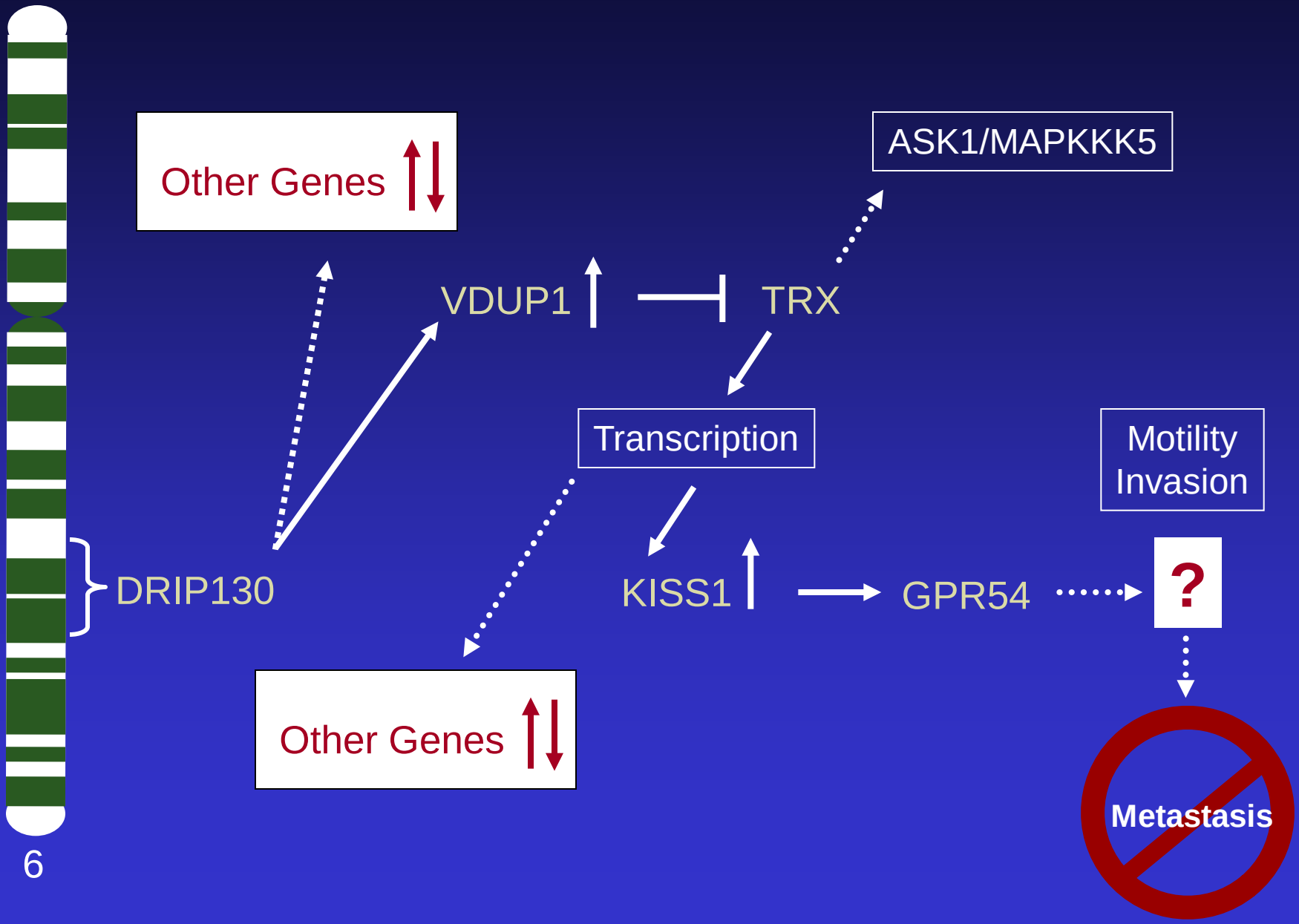
Possible Mechanisms for Loss of KAI-1 Function

- Mutation -never observed
- Down regulation of mRNA - common in many cancers
- DNA methylation of promoter- not observed
- **Posttranslational modification - glycosylation differences observed in some tumors**
- **Loss of function in KAI-1 partners or downstream effectors - not fully tested**

