

La transizione epitelio-mesenchimale

La cascata invasione-metastasi

DISSEMINAZIONE

COLONIZZAZIONE

Physical translocation from primary tumor to distant organ

Colonization

A Acquisition of invasive phenotype

B

Local invasion cells invade into surrounding stroma, then intravasate to enter hematogenous circulation

C

CTCs transit to distant organ

E


Survival at secondary site


D


CTCs extravasate and invade into the parenchyma of foreign tissue

F


Adaptation and proliferation to form metastases

 Differentiated cancer cell

 Transitioning cancer cell

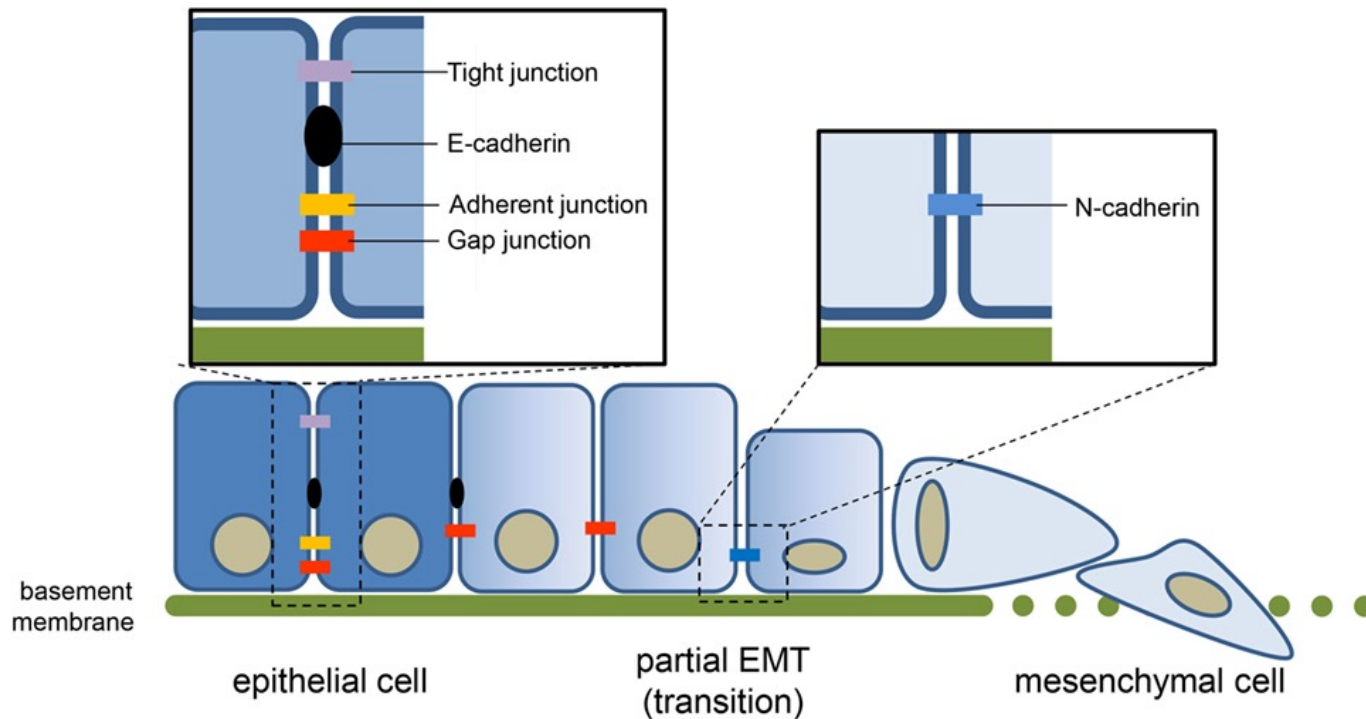
 Disseminated cancer cell

 Stromal cell

 Inflammatory cell

EMT

MET



EMT inducers



epithelial to mesenchymal transition

Phenotype

Loss of differentiation
Junctions dissociation
Loss of apical-basal polarity

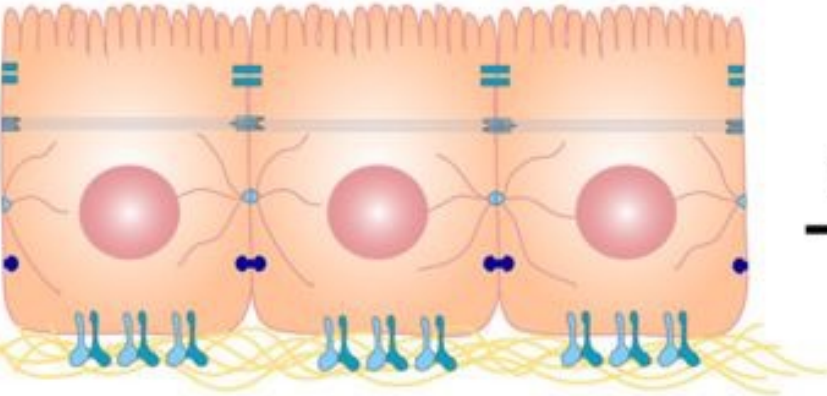
Loss of epithelial markers
(e.g. E-cadherin, claudins, cytokeratin)
Increase of transcription factors
(e.g. Snail, Slug, ZEB, Twist)

Cytoskeleton reorganization
Migration
Secretion of MMPs
Basement membrane degradation
Invasion

Acquisition of mesenchymal markers
(N-cadherin, vimentin, α -SMA)
Increase of transcription factors
(e.g. Slug)

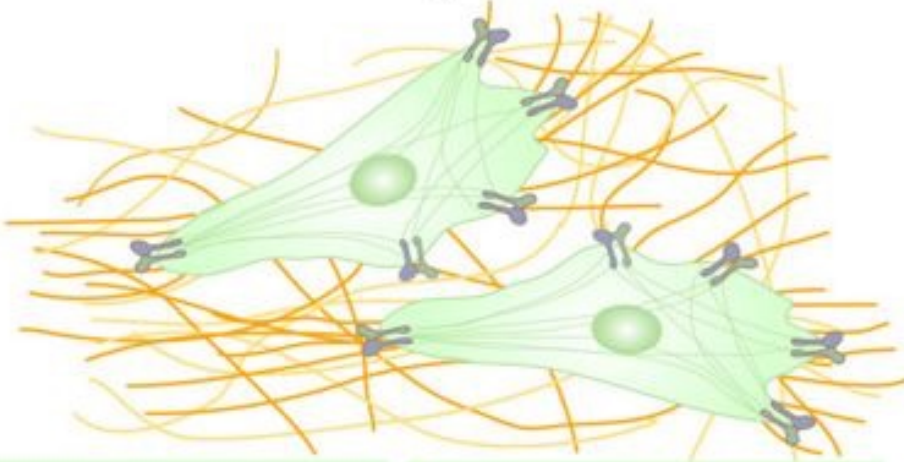
MARKERS di EMT

Epithelial cells



EMT
→

Mesenchymal cells



Proteome	Phenotypic Markers
E-cadherin Occludins Cytokeratins	Cuboidal shape Presence of cell junctions Apicobasal polarity

Proteome	Phenotypic Markers
N-cadherin Vimentin α SMA	FSP-1 Fibronectin Collagen I
	Spindle-like shape Increased contractility Increased matrix deposition

Ruoli fisiologici e patologici della EMT

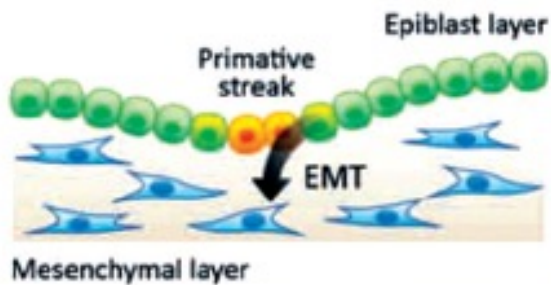
Embriogenesi

Riparazione delle ferite
(*Wound healing*)

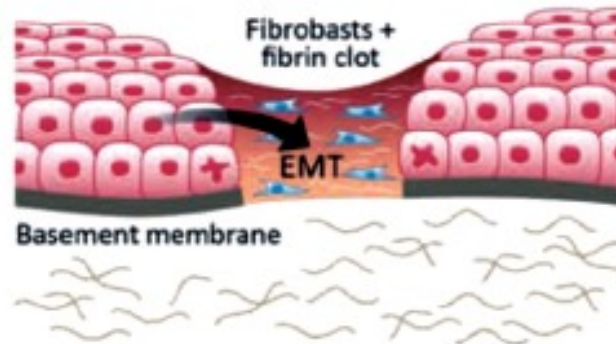
Cancro

Fibrosi

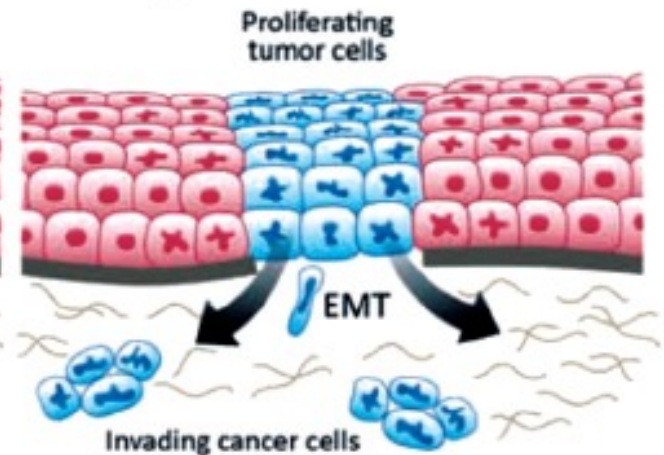
Type 1: Development



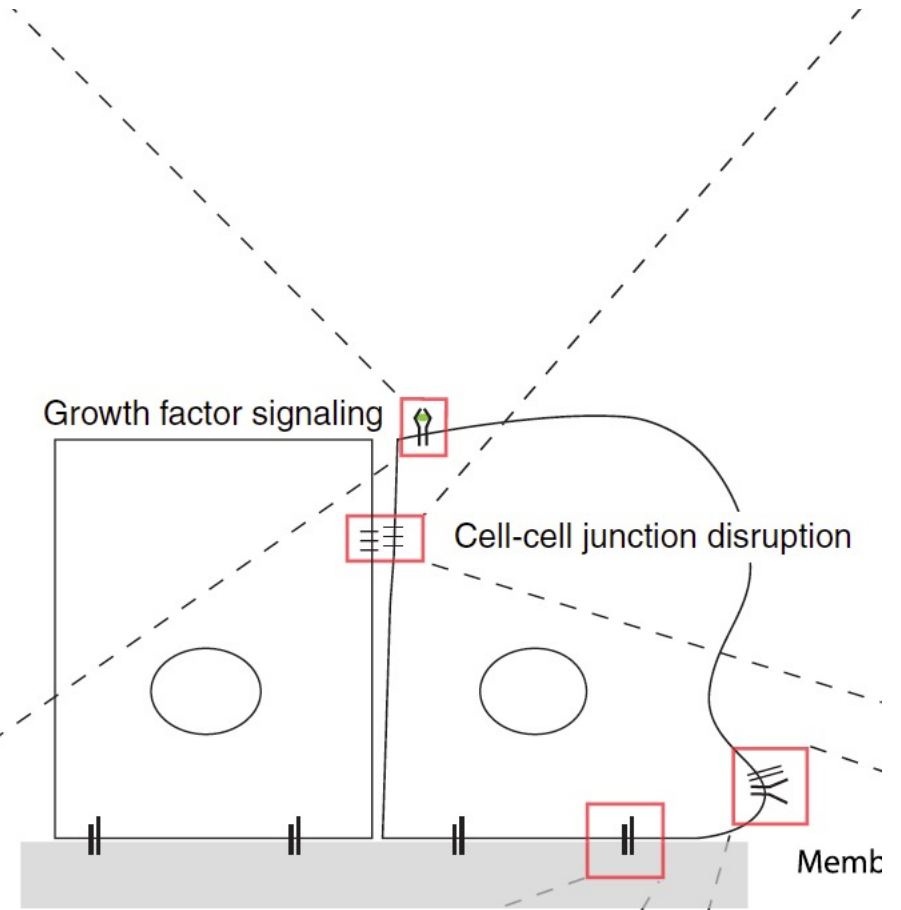
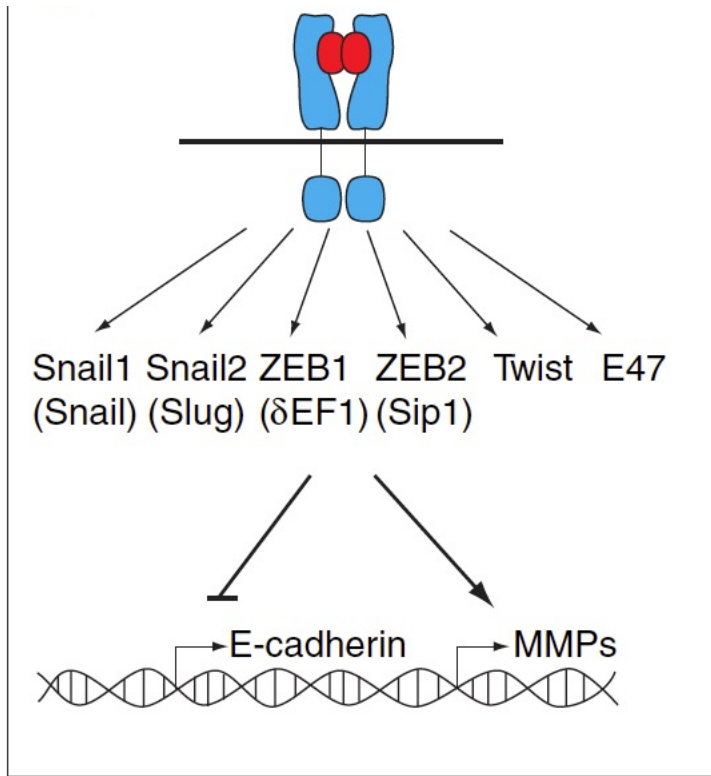
Type 2: Wound Healing



