Corso di Biologia Cellulare del Cancro AA 2019-2020

L'INFIAMMAZIONE PRO-TUMORALE

Indipendenza dai segnali proliferativi

Resistenza ai segnali inibitori della proliferazione

Riprogrammazione del metabolismo cellulare

Evasione dal sitema immunitario

Evasione dai meccanismi oncosoppressivi intrinseci

Potenziale replicativo illimitato

Mutazione e instabilità genomica

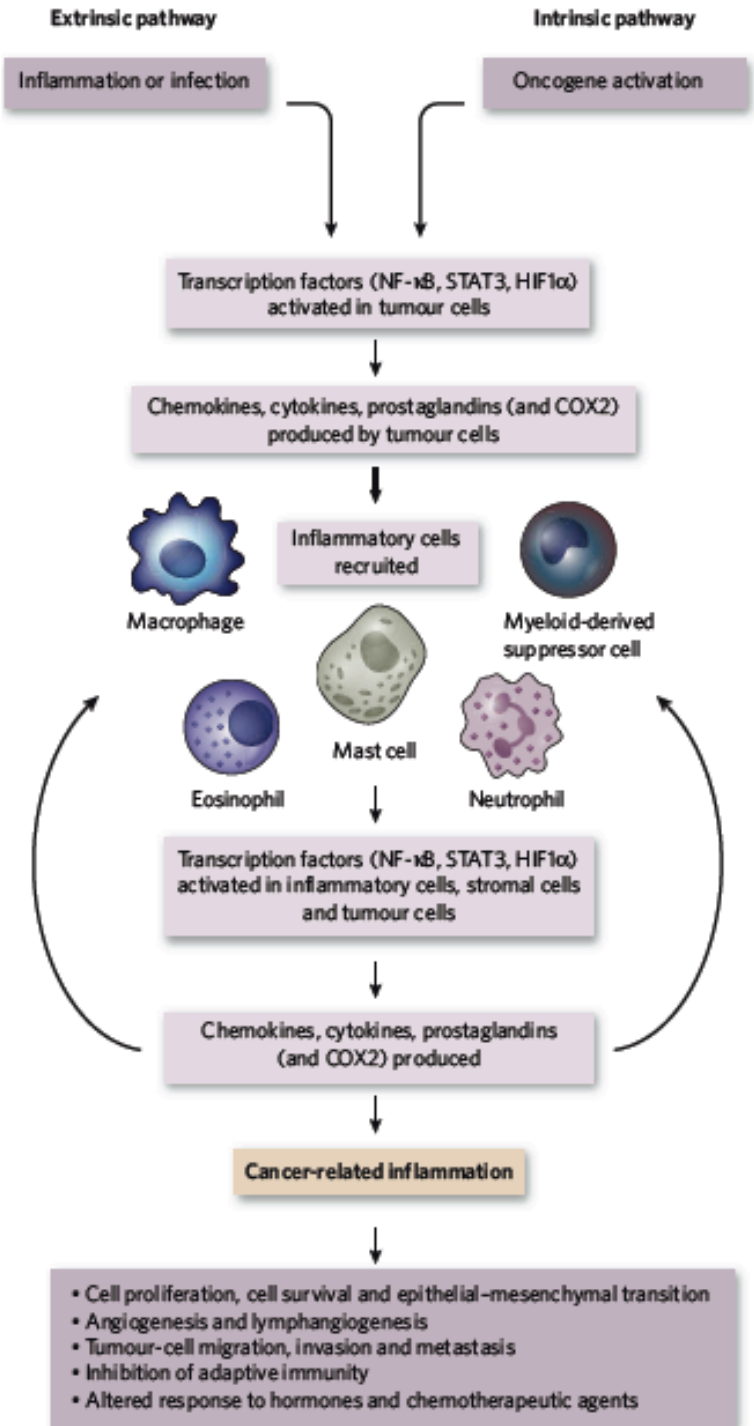
Infiammazione pro-tumorale

Angiogenesi

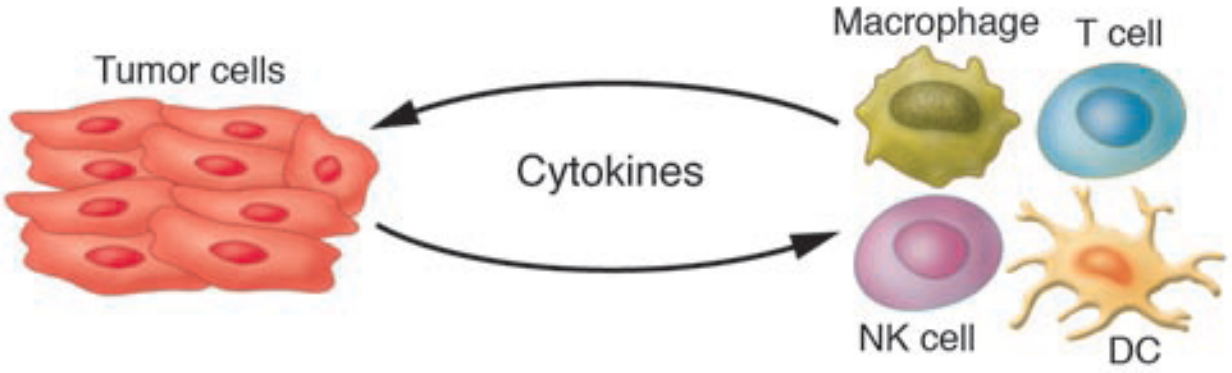
Invasione e metastasi

Box 1 | The evidence that links cancer and inflammation

- Inflammatory diseases increase the risk of developing many types of cancer (including bladder, cervical, gastric, intestinal, oesophageal, ovarian, prostate and thyroid cancer).
- Non-steroidal anti-inflammatory drugs reduce the risk of developing certain cancers (such as colon and breast cancer) and reduce the mortality caused by these cancers.
- Signalling pathways involved in inflammation operate downstream of oncogenic mutations (such as mutations in the genes encoding RAS, MYC and RET).
- Inflammatory cells, chemokines and cytokines are present in the microenvironment of all tumours in experimental animal models and humans from the earliest stages of development.
- The targeting of inflammatory mediators (chemokines and cytokines, such as TNF-α and IL-1β), key transcription factors involved in inflammation (such as NF-κB and STAT3) or inflammatory cells decreases the incidence and spread of cancer.
- Adoptive transfer of inflammatory cells or overexpression of inflammatory cytokines promotes the development of tumours.


Extrinsic pathway
Intrinsic pathway
Inflammation or infection
Oncogene activation
Transcription factors (NF-κB, STAT3, HIF1α) activated in tumour cells
Chemokines, cytokines, prostaglandins (and COX2) produced by tumour cells
Inflammatory cells recruited
Macrophage
Myeloid-derived suppressor cell
Mast cell
Eosinophil
Neutrophil
Transcription factors (NF-κB, STAT3, HIF1α) activated in inflammatory cells, stromal cells and tumour cells
Chemokines, cytokines, prostaglandins (and COX2) produced
Cancer-related inflammation
• Cell proliferation, cell survival and epithelial–mesenchymal transition
• Angiogenesis and lymphangiogenesis
• Tumour-cell migration, invasion and metastasis
• Inhibition of adaptive immunity
• Altered response to hormones and chemotherapeutic agents

Cellule immuni-infiammatorie infiltranti il tumore



Infiltrato immune

Limita la progressione tumorale

Th1 lymphocytes
M1 macrophages
N1 neutrophils
T cytotoxic lymphocytes
NK cells

Involved in innate & adaptive immune response to pathogens

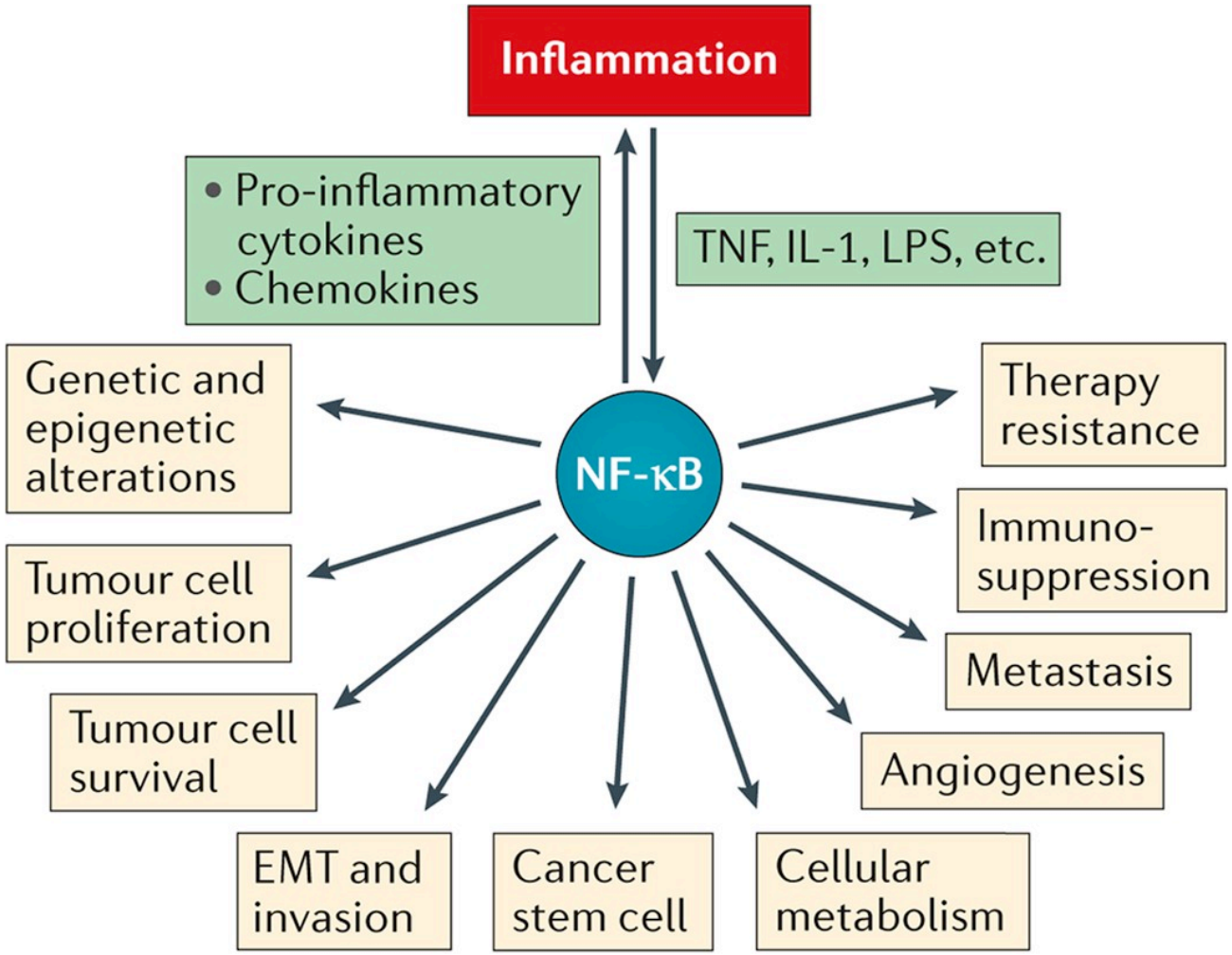
Stimola la progressione tumorale

Th2 lymphocytes
M2 macrophages
N2 neutrophils
Myeloid progenitors
Regulatory T cells

Involved in wound healing & tissue housecleaning

Mantovani and Sica, 2012 - Lin and Karin, 2007 - Zou, 2005 - Grivennikov, 2010

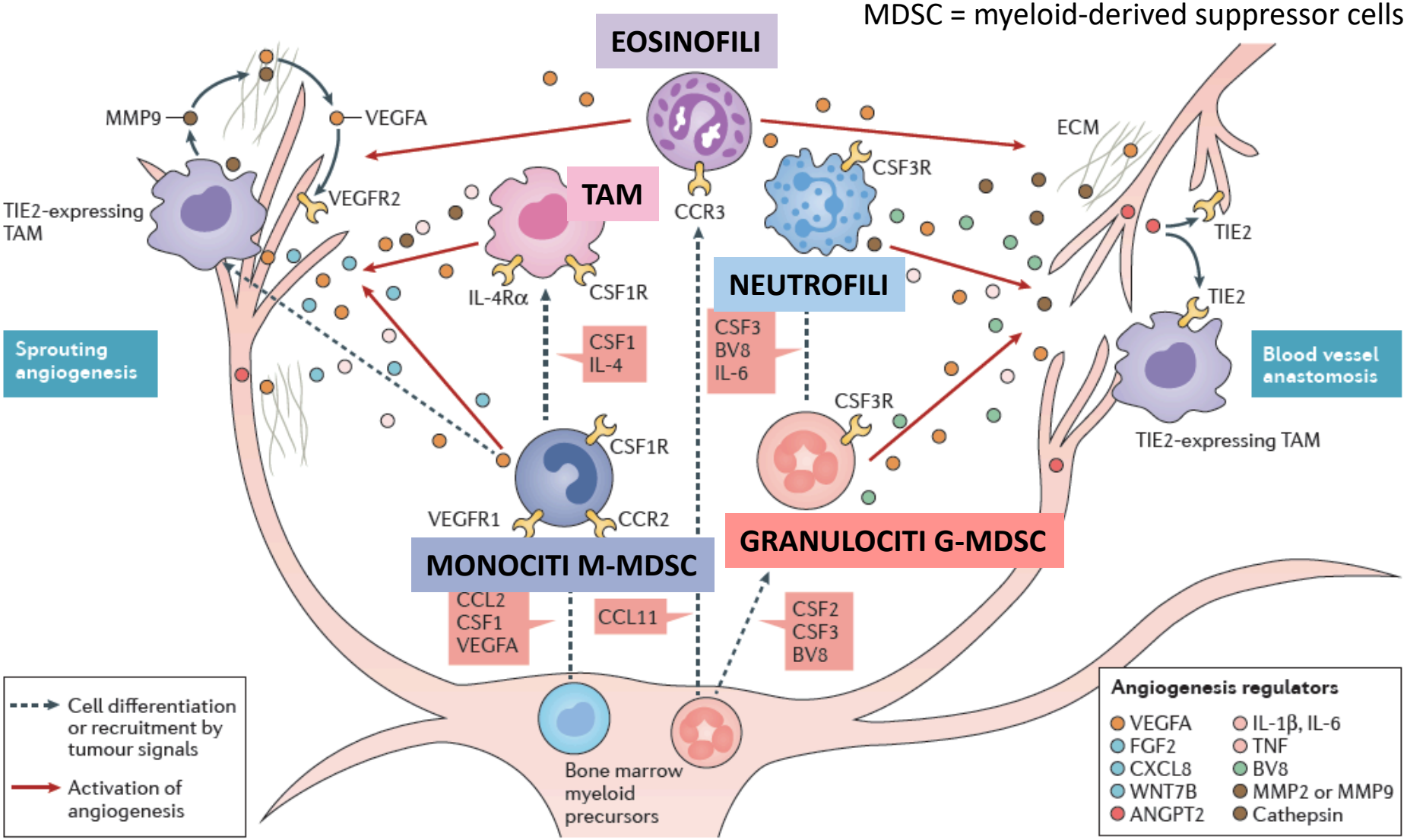
Citochine prodotte da cellule del SI inducono NF-κB pathway in cellule tumorali