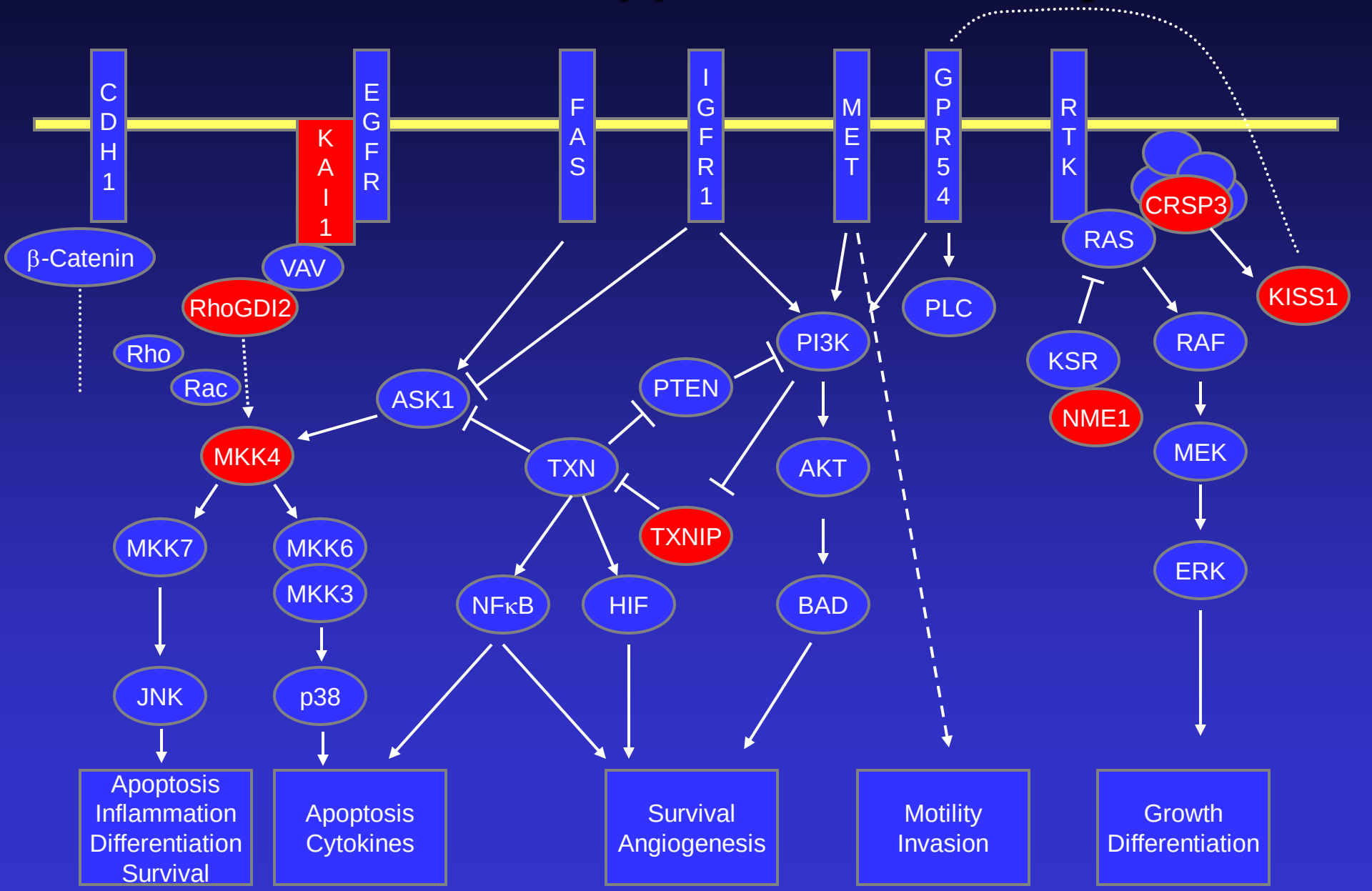
Regulators of KAI-1 expression in cancer cells