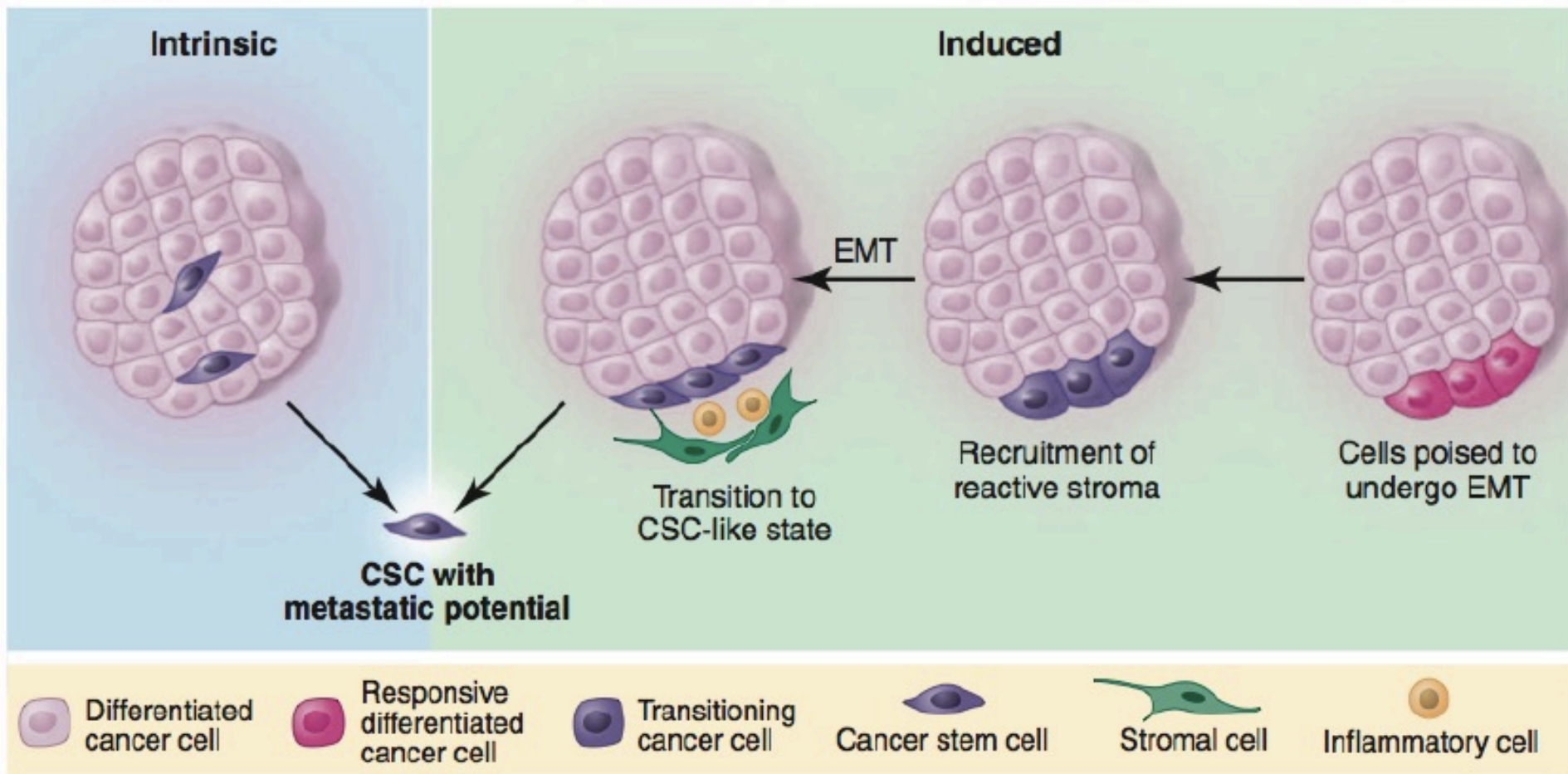
Type 3: Cancer Invasion



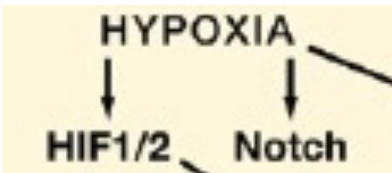
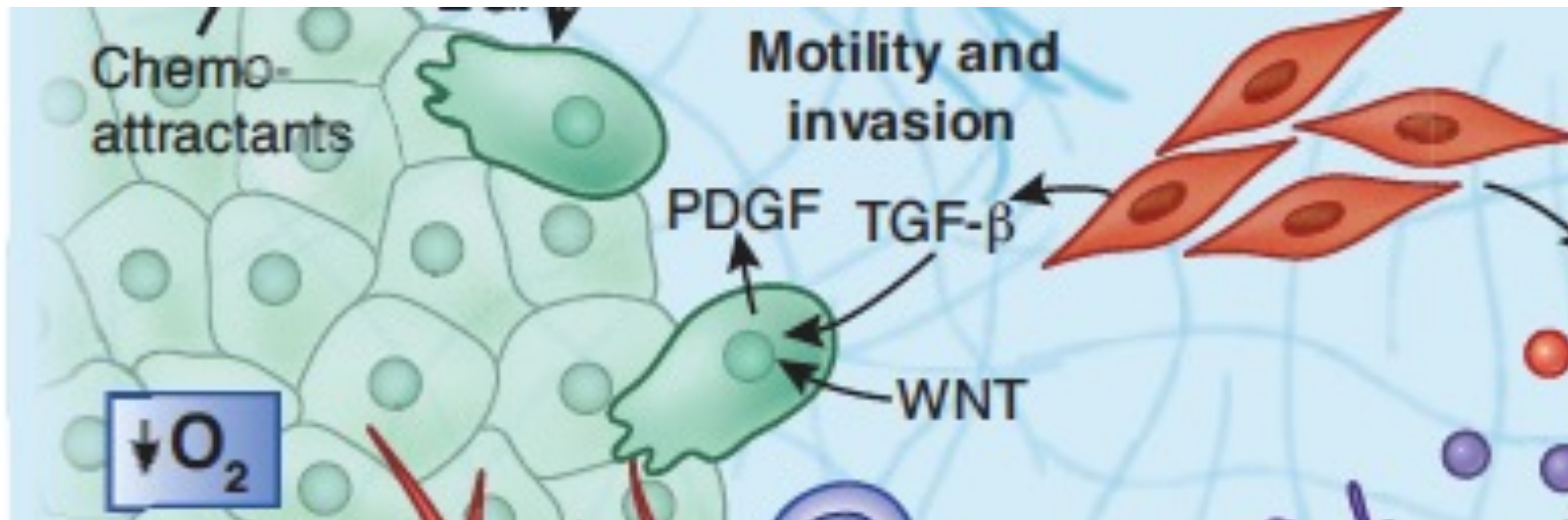
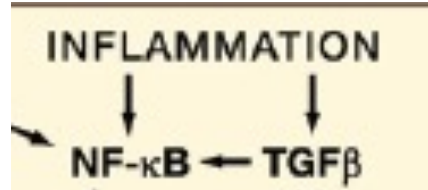
Gli effettori del programma genico della EMT: TWIST SNAIL e ZEB:



**Il programma di EMT conferisce caratteristiche STAMINALI
generando TUMOR INITIATING CELLS (CSCs)
essenziali per la metastasi e chemioresistenti**



ORIGINE DEI SEGNALI ESTRINSECI DI EMT



Stroma(CAFs)
TFGβ e WNT

Regolazione della EMT da oncogeni e segnali eterotipici

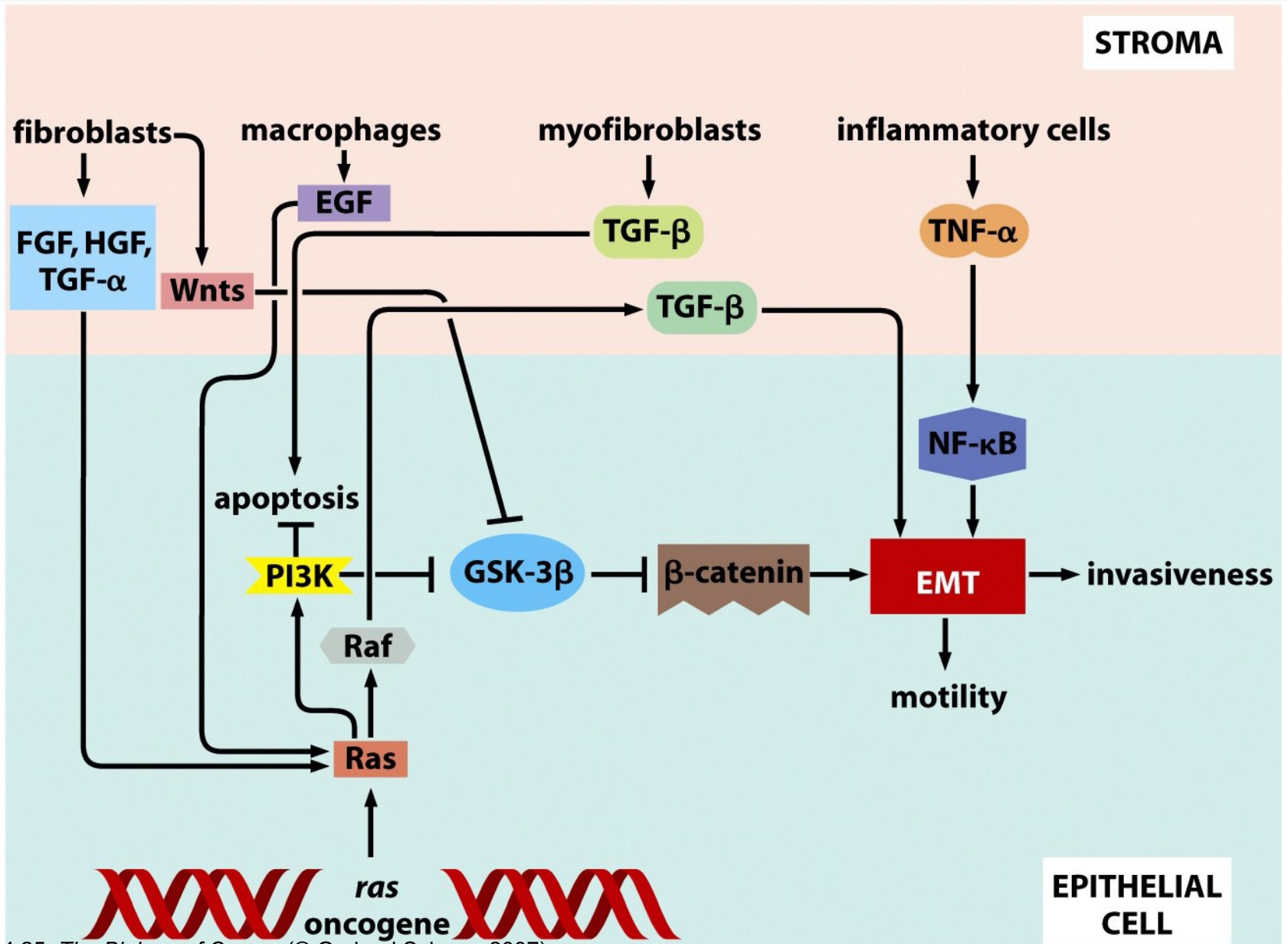
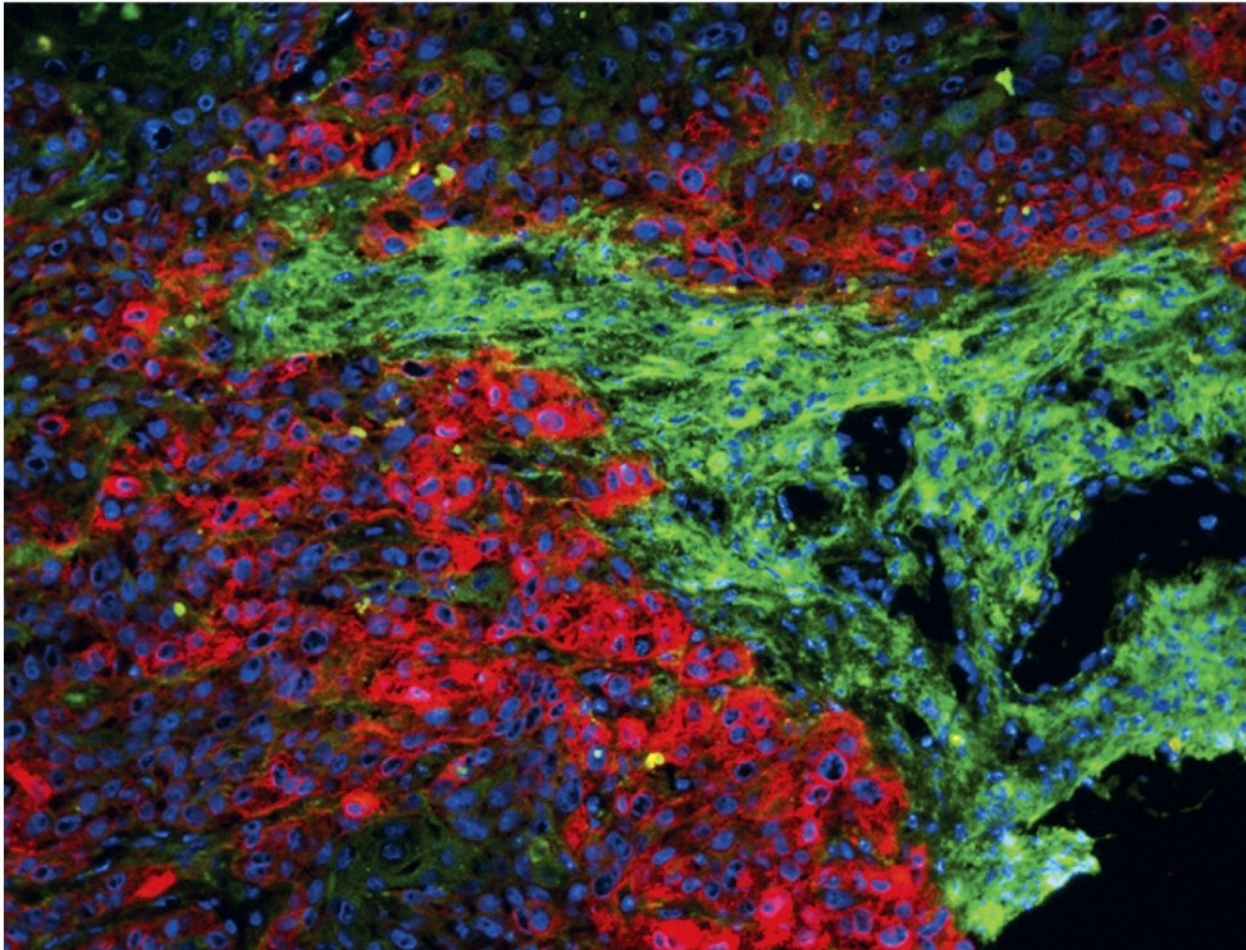


Figure 14.25 *The Biology of Cancer* (© Garland Science 2007)

TGF- β (stromal cells)

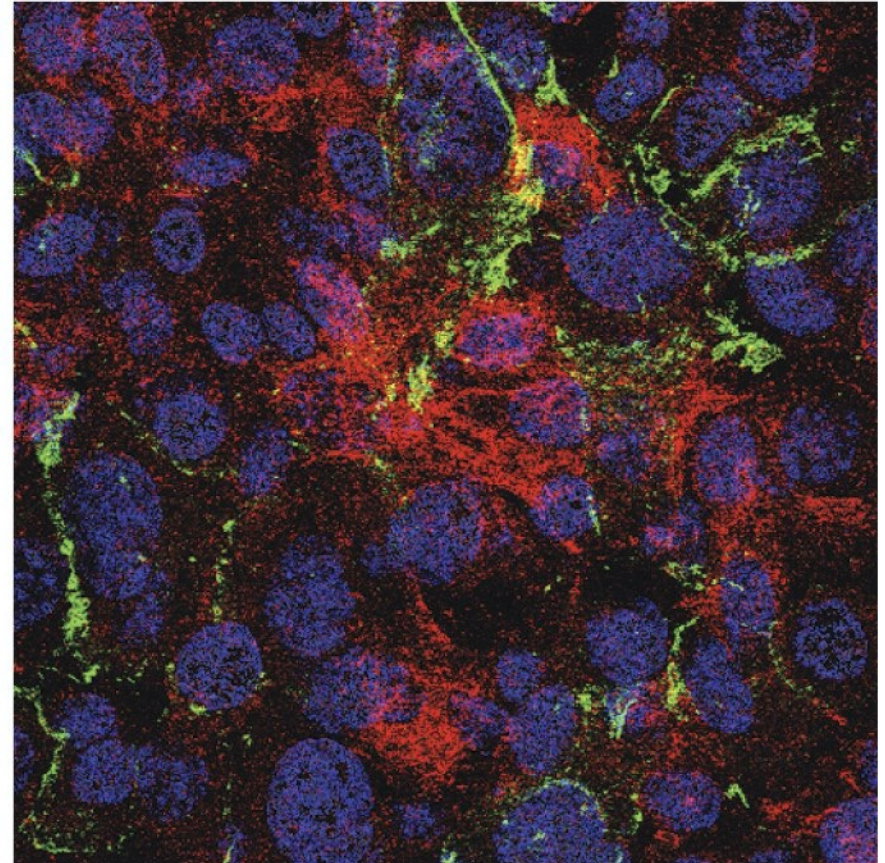
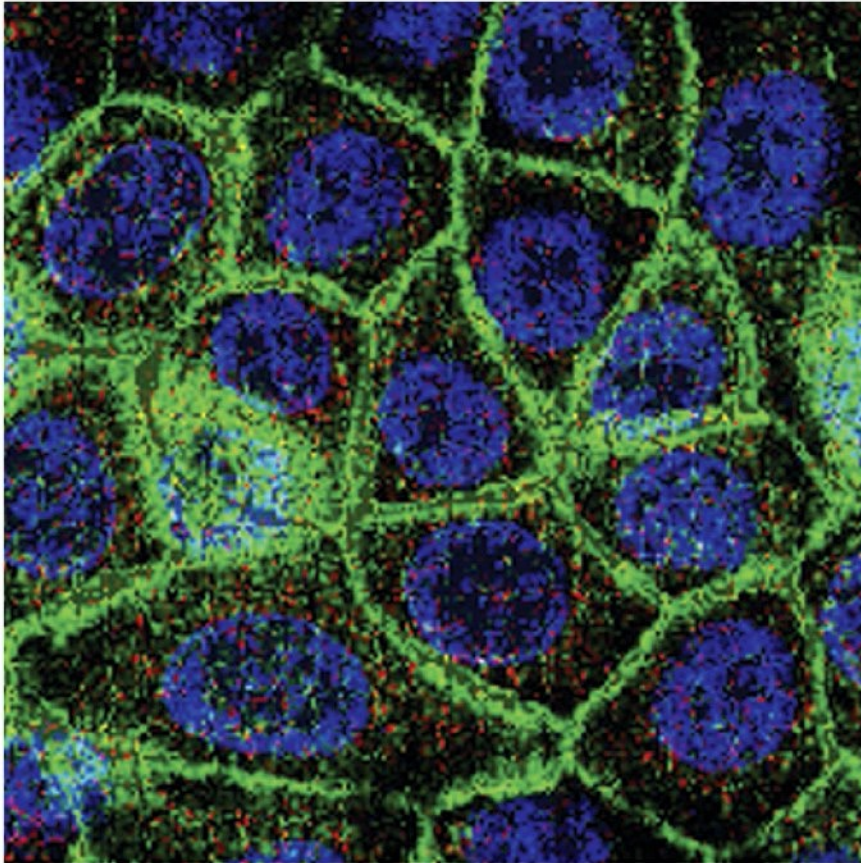


