

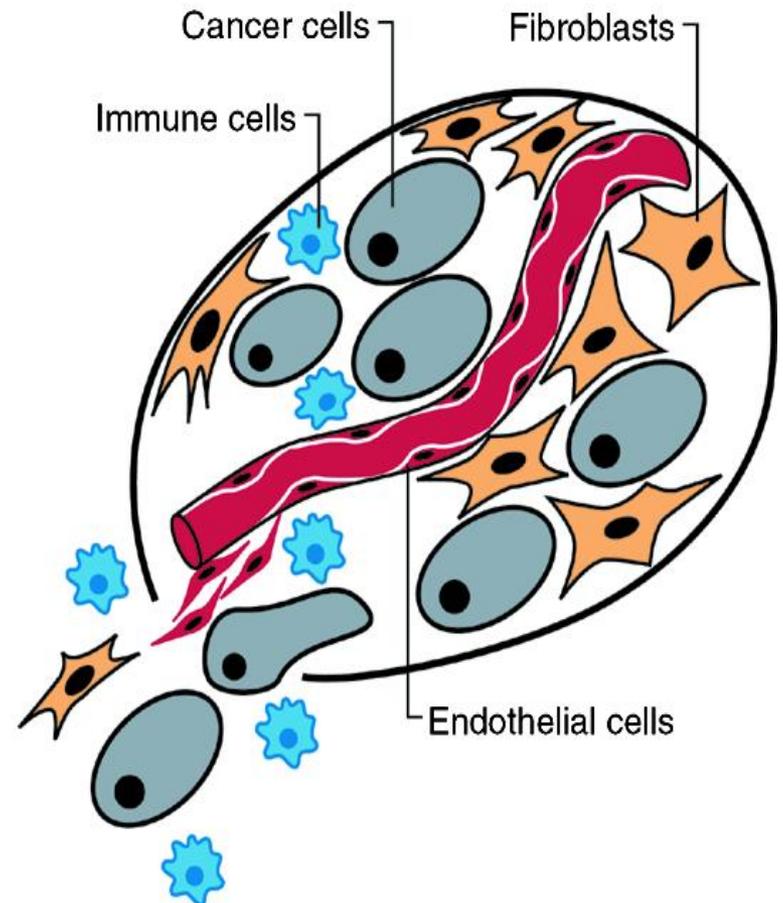
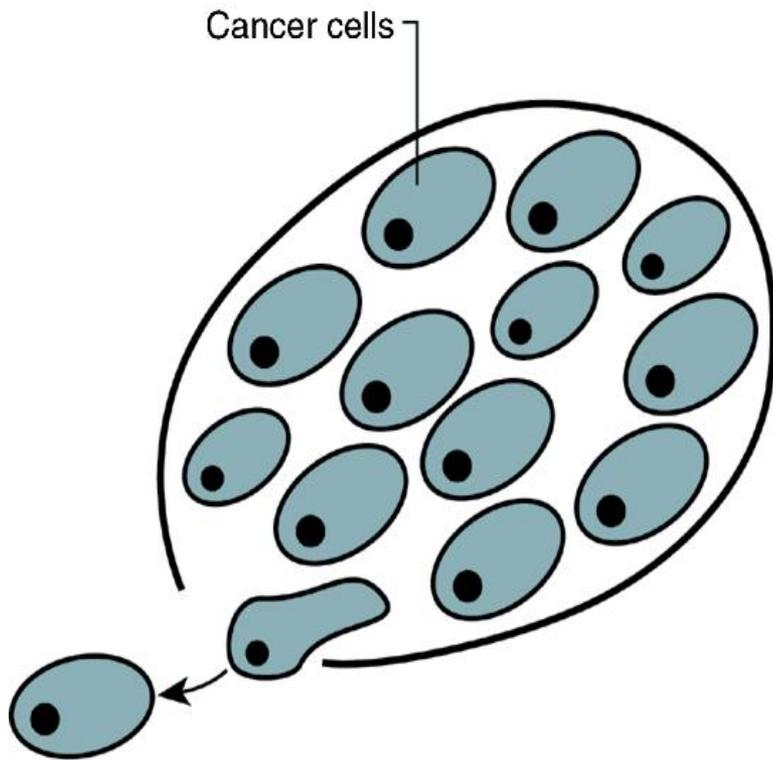
Corso di Biologia Cellulare del Cancro

AA 2019-2020

IL MICROAMBIENTE TUMORALE

I tumori sono organi

Visione riduzionista



La componente stromale dei tumori

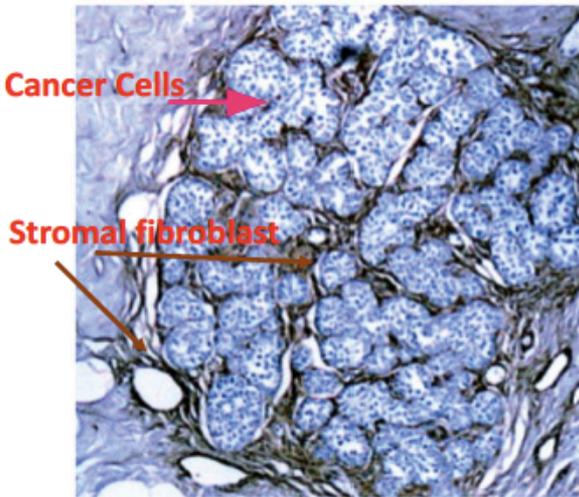


Figure 15.3c The Biology of Cancer (© Garland Science 2014)

Lobular CA in situ of the Breast

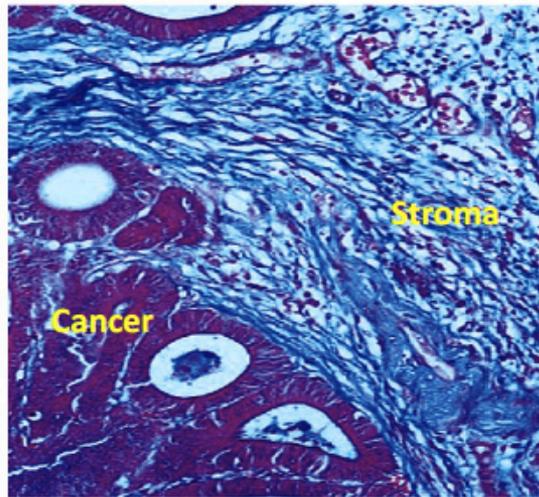


Figure 13.1d The Biology of Cancer (© Garland Science 2014)

Adenocarcinoma of the stomach

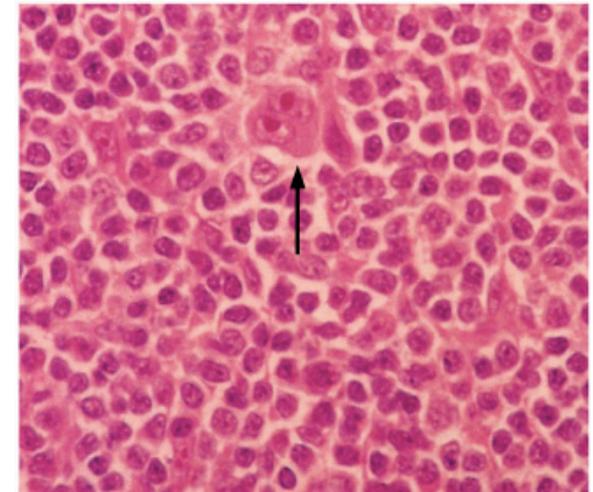


Figure 13.1a The Biology of Cancer (© Garland Science 2014)

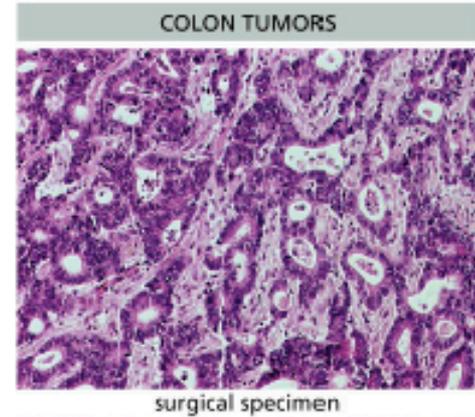
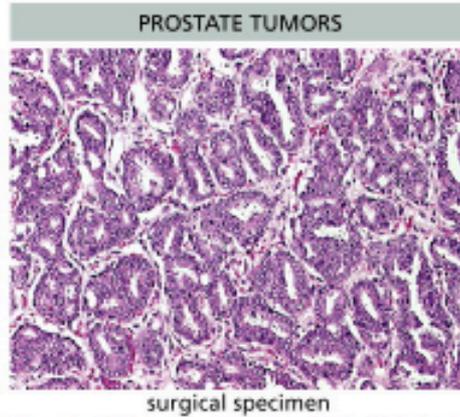
In Hodgkin disease rare cancer cells
Surrounded by normal lymphocytes

I classici modelli tumorali in vitro/ in vivo non ricapitolano la complessità dei tessuti tumorali

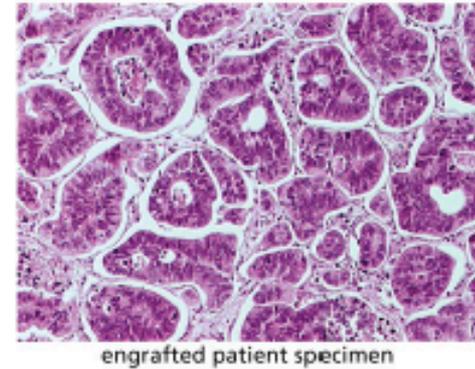
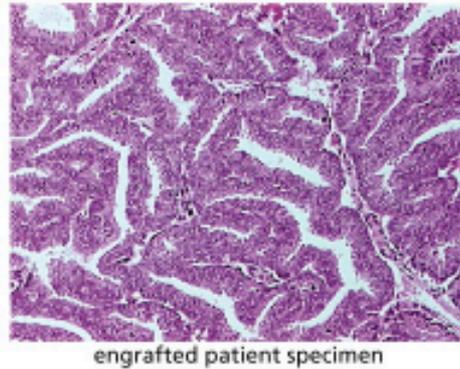
Tumore primario

A

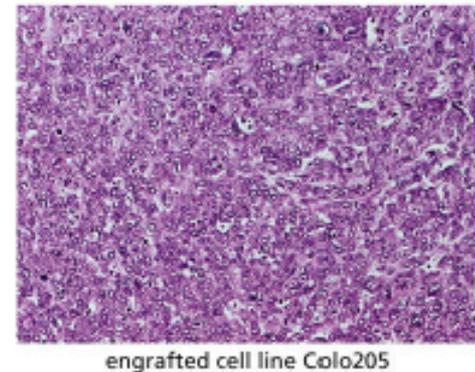
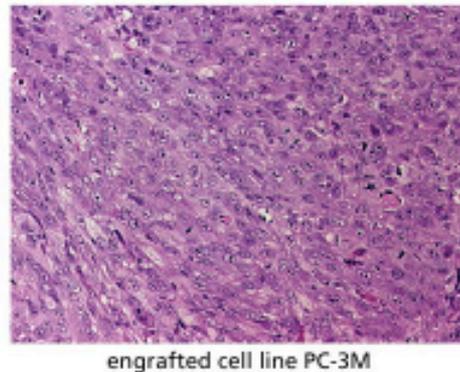
or



PDX: tumore trapiantato in animale immunocopromesso

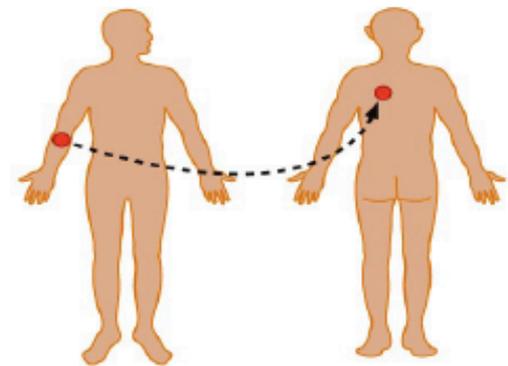


Trapianto di una linea cellulare tumorale in animale immunocopromesso

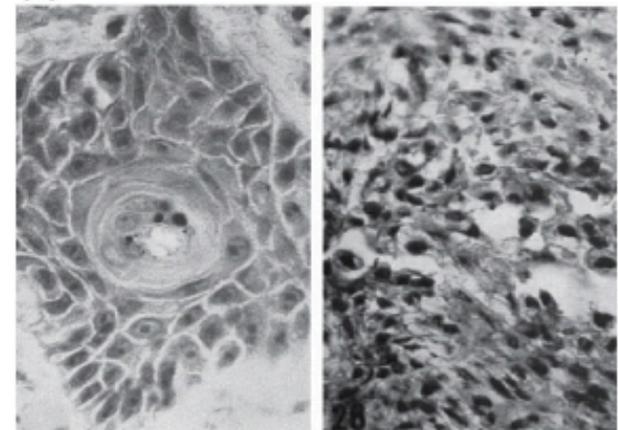


Un microambiente permissivo supporta la progressione tumorale

Trapianto autologo di carcinomi cutanei:
Solo le cellule tumorali trapiantate
assieme al proprio stroma
colonizzano il nuovo sito