- 5-AzadC
- Phorbol esters
- Nerve growth factor
- TNF/NFkB

A Metastasis Suppression Pathway



Metastasis Suppressor Pathways



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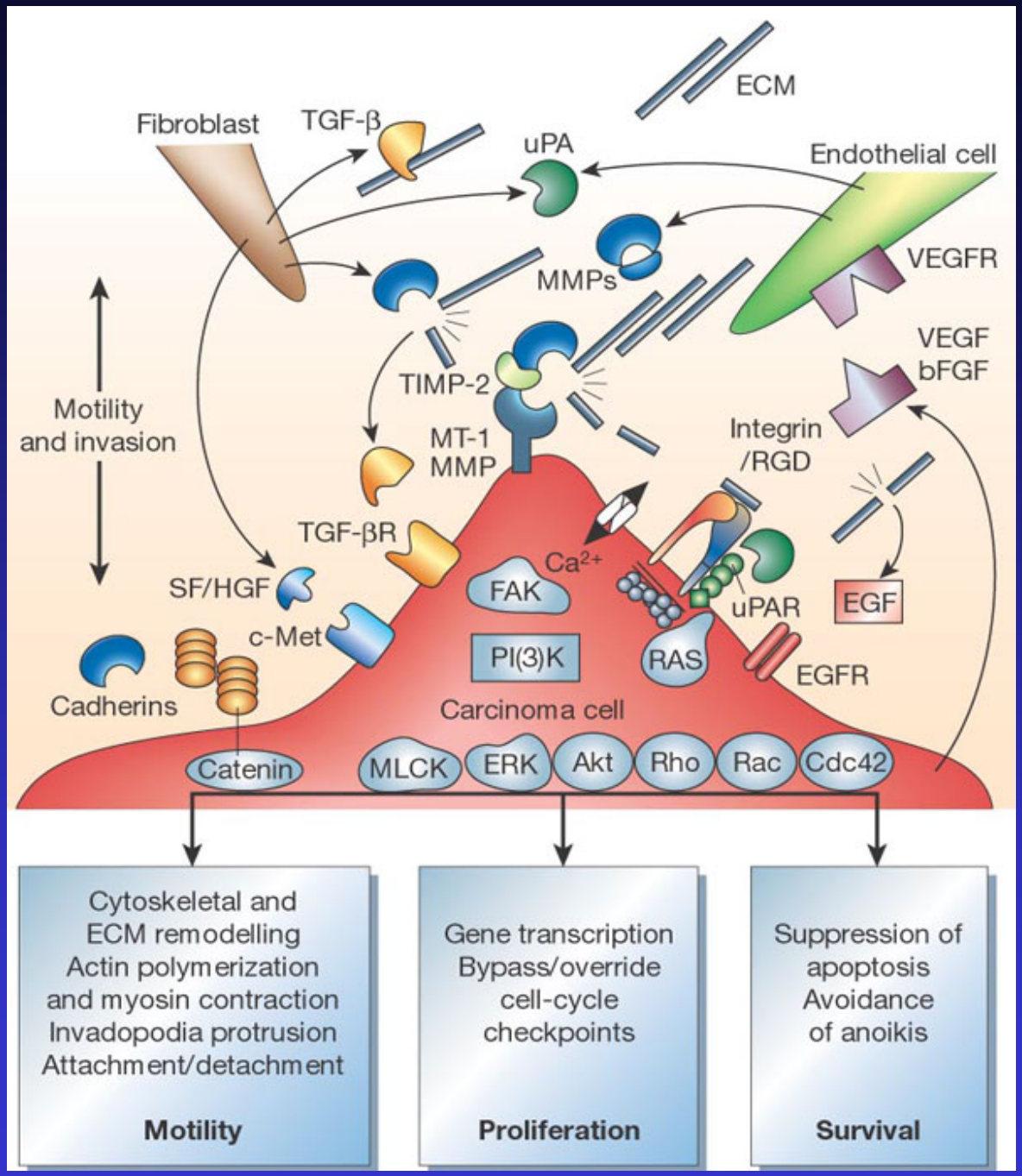
- Interactions and possible pathways of genes involved in metastasis are observed.
- Negative regulators of metastasis often exhibit epigenetic silencing in cancers.
- Negative regulators of metastasis exhibit plasticity of expression and function

Hard Clinical Truths About Metastasis

1. Upwards of 70% of patients may have overt or occult metastases at diagnosis.
2. Acquisition of the invasive and metastatic phenotype is an early event in cancer progression.
3. Millions of tumor cells are shed daily into the circulation.
4. Angiogenesis is a ubiquitous and early event that is necessary for and promotes metastatic dissemination.

Lucky Clinical Truths About Metastasis

1. Both malignant invasion and angiogenesis use the same “hardware” and “software” programs.
2. Less than 0.01% of circulating tumor cells successfully initiate a metastatic focus.
3. Circulating tumor cells can be detected in patients who do not develop overt metastatic disease.
4. Metastases may be as susceptible to anti-cancer therapy as primary tumors.


ECM
Fibroblast
TGF-β
uPA
Endothelial cell
VEGFR
MMPs
VEGF
bFGF
TIMP-2
Motility
and invasion
MT-1
MMP
Integrin
/RGD
TGF-βR
Ca2+
SF/HGF
FAK
uPAR
EGF
c-Met
PI(3)K
RAS
EGFR
Cadherins
Carcinoma cell
Catenin
MLCK
ERK
Akt
Rho
Rac
Cdc42
Cytoskeletal and
ECM remodelling
Actin polymerization
and myosin contraction
Invadopodia protrusion
Attachment/detachment
Motility
Gene transcription
Bypass/override
cell-cycle
checkpoints
Proliferation
Suppression of
apoptosis
Avoidance
of anoikis
Survival