$\alpha_v\beta_6$ integrin (epithelial cells)

E-cadherin

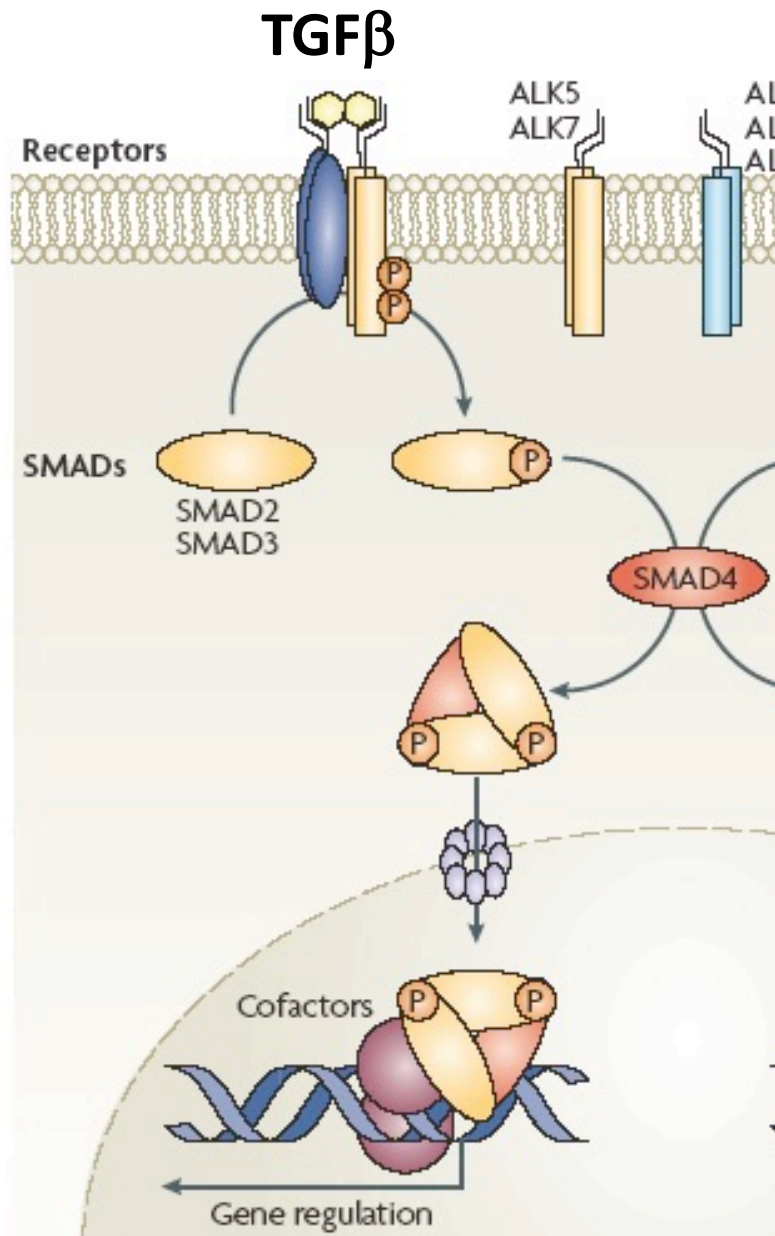
nuclei

vimentin

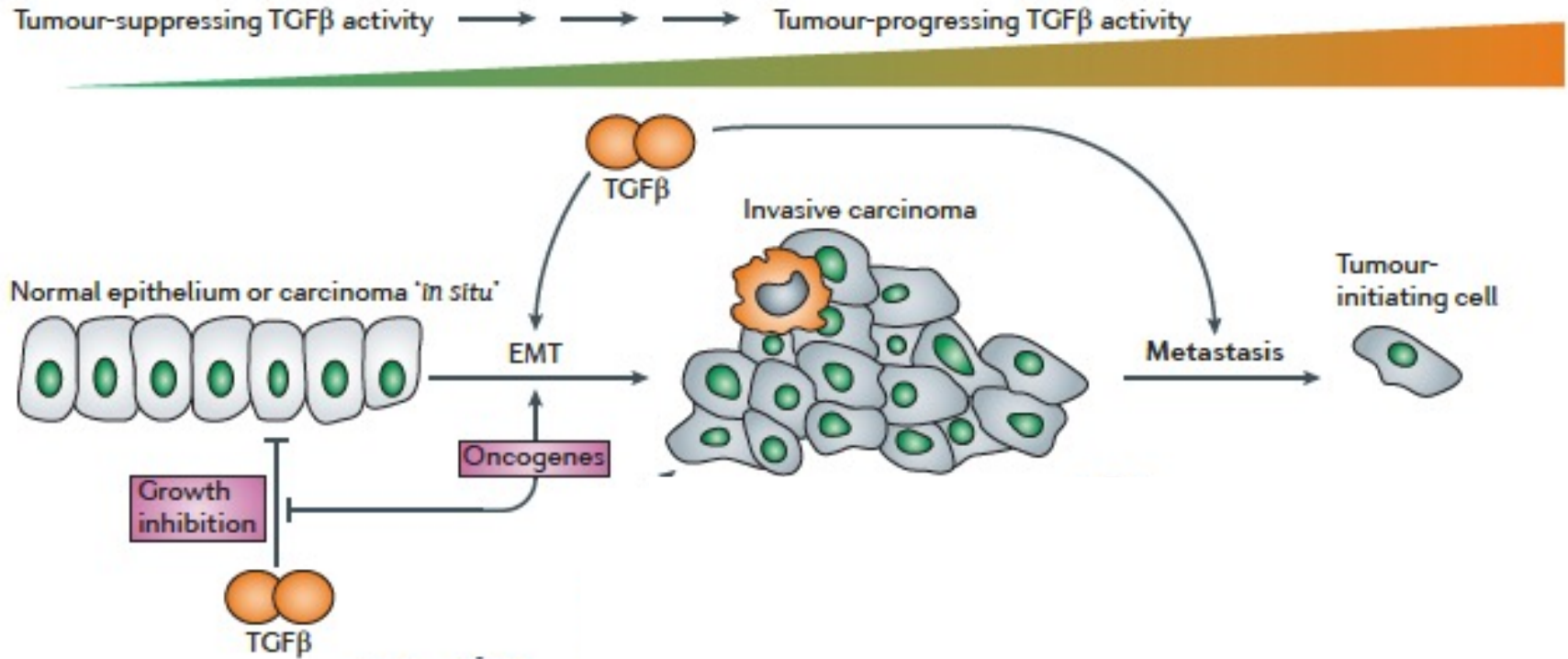


TGF- β for 7 days \longrightarrow

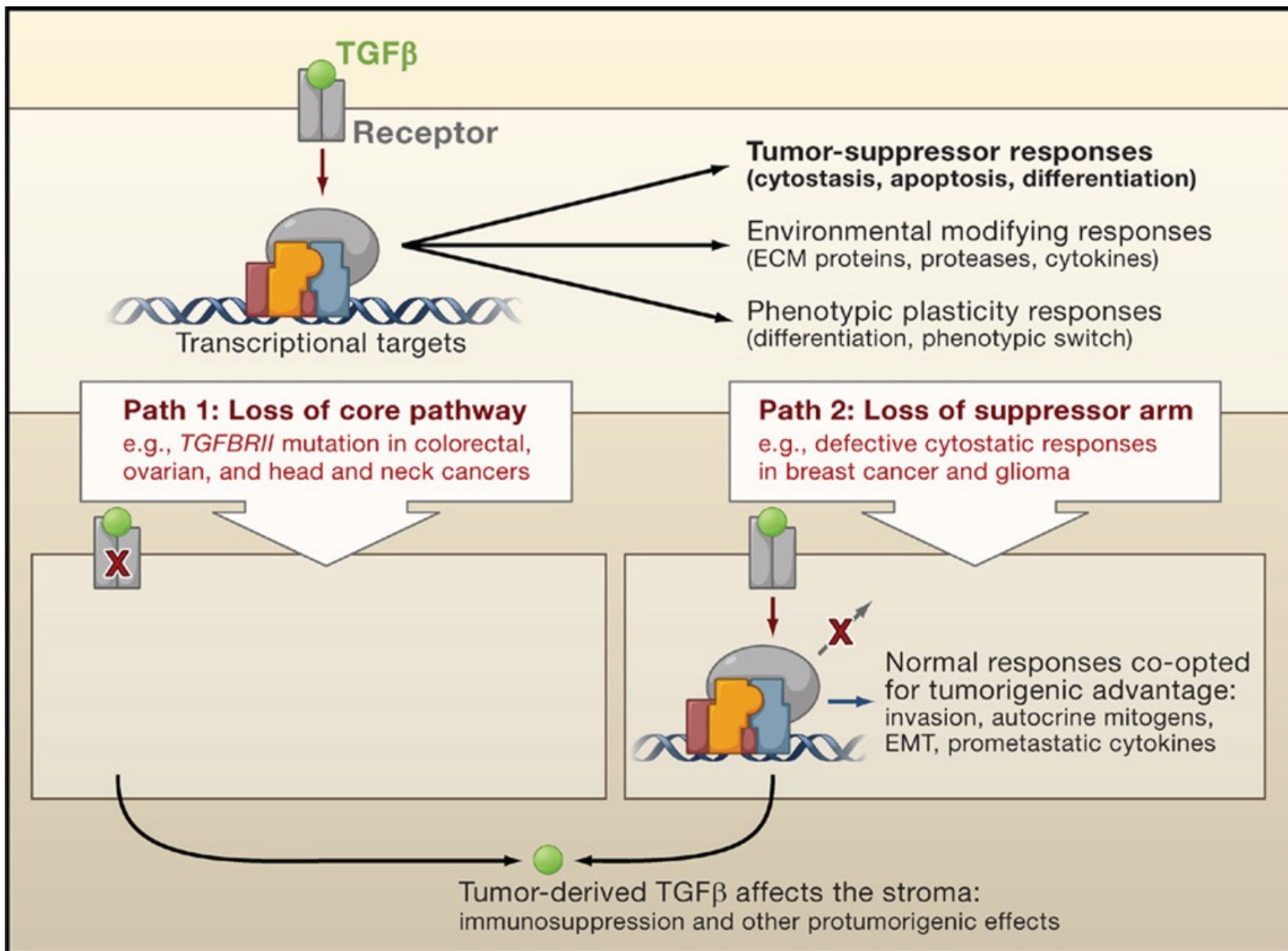
La pathway di TGFβ



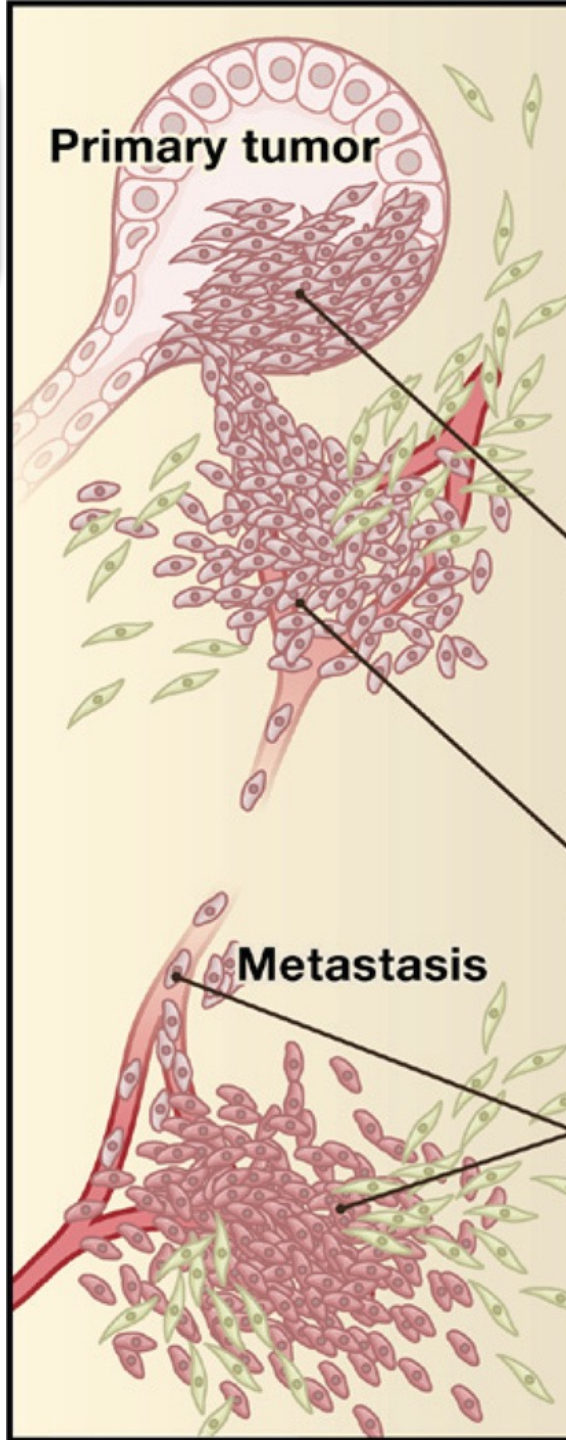
Il doppio ruolo della pathway di TGF β nel cancro



Alterazioni della pathway di TGF β nel cancro



Il doppio ruolo della pathway di TGF β nel cancro



Roles of TGF β in cancer

Premalignant state

Tumor-suppressive effects: cytostasis, differentiation, apoptosis

Suppression of tumorigenic inflammation

Suppression of stroma-derived mitogens

Malignant progression (Loss of tumor suppression)