(B)



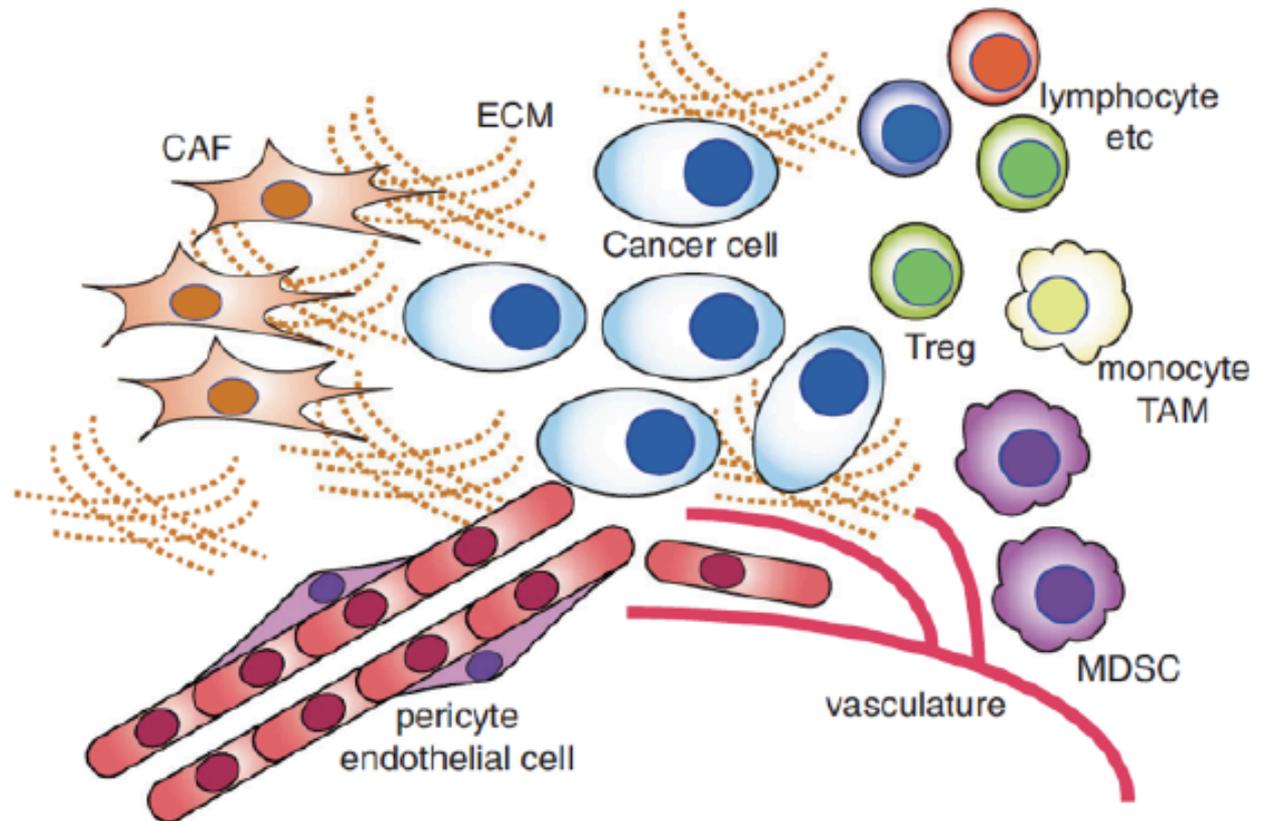
with stroma

without stroma

Figure 13.5 The Biology of Cancer (© Garland Science 2014)

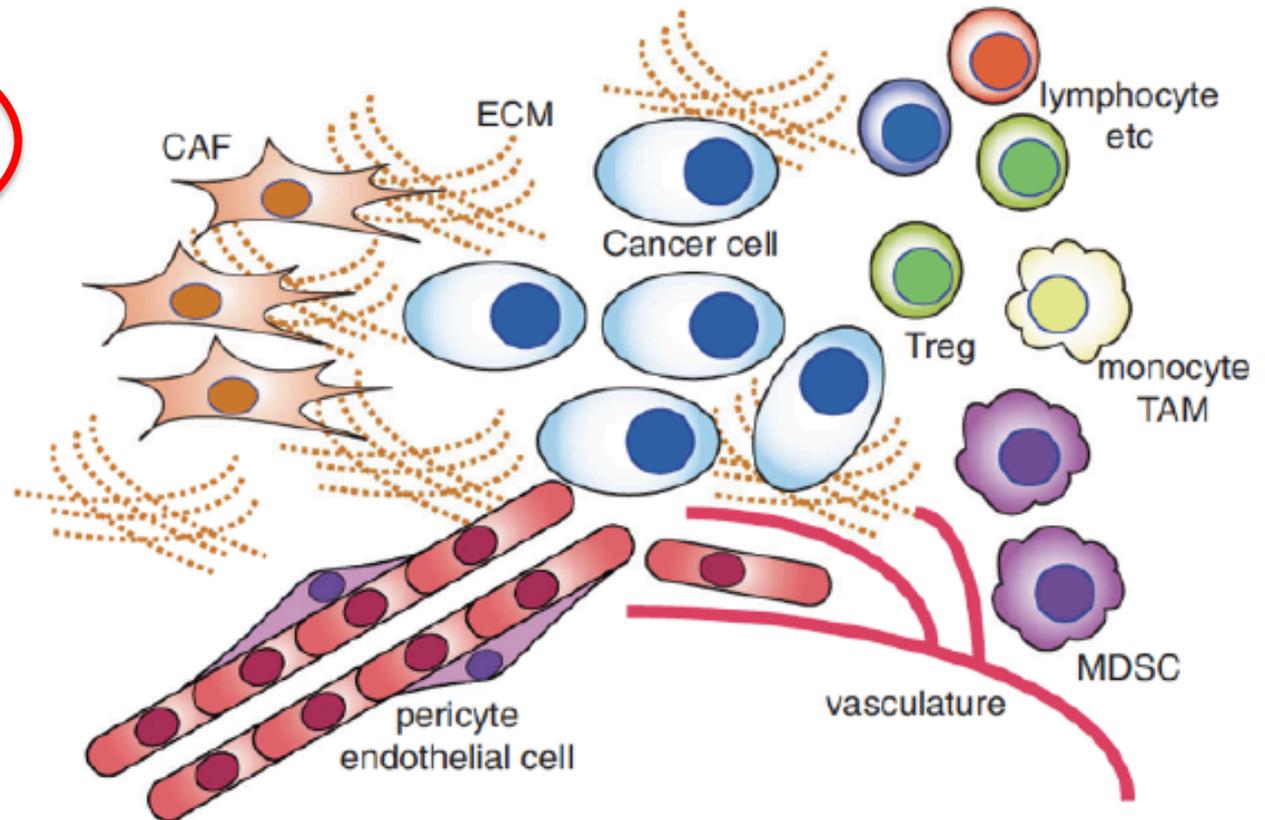
Il crosstalk tra le cellule tumorali e lo stroma

- Extracellular Matrix deposition and stiffening;
- Fibroblast activation (CAF);
- Neo-angiogenesis;
- Recruitment of cells from bone marrow.



Il crosstalk tra le cellule tumorali e lo stroma

- Extracellular Matrix deposition and stiffening;
- Fibroblast activation (CAF);
- Neo-angiogenesis;
- Recruitment of cells from bone marrow.