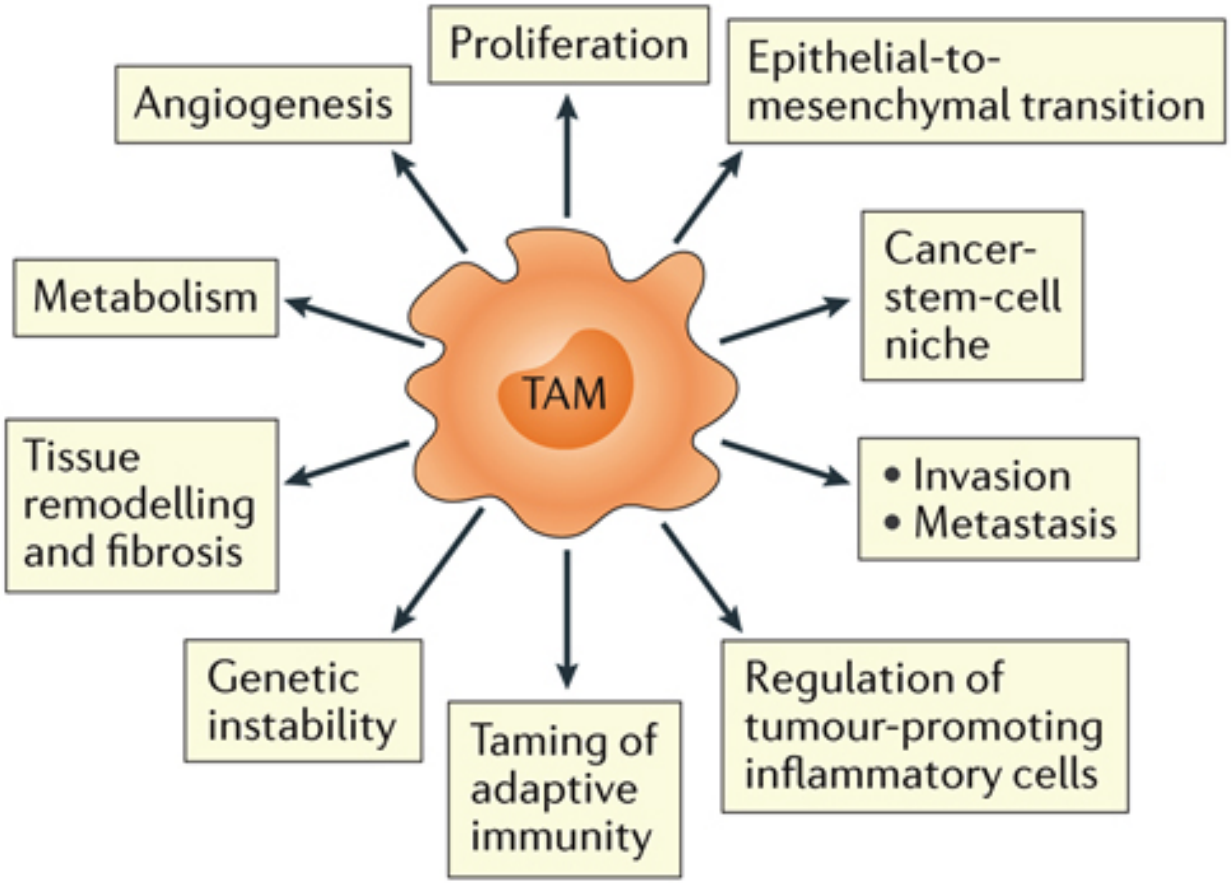
Nature Reviews | Immunology

Cellule della linea mieloide nel microambiente tumorale

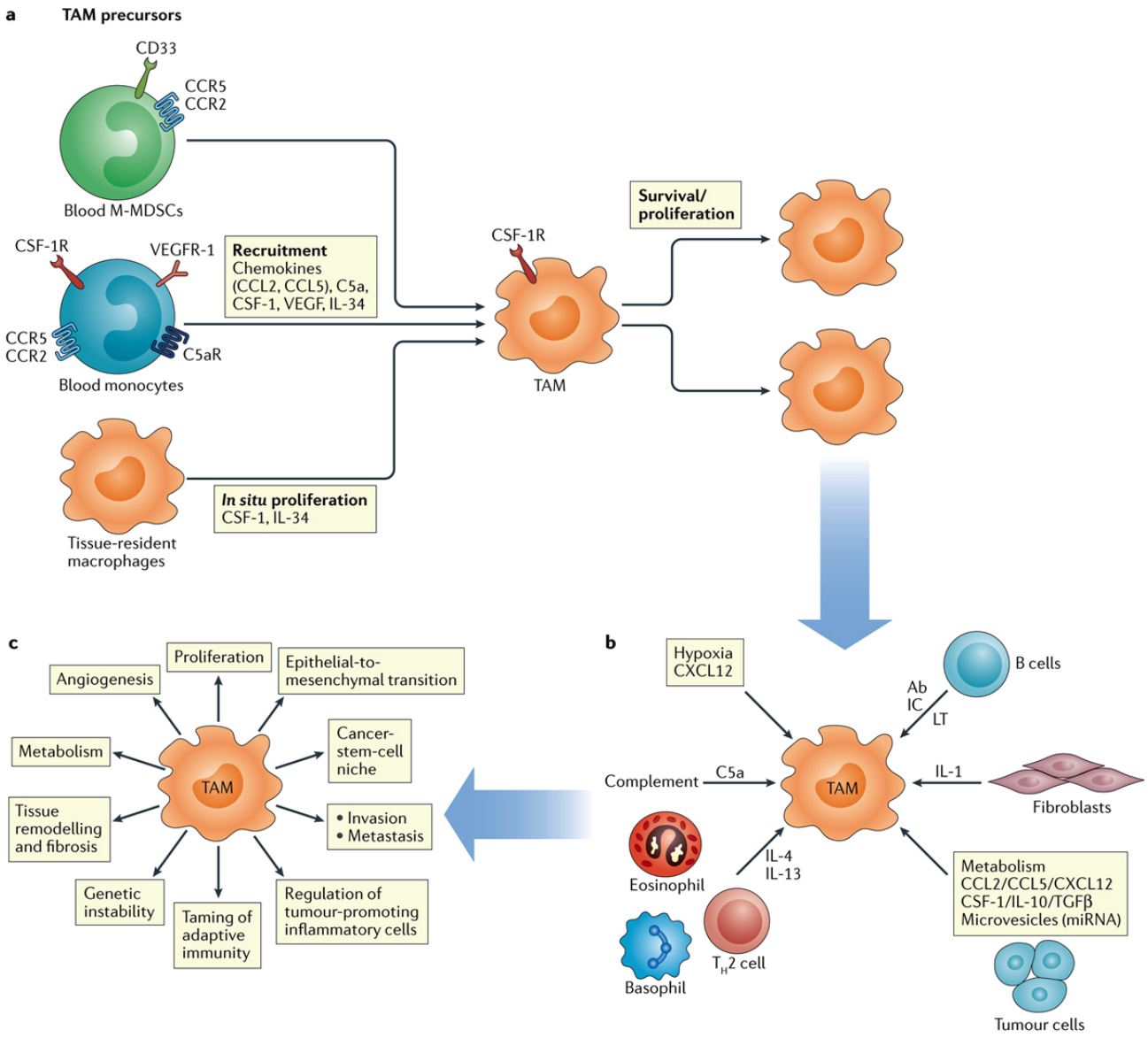
MDSC = myeloid-derived suppressor cells



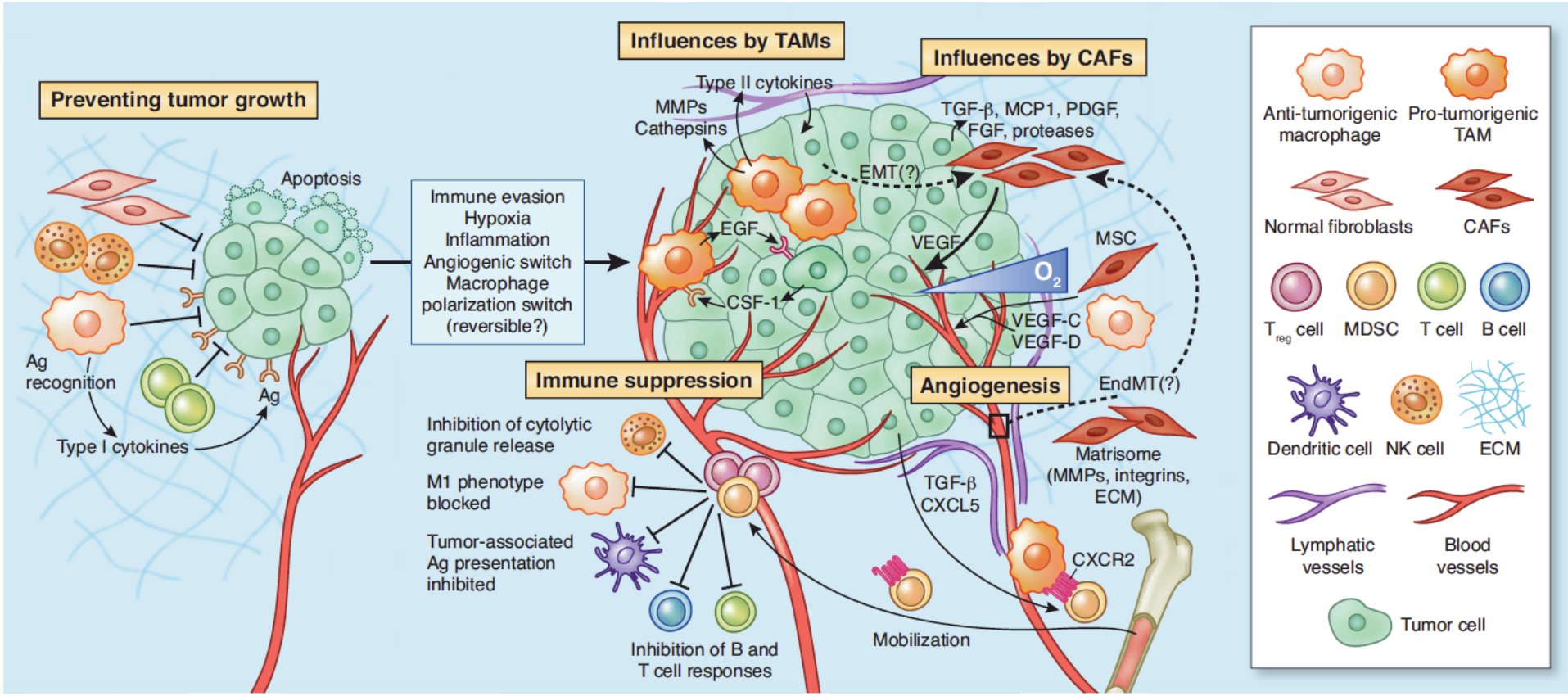
TAM = TUMOR-ASSOCIATED MACROPHAGES



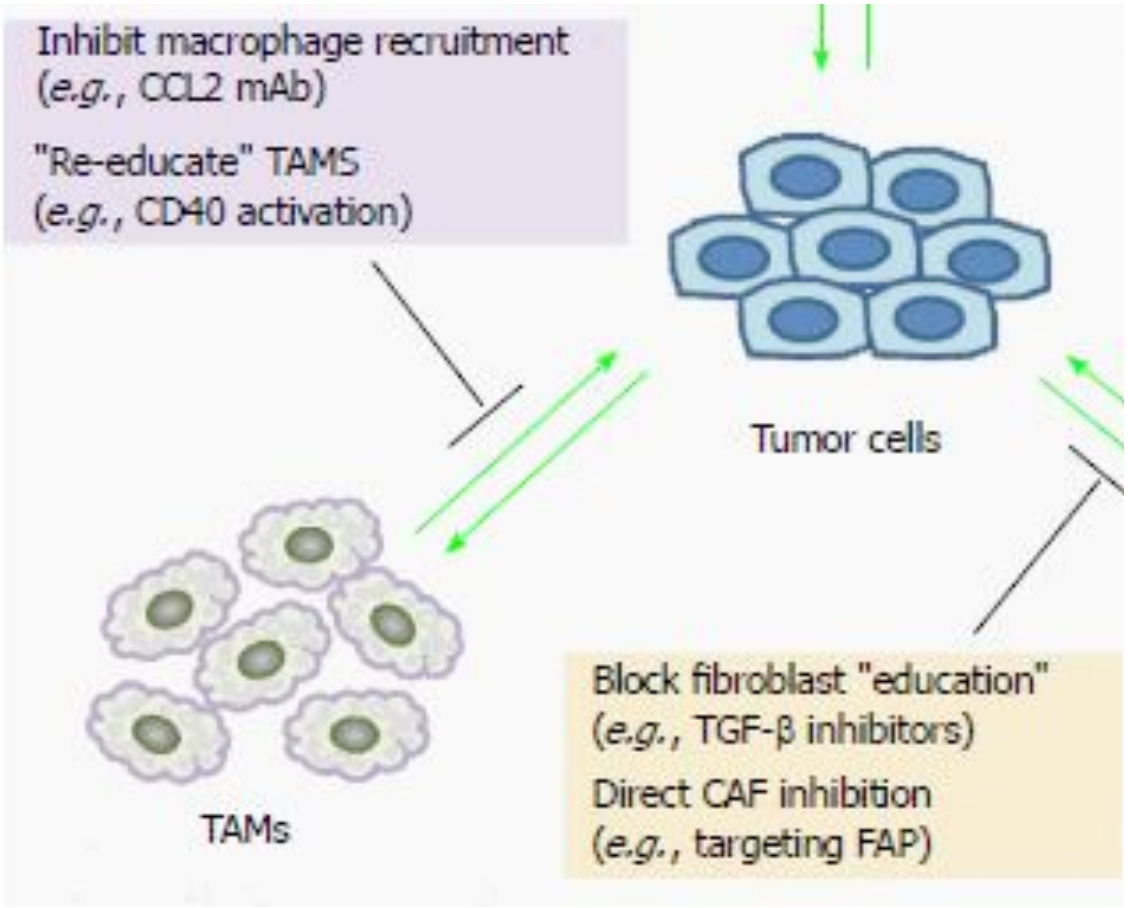
TAM = TUMOR-ASSOCIATED MACROPHAGES



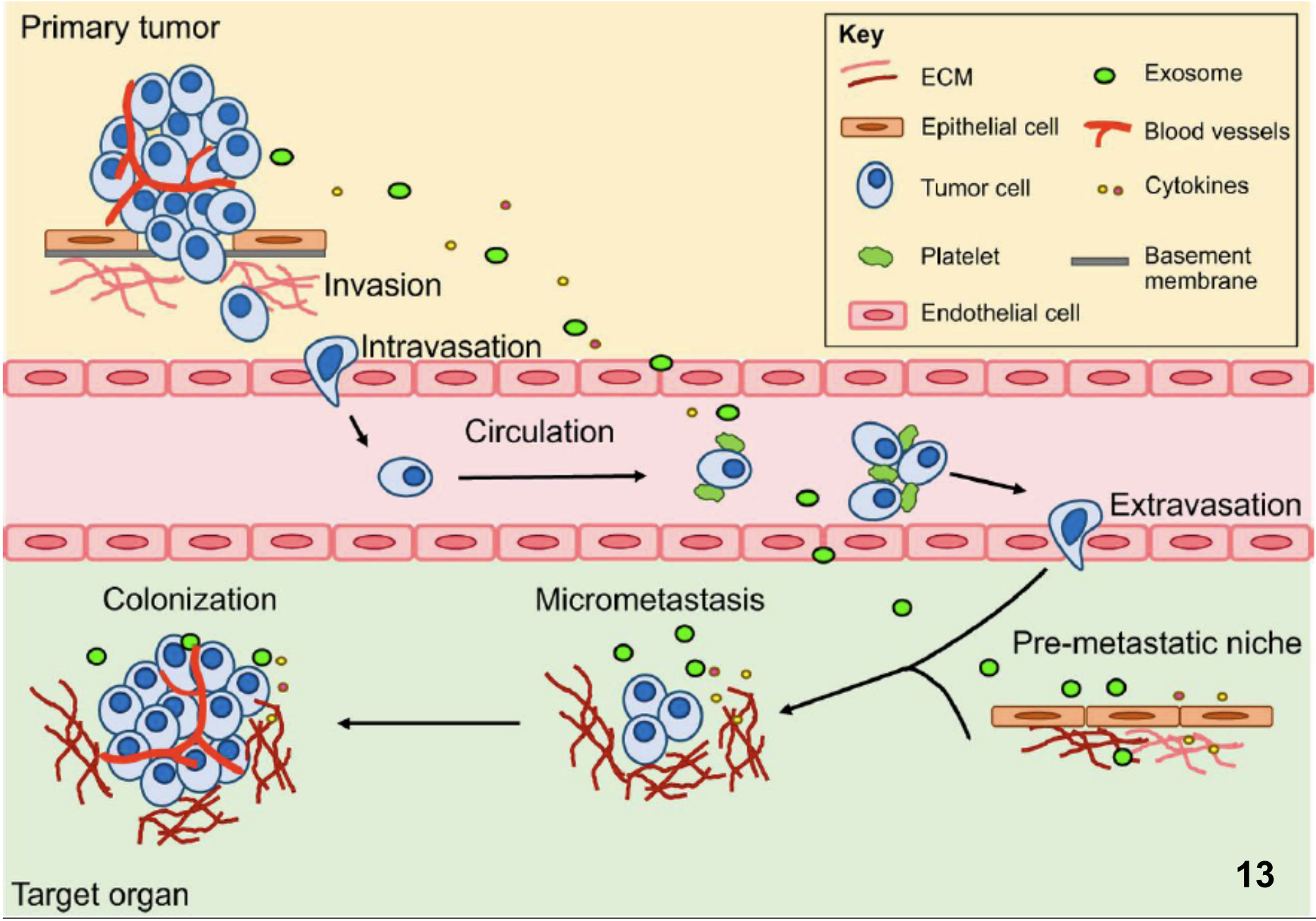
Nature Reviews | Clinical Oncology


Preventing tumor growth
Apoptosis
Ag recognition
Ag
Type I cytokines
Immune evasion
Hypoxia
Inflammation
Angiogenic switch
Macrophage polarization switch
(reversible?)
Influences by TAMs
Type II cytokines
MMPs
Cathepsins
EGF
CSF-1
Influences by CAFs
TGF-β, MCP1, PDGF,
FGF, proteases
EMT(?)
VEGF
MSC
O2
VEGF-C
VEGF-D
Angiogenesis
EndMT(?)
Matrisome
(MMPs, integrins,
ECM)
TGF-β
CXCL5
CXCR2
Immune suppression
Inhibition of cytolytic
granule release
M1 phenotype
blocked
Tumor-associated
Ag presentation
inhibited
Inhibition of B and
T cell responses
Mobilization
Anti-tumorigenic
macrophage
Pro-tumorigenic
TAM
Normal fibroblasts
CAFs
Treg cell
MDSC
T cell
B cell
Dendritic cell
NK cell
ECM
Lymphatic
vessels
Blood
vessels
Tumor cell

Terapie dirette contro il microambiente



LA CASCATA INVASIONE-METASTASI


Primary tumor
Key
ECM
Exosome
Epithelial cell
Blood vessels
Tumor cell
Cytokines
Platelet
Basement membrane
Endothelial cell
Invasion
Intravasation
Circulation
Extravasation
Colonization
Micrometastasis
Pre-metastatic niche
Target organ

Il processo metastatico

- E' il processo attraverso il quale si formano **tumori secondari**, in nuovi siti, diversi da quello originale.

- Durante la cascata metastatica le cellule tumorali subiscono/attivano una serie di cambiamenti più o meno stabili, la cui conseguenza è la generazione di cellule con **competenza metastatica**.

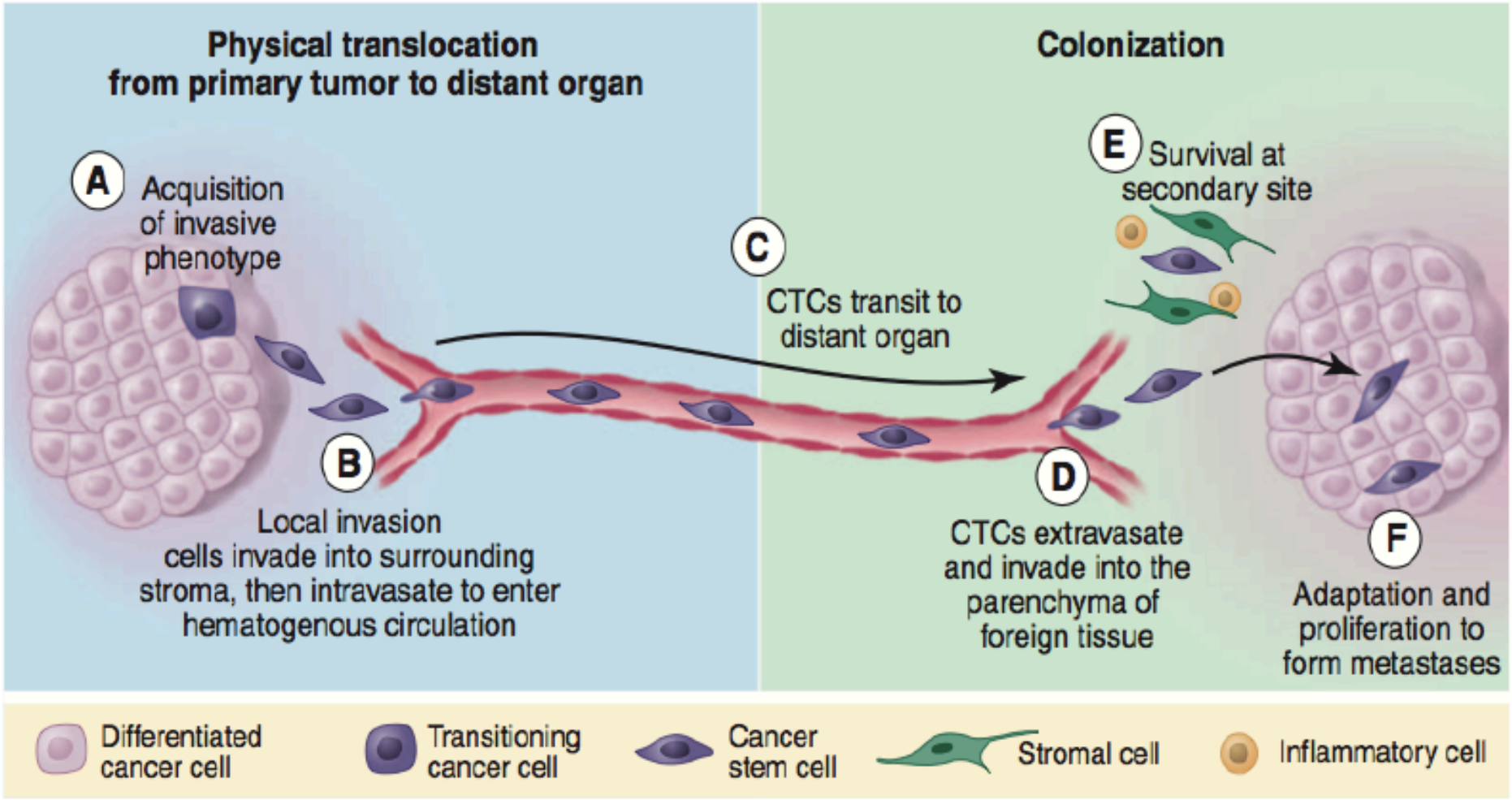
- È importante ricordare che questo processo dipende da interazioni con il microambiente.