Evasion of immune surveillance

Autocrine mitogen production

Motility

Invasiveness and dissemination

Epithelial-mesenchymal transition

Myofibroblast mobilization

Cancer cell priming for metastasis

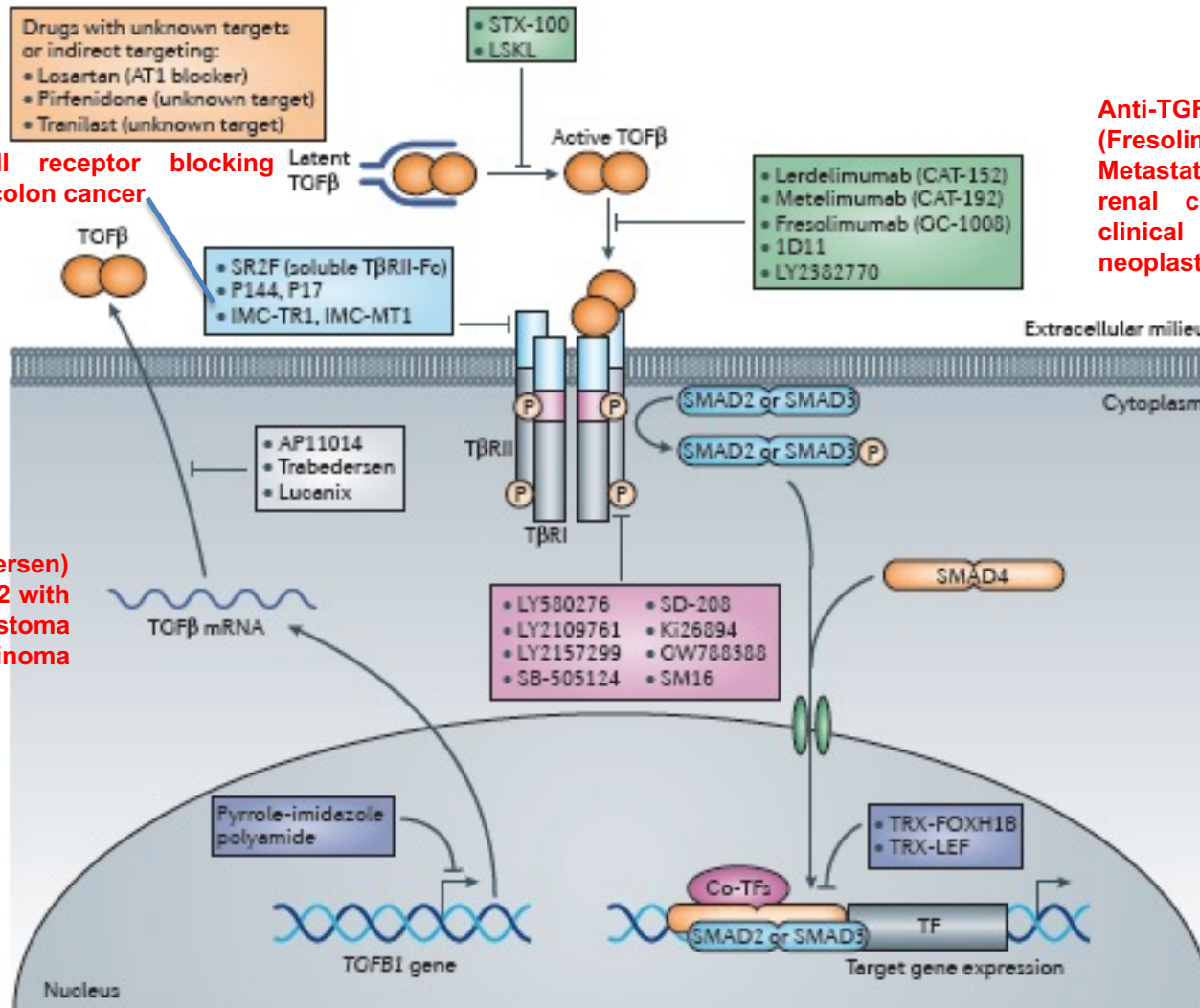
Metastatic colonization

Extravasation

Osteoclast mobilization

Microenvironmental-modifying factors: cytokines, proteases

Strategie farmacologiche per inibire la TGF- β pathway nel cancro e nelle malattie fibrotiche

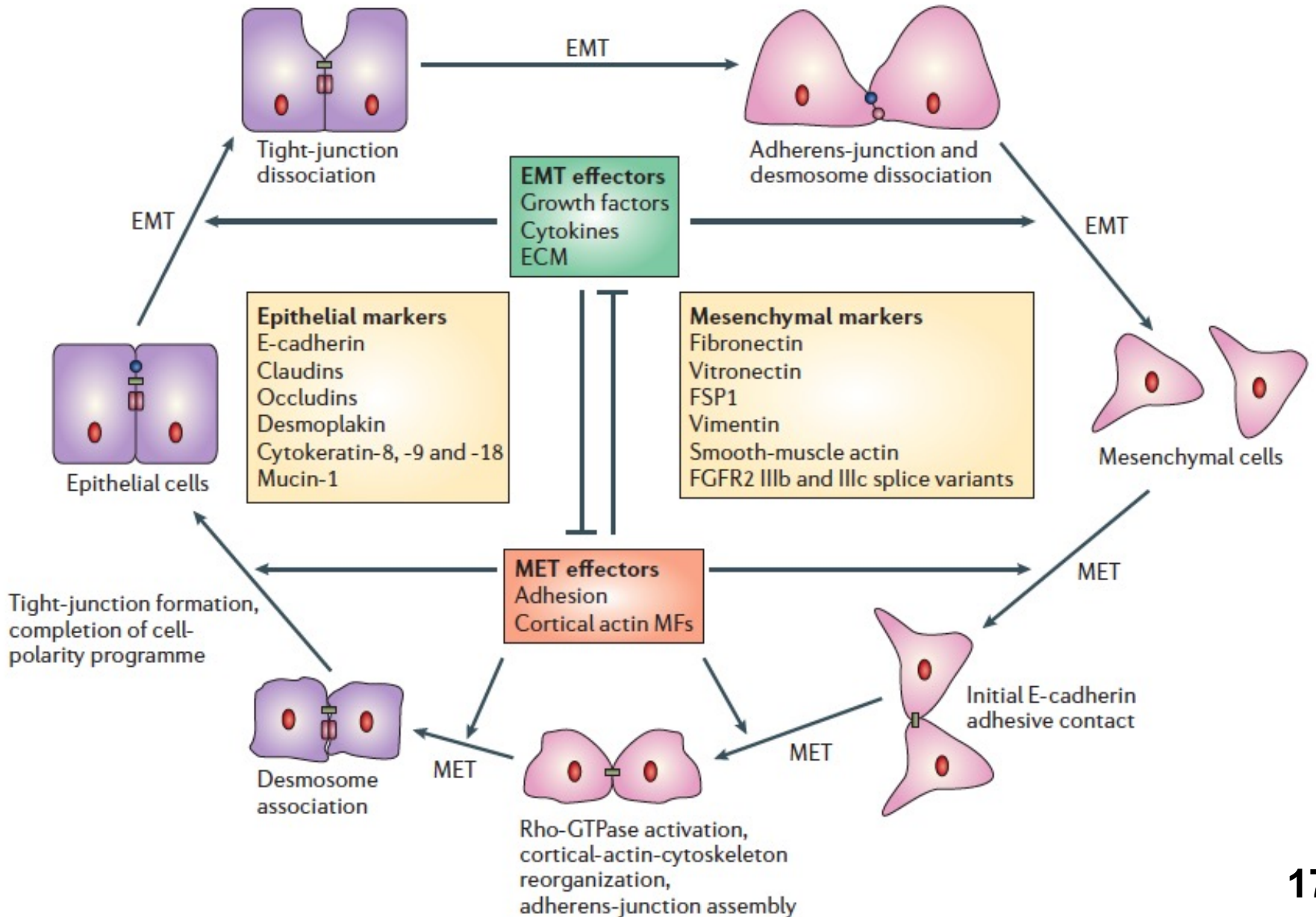


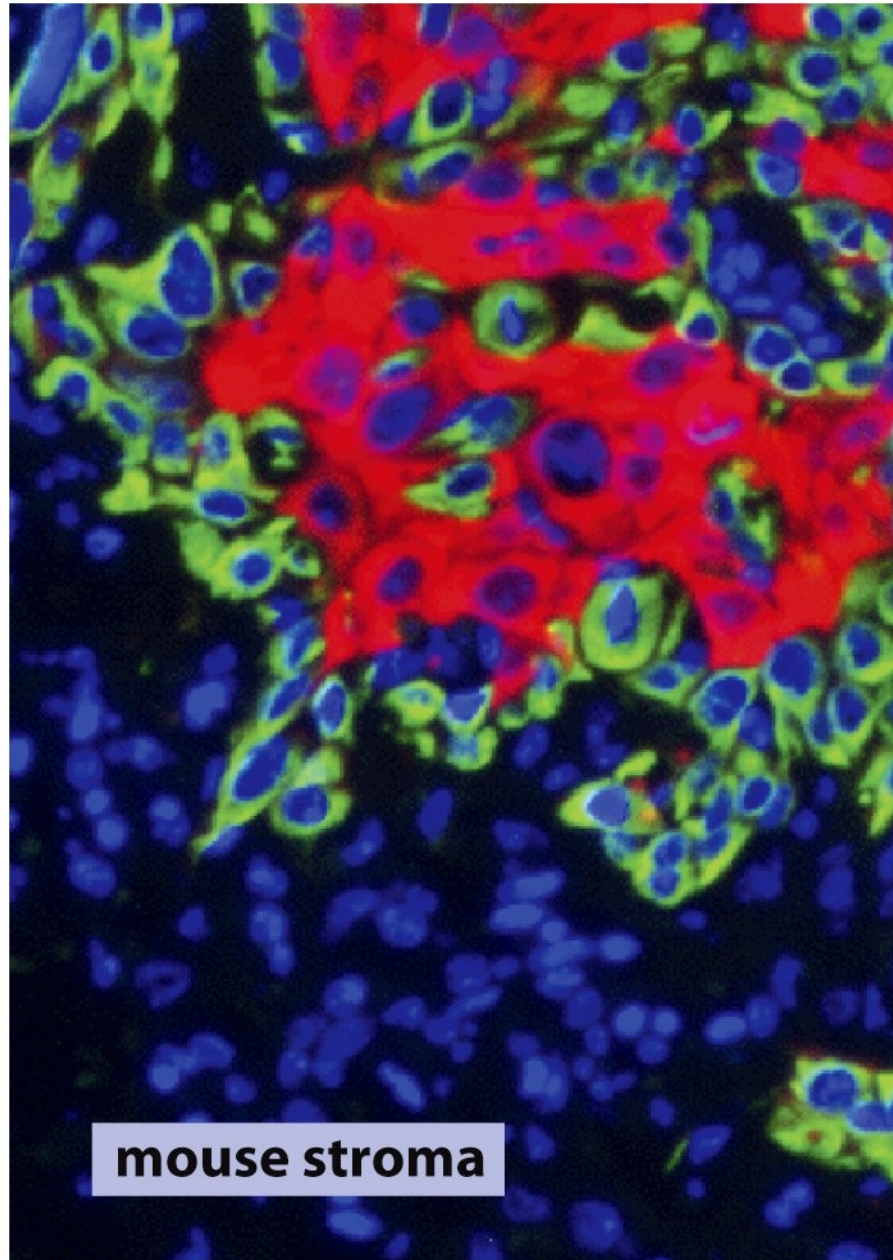
e.g. (IMC-TR-1) TGF β II receptor blocking antibody for breast and colon cancer

Anti-TGFbeta1 antibody e.g. (Fresolimumab) Metastatic melanoma and renal carcinoma (phase I clinical trial) and non-neoplastic applications

e.g. (AP1209 = Trabedersen) Targeting excess TGF β 2 with ASO-RNAi in glioblastoma and pancreatic carcinoma (phase III clinical trial)

SWITCH FENOTIPICO EMT/MET





**(human)
cytokeratin**

**(human)
vimentin**

mouse stroma

Figure 14.19c *The Biology of Cancer* (© Garland Science 2007)

Lo SWITCH delle caderine facilita l'invasione

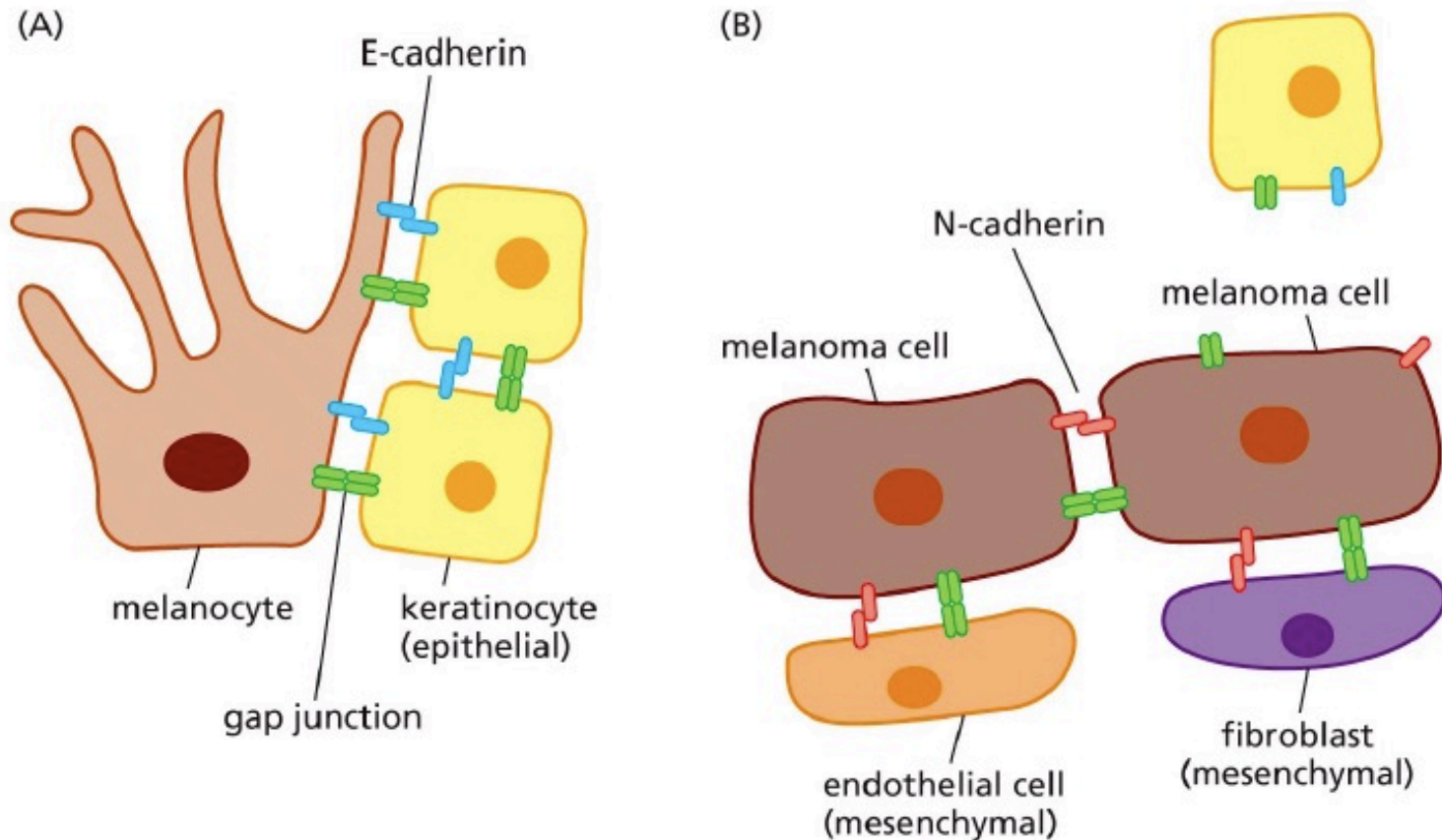
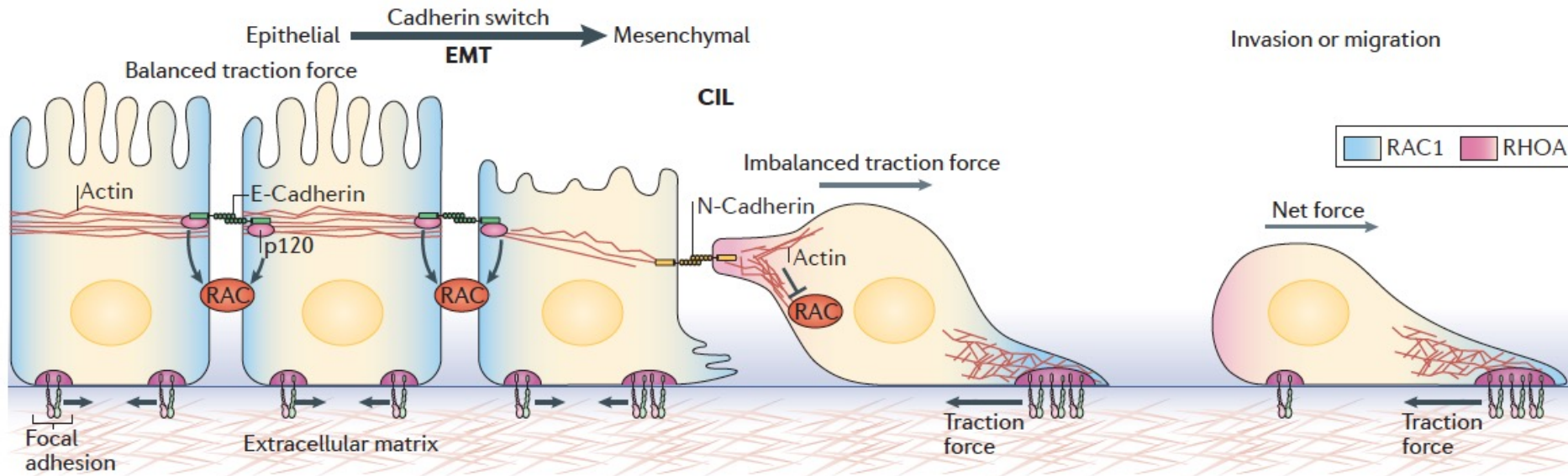


Figure 14.16 The Biology of Cancer (© Garland Science 2014)

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.