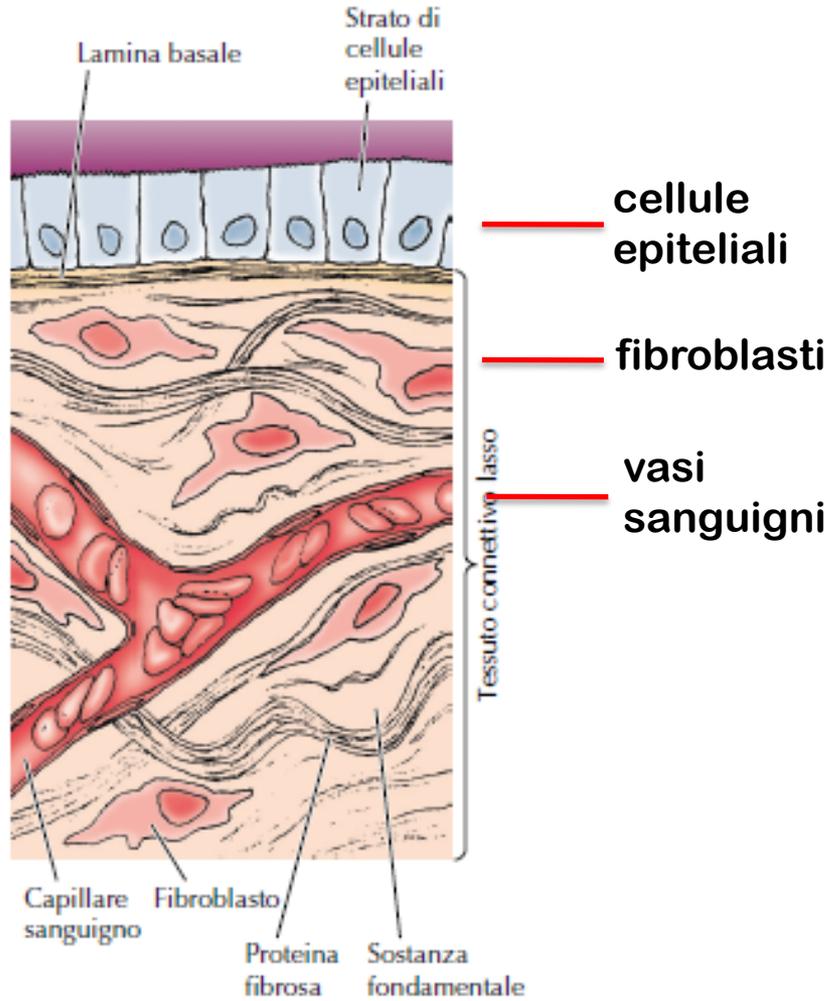


**IL MICROAMBIENTE TUMORALE:
LA MATRICE EXTRACELLULARE
E LA MECCANOTRASDUZIONE**

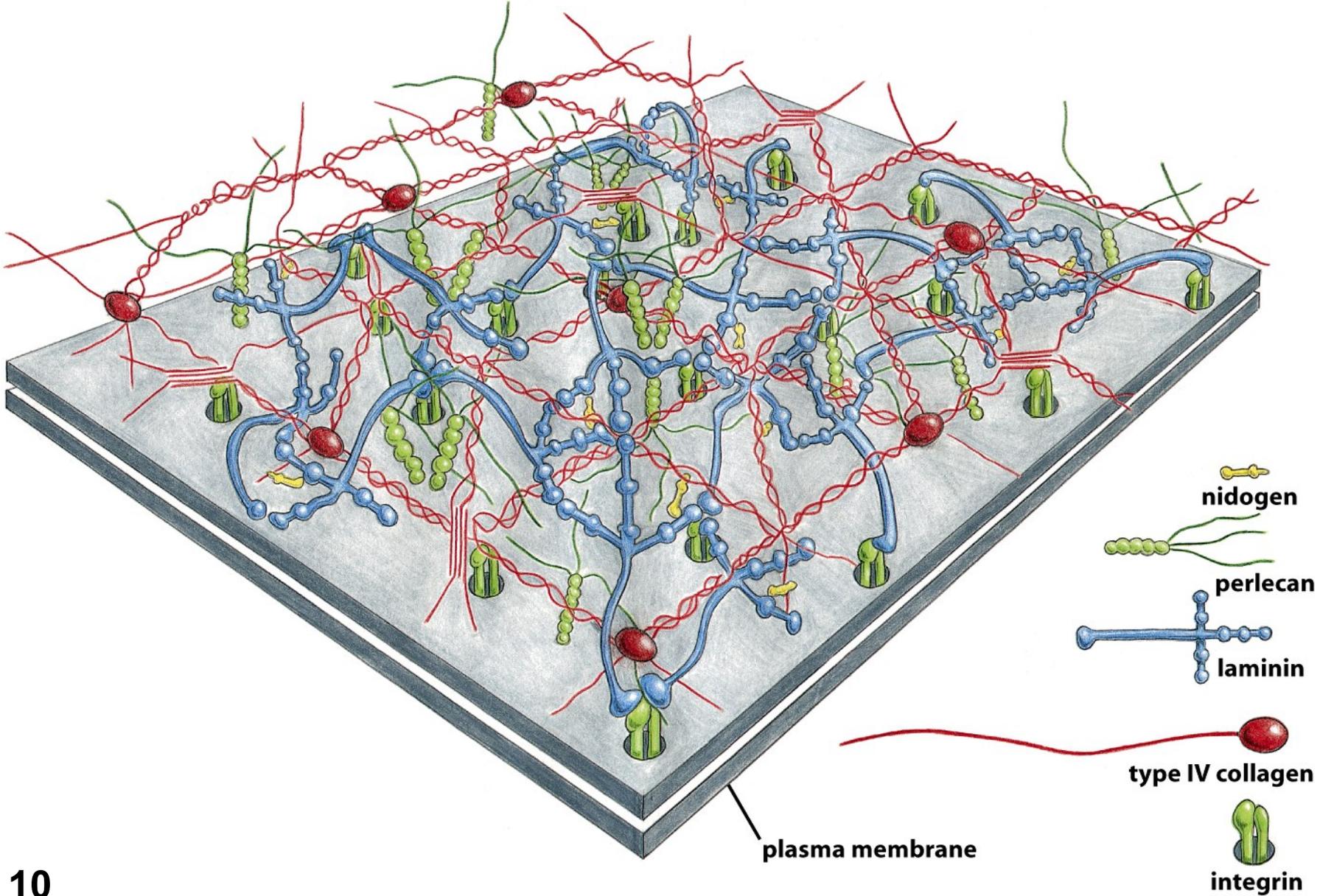
La matrice extracellulare: ECM

Lamina basale

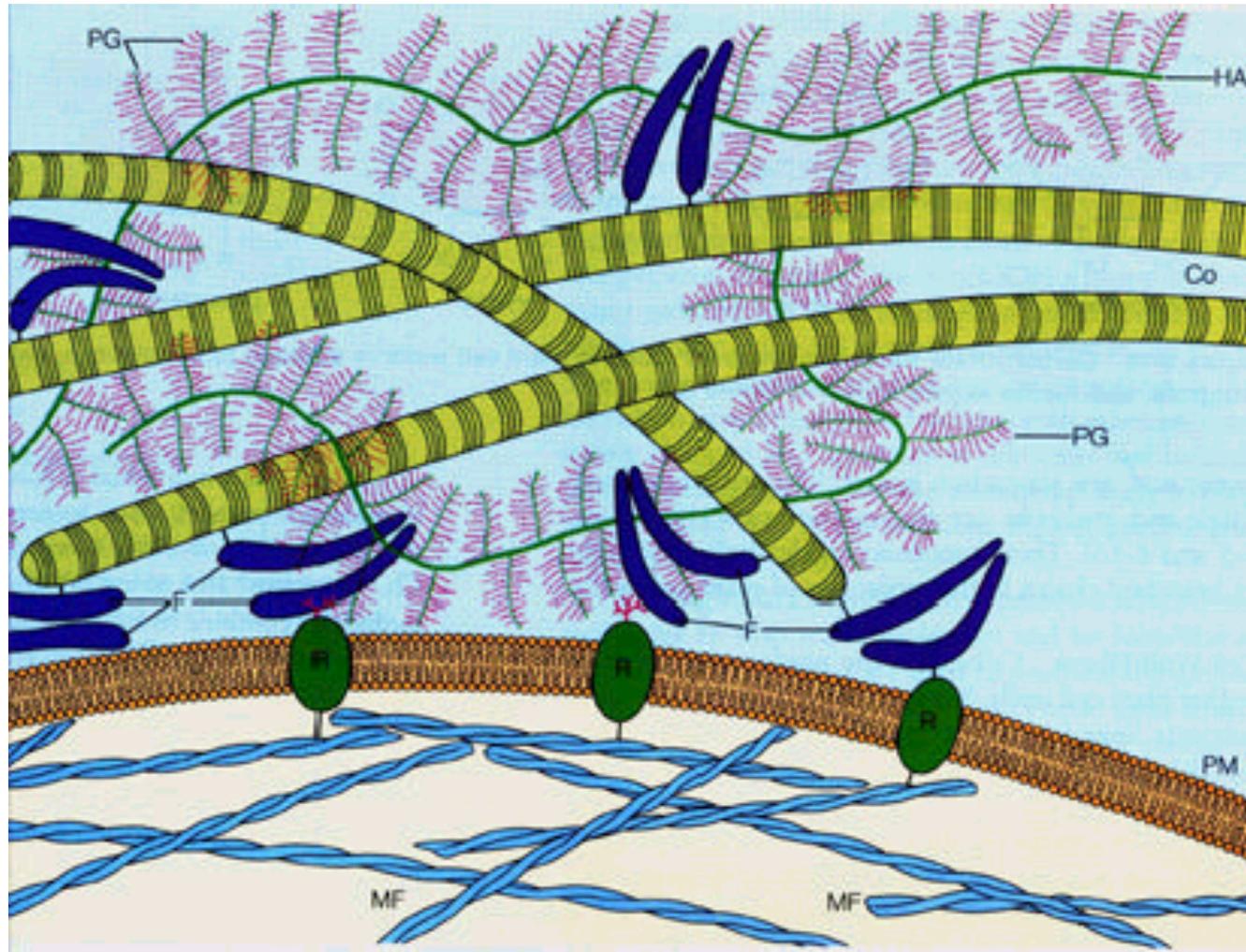
Matrice interstiziale



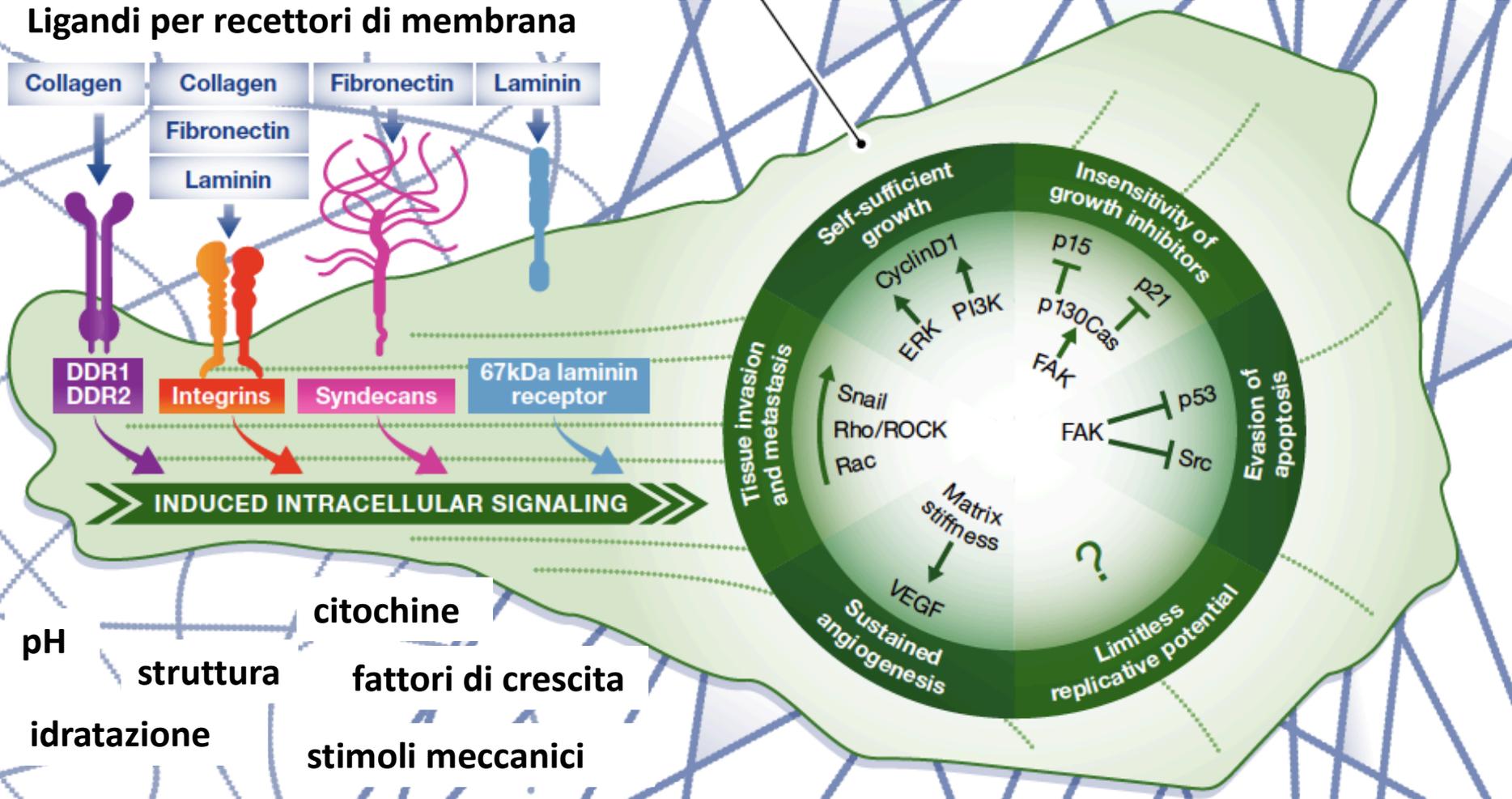
La lamina basale:



La matrice interstiziale



La progressione tumorale avviene in una matrice che evolve in modo dinamico



La matrice extracellulare: ECM

The extracellular matrix regulates tissue development and homeostasis, and its dysregulation contributes to neoplastic progression.

The extracellular matrix serves not only as the **scaffold** upon which tissues are organized but provides critical **biochemical and biomechanical cues that direct cell growth, survival, migration and differentiation and modulate vascular development and immune function.**

biophysical and biochemical cues from the tumor-associated extracellular matrix influence cancer hallmarks and are therefore critical for malignancy.