- **Mortalità**: > 90% della mortalità associata al cancro si può attribuire alla formazione di metastasi.

- **Terapia**: la malattia metastatica è difficile da curare essendo di natura sistemica e spesso le cellule disseminate **sono resistenti agli agenti terapeutici**. Inoltre, le metastasi possono essere diverse geneticamente e fenotipicamente dal tumore primario.

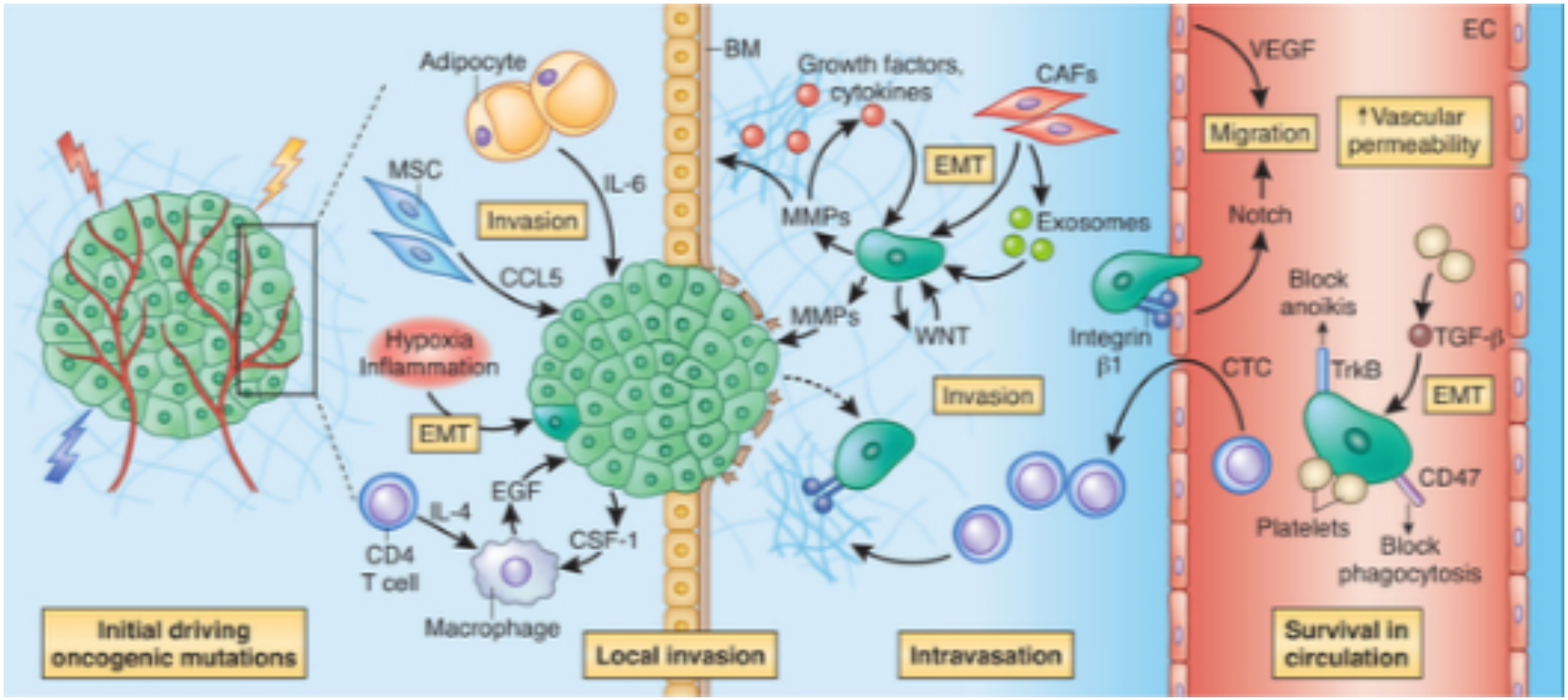
Il processo metastatico in due momenti principali

DISSEMINAZIONE **COLONIZZAZIONE**



EMT **MET**

Le fasi iniziali della disseminazione



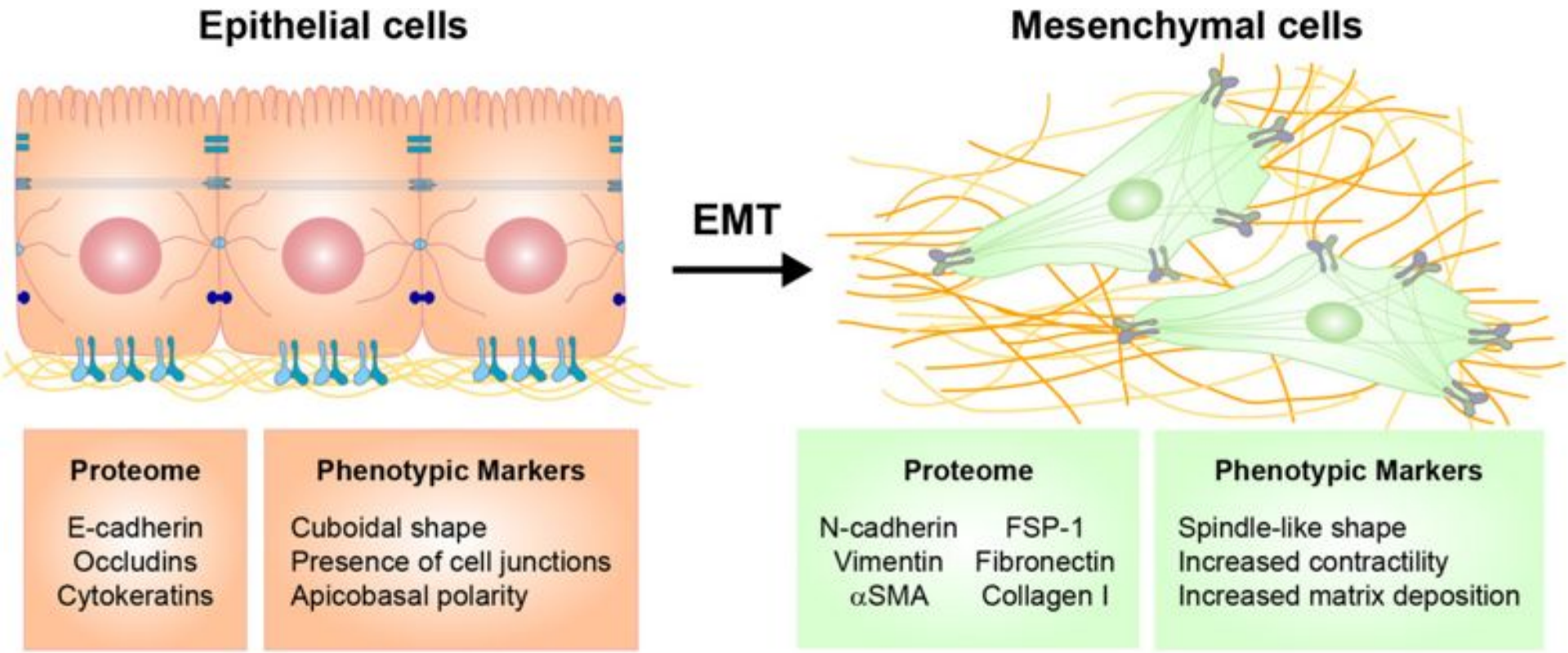
Wan, Pantel & Kang, Nat Med 2013

EMT: dedifferenziamento e acquisizione di un fenotipo motile e dotato di staminalità

Disgregazione della Membrana Basale e invasione locale Proteasi extracellulari (ad es. **MMPs**) degradano componenti della Membrana Basale e dello stroma e promuovono l'attivazione di citochine e fattori di crescita stromali (TGFb, VEGF, etc.) o legati alla membrana cellulare.

Intravasazione = Cellule tumorali invasive entrano nel sistema circolatorio attraversando l'endotelio di vasi sanguigni o linfatici. I vasi tumorali sono più permeabili e la **migrazione transendoteliale** è facilitata.

La TRANSIZIONE EPITELIO-MESENCHIMALE



Regolazione della EMT da oncogeni e segnali eterotipici

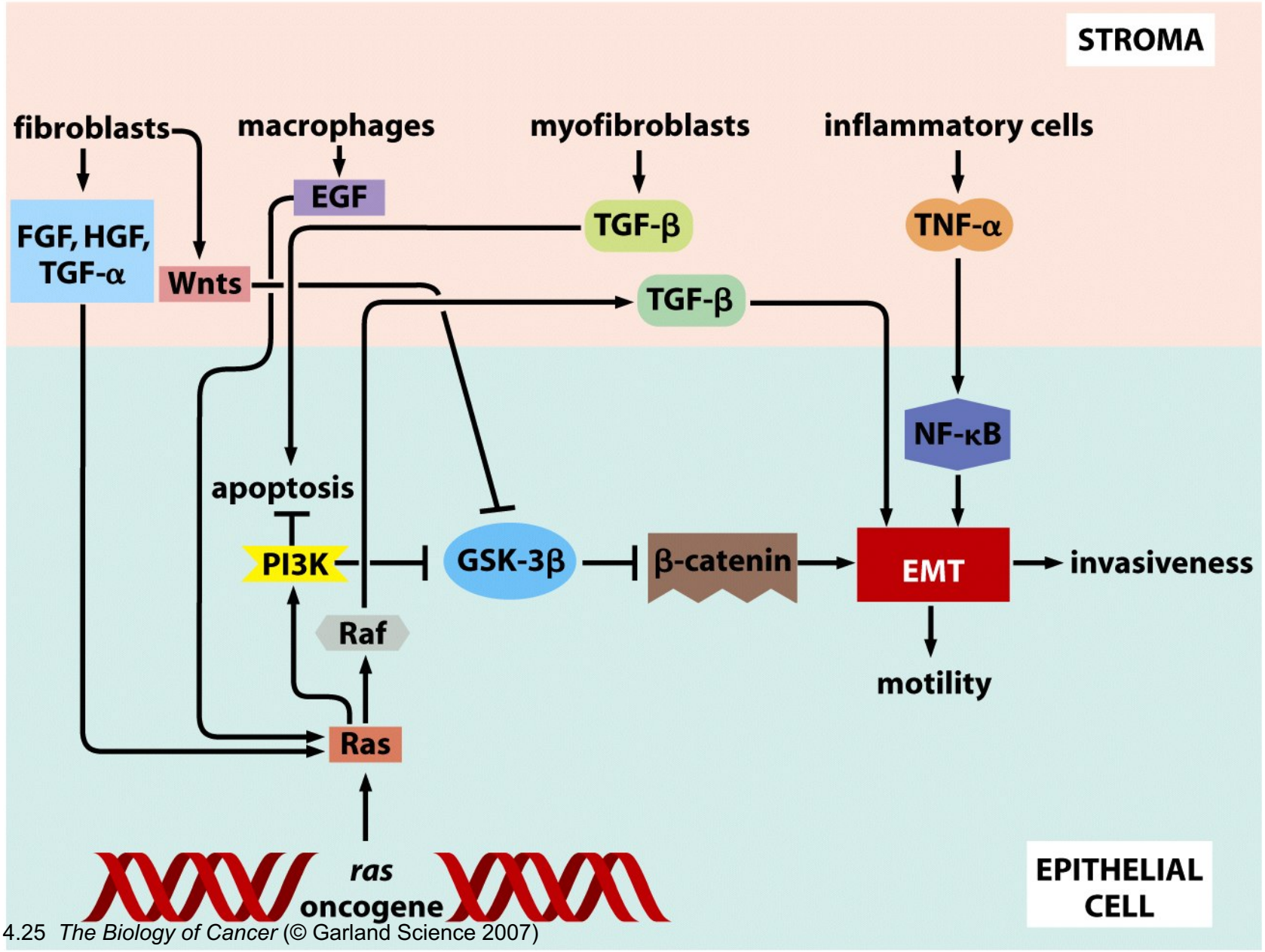
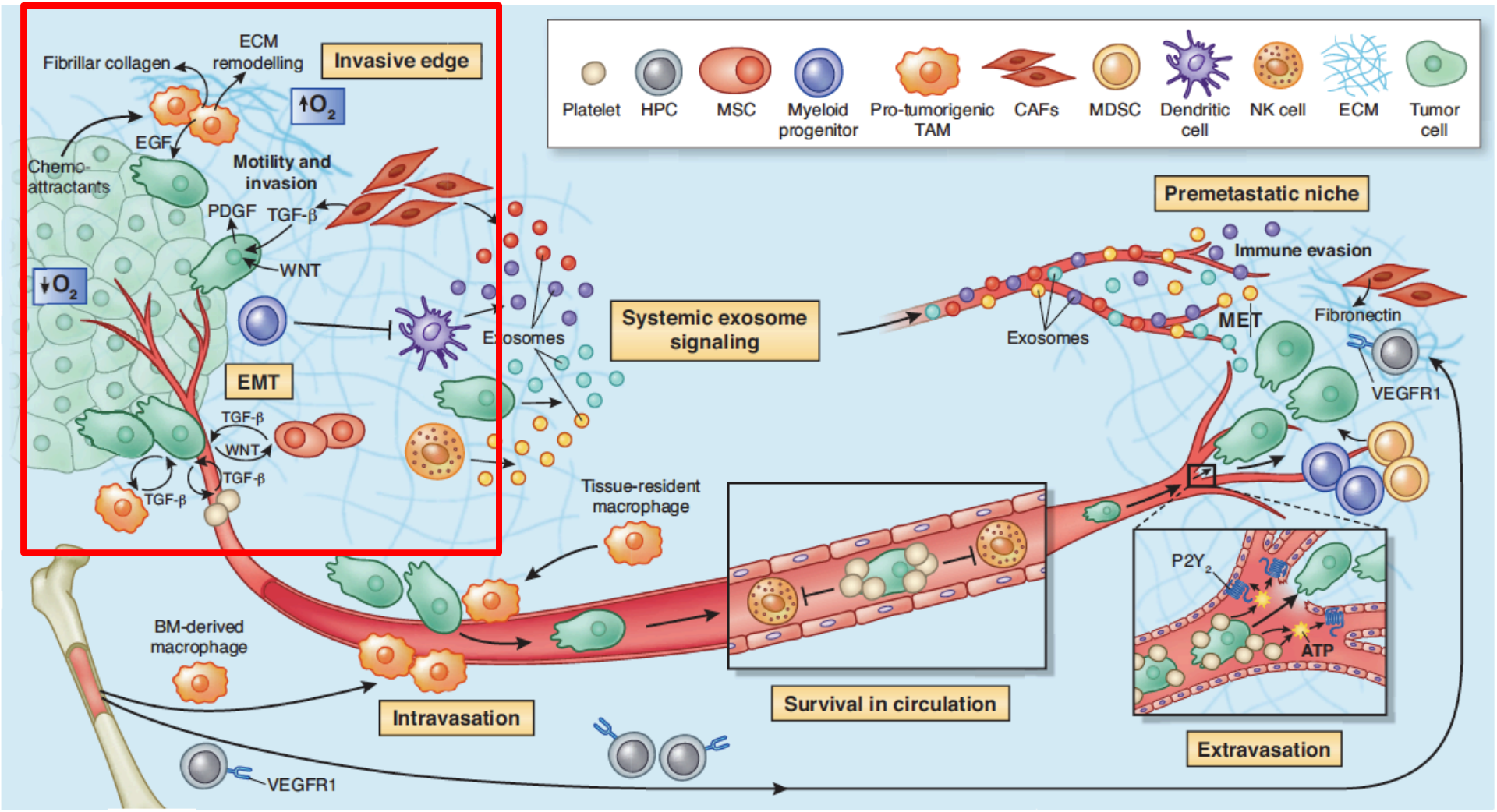
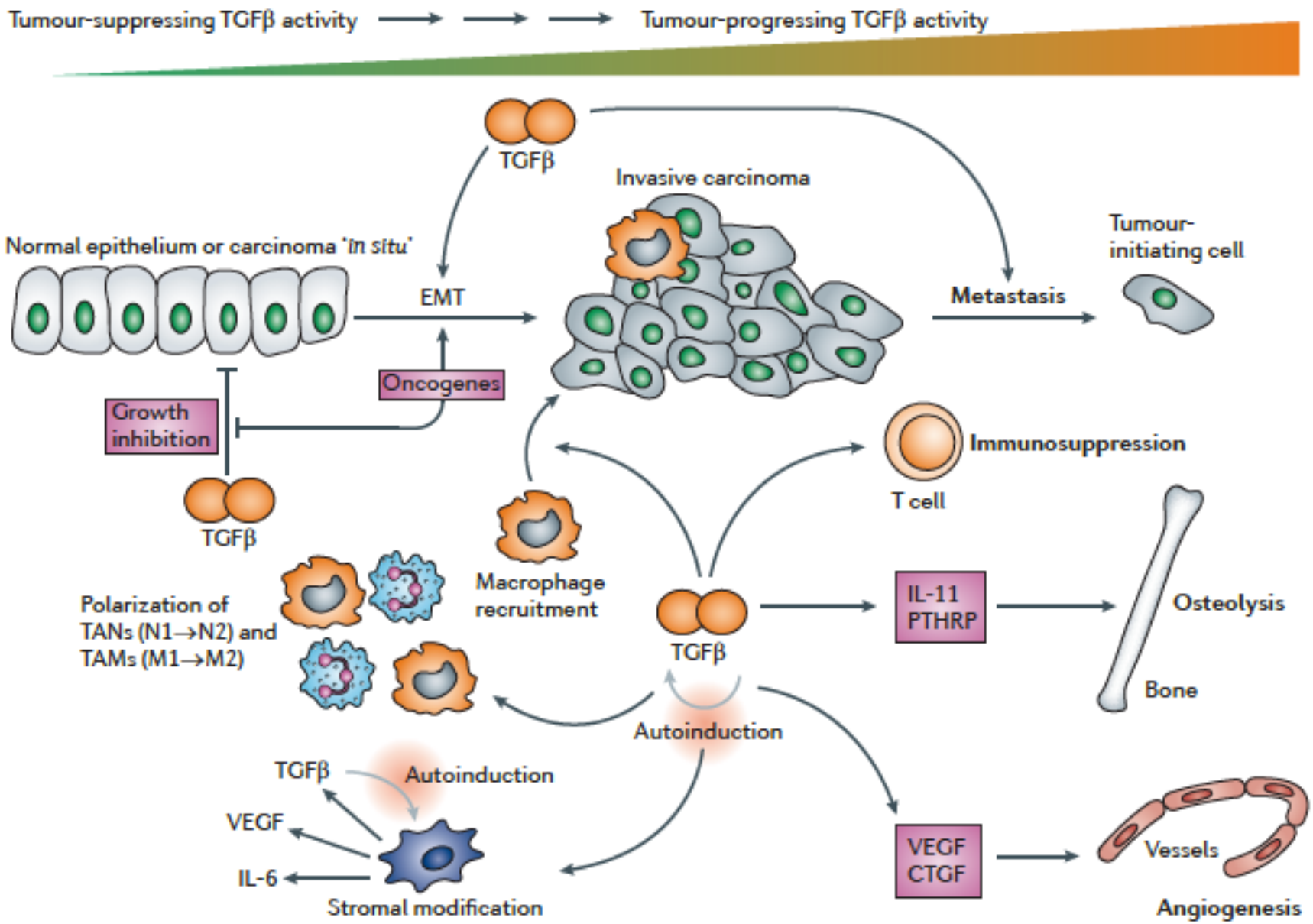