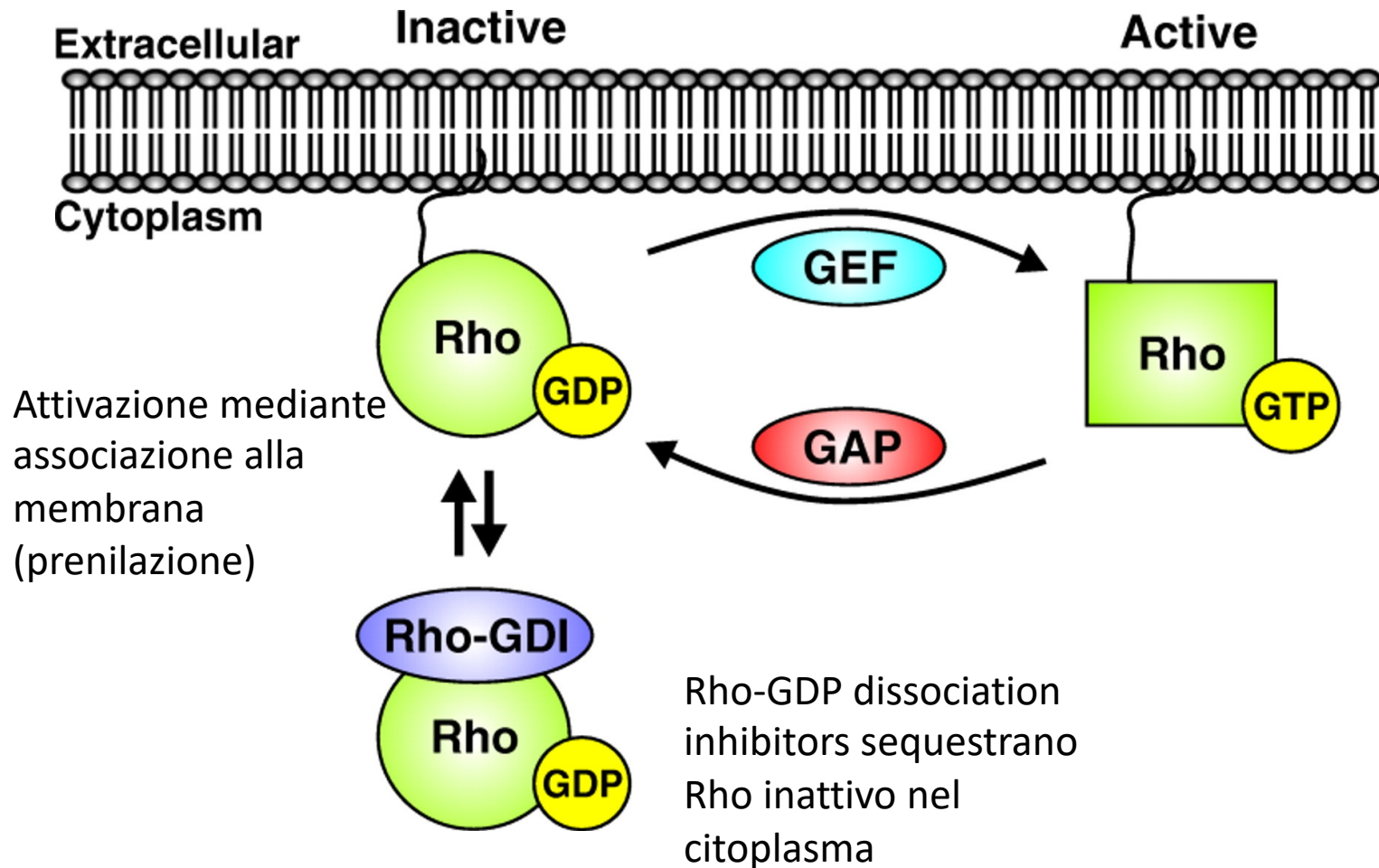
Lo switch delle caderine facilita la perdita di polarità e rimuove l'inibizione della migrazione



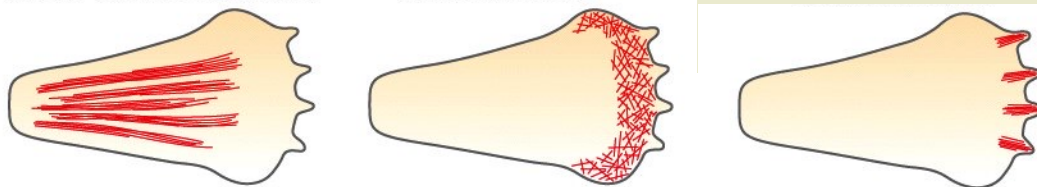
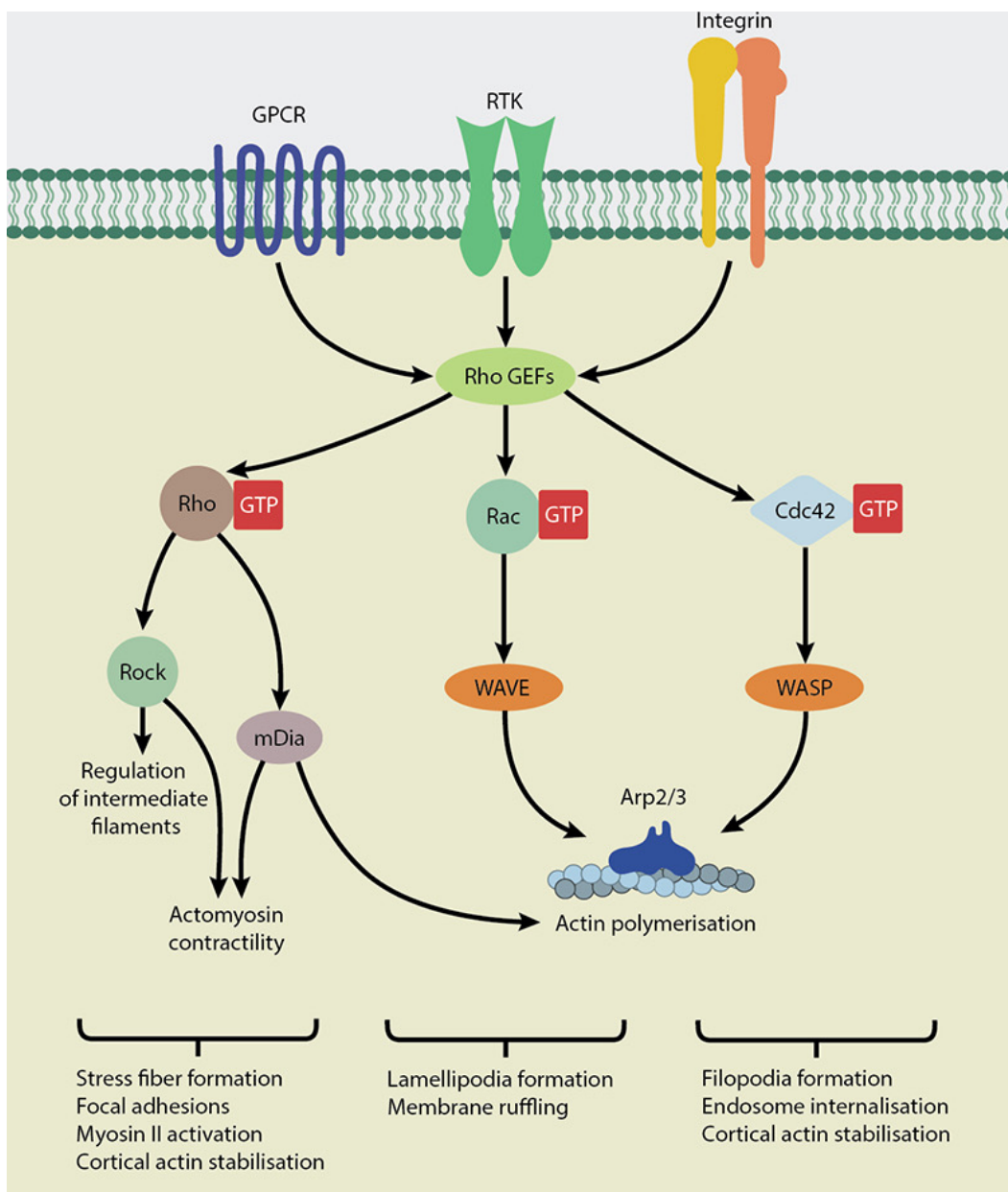
E-Cadherin suppresses EMT by signalling to other adhesion components, such as p120 catenin, which polarizes the small GTPase **RAC1** towards cell-cell junctions.

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

IL CICLO DI ATTIVAZIONE DELLE GTPasi della famiglia di RHO

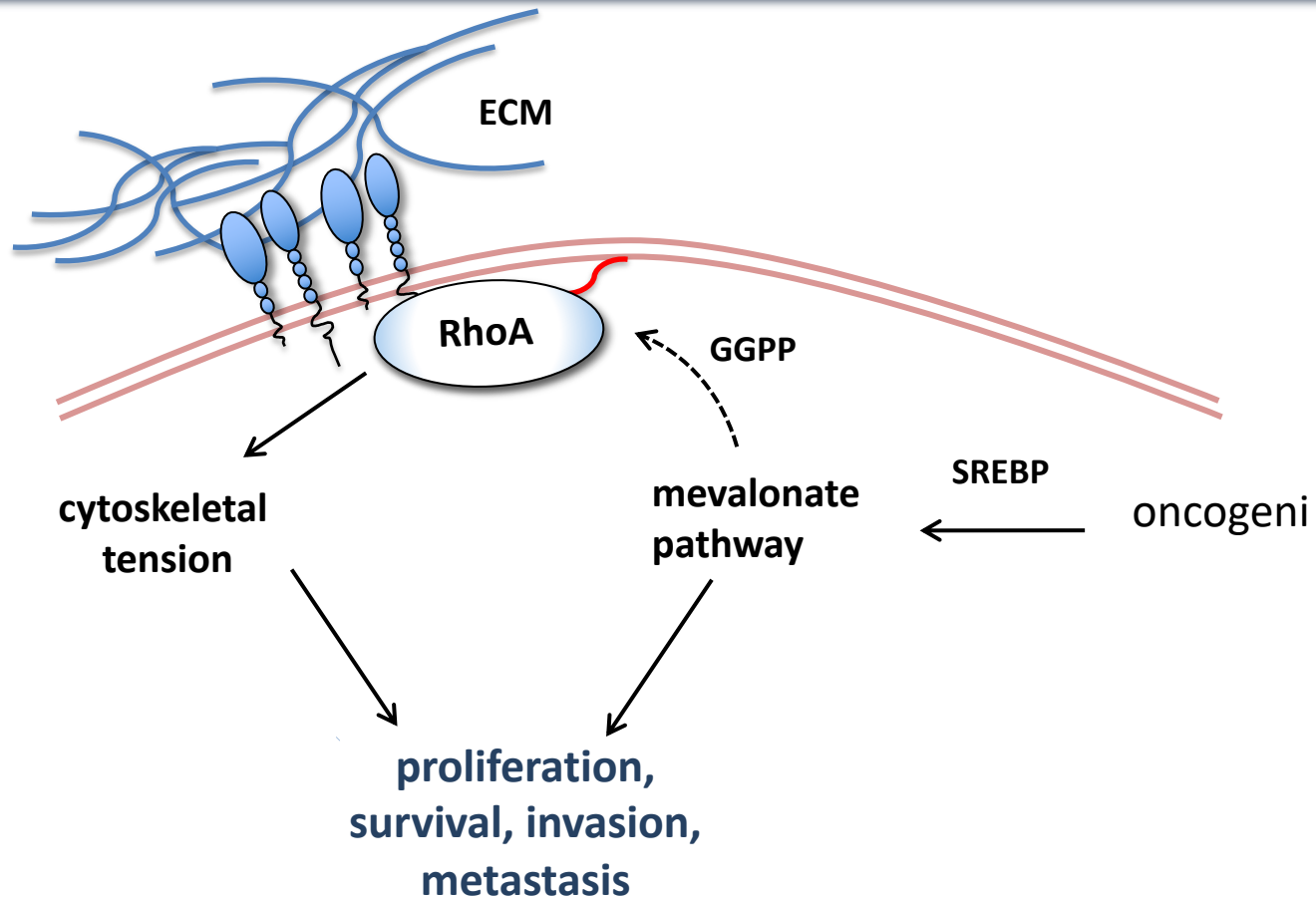


Stephan Huveneers, and Erik H. J. Danen *J Cell Sci* 2009;122:1059-1069

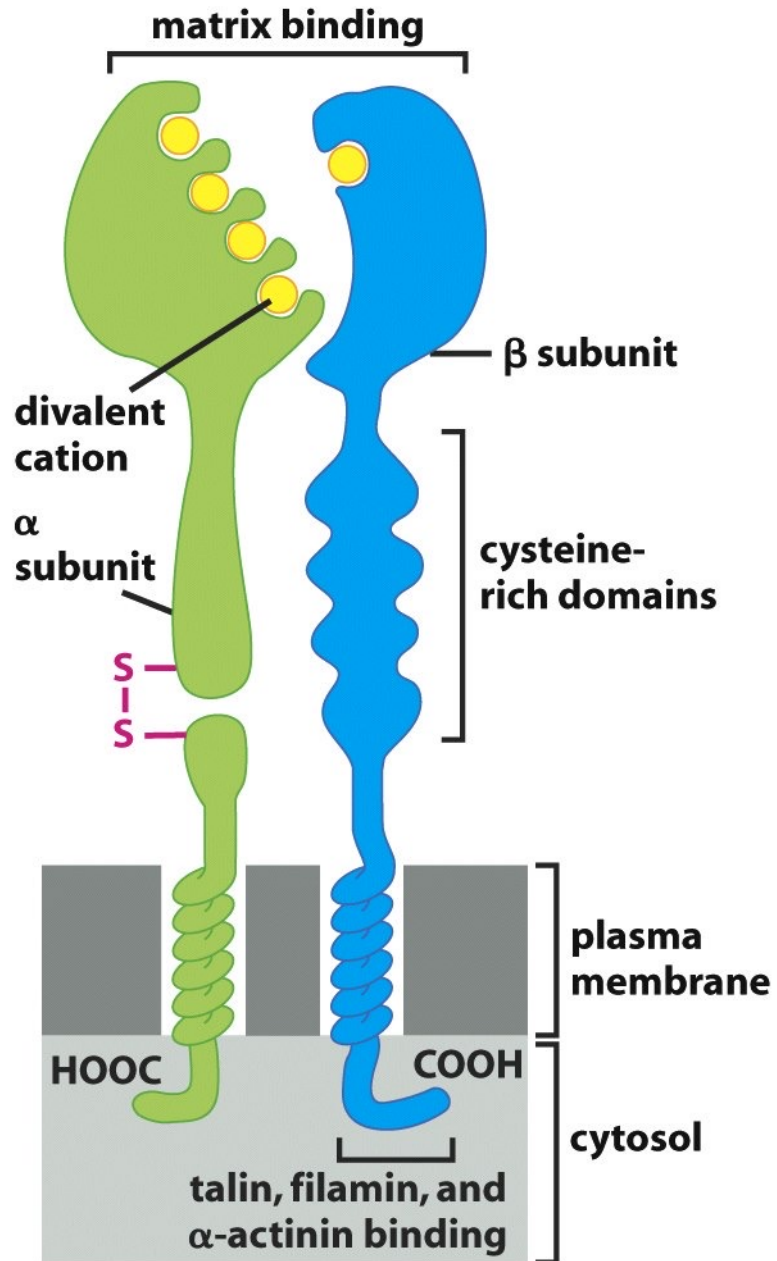


La via del mevalonato potenzia le attività tumorigeniche di RhoA favorendo la sua localizzazione alle adesioni focali via