While genetic modifications in tumor cells undoubtedly initiate and drive malignancy, **cancer progresses within a dynamically evolving extracellular matrix**

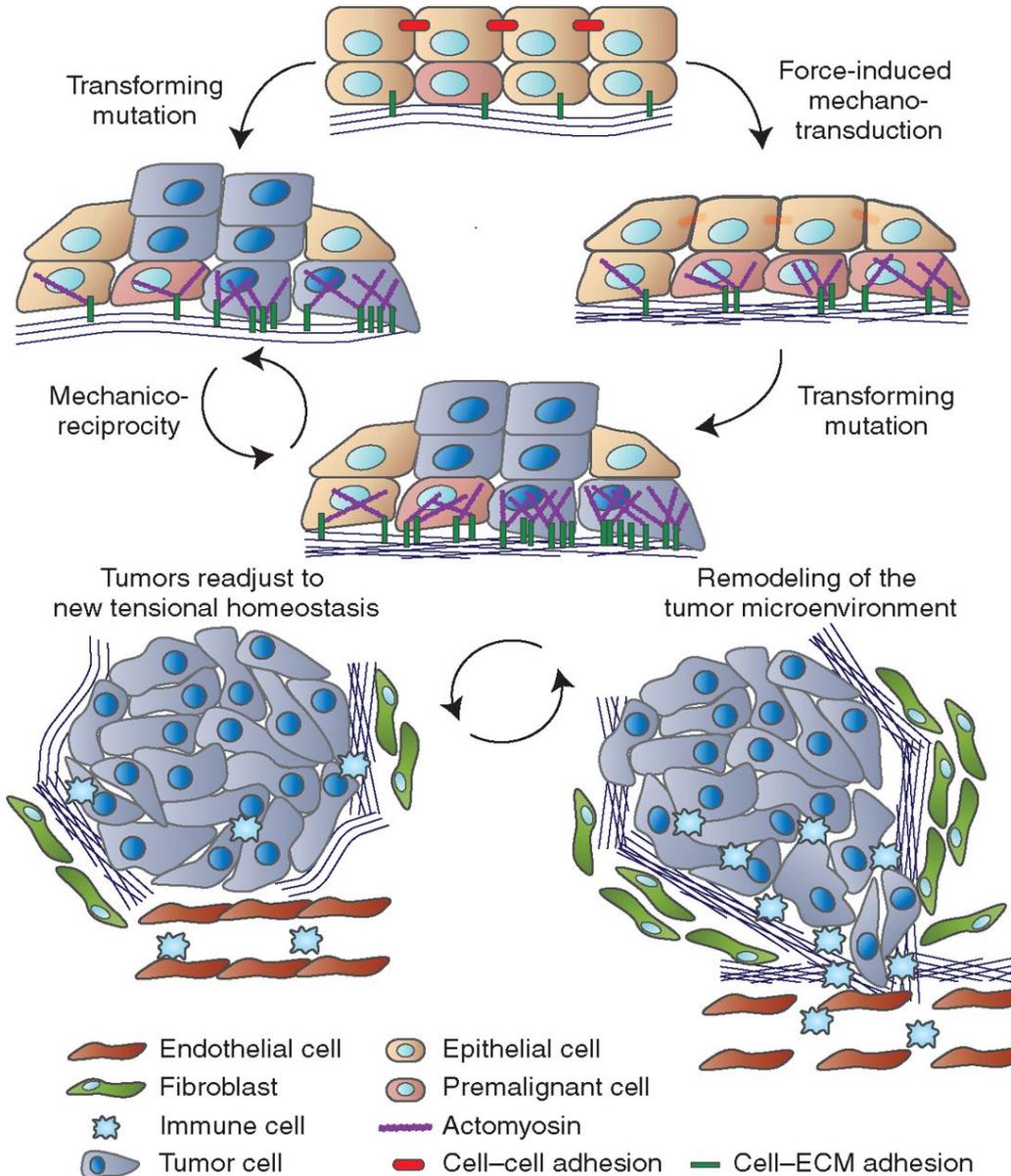
The ECM is composed of a complex network of macromolecules that assemble into three-dimensional supramolecular structures with distinct biochemical and biomechanical properties that regulate cell growth, survival, motility and differentiation by **ligating specific receptors such as integrins, syndecans and discoidin receptors.**

The ECM also provides the structural foundation for tissue function and mechanical integrity, regulates the **availability of growth factors** and **cytokines** and maintains the hydration and pH of the local microenvironment.

A critical aspect of the ECM is that it is **dynamically remodeled** and specifically tailored to the structure/function of each organ, and its composition, biomechanics and anisotropy are exquisitely tuned to reflect the physiological state of the tissue.

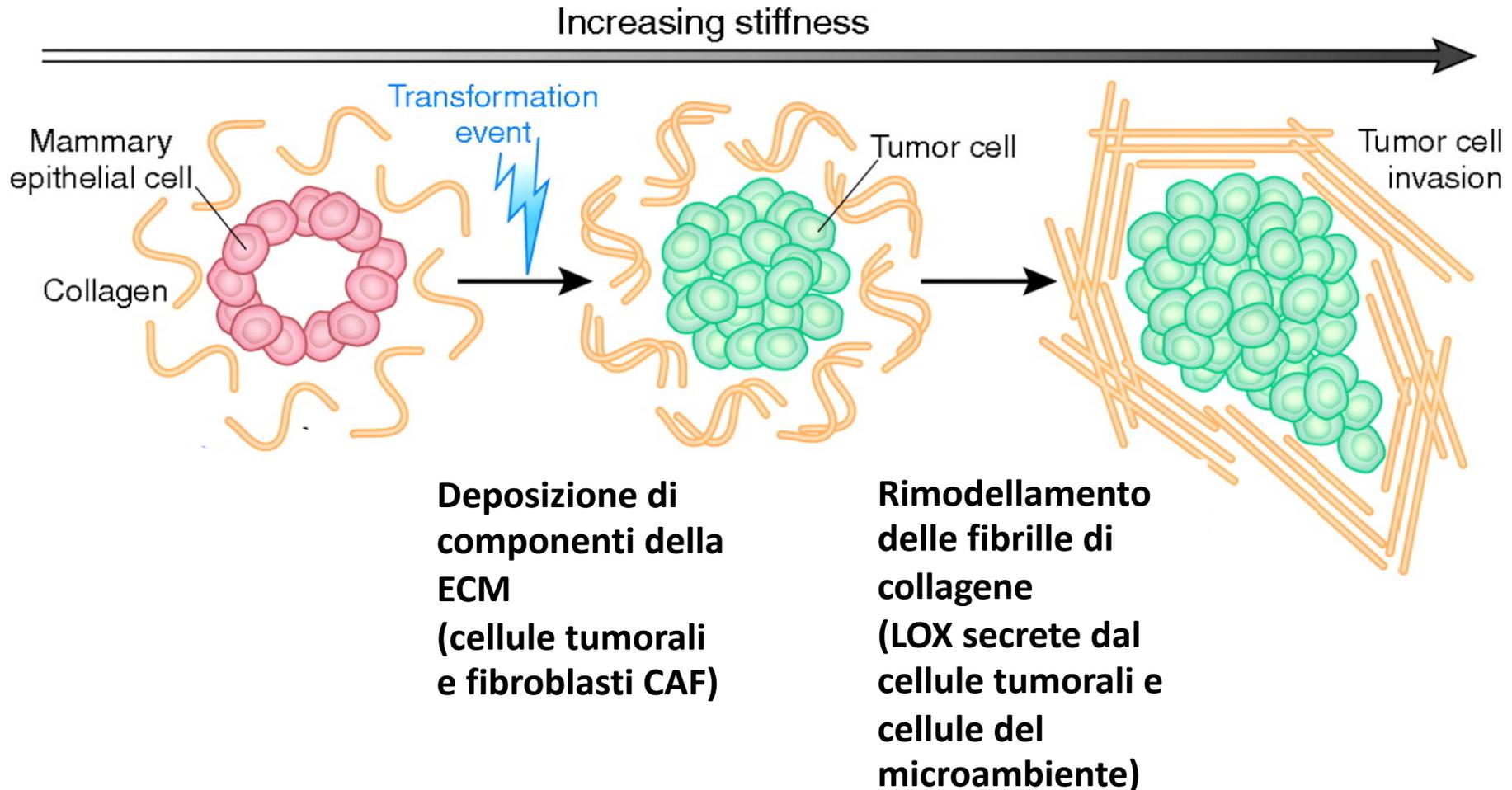
- **Tumors often display desmoplasia**, and this **fibrotic state** is characterized by increased deposition, an altered organization and enhanced post-translational modifications of ECM proteins.
- **Fibrosis can also predispose a tissue to malignancy;**
patients with **cirrhosis of the liver** or cystic fibrosis, conditions that are characterized by abnormal accumulation of collagen, have an increased risk of developing cancer.
Moreover, increased mammographic density, which associates with increased collagen deposition, correlates with an elevated risk of developing breast cancer.
- **Major drivers** of changes to the mechanical properties of the extracellular matrix include:
 - **phenotypically converted myofibroblasts,**
 - **transforming growth factor beta (TGFb),**
 - **and matrix cross-linking.**

Desmoplasia ed evoluzione tumorale

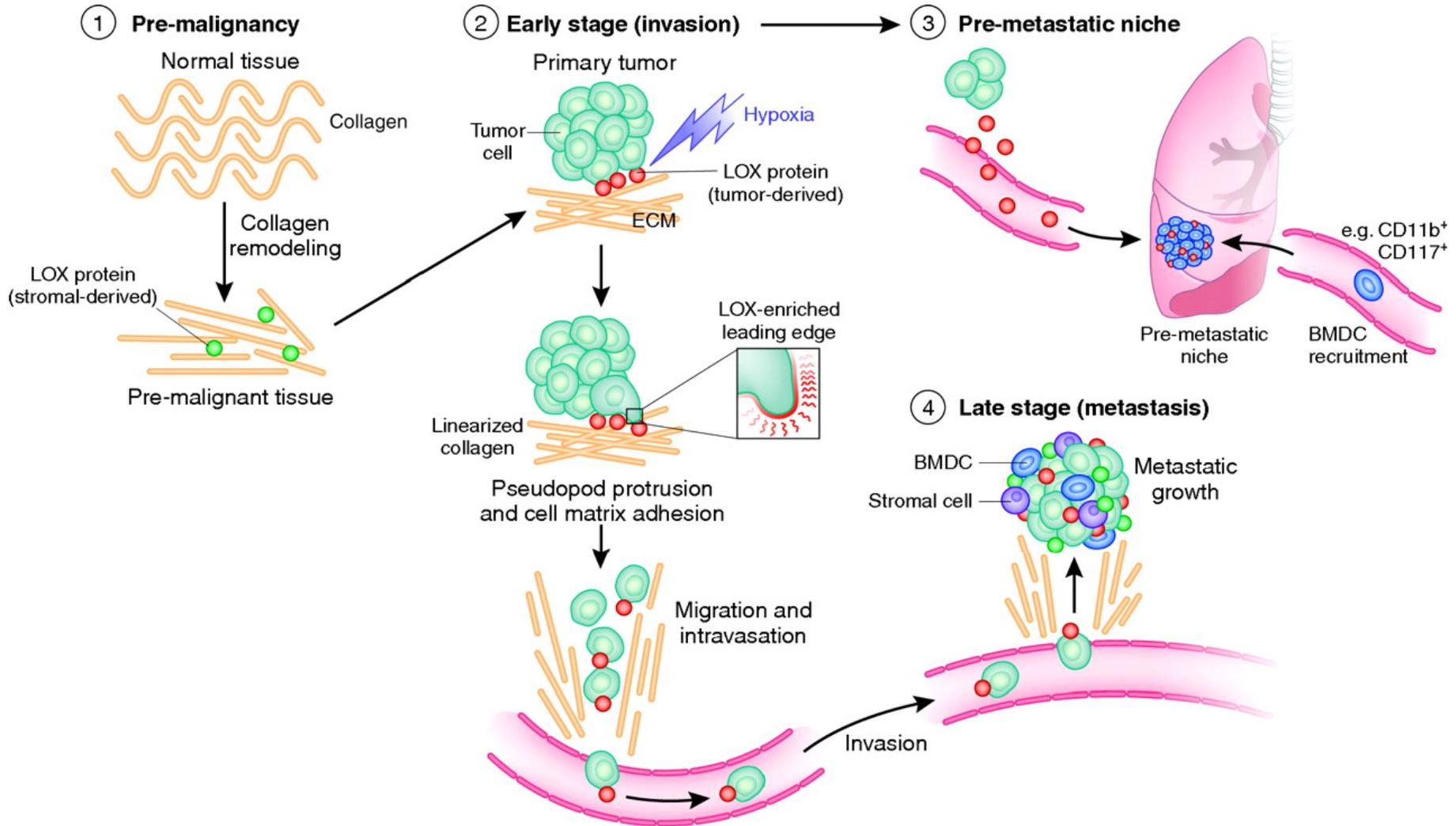


Jason J. Northey et al.
 Cancer Discov
 2017;7:1224-1237

Rimodellamento della ECM nella tumorigenesi



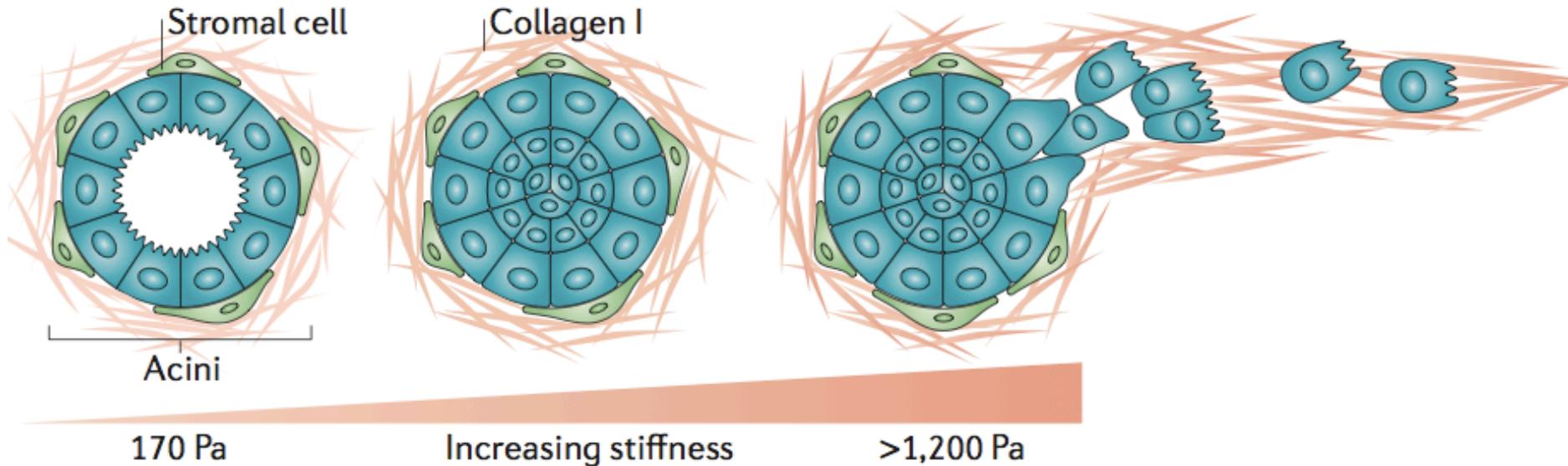
Le lisil-ossidasi LOX nel rimodellamento della ECM