Figure 14.25 *The Biology of Cancer* (© Garland Science 2007)

Interazioni con il microambiente nella cascata metastatica



Il doppio ruolo della pathway di TGFβ nel cancro



Akhurst & Hata, Nat Rev Drug Disc 2012

Lo SWITCH delle caderine facilita la metastasi

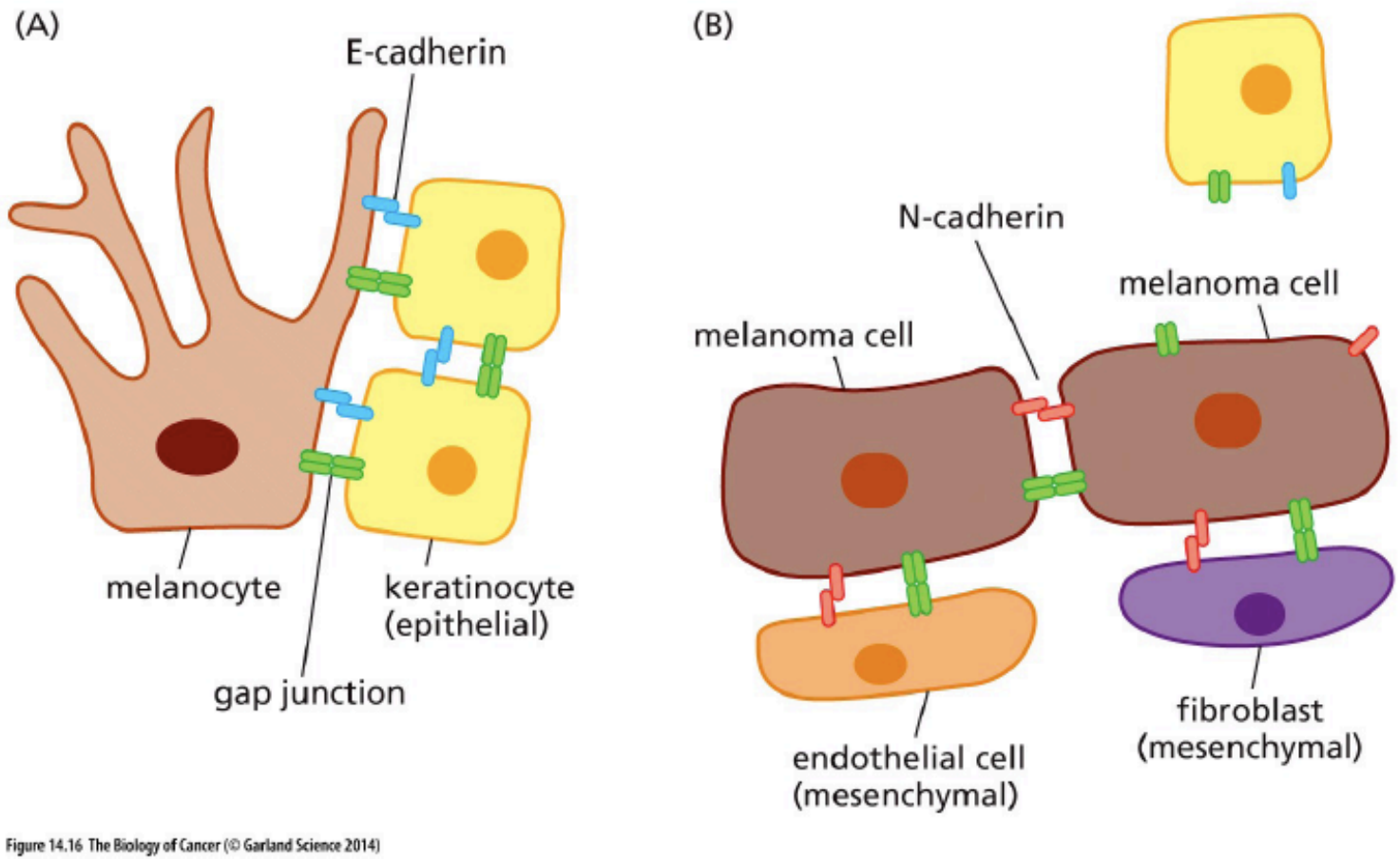
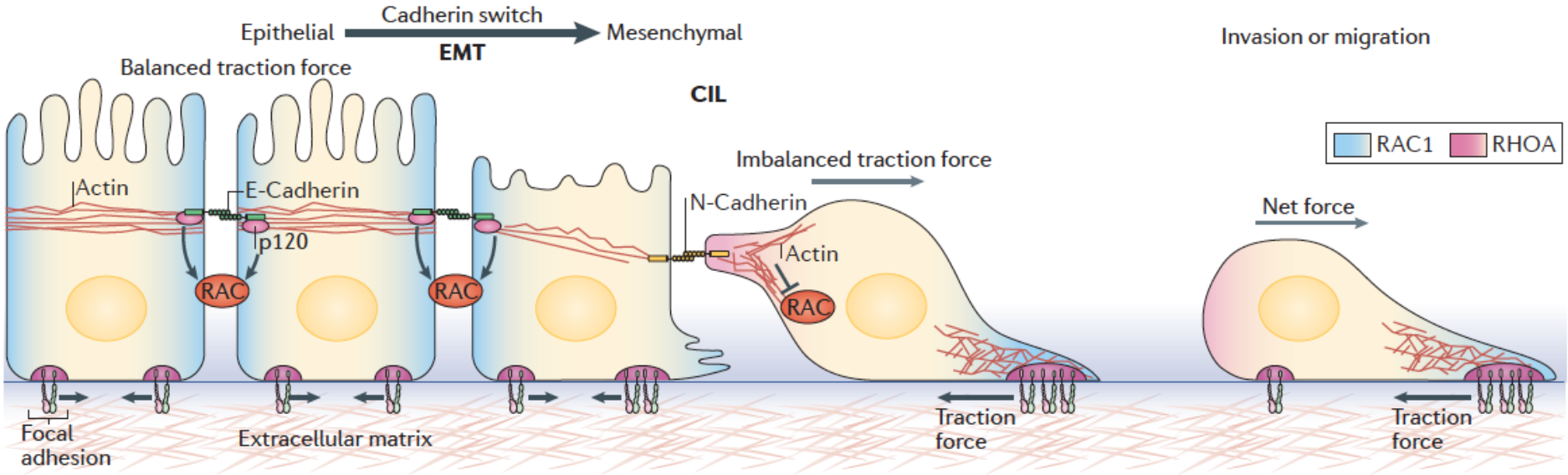


Figure 14.16 The Biology of Cancer (© Garland Science 2014)

When melanocytes become transformed in melanoma cells they shift from **E-Cadherin to N- Cadherin** thus extricating from keratinocytes and making more interactions via N-cadherin to stromal cells facilitating cell migration and invasion.

Lo switch delle caderine facilita la perdita di polarità e dell'inibizione della migrazione da contatto



E-Cadherin suppresses EMT and CIL by signalling to other adhesion components, such as p120 catenin, which polarizes the small GTPase **RAC1 towards cell–cell junctions.**

N-cadherin expression promotes polarization of **RAC1 activity towards the leading edge** of cells to generate asymmetric traction stress.

Stramer et al., Nature Revies on Cancer 2016

RUOLO DEL CITOSCHELETRO NELLA MIGRAZIONE

Filopodi/invadopodi

Lamellipodio/ margine guida

Fibre di stress (contrazione e avanzamento)

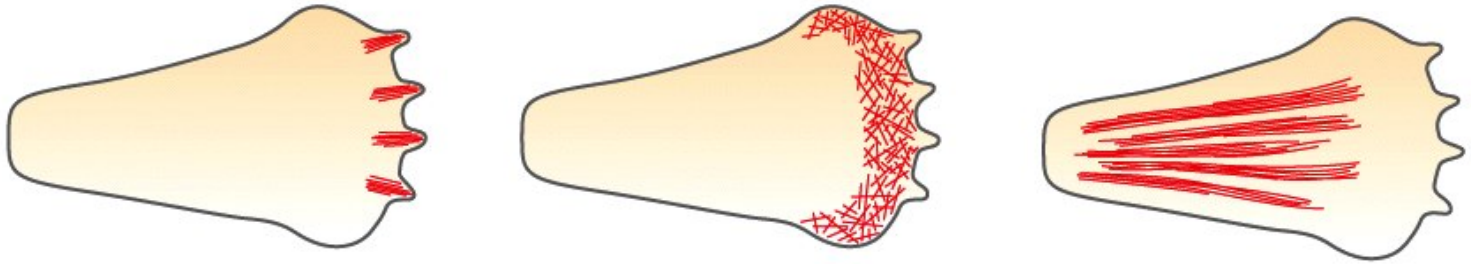
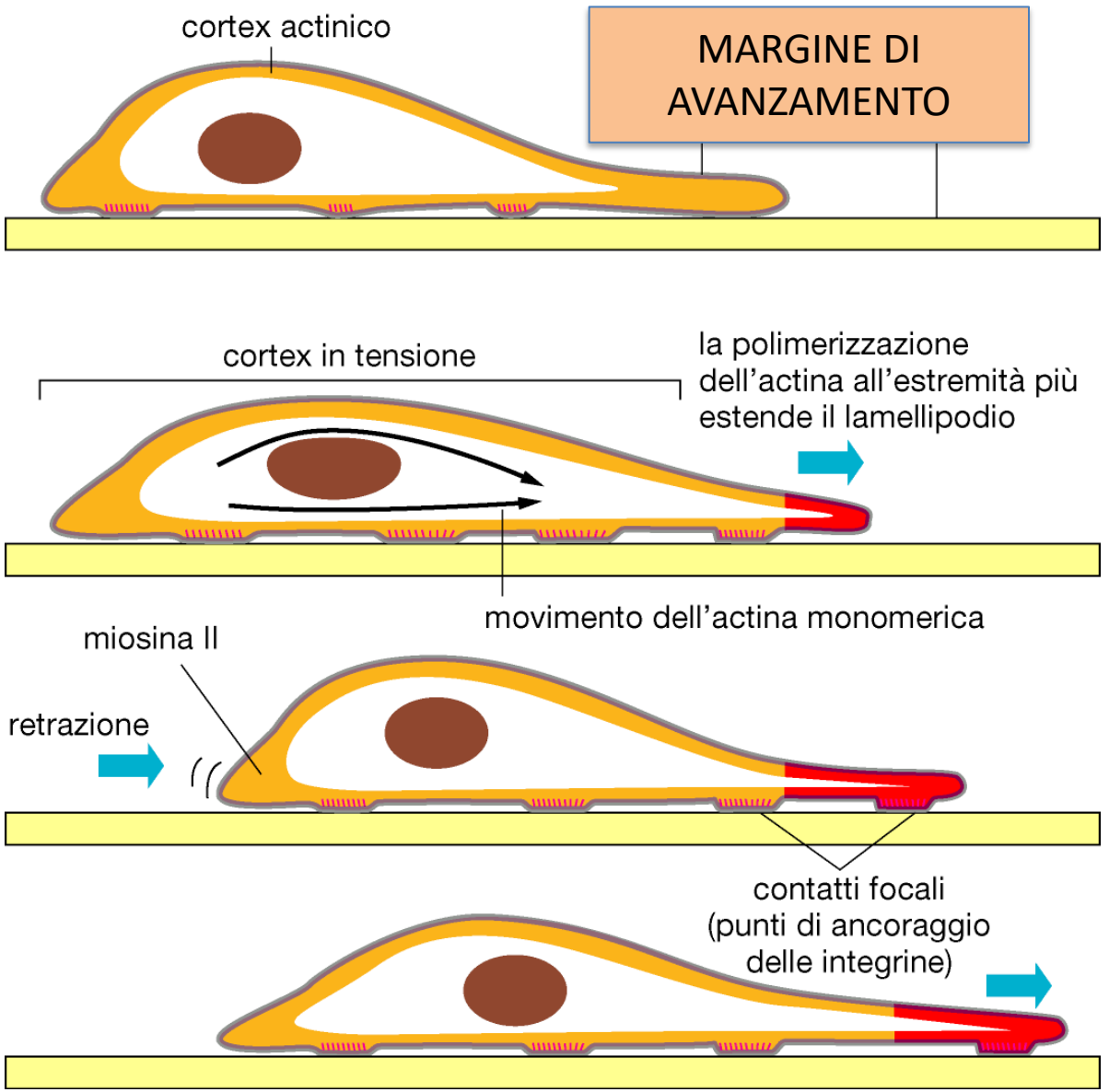


Figure 17-42
Molecular Cell Biology, Sixth Edition

migrazione cellulare



Il pattern di espressione delle integrine al leading edge (bordo anteriore invasivo) è diverso in cellule dotate di capacità migratoria.
L'attivazione del recycling delle integrine permette di smantellare i contatti focali posteriori per far avanzare il corpo cellulare formandone di nuovi al bordo anteriore