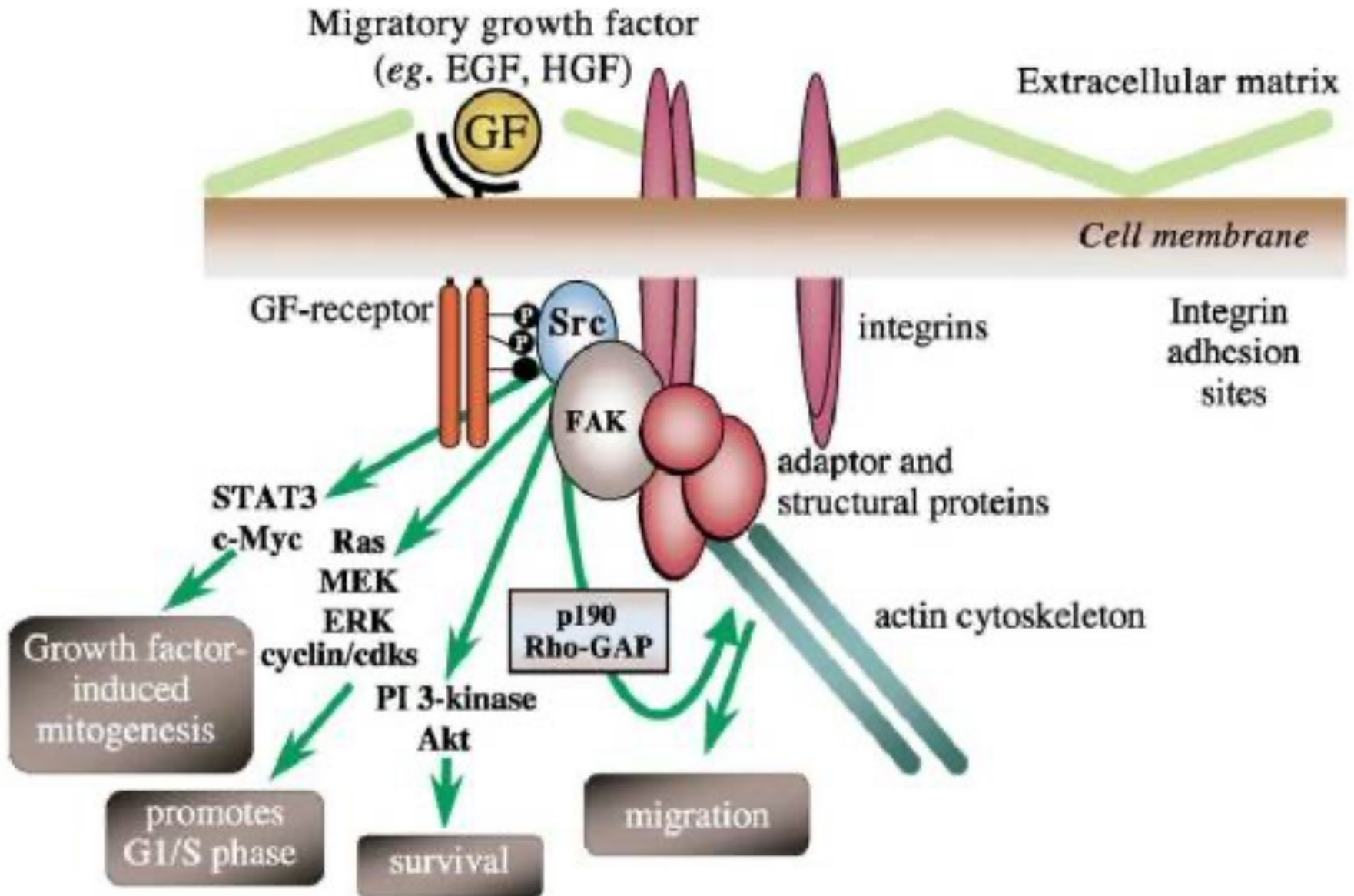
GGPP



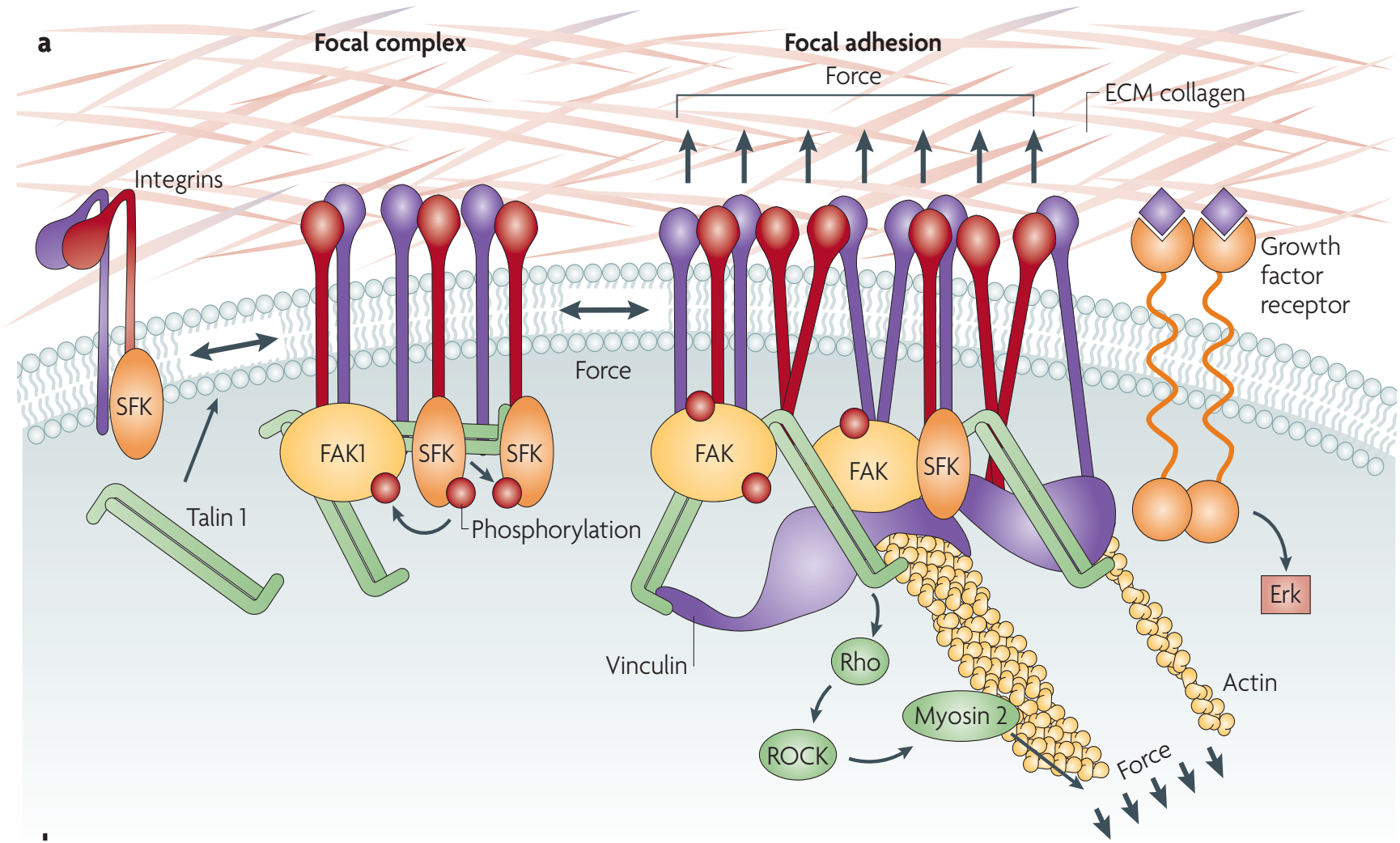
Le integrine mediano le interazioni cellula-ECM



Src è coinvolto in diverse vie di trasduzione attivate da recettori di membrana

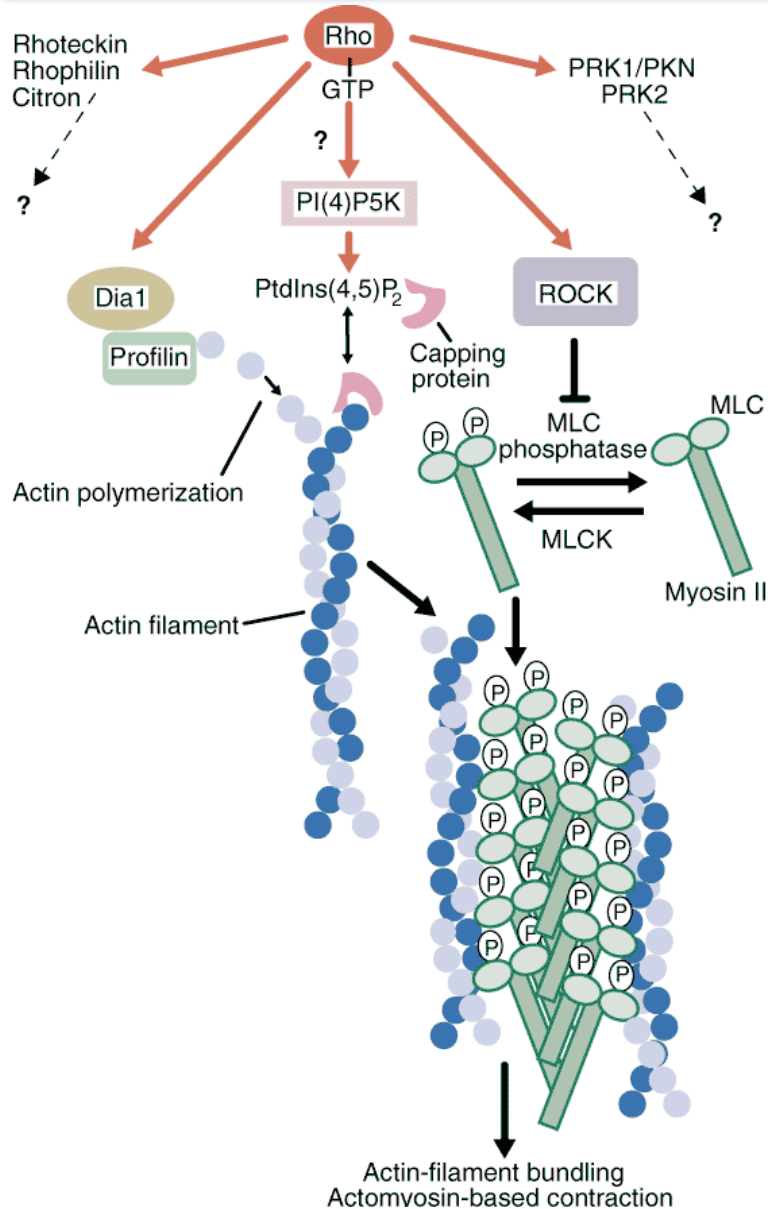


Attivazione del signaling delle integrine alle adesioni focali



Impatto su: sopravvivenza, proliferazione e progressione tumorale

Le GTPasi Rho e la dinamica del citoscheletro actomiosinico



La GTPasi Rho induce
polimerizzazione della F actina e
contrattilità actomiosinica

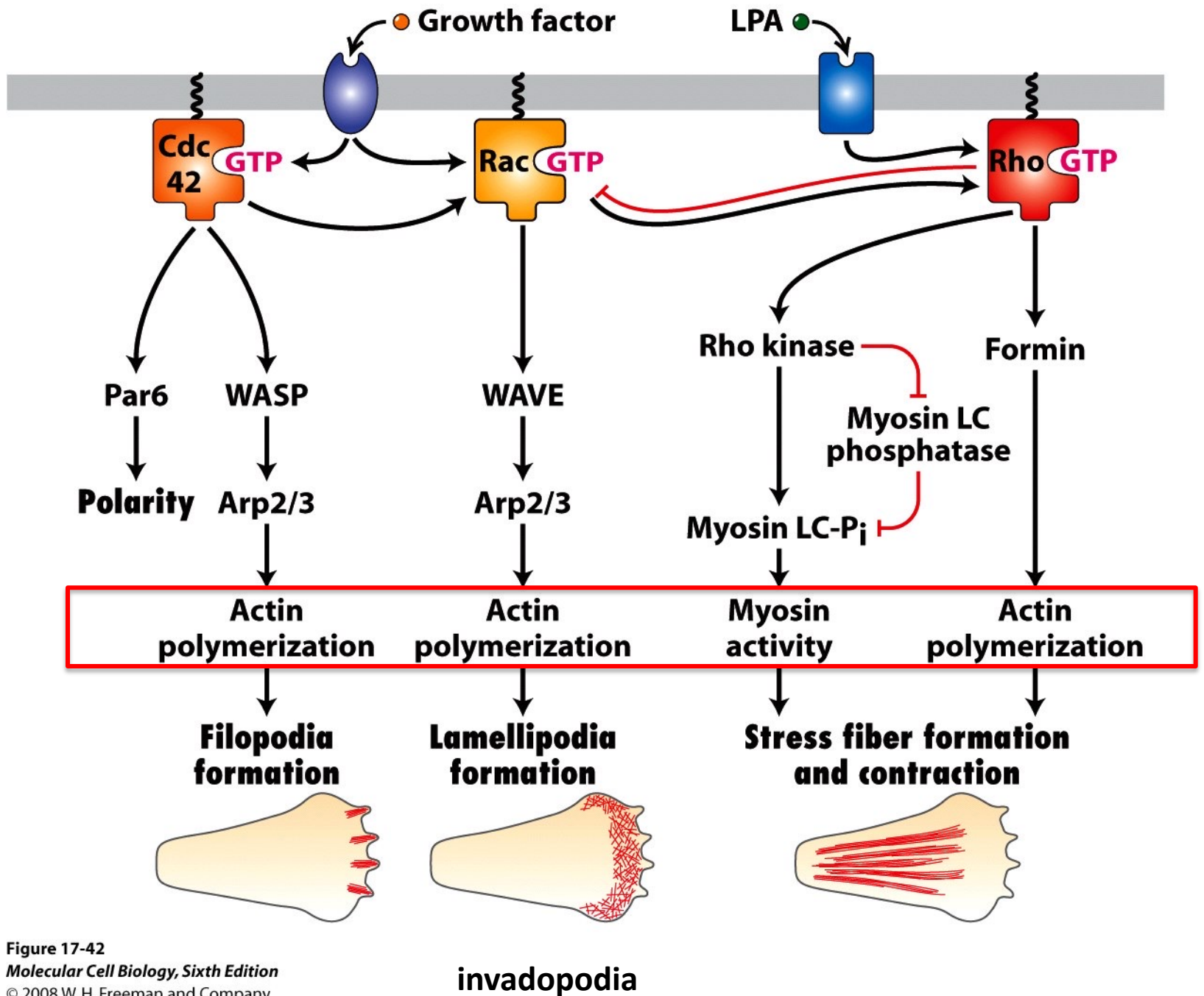


Figure 17-42
Molecular Cell Biology, Sixth Edition
 © 2008 W. H. Freeman and Company