APPROCCI TERAPEUTICI: LOX inhibitors

- The lysyl oxidase (LOX) family of proteins are secreted amine oxidases, the primary function of which is the covalent crosslinking of collagens and elastin in the extracellular matrix. The function of these enzymes is required for the structural integrity of many tissues.
- Preclinical studies involving the targeting of LOX or LOXL2 by small irreversible competitive inhibitors, as well as specific function-blocking antibodies to prevent metastasis, have been efficacious. So far, no detrimental side effects or tumour progression (owing to the proposed tumour suppressive roles of these proteins) have been noted with the use of specific antibody inhibitors.
- Targeting the LOX family is an exciting prospect in the development of new drugs to prevent the progression and metastasis of cancer.

Alterazioni delle proprietà meccaniche e dell'architettura della ECM regolano il comportamento cellulare



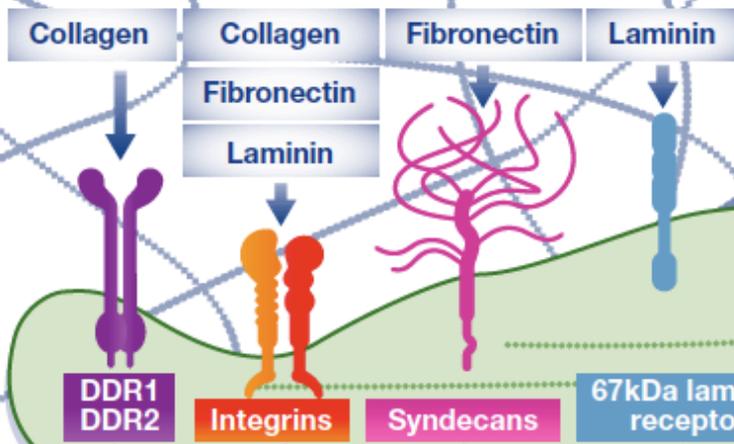
Soft ECM

Stiff ECM

Inibizione dell'apoptosi = mancata
formazione del lume
Destabilizzazione delle giunzioni aderenti
Perdita di polarità e adesione = ECM
Migrazione e invasione

La ECM supporta il signaling di proliferazione e sopravvivenza

Ligandi per recettori di membrana



INDUCED INTRACELLULAR SIGNALING

pH

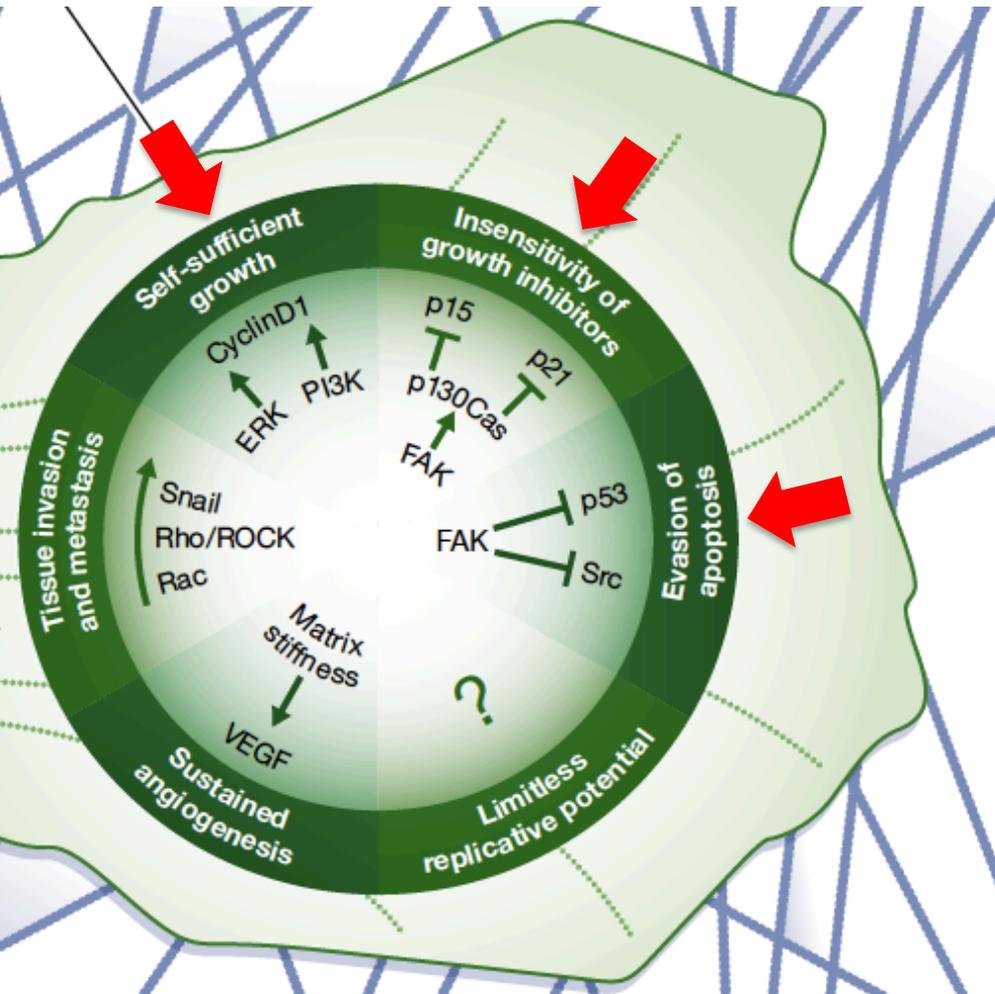
struttura

idratazione

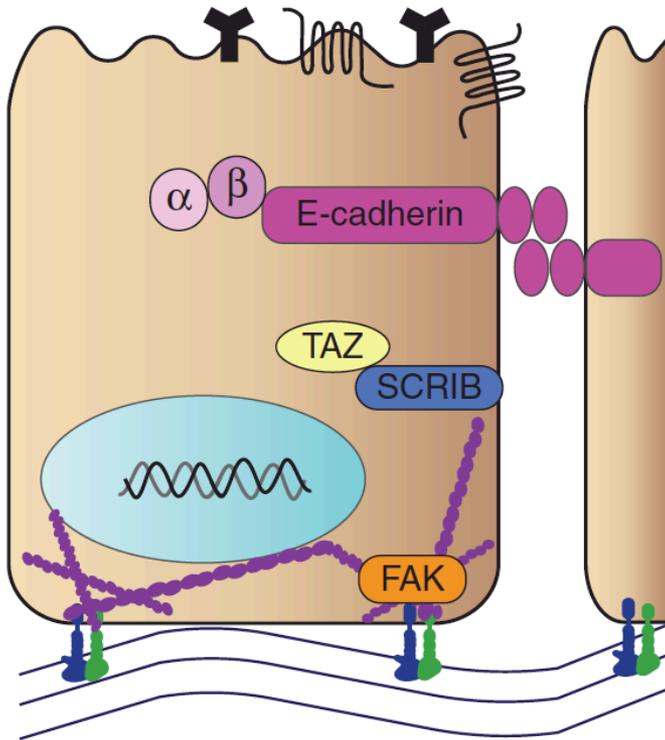
citochine

fattori di crescita

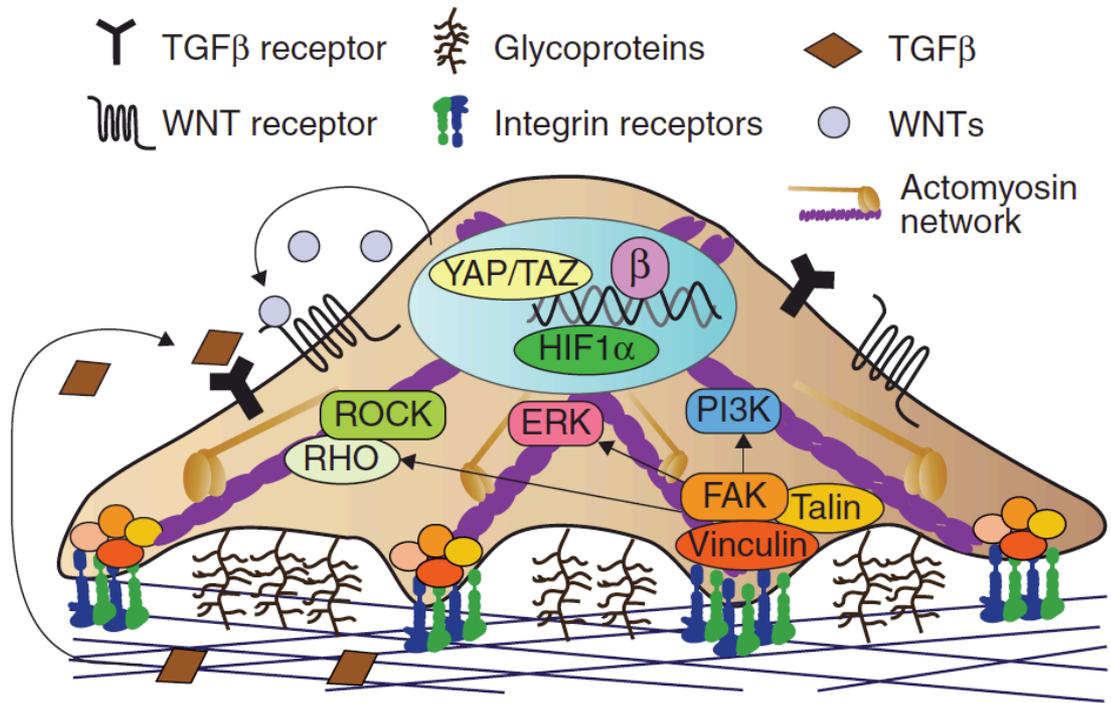
stimoli meccanici



Il signalling delle integrine attiva pathways di proliferazione e sopravvivenza



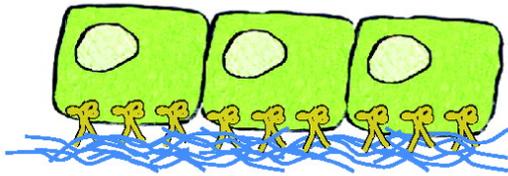
Low tension



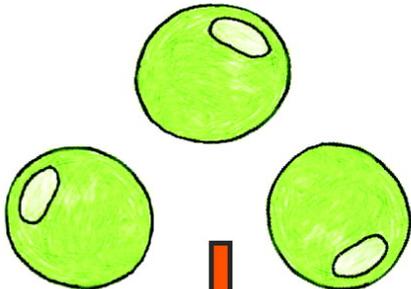
High tension

- Y TGFβ receptor
- Wavy WNT receptor
- Branching Glycoproteins
- Integrin receptors
- ◇ TGFβ
- WNTs
- Actomyosin network