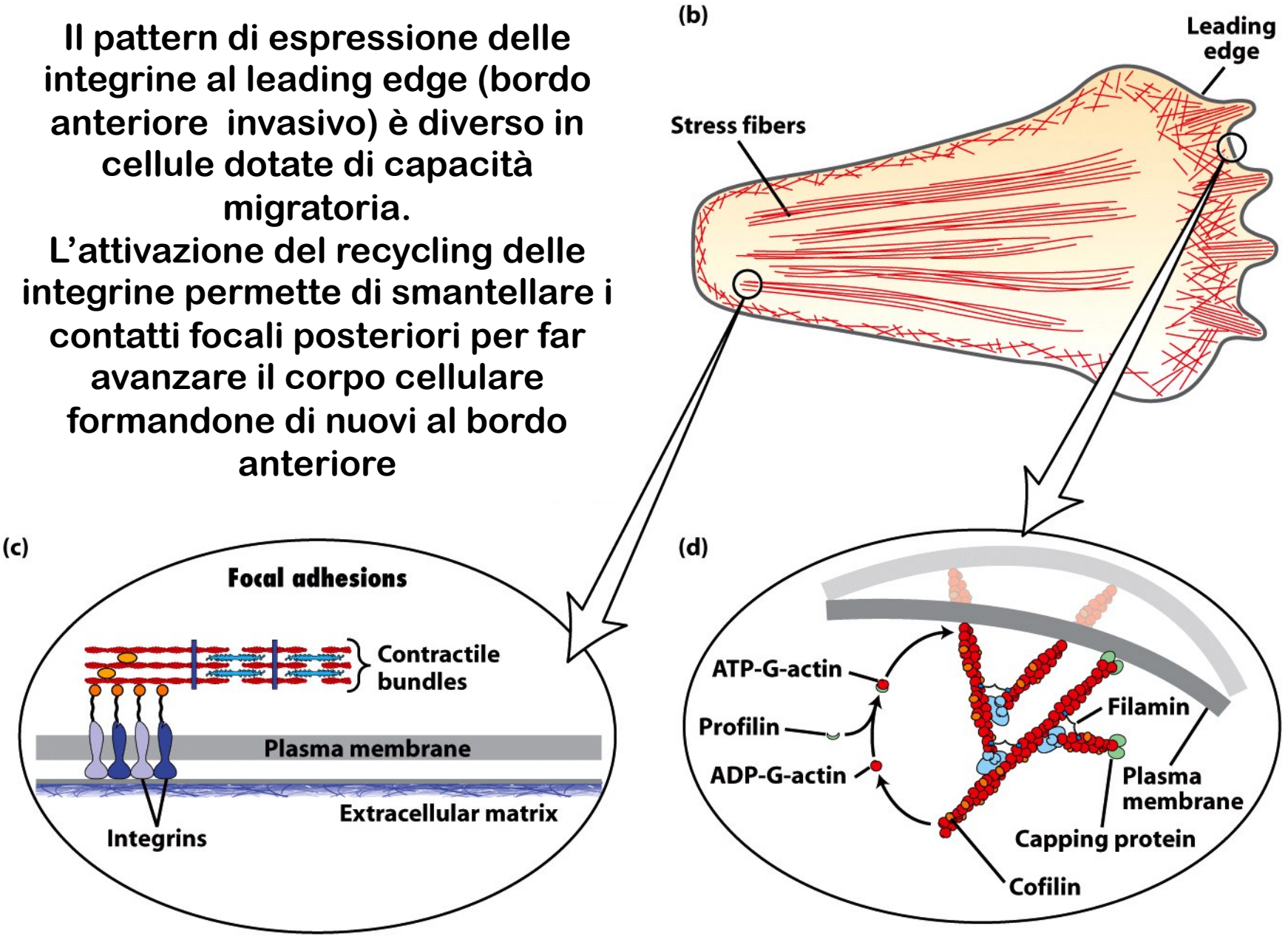


Figure 17-39
Molecular Cell Biology, Sixth Edition

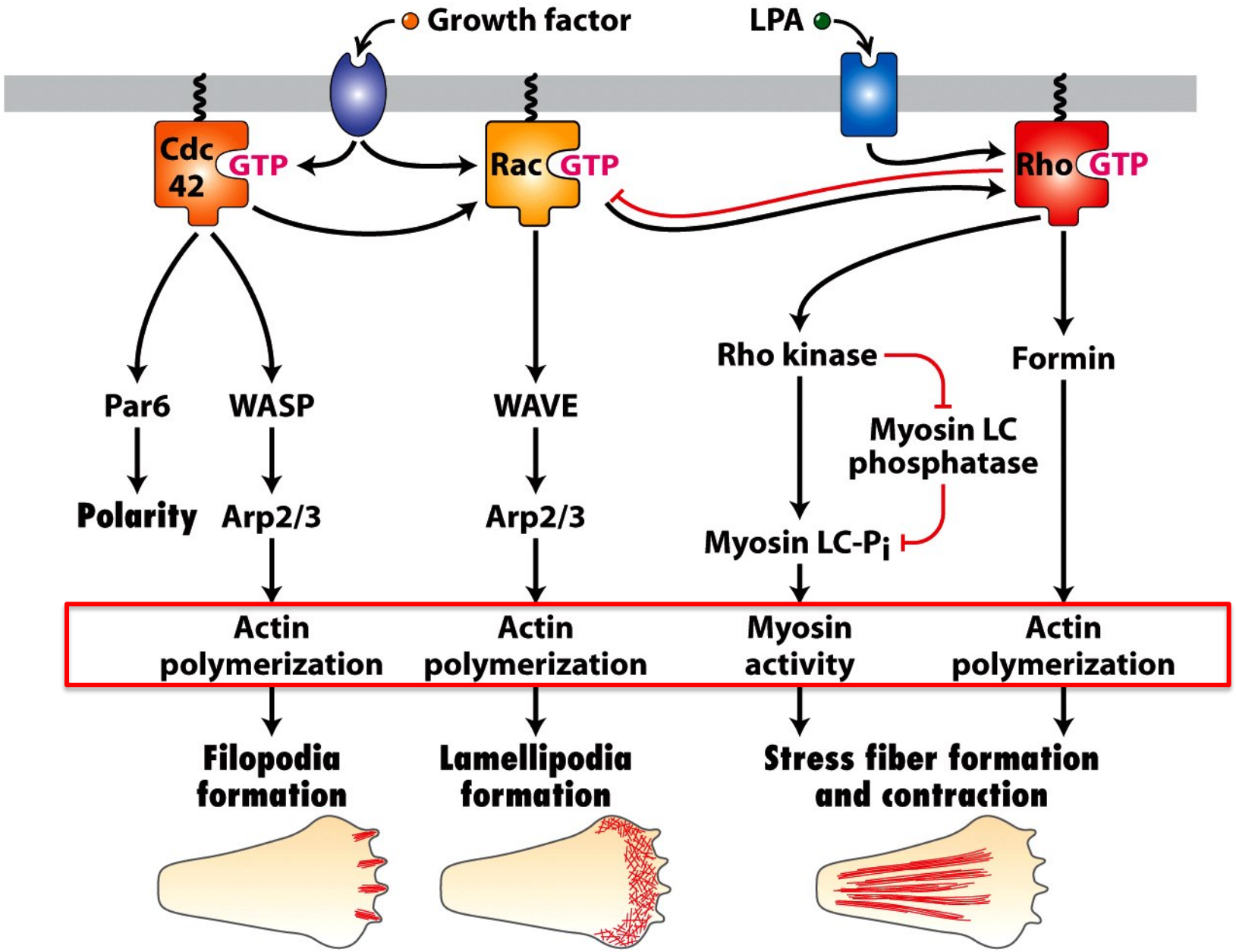
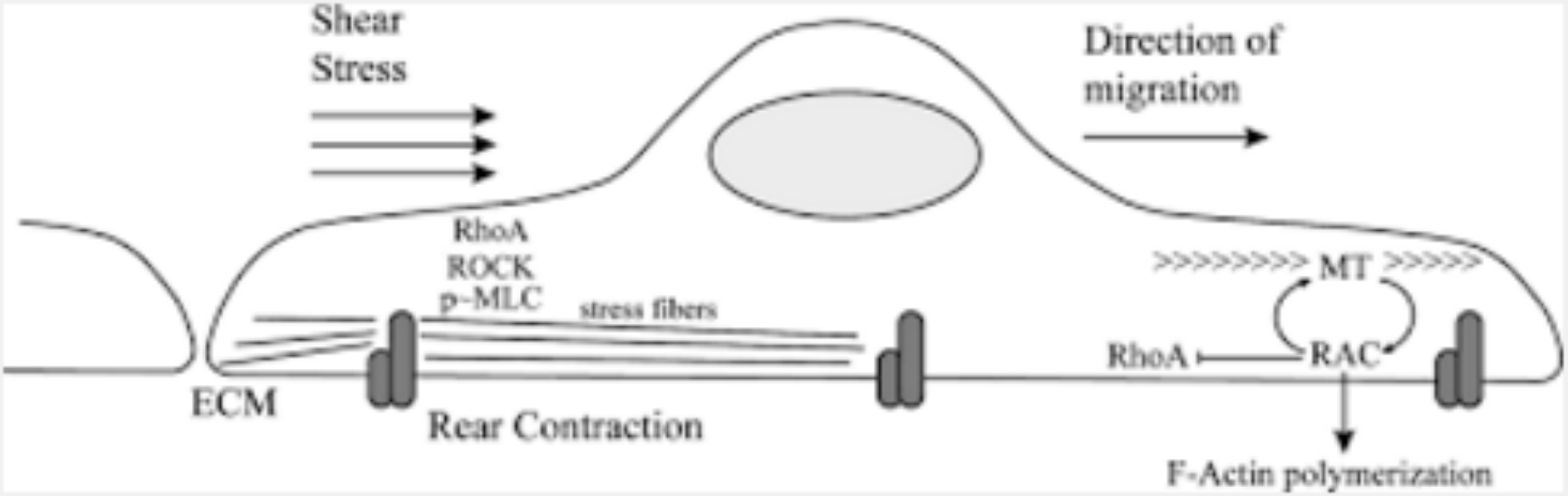


Figure 17-42
Molecular Cell Biology, Sixth Edition

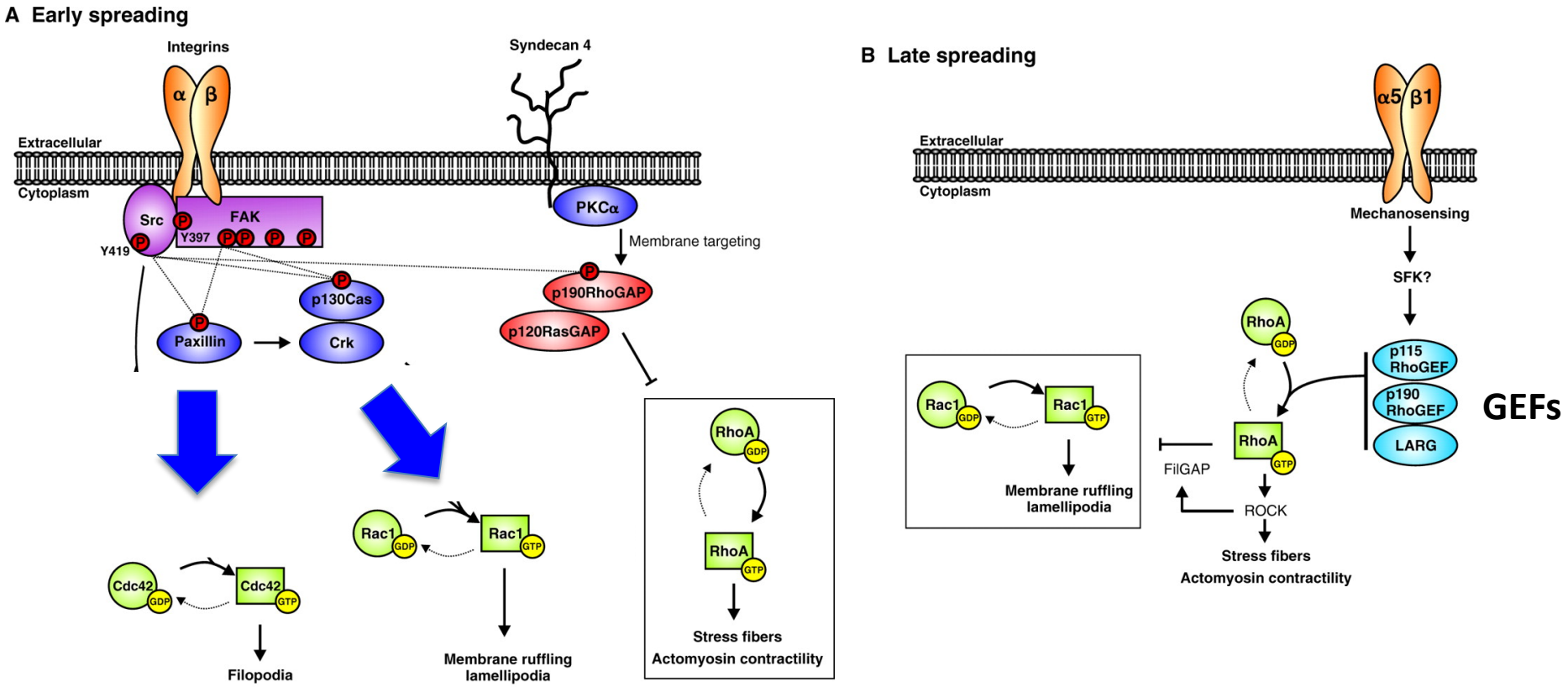
invadopodia



Le GTPasi Rho, Rac e cdc42 e controllano la organizzazione dell'actina e delle adesioni focali.

La migrazione dipende da **attivazione localizzata di qs proteine in piccoli, limitati domini di membrana**.

REGOLAZIONE DI RHO DA SIGNALING DELLE INTEGRINE DURANTE LA MIGRAZIONE



Stephan Huveneers, and Erik H. J. Danen J Cell Sci 2009;122:1059-1069

Journal of Cell Science

The **invasion** of transformed cells into the adjacent parenchyma requires the acquisition of a **motile phenotype**. Actin-rich protrusions, termed **invadopodia**, which require integrin-mediated adhesion and focal adhesion formation, direct tumor cell invasion through **localized MMP-mediated matrix degradation**.
ECM stiffness **promotes invadopodia formation** and enhances tumor cell invasion by **driving focal adhesion assembly**.
Once the physical barriers surrounding a benign tumor are compromised, **tumor cell migration is driven through elevated activity of Rho and Rac GTPases**, which stimulate actin assembly and turnover and actomyosin-dependent cell tension.

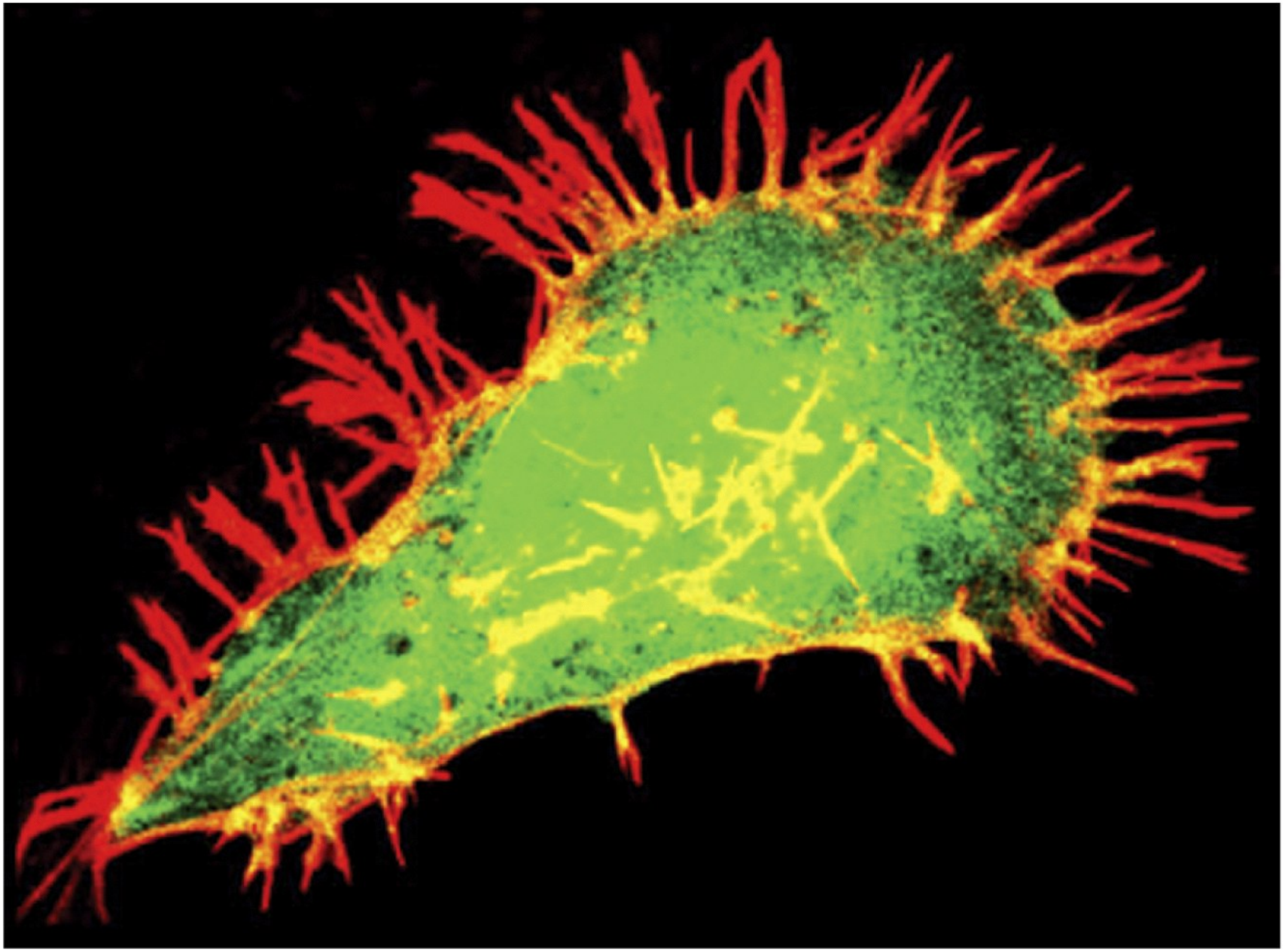


Figure 14.37a *The Biology of Cancer* (© Garland Science 2007)

INVASIONE: rottura della lamina basale

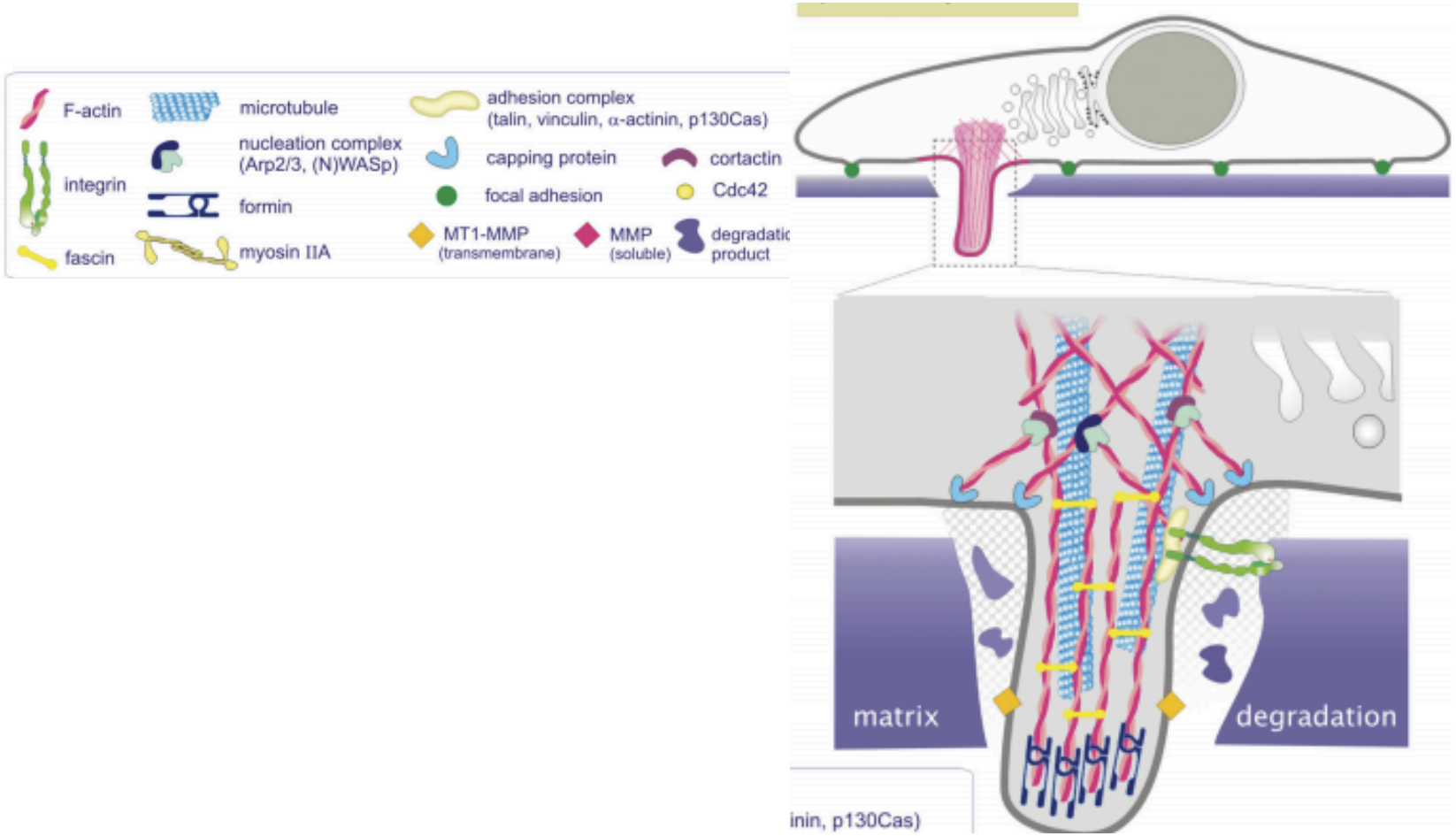
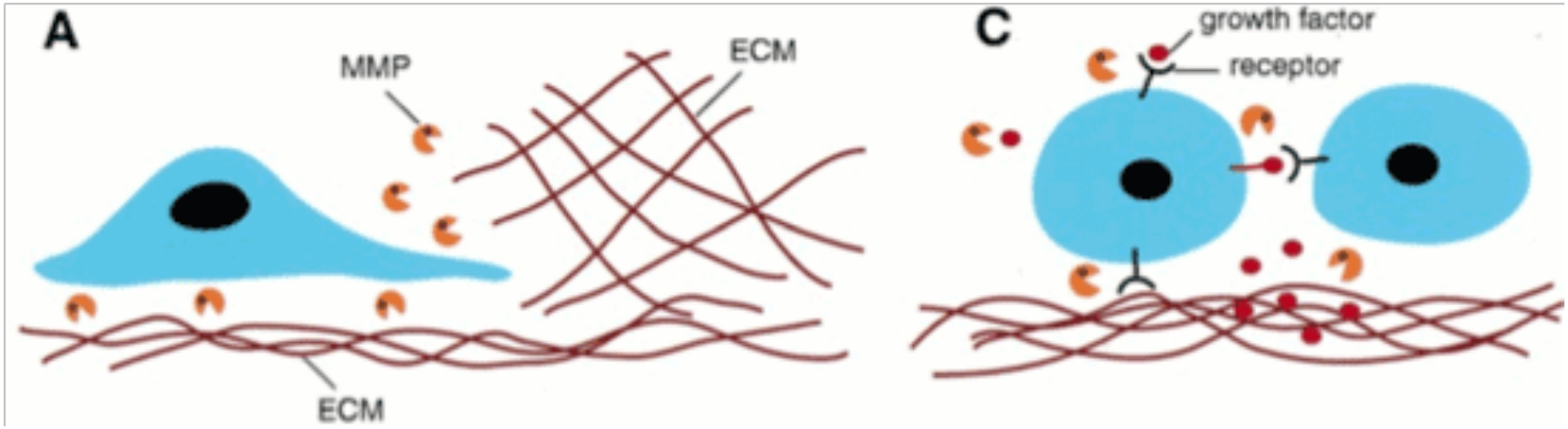


Figure 2. Schematic representation of podosomes and invadopodia. Podosomes consist of a core column of actin filaments that extends upwards from the ventral cell surface into the cytoplasm whereas invadopodia are long filopodial-like membrane extensions that penetrate into the ECM. (**A**) Schematic representation of a podosome in a macrophage: Cdc42/WASp/Arp2/3 drives actin polymerization at the face of the actin core, generating forces that push against the core and consequently promotes podosome growth. Radial actin filaments emanate from the actin core and link it to cell surface integrins and associated proteins that form the basis of the adhesive ring complex. (**B**) Schematic representation of an invadopodium in a cancerous cell: The N-WASp/Arp2/3 complex is present at the base and along the length of shafts of invadopodia but absent from the tips. The Rho effector mDia is involved in both initiation of invadopodia formation through actin nucleation and subsequent growth of invadopodia through the elongation of actin filaments. The protease activity is essential for the assembly of protrusive actin structures. Invadopodia lack the strict organization of core and ring structure found in podosomes.