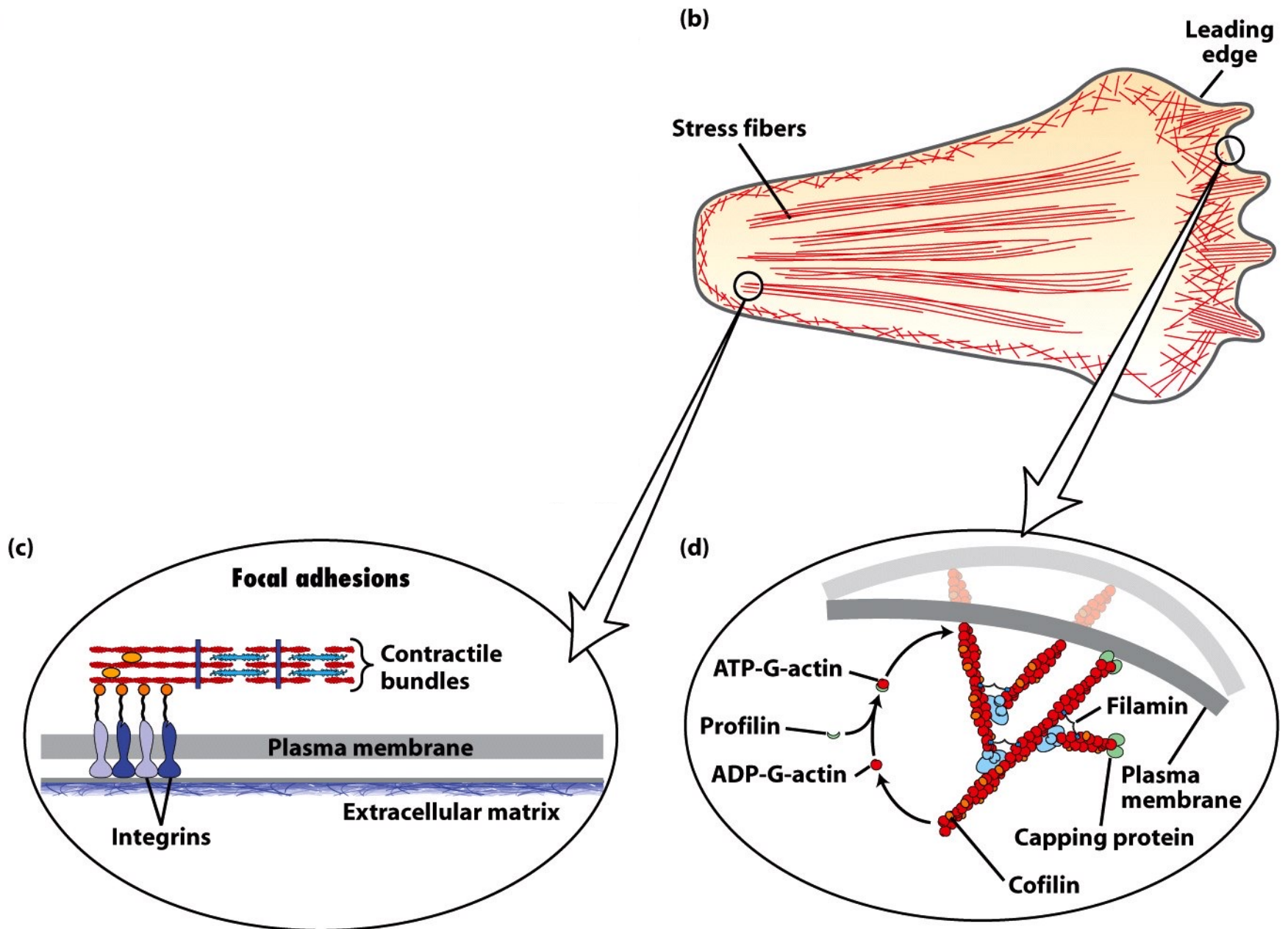
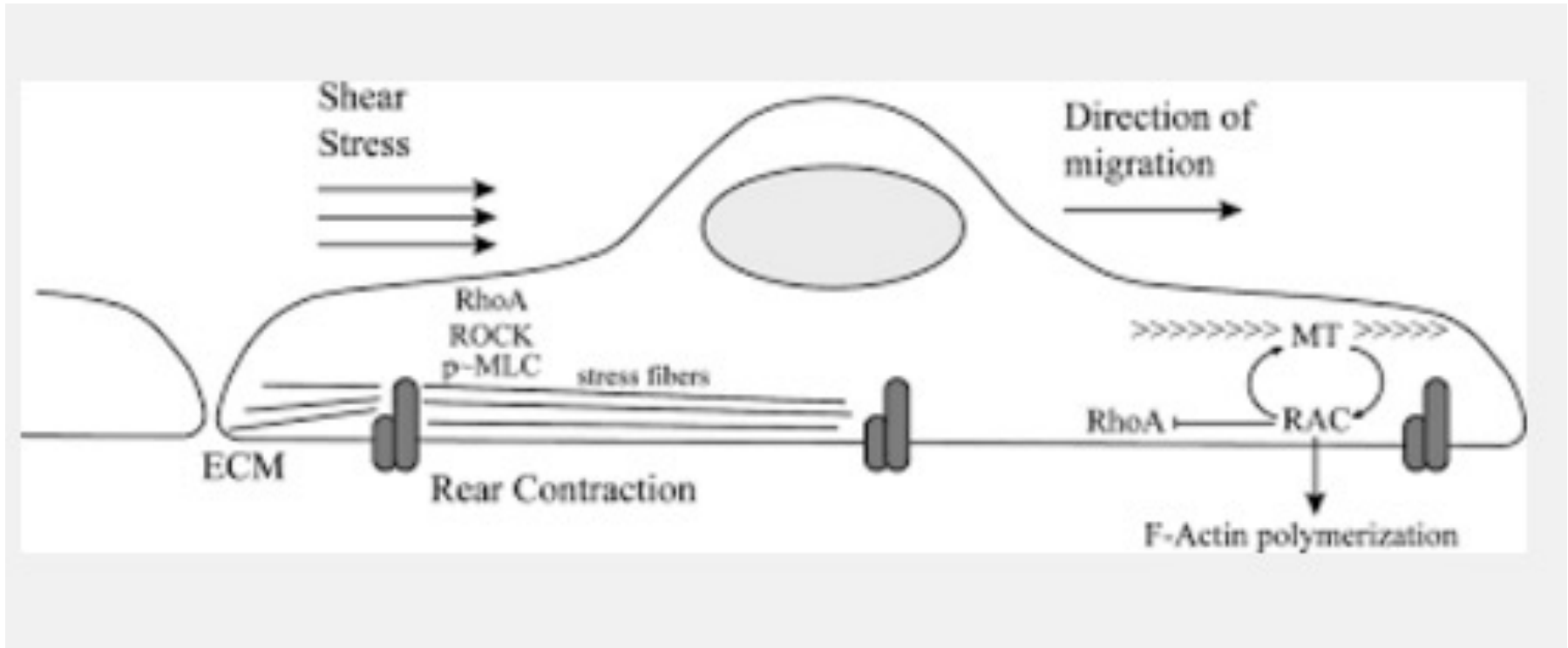


Figure 17-39
Molecular Cell Biology, Sixth Edition
 © 2008 W.H. Freeman and Company

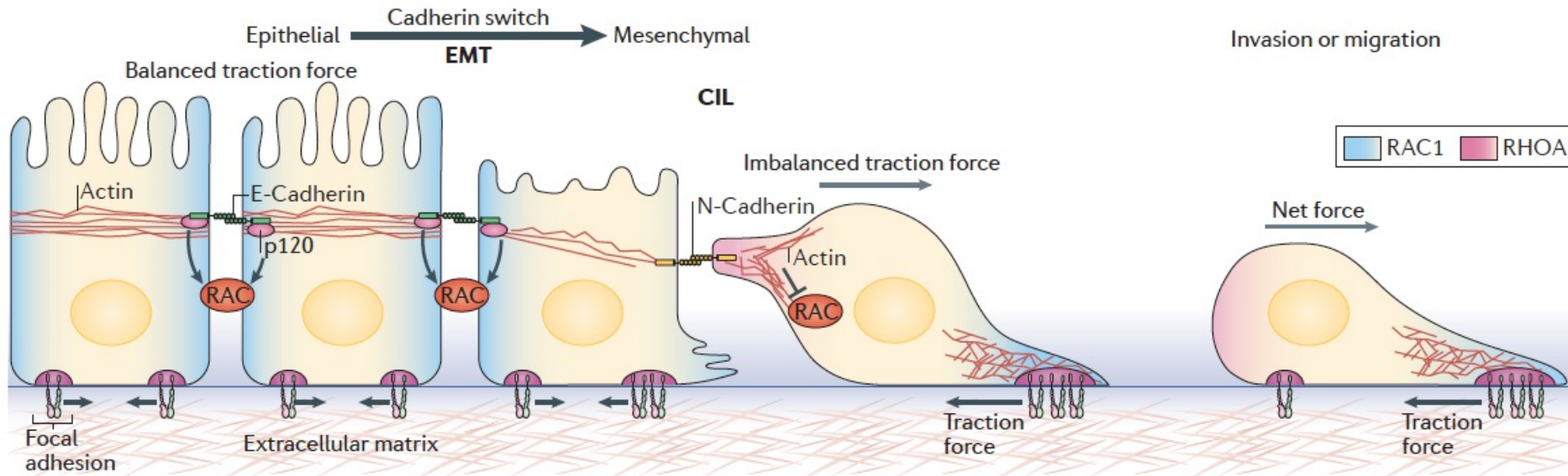
La migrazione dipende da attivazione localizzata di specifiche proteine della famiglia Rho



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.**

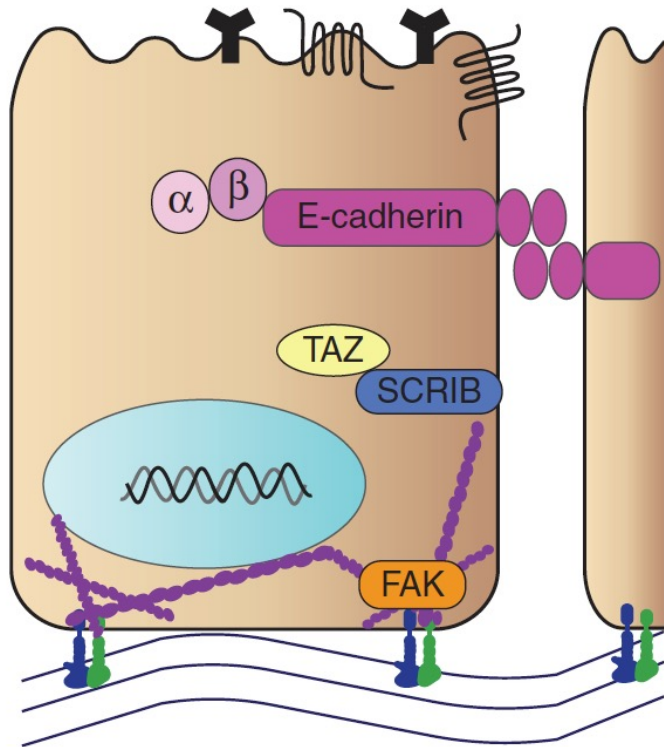
Lo switch delle caderine facilita la perdita di polarità e rimuove l'inibizione della migrazione



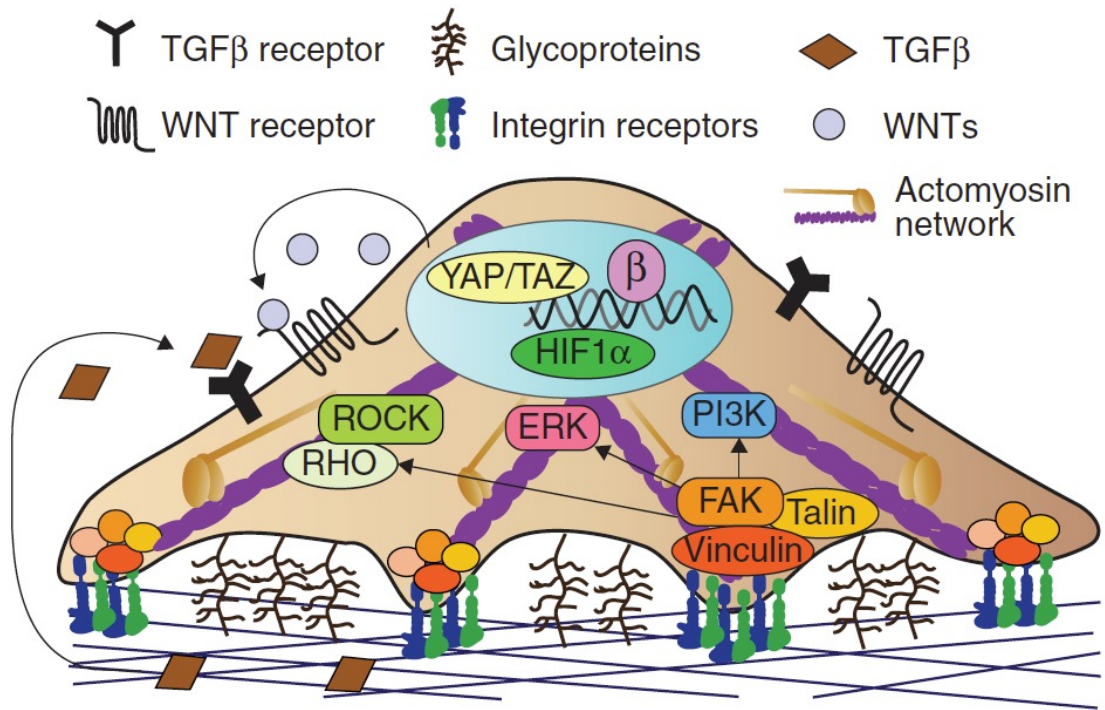
E-Cadherin suppresses EMT by signalling to other adhesion components, such as p120 catenin, which polarizes the small GTPase **RAC1** towards cell-cell junctions.

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

L'attivazione di Rho aumenta la tensione del citoscheletro



Low tension



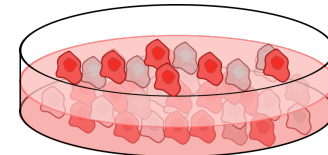
High tension

Il riposizionamento dei farmaci

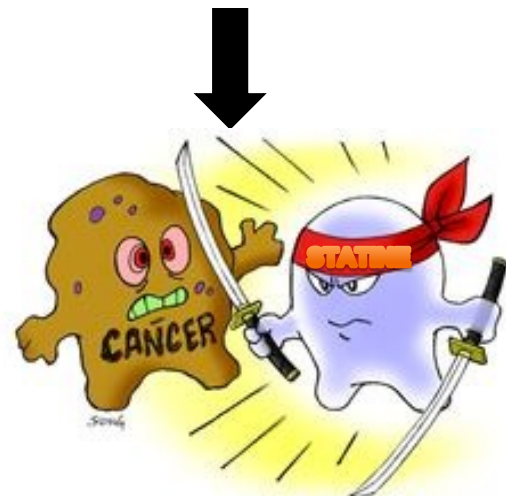
Farmaci approvati da EMA/FDA e commercializzati per **qualsiasi** malattia



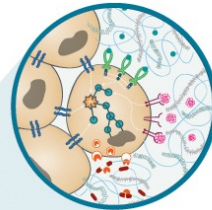
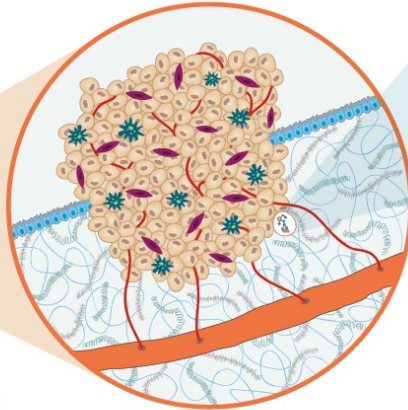
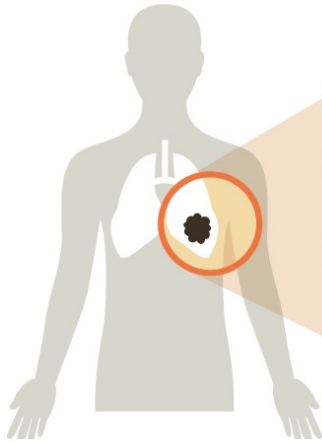
Testare in vitro l'efficacia antitumorale dei farmaci



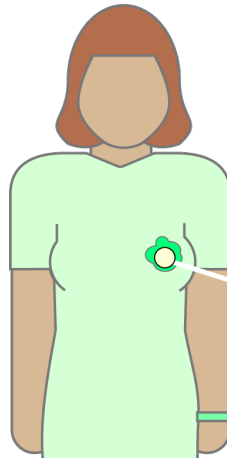
Es. Identificazione delle **statine** (anticolescerolemici) come potenziali farmaci antitumorali



Paziente con tumore alla mammella



Trial clinico per l'efficacia delle statine in pazienti con tumore al seno in combinazione con le terapie standard



Screening per il riposizionamento di farmaci

Modello tumorale

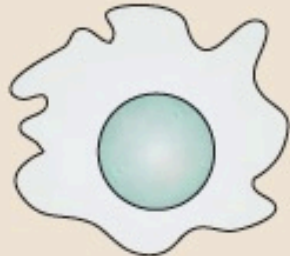
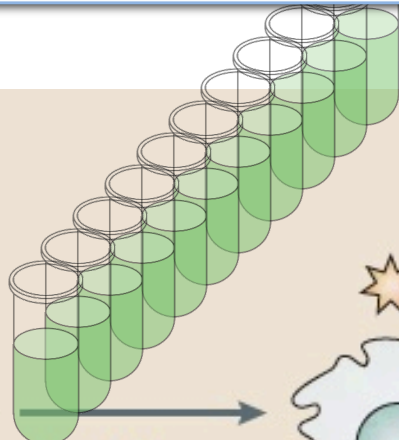
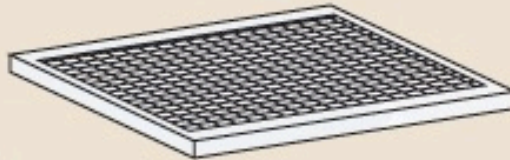