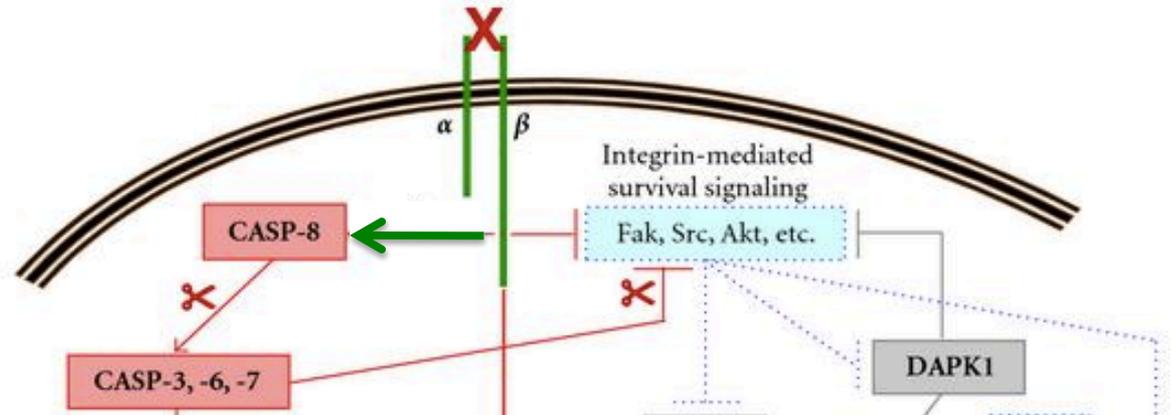
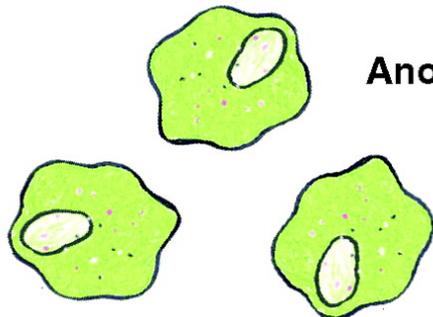
Sopravvivenza: il signaling delle integrine inibisce l'anoikis.



Loss of
integrin
attachment

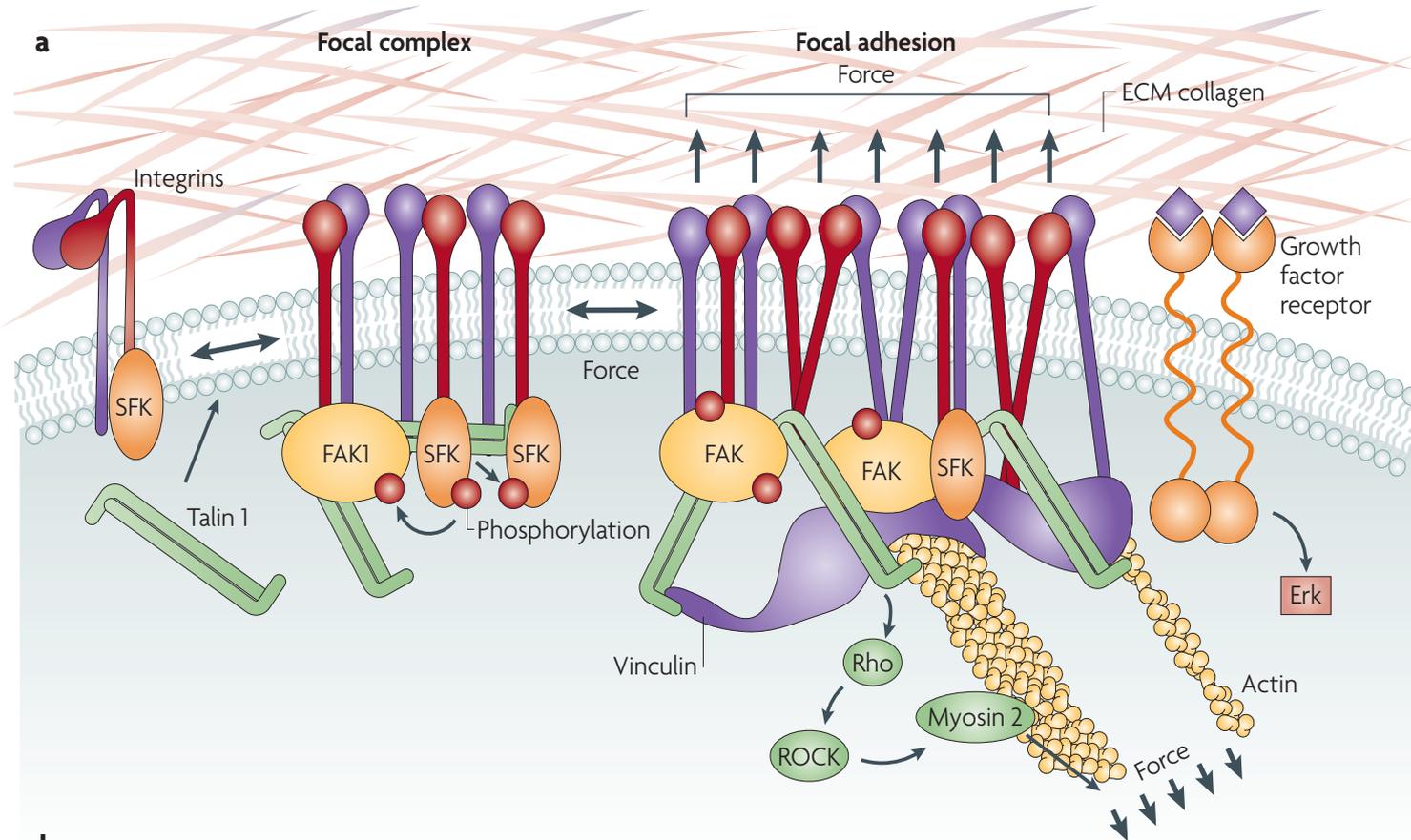


Anoikis



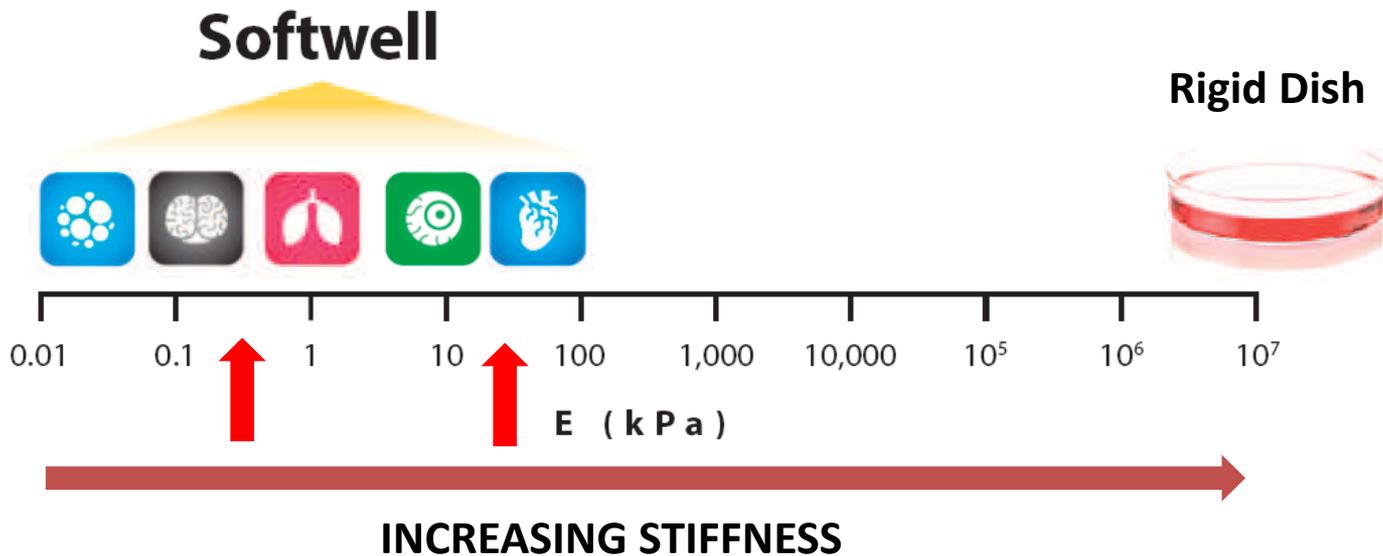
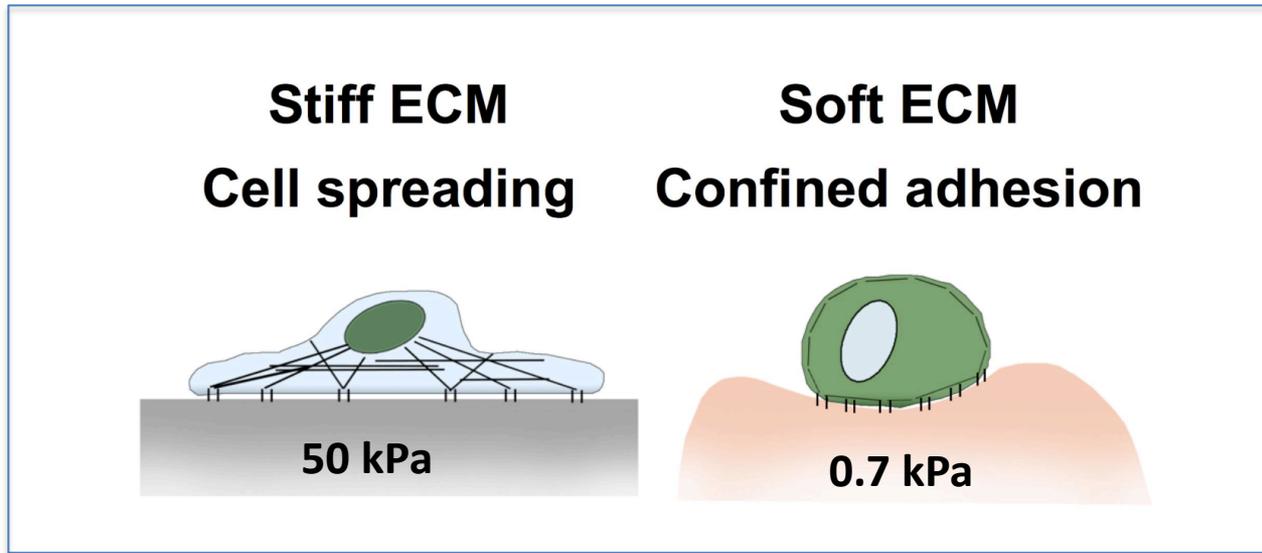
Se prive del loro ligando le integrine reclutano e attivano la caspasi 8 alla membrana

RhoA agisce da meccanotrasduttore di stimoli dalla ECM attraverso il controllo della tensione del citoscheletro actomiosinico



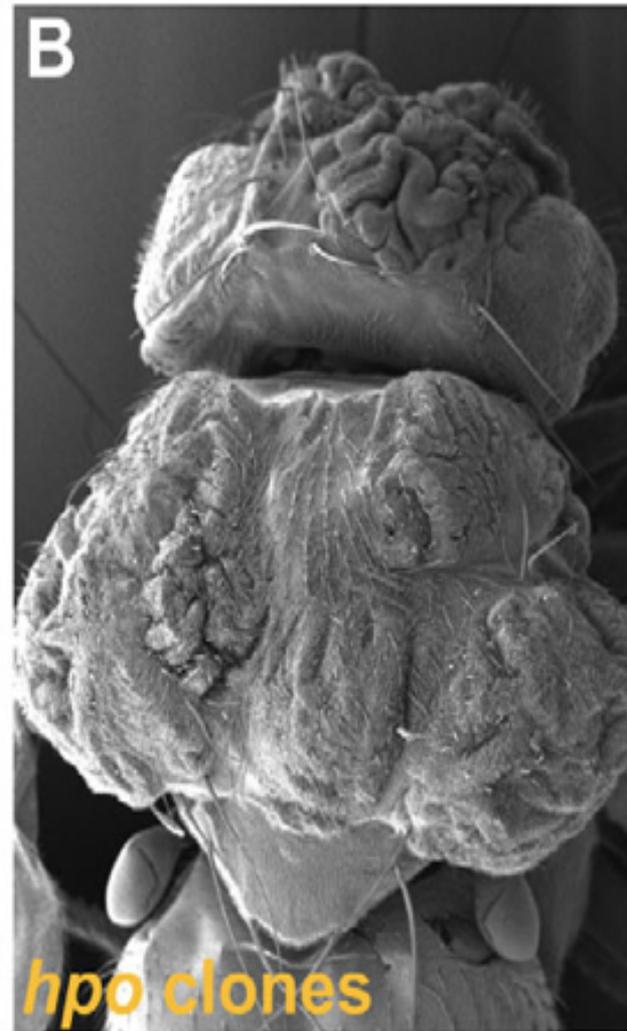
Impatto su: sopravvivenza, proliferazione e progressione tumorale