La degradazione localizzata della ECM favorisce la disseminazione

Serin proteasi e metalloproteasi



Le MMPs liberano fattori di crescita e TGFβ sequestrati nella ECM

3 famiglie (24 membri):

MMPs, ADAM, ADAMTS — **famiglie di enzimi zinco-dipendenti con diversa specificità di substrato**

INIBITORI: TIMPs (endogenous tissue inhbitors)

Gli inibitori delle metalloproteasi MPIs

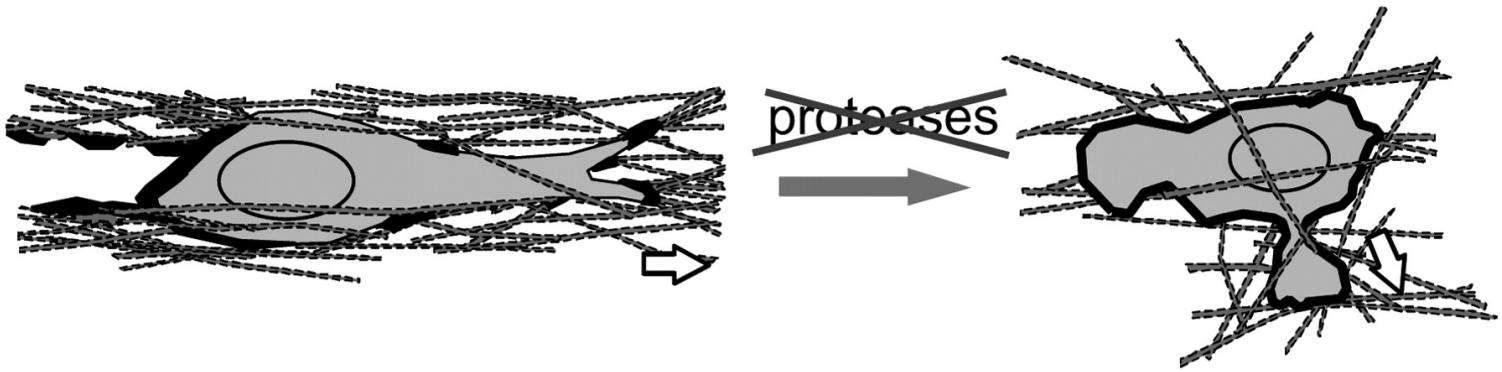
Review

Molecular Cancer Therapeutics

Matrix Metalloproteinase Inhibitors in Cancer Therapy: Turning Past Failures Into Future Successes

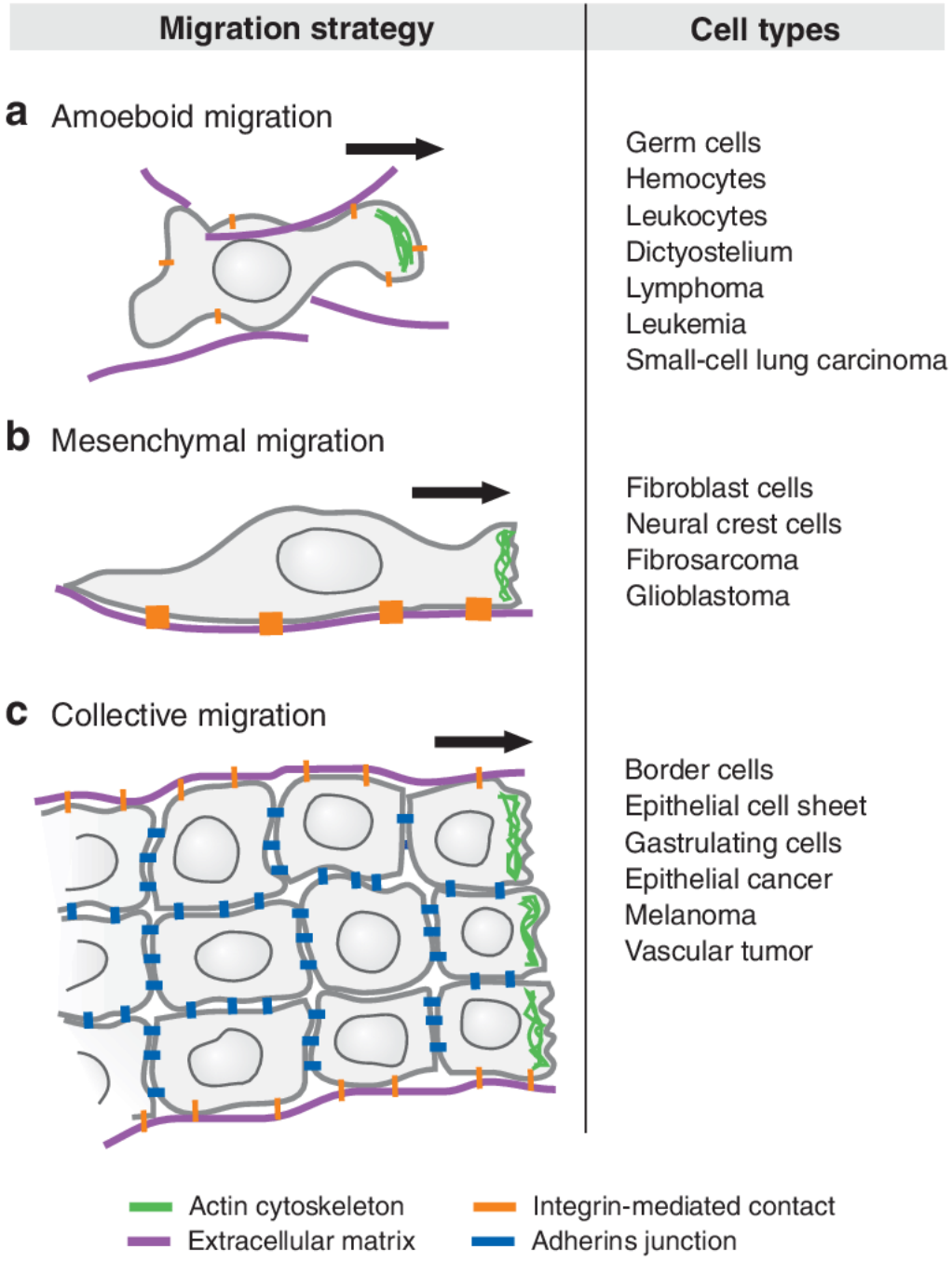
Arthur Winer, Sylvia Adams, and Paolo Mignatti

MMPs e modalità di migrazione

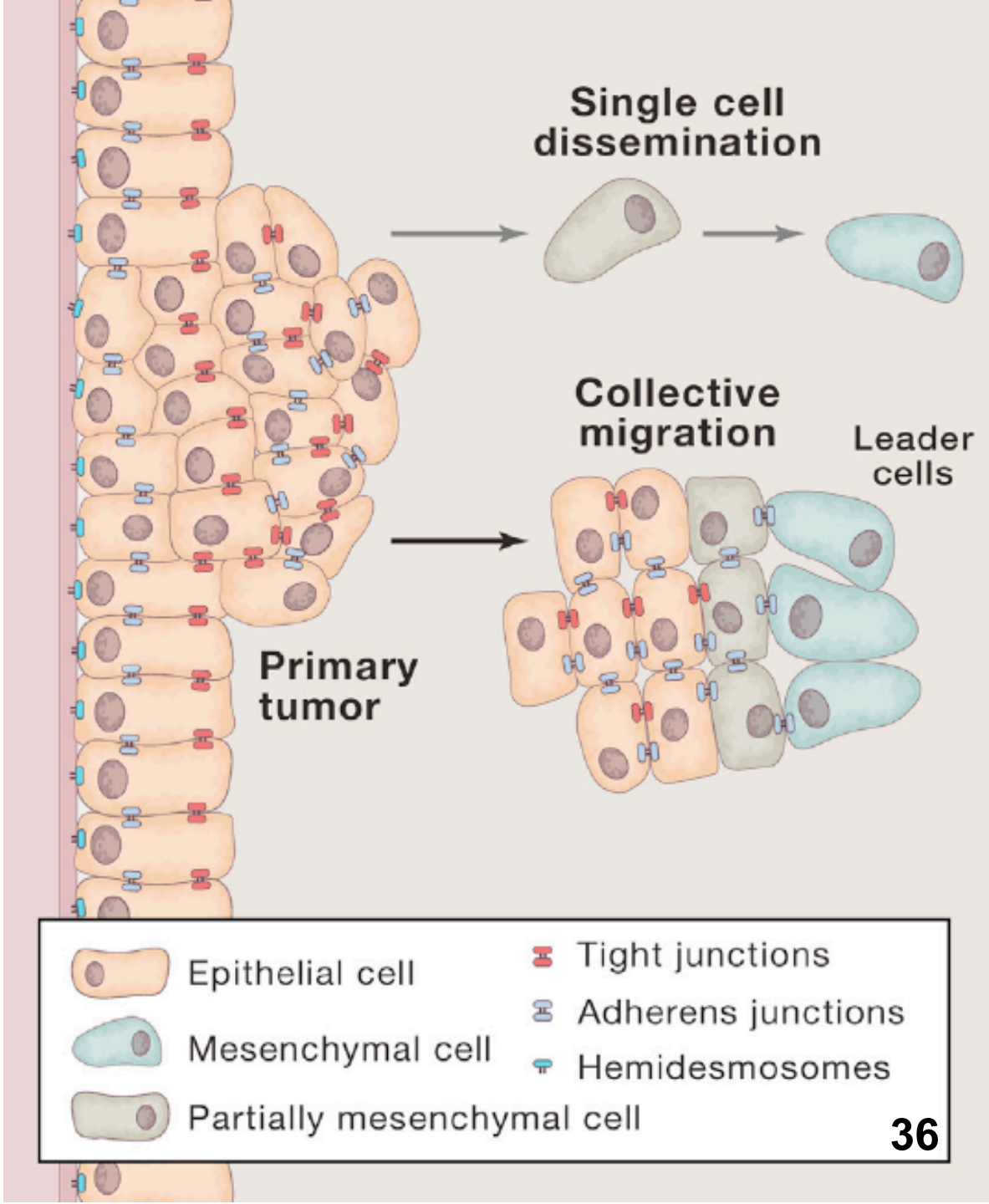


	mesenchymal	amoeboid
migration strategy	path generating	path finding
mechanism for overcoming tissue barriers	proteolytic matrix defect	morphological adaptation (constriction ring)
composition of cell-matrix interactions	focalized; integrins and MT1-MMP coclustered	diffuse; integrins non-clustered; MT1-MMP internalized and dissociated from integrins

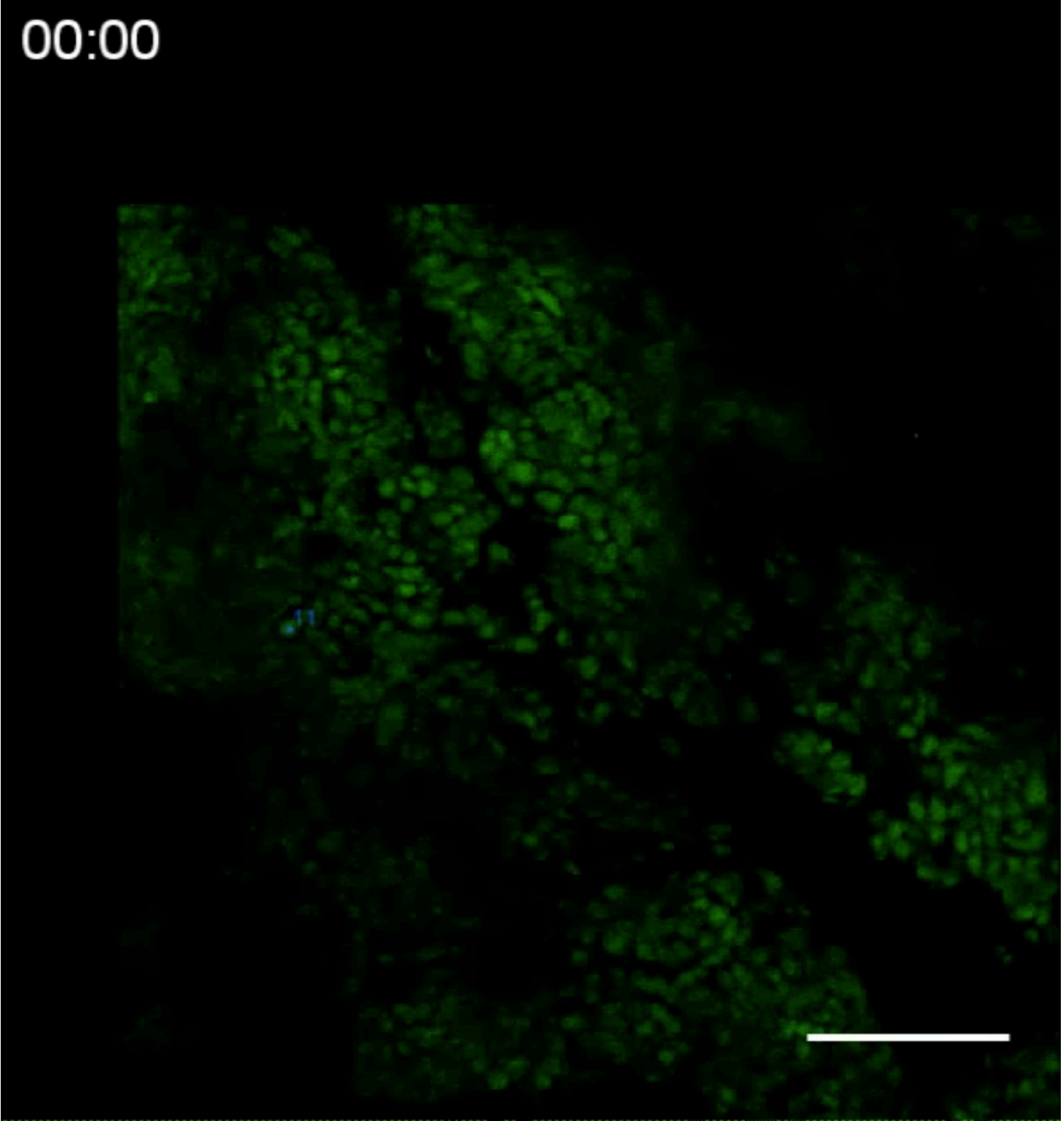
Katarina Wolf et al. J Cell Biol 2003;160:267-277


Migration strategy
Cell types
a Amoeboid migration
Germ cells
Hemocytes
Leukocytes
Dictyostelium
Lymphoma
Leukemia
Small-cell lung carcinoma
b Mesenchymal migration
Fibroblast cells
Neural crest cells
Fibrosarcoma
Glioblastoma
c Collective migration
Border cells
Epithelial cell sheet
Gastrulating cells
Epithelial cancer
Melanoma
Vascular tumor
Actin cytoskeleton
Extracellular matrix
Integrin-mediated contact
Adherins junction