Plate cells onto clear bottom 384-well plate

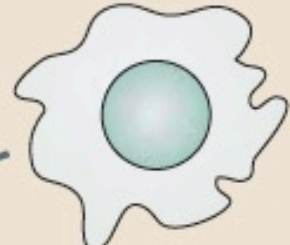
“Librerie” di farmaci



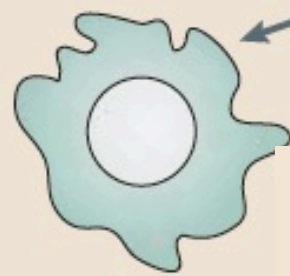
Transfer compounds onto cells



Compound treatment



Readout: inattivazione di oncogeni



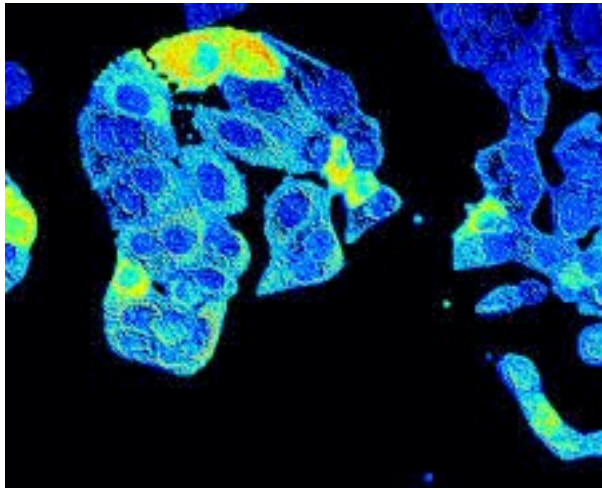
for hits



Validazione: analisi fenotipica mediante opportuni saggi

Compound and target identification

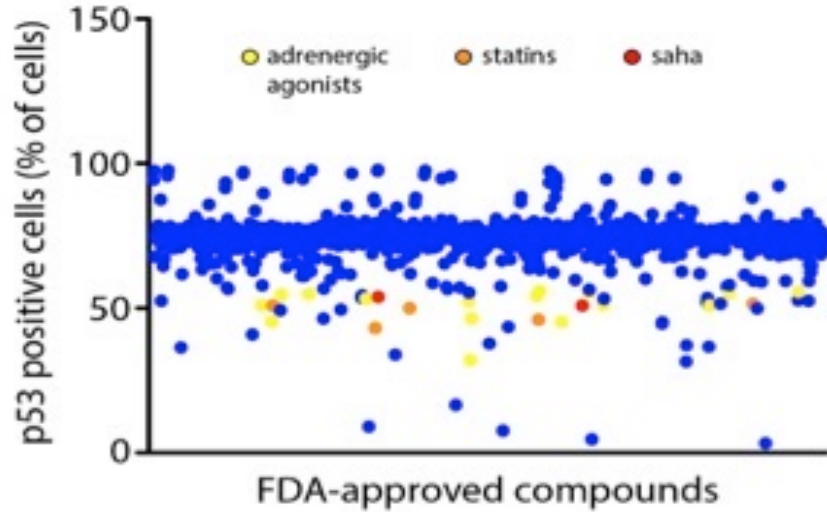
High content screening systems



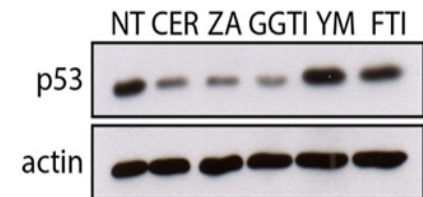
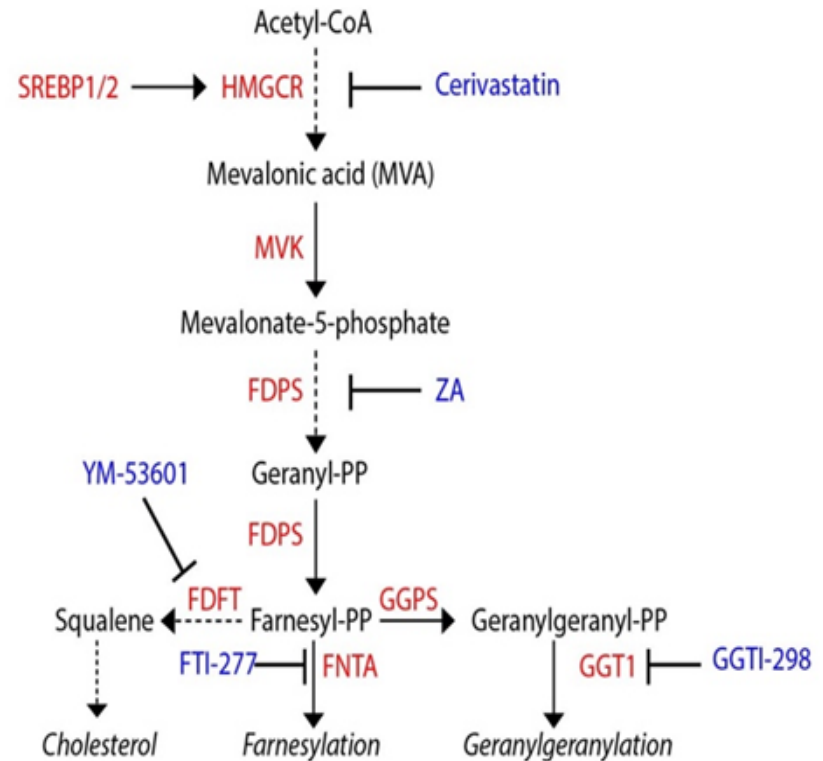
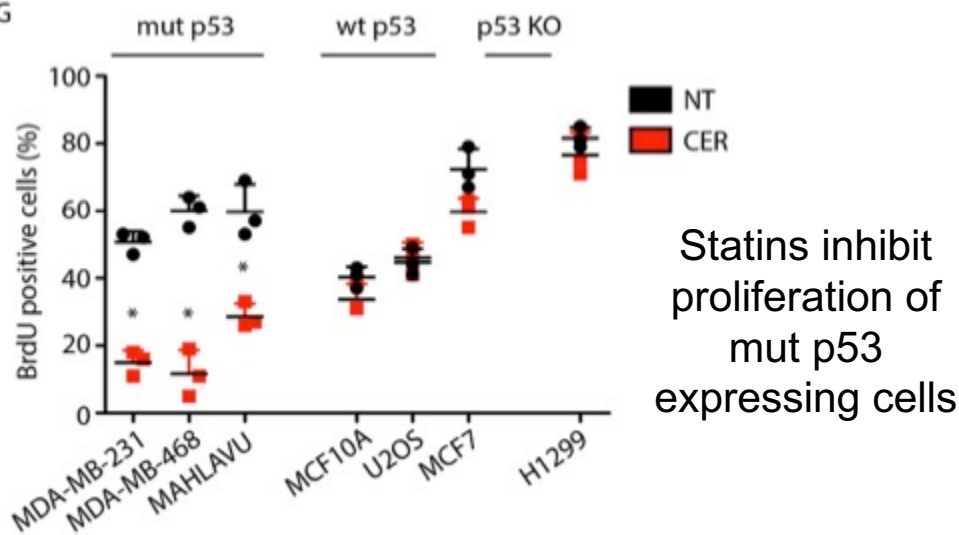
Next generation confocal high content screening system, designed to reliably discriminate phenotypes of complex cellular models, such as primary cells and 3D microtissue, integrated with automated microplate loader and liquid handling robot station for automated transfection of cells in 96- and 384-well microplates and assay preparation

Identification of molecules affecting mutant p53 protein levels by high content high-throughput screening

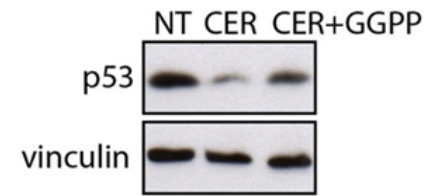
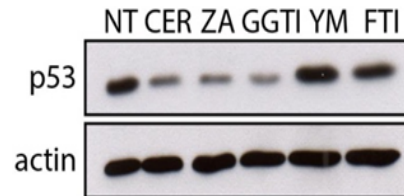
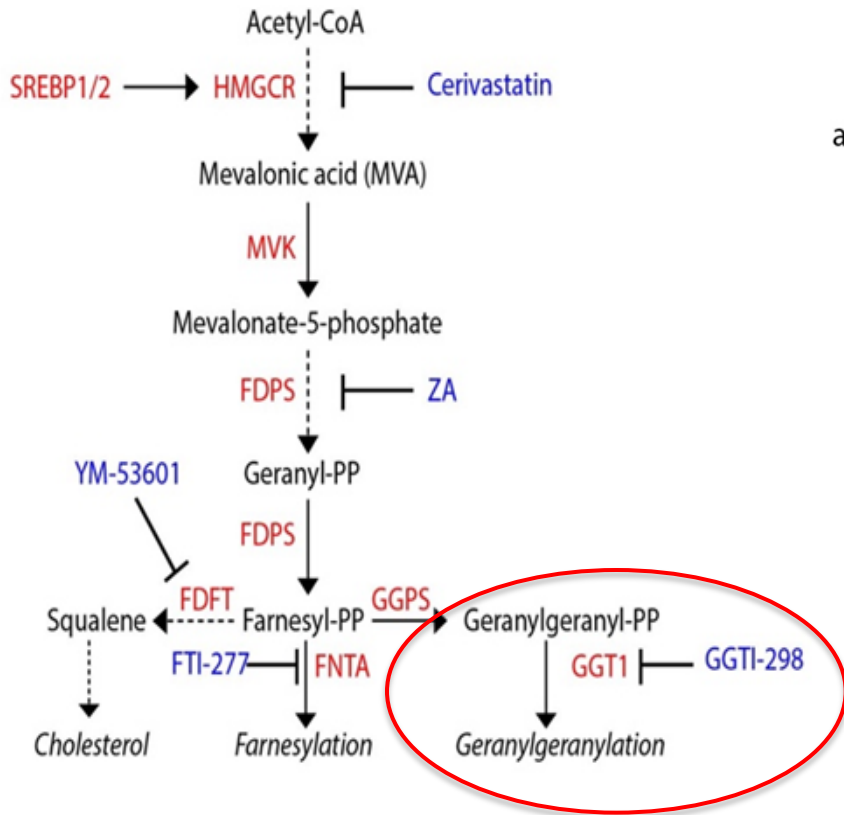
A



G

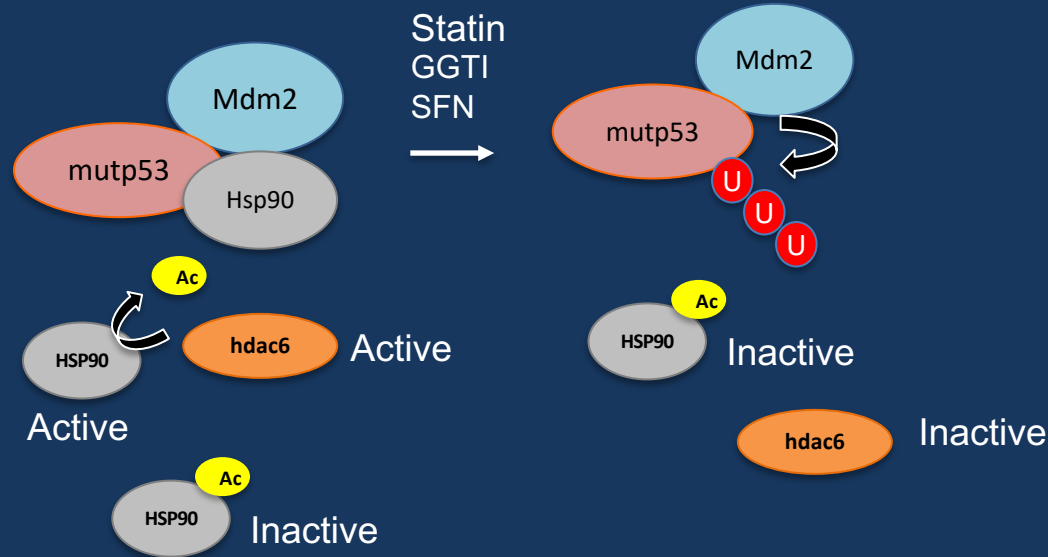


Geranyl-geranylation is required for mutp53 stabilization



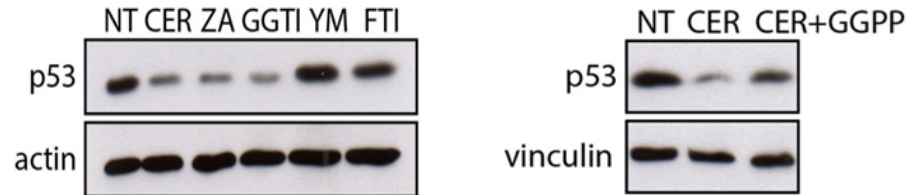
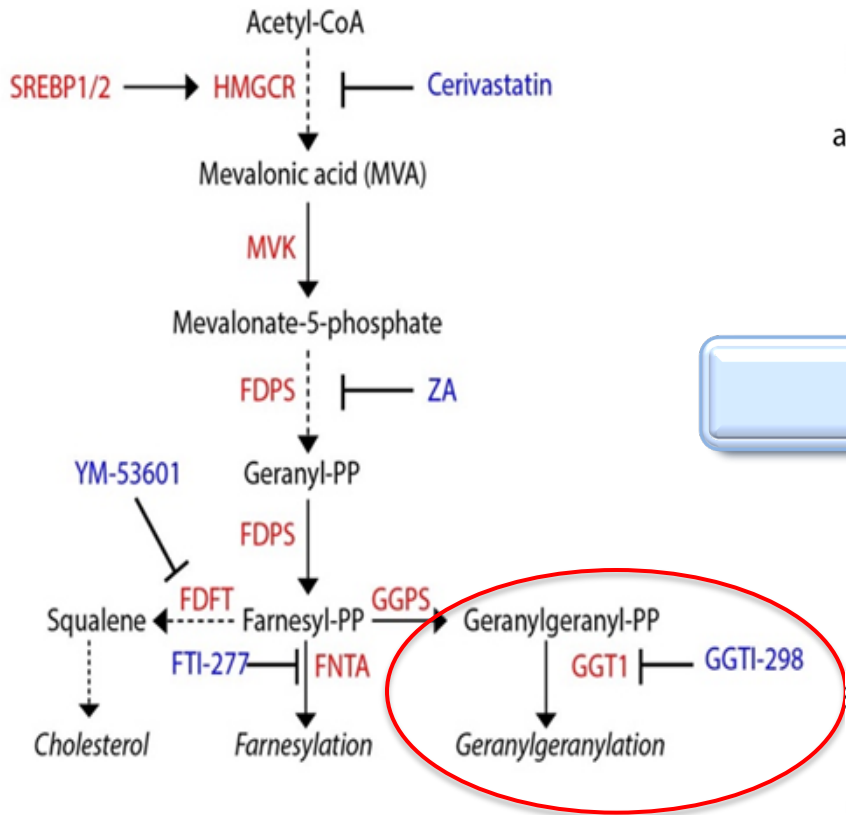
Key findings

- ✓ Statins and other MVA pathway inhibitors are potent mutant p53 inhibitors.
- ✓ Statins cause mutant p53 degradation through MDM2 by inactivating Hsp90 and HDAC6

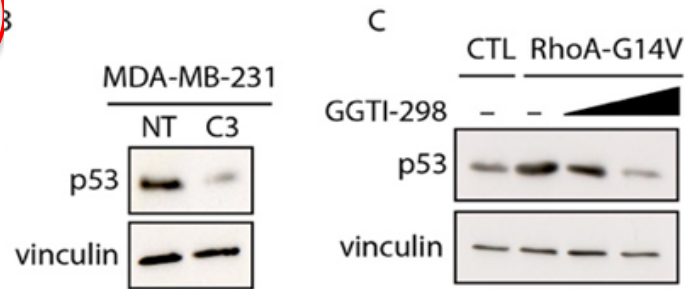


- ✓ The MVA pathway sustains mutant p53 accumulation in cancer cells.
- ✓ Loss of Geranyl-Geranyl-Phyrophosphate induces mutant p53 degradation.

Geranyl-geranylation is required for mutp53 stabilization



And the substrate is... RhoA



L'attivazione di RhoA induce la stabilizzazione di mp53