Meccanotrasduzione: risposte cellulari alla rigidità della ECM

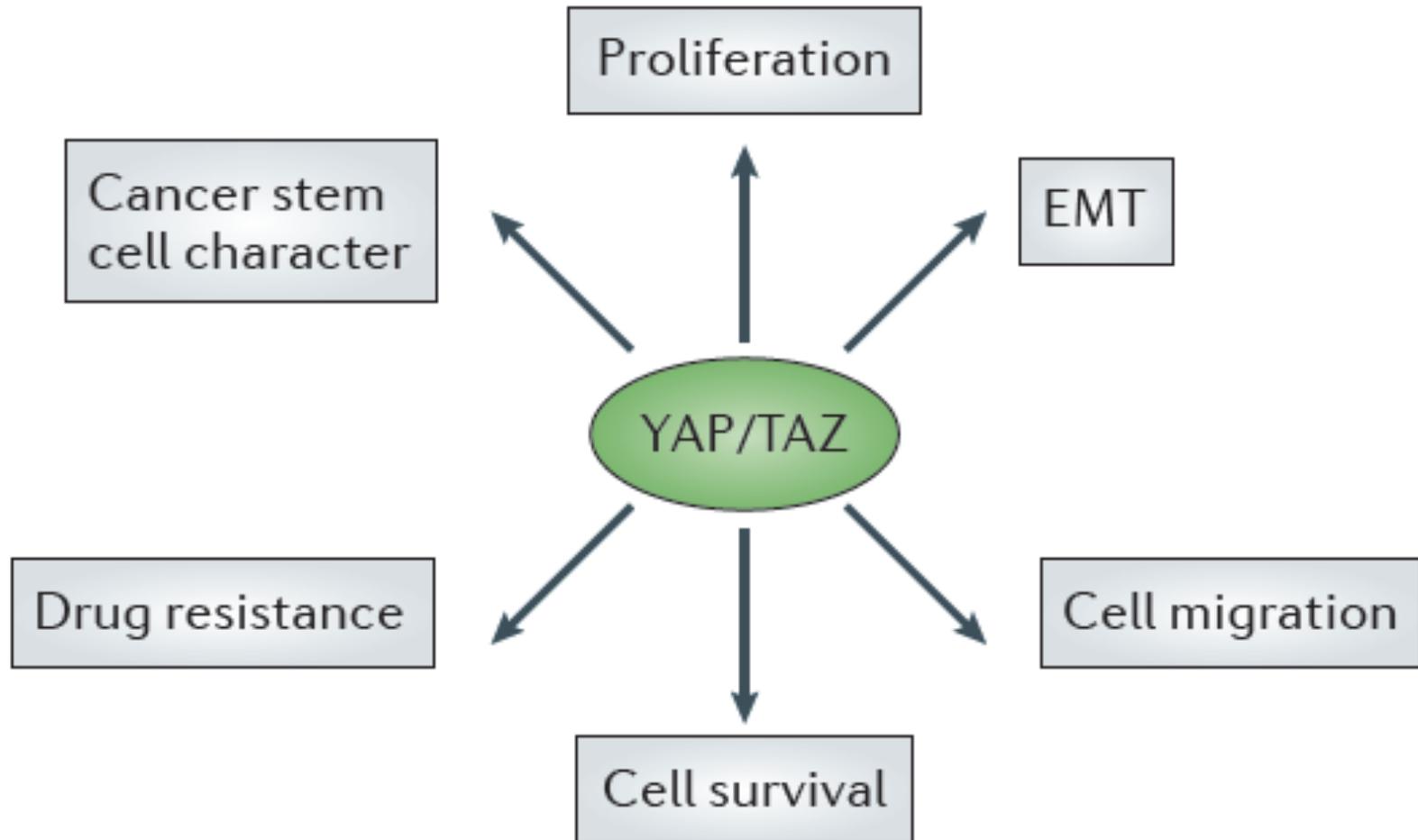


**La ECM stimola la proliferazione e la staminalità attraverso
l'attivazione degli oncogeni YAP/TAZ**

**YAP/TAZ sono effettori della HIPPO pathway,
coinvolta nella regolazione dell'omeostasi tissutale**

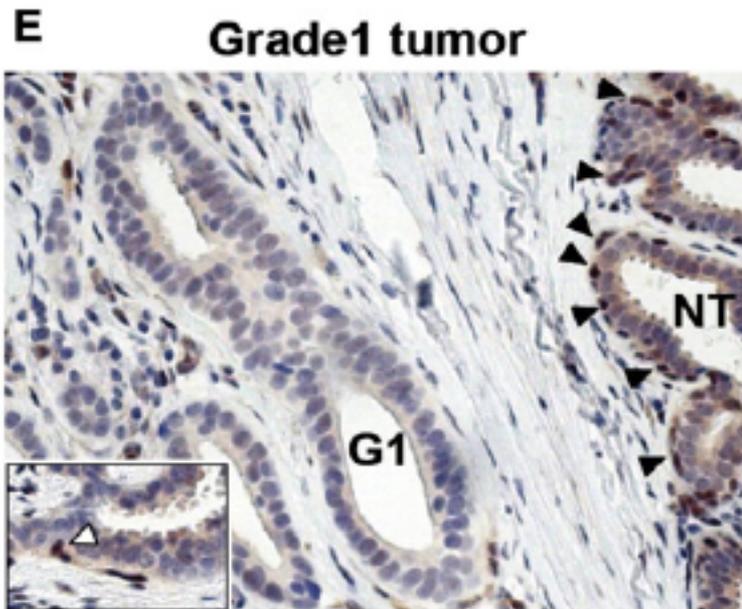


Roles of YAP/TAZ in cancer

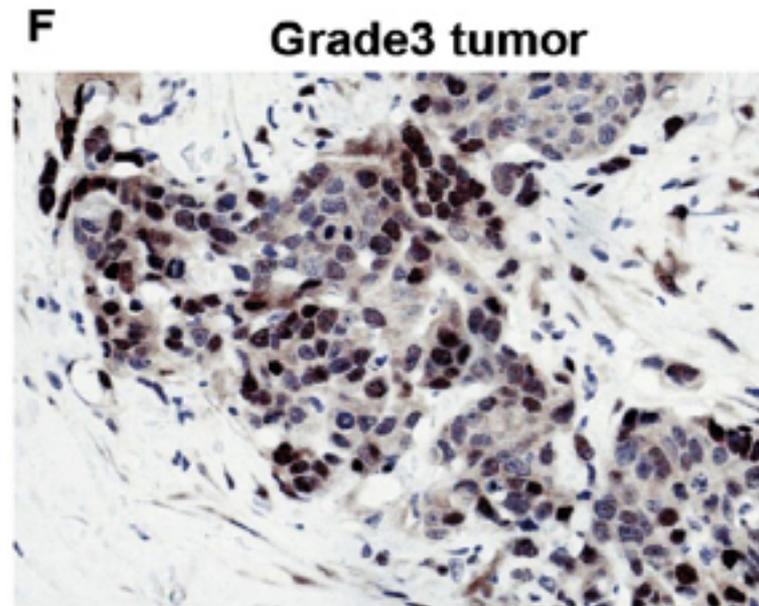


The Hippo Transducer TAZ Confers Cancer Stem Cell-Related Traits on Breast Cancer Cells

Michelangelo Cordenosi,^{1,*} Francesca Zanconato,¹ Luca Azzolin,¹ Mattia Forcato,² Antonio Rosato,³ Chiara Frasson,⁴ Masafumi Inui,¹ Marco Montagner,¹ Anna R. Parenti,⁵ Alessandro Poletti,⁶ Maria Grazia Daidone,⁷ Sirio Dupont,¹ Giuseppe Basso,⁴ Silvio Bicciato,² and Stefano Piccolo^{1,*}



TAZ



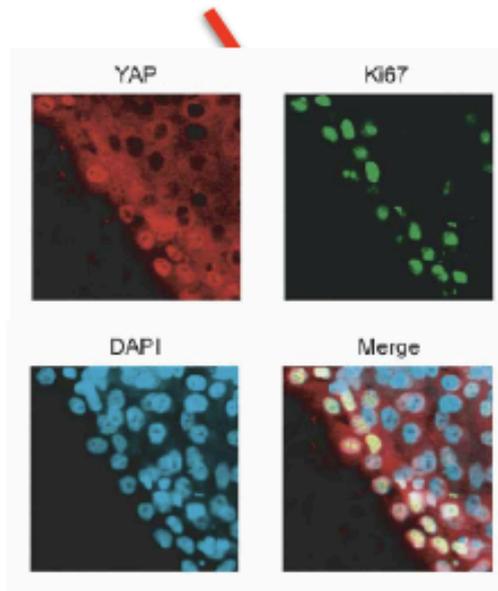
TAZ

YAP/TAZ sono sensori delle caratteristiche fisiche della cellula: forma, polarità, adesione e forze meccaniche (architettura tissutale)

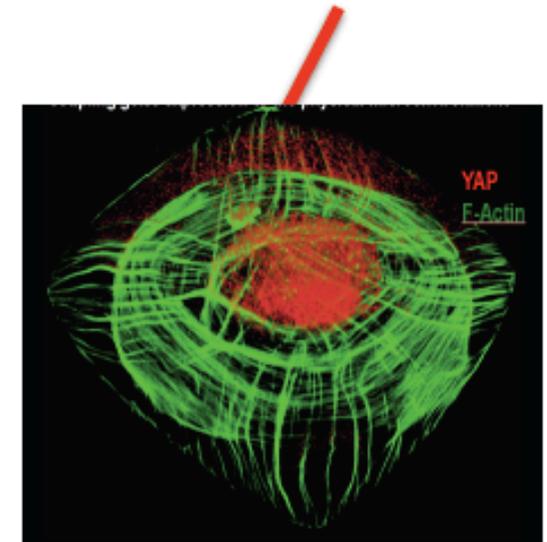
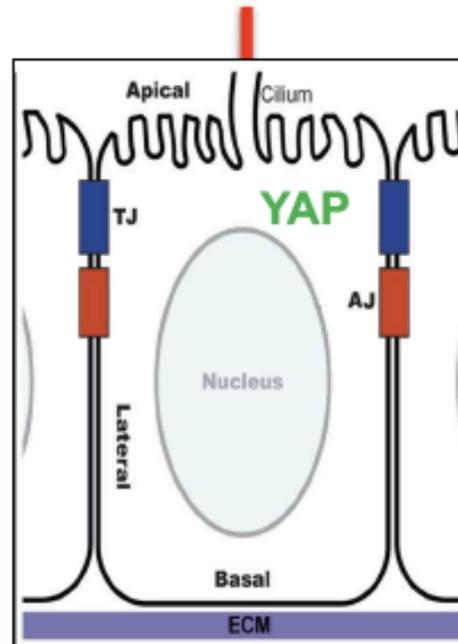
Contact Inhibition