La migrazione collettiva

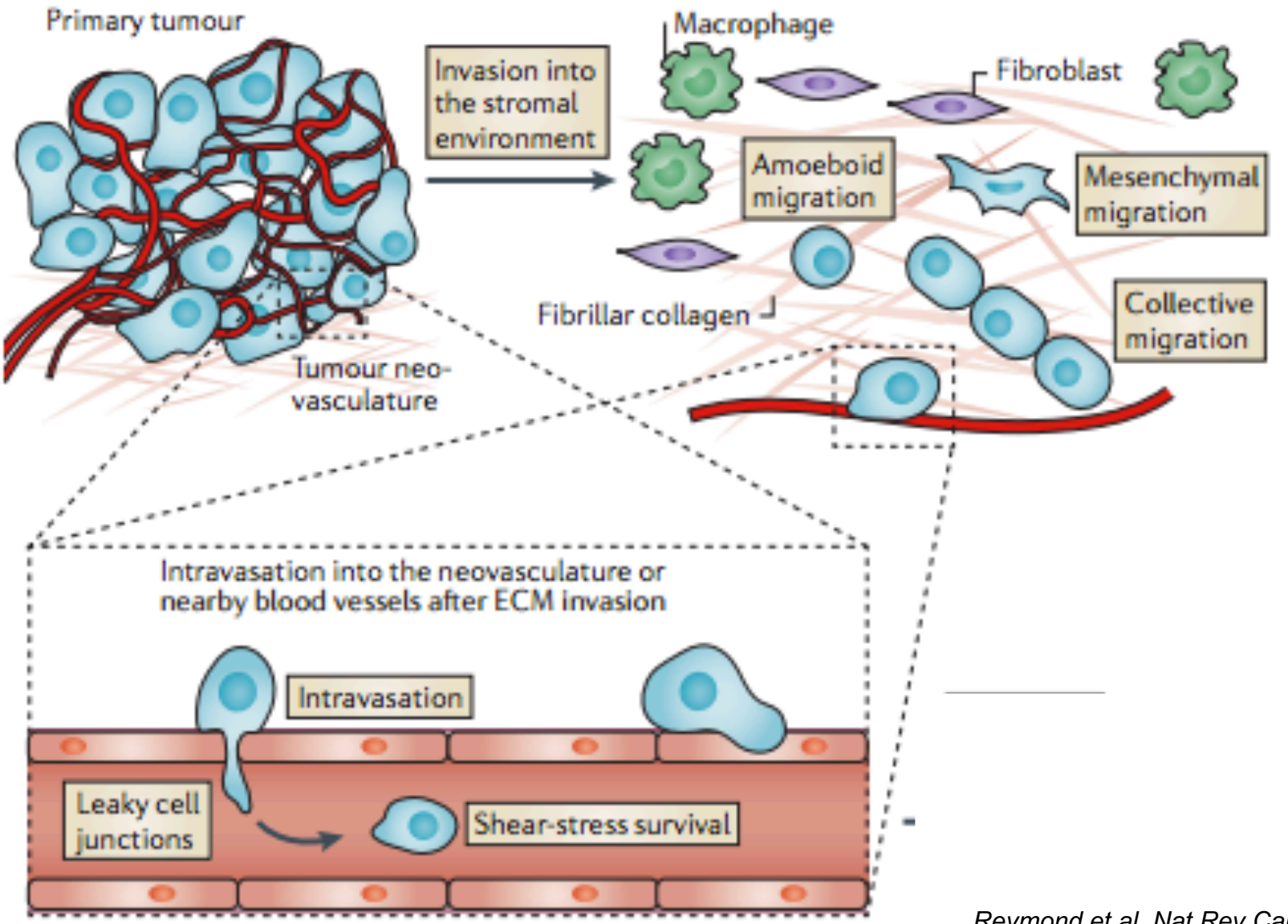


Lambert et al Cell 2017



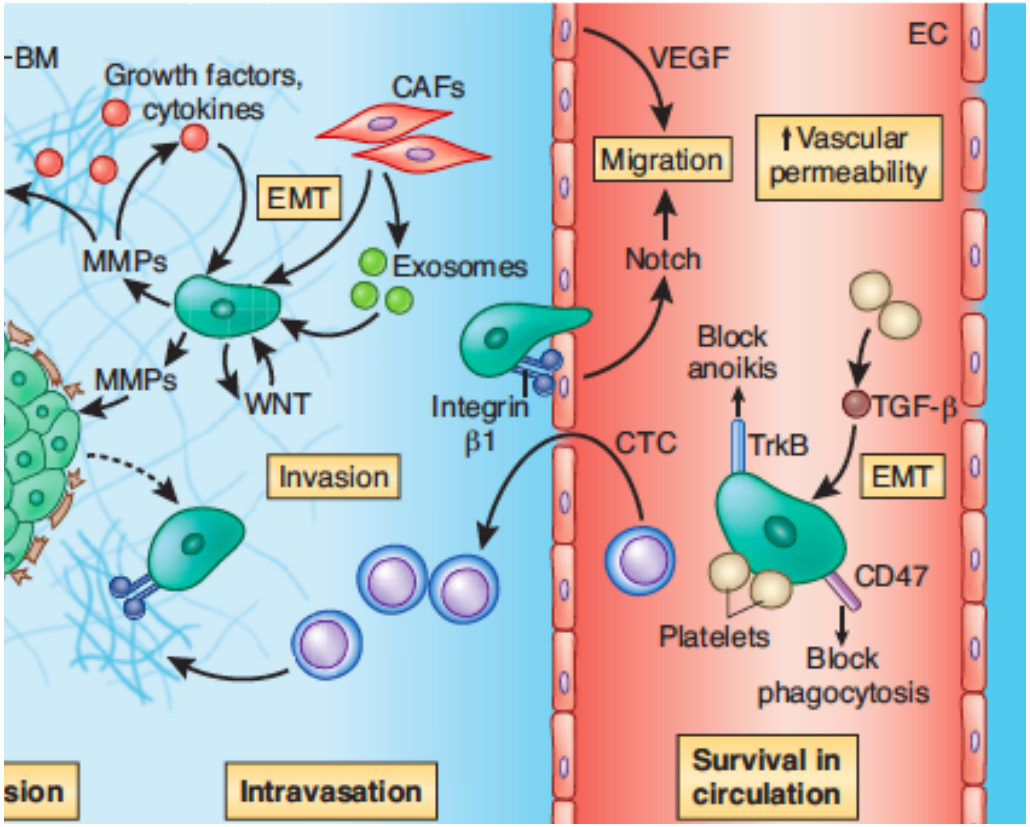
Journal of Cell Science 2019 132: jcs 220277 doi: 10.1242/jcs.220277

Invasione dello stroma e intravasazione



Reymond et al. Nat Rev Cancer 2013

Intravasazione e sopravvivenza nel circolo



Intravasazione:

- Facilitata dall'interazione tra cellula tumorale (**N-caderina**) e endoteliali e da **fattori secreti** dal microambiente tumorale: angiopoietin-like 4 (ANGPTL4), TGF-β, epiregulin (EREG), cytochrome c oxidase subunit 2 (COX2), MMP1, MMP2, MMP3, ANGPT2, MMP10 e VEGF.
- Facilitata dalla particolare struttura dei vasi tumorali

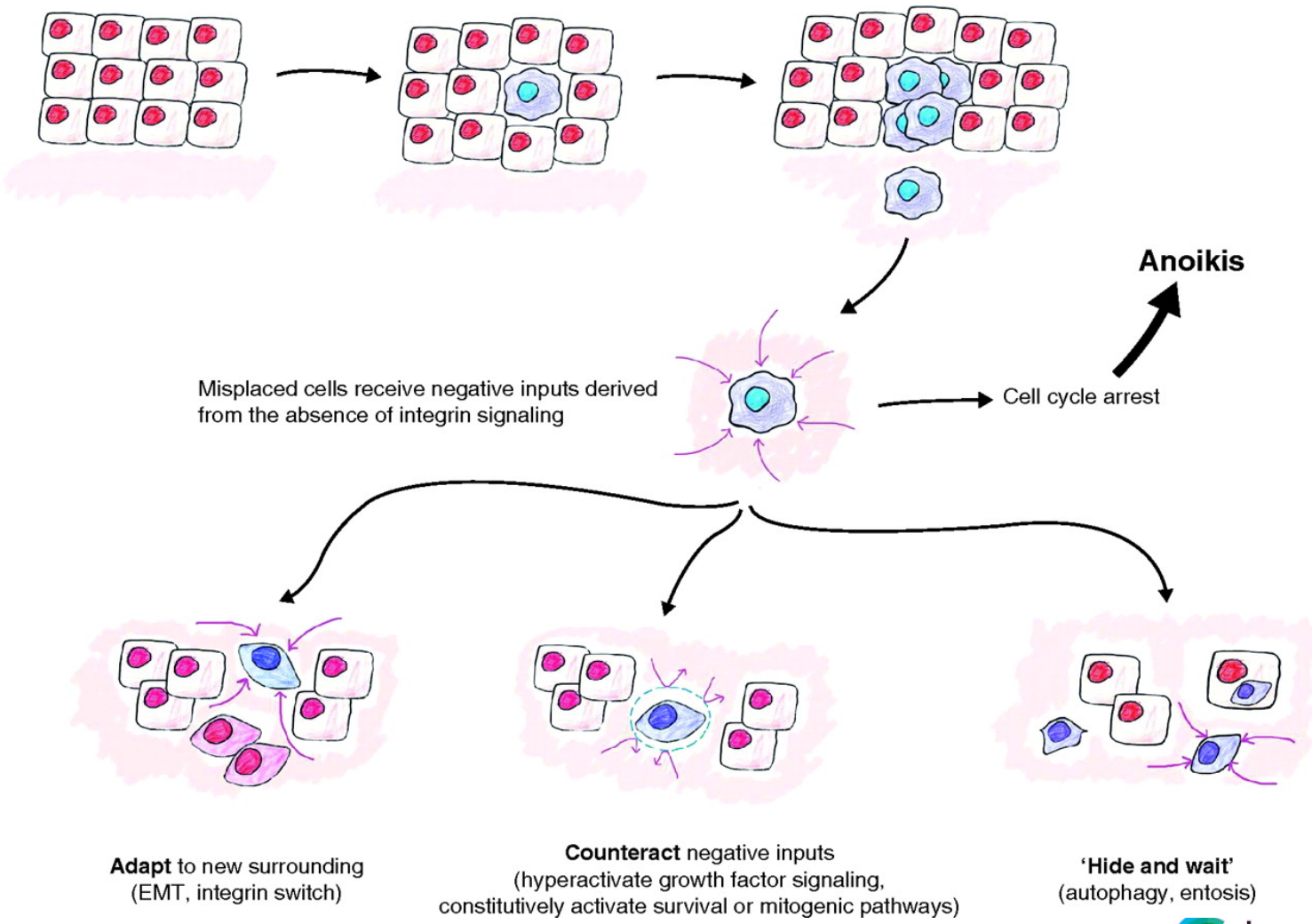
Sopravvivenza nella circolazione

Superamento dell'**anoikis** (causa **assenza di adesioni e forze emodinamiche**) attraverso:

a) attivazione di pathways di **sopravvivenza** (Akt/PKB);

b) **evasione** dal sistema immunitario (CD47) anche interagendo con **piastrine** a formare degli emboli (L-, P- selectin).

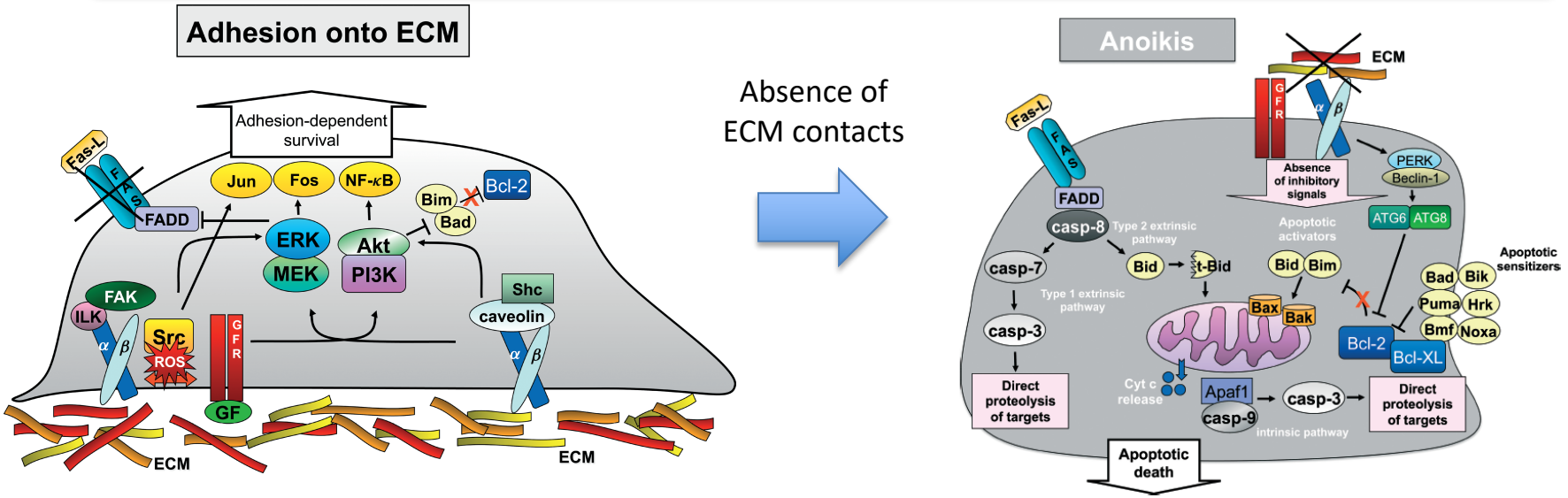
Acquisizione dell'indipendenza dall'ancoraggio

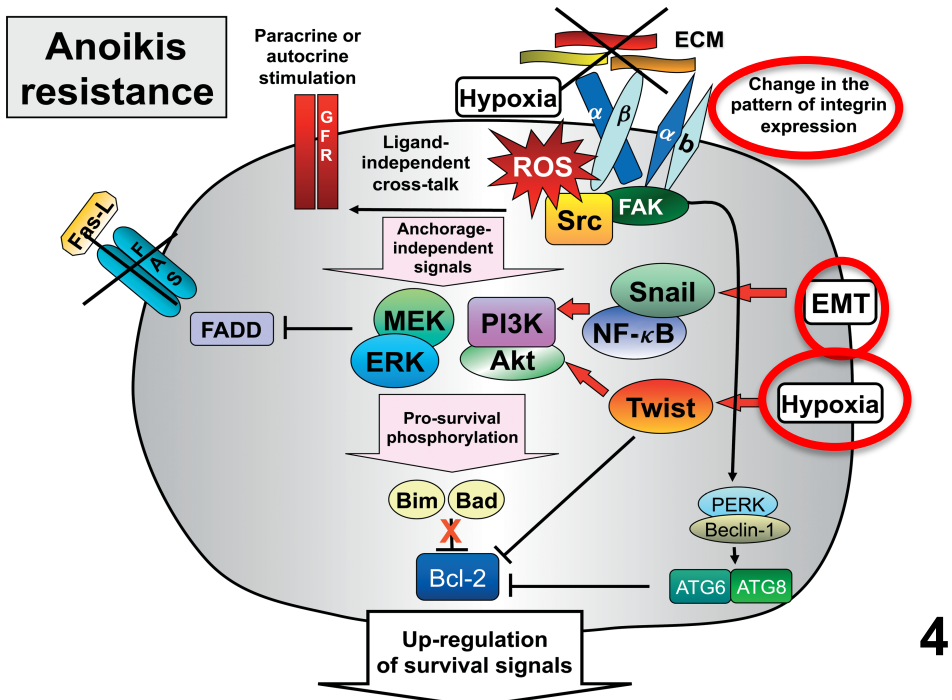


Marta C. Guadamillas et al. J Cell Sci 2011;124:3189-3197

Journal of Cell Science

Acquisizione dell'indipendenza dall'ancoraggio

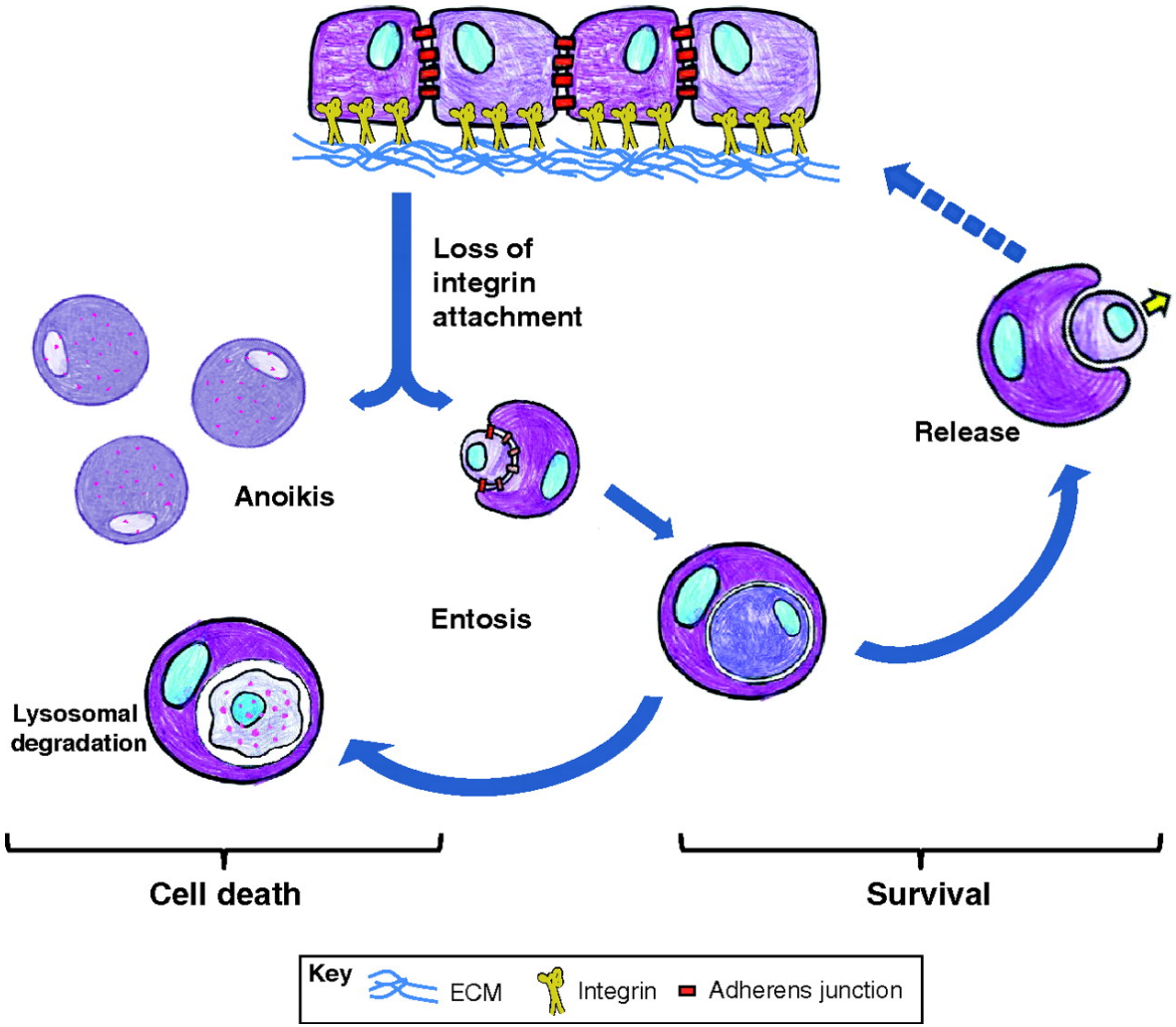




Integrin engagement by ECM triggers several pro-survival pathways through the activation of key players, such as **FAK**, integrin-linked kinase (ILK), **Src** tyrosine kinase, **PI3K**, ERK and the adaptor protein Shc, finally leading to the transcription of Jun, Fos and NF-κB.

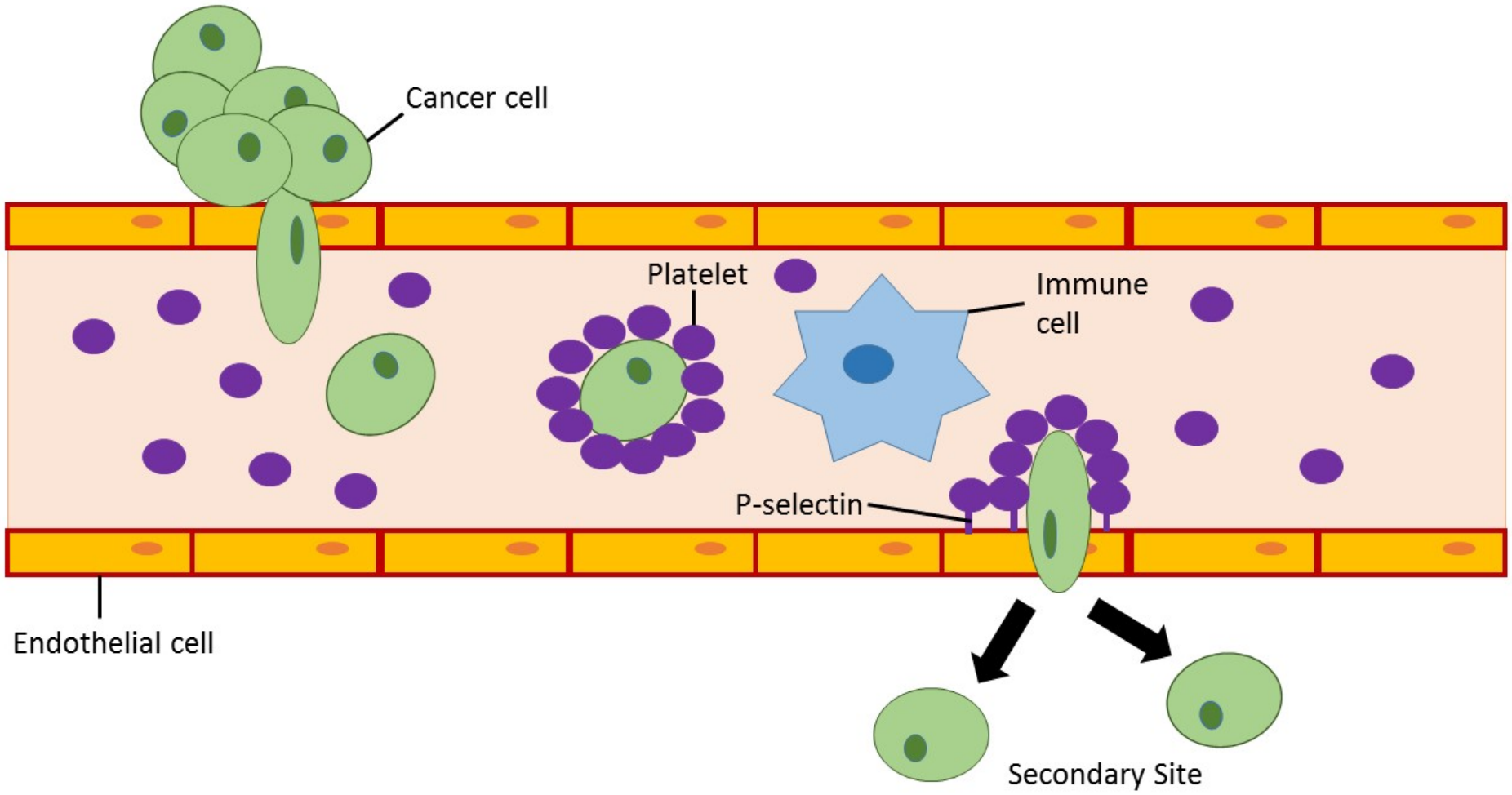
In addition, pro-apoptotic proteins are inhibited, preventing both the extrinsic and intrinsic pathways of cell death. **Growth factor receptors collaborate with integrin in promoting cell survival, largely converging on the same pathways.**

L'entosi può proteggere le cellule tumorali in circolo



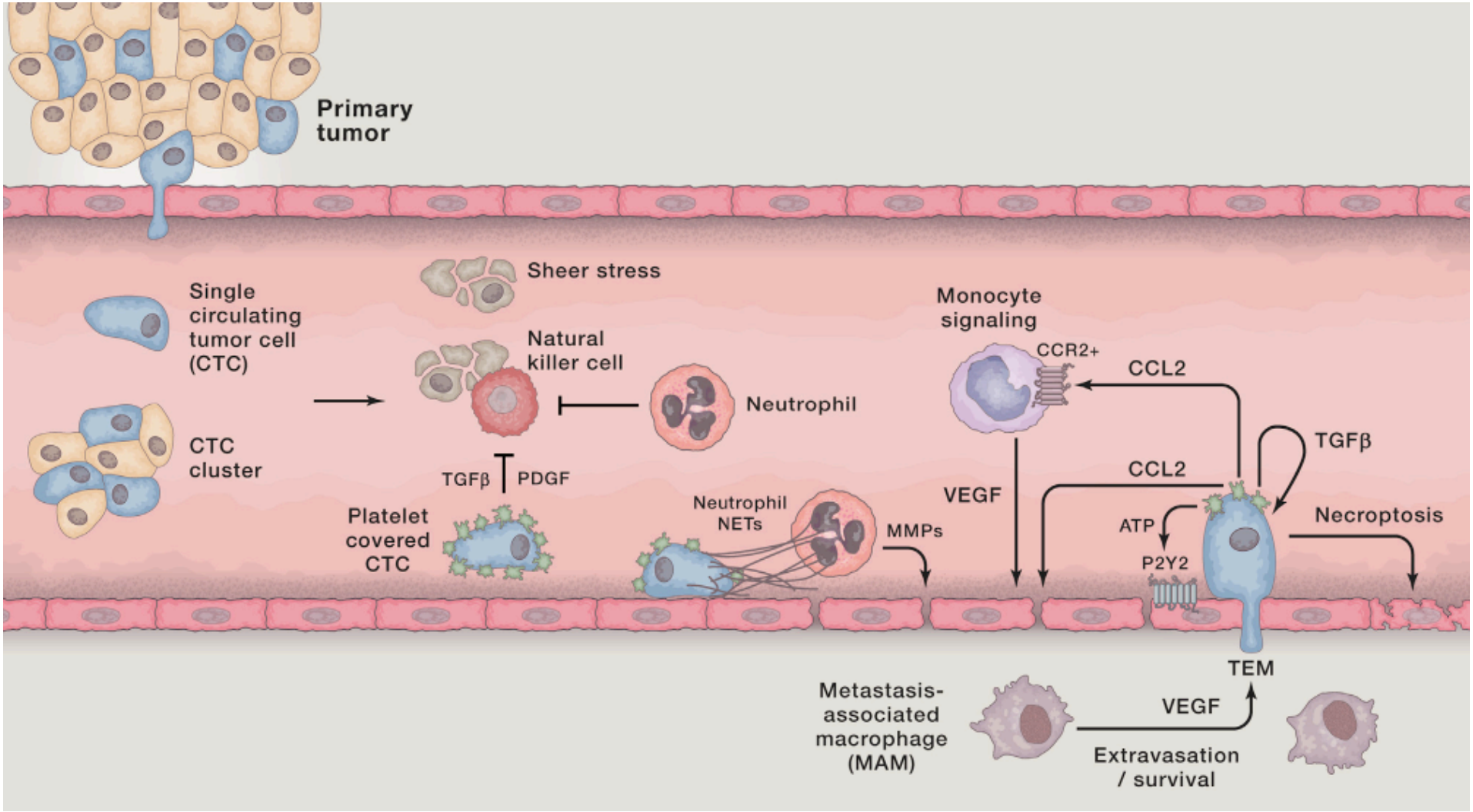
Marta C. Guadamillas et al. J Cell Sci 2011

Sopravvivenza in circolo ed extravasazione



L'interazione con le piastrine sostituisce l'assenza dello stroma nel circolo

Interazioni in transito



L'interazione con le piastrine sostituisce l'assenza dello stroma nel circolo

Lambert et al Cell 2017

CTCs rapidly associate with platelets, an interaction that is triggered by tissue factors displayed on the surface of the carcinoma cells.

• Unbalance in the normal homeostatic controls on coagulation resulting in certain clotting symptoms that are seen in patients with cancer, specifically microthrombi, disseminated intravascular coagulation, large pulmonary emboli

• Platelets facilitate also tumor metastasis by bioactive molecules impacting on cancer progression.

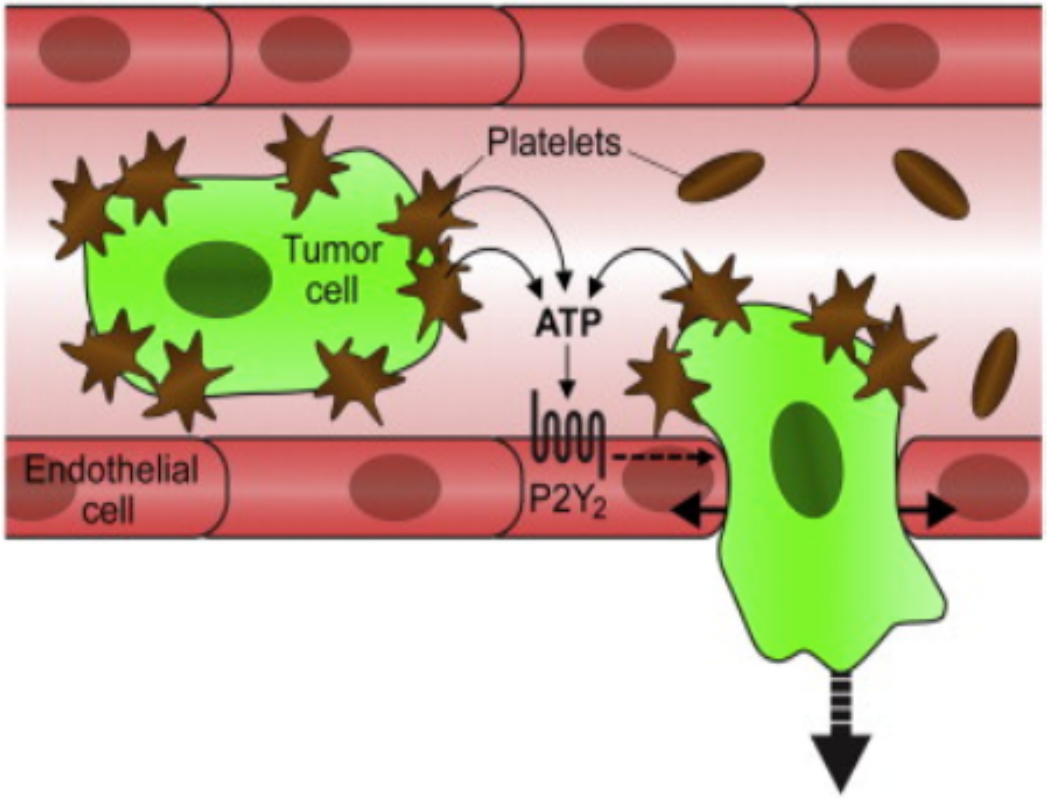
Platelets prevent tumor cell recognition and lysis by NK via **TGFb and PDGF** produced by platelets.

They may physically shield cancer cells from NK by a formation of a **fibrinogen coat** on CTC. (this is an important benefit from platelets to CTC).

• TGFb secreted from platelets can stimulate cancer cells to sustain EMT.

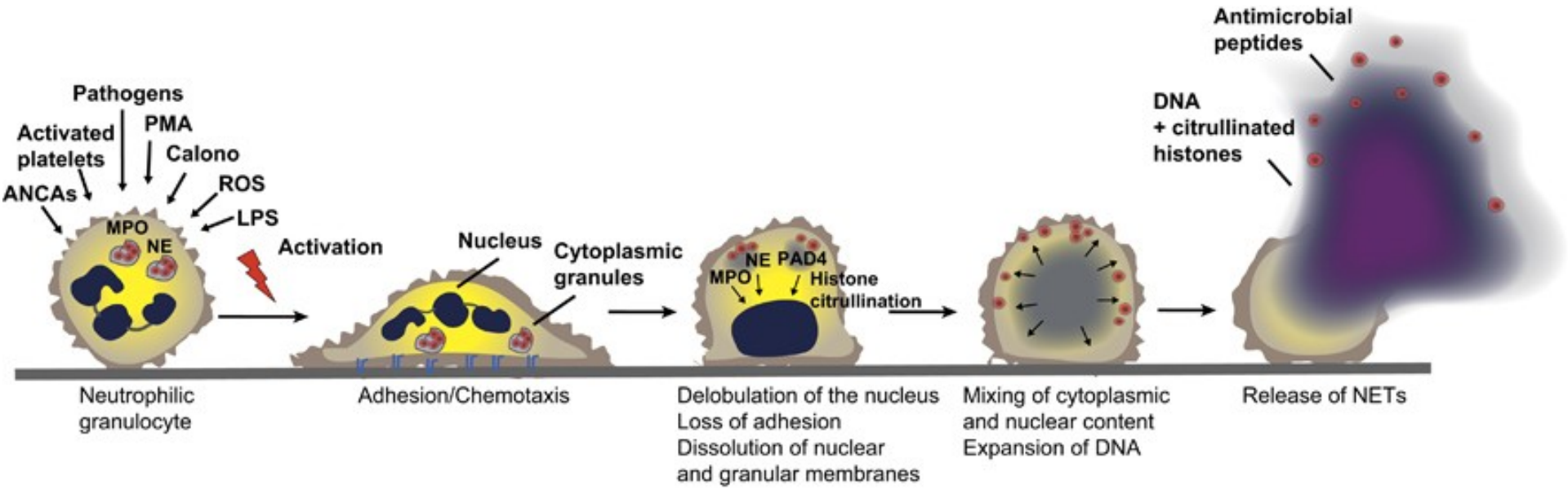
The **interaction with platelets thus substitutes the absence of stroma during the journey into the blood circulation. Without** this interactions the metastating cells could revert to **MET thus loosing EMT and tumor initiating traits capability necessary for extravasation and colonization.**

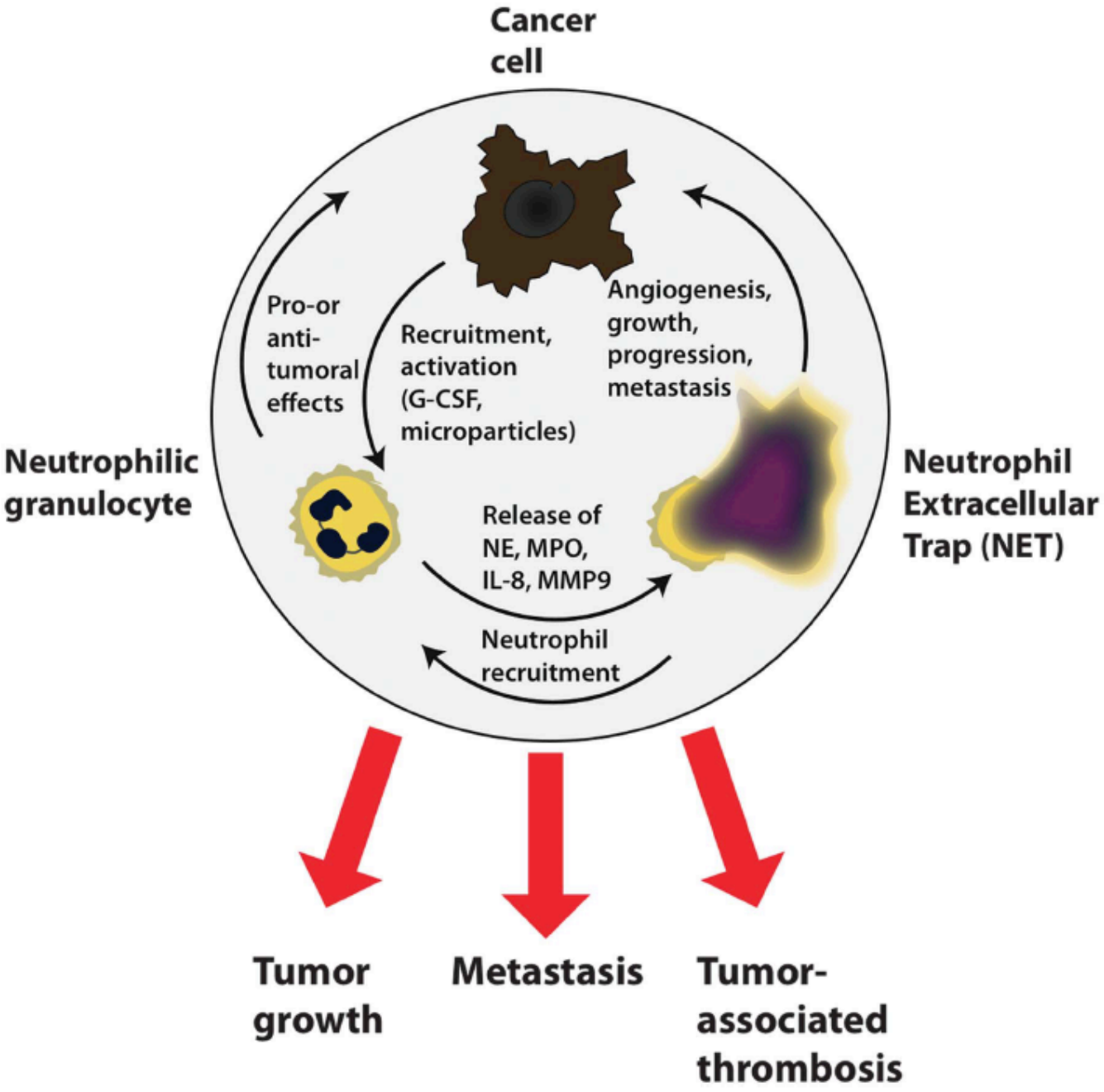
Migrazione transendoteliale ed extravasazione



Platelets activated by cancer cells may signal to endothelial cells via ATP and make endothelial cells more permeable via PyP2 Receptor
In addition **selectin present on platelets may interact with endothelial cells trapping platelets/CTC clusters to** the walls of the vasculature.

Neutrophil extracellular traps (NETs)




Cancer cell
Pro-or anti-tumoral effects
Recruitment, activation (G-CSF, microparticles)
Angiogenesis, growth, progression, metastasis
Neutrophilic granulocyte
Neutrophil Extracellular Trap (NET)
Release of NE, MPO, IL-8, MMP9
Neutrophil recruitment
Tumor growth
Metastasis
Tumor-associated thrombosis