Cell Polarity

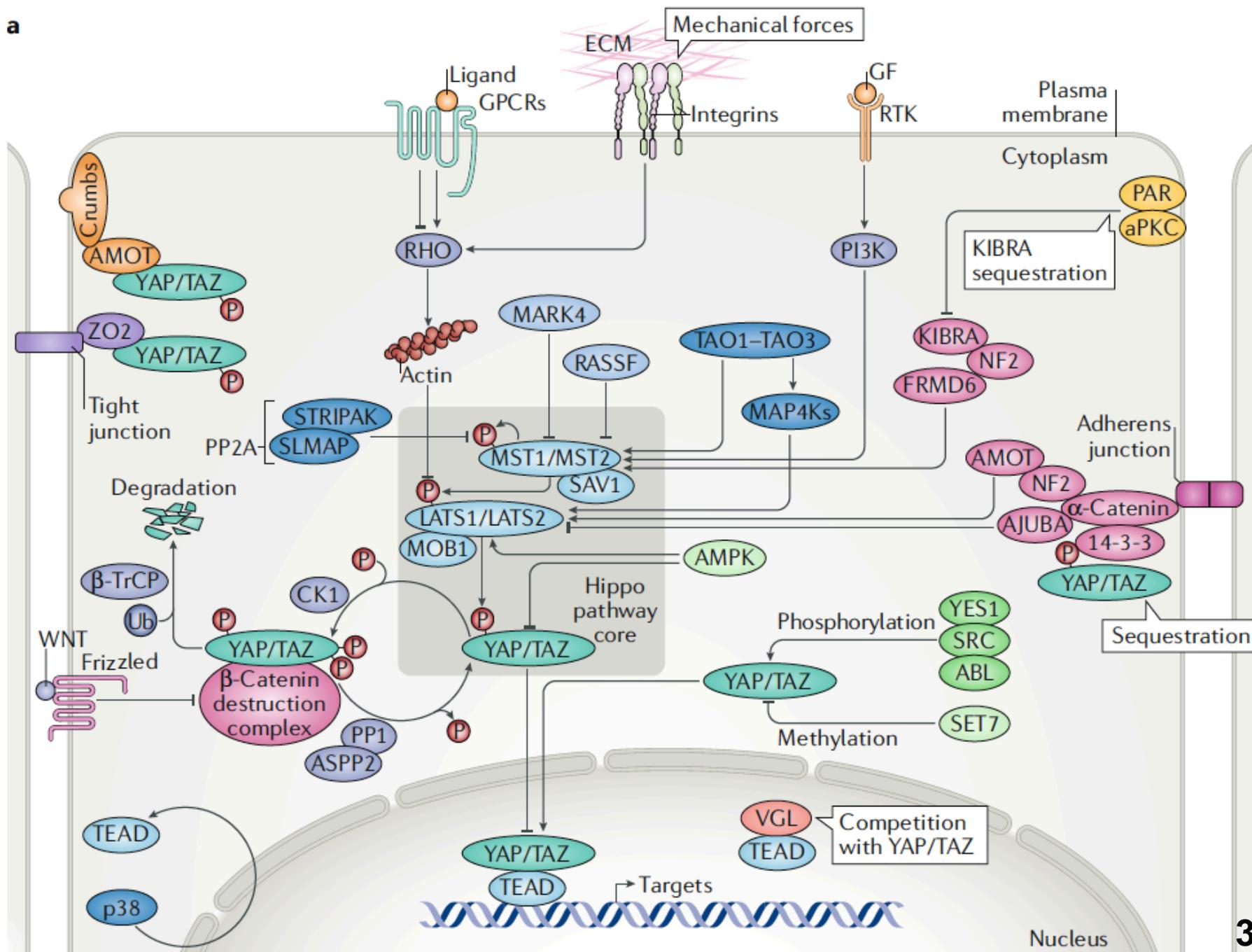
Mechanical Signals

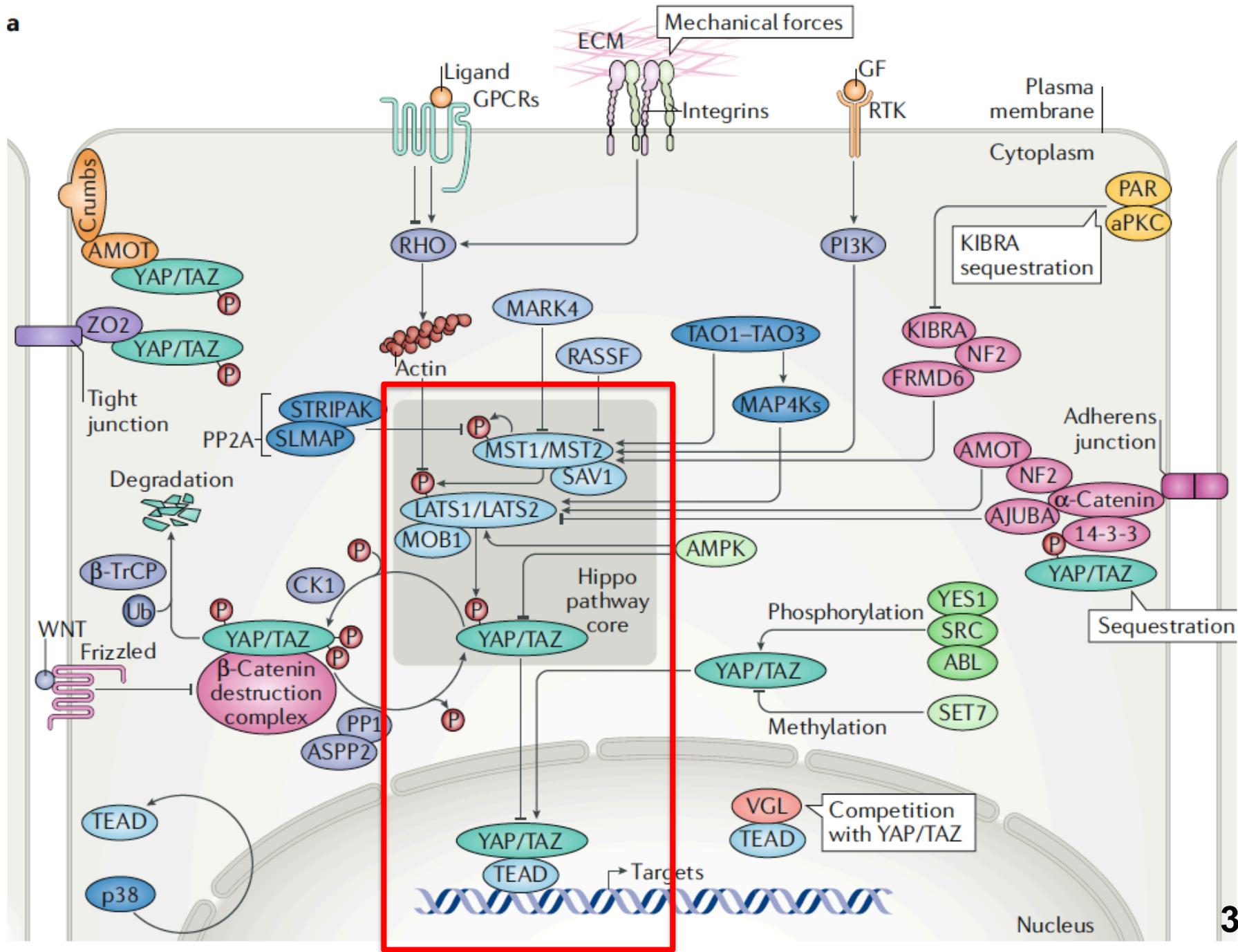


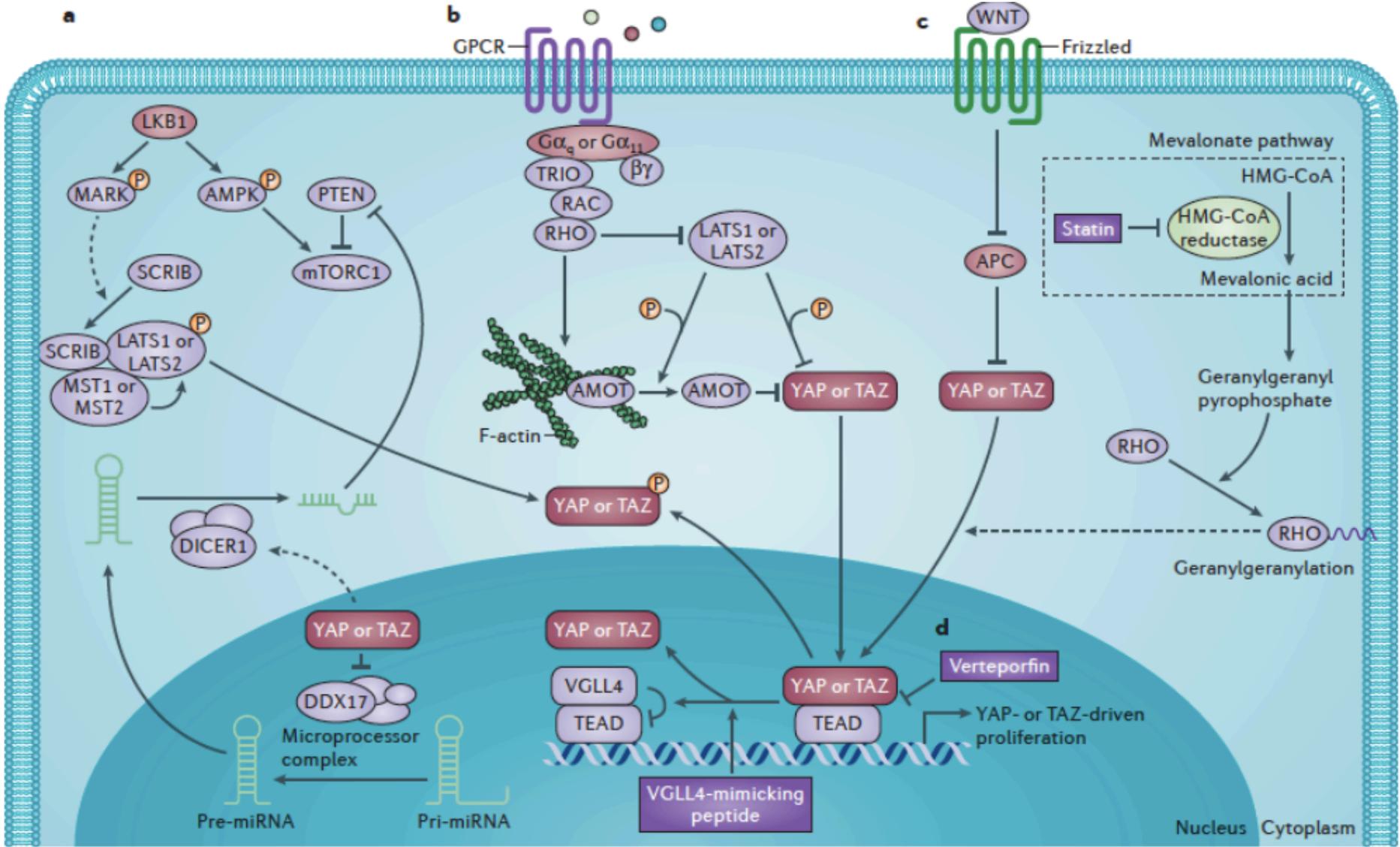
Zhao et al. Gene and Development 2007



YAP/TAZ

a

a



The Hippo pathway is regulated by **tissue architecture**, defined by cell-cell adhesion and cell polarity (Pan D 2010).

At cell-cell junctions, bound to each other, are in fact found NF2/Merlin and Expanded (Ex1/Ex2), that through the protein Kibra interact with MST1/2, leading to the activation of the entire pathway (Hamaratoglu F et al 2006). The tumor suppressor NF2/Merlin acts as an inhibitor of YAP/TAZ and its genetic mutations correlate with neurofibromatosis and other cancers types of the nervous system (Gusella JF et al. 1999).

Another mechanism of Hippo activation is represented by the **preservation of cell apicobasal polarity, with Scribble as the central actor**. At the basolateral plasma membrane, **Scribble forms a complex with Hippo pathway kinases**, stimulating their action. It was observed that **Scribble delocalization, induced for example by epithelial-to-mesenchymal transition** in transformed cells, or by the loss of LKB1 (a tumor suppressor involved in stabilizing the complex between Scribble and the Hippo kinases), results in YAP/TAZ activation (Cordenonsi M et al. 2011, Mohseni M et al. 2014). Furthermore, at the apical membrane is also found the transmembrane protein **Crumbs that can inhibit YAP/TAZ** by causing their cytoplasmic retention. The action of Crumbs may additionally **stimulate AMOT**, which in turn can either sequester YAP/TAZ or activate the Hippo pathway. Interestingly, **AMOT interacts with and is inactivated by actin stress fiber formation**, coupling actin cytoskeleton organization with regulation of Hippo pathway functions. Another protein related to the actin cytoskeleton that contributes to YAP/TAZ regulation is **α -catenin**. In particular, **the cell-adhesion signaling transmitted by E-cadherin/ α -catenin association activates LATS**, provoking YAP/TAZ inhibition (Piccolo S et al. 2014).

The regulation of YAP/TAZ activity in mammals includes also Hippo-independent mechanisms, which include, among others, metabolic and mechanical factors, G-protein coupled and Wnt receptors.

The activity of YAP and TAZ can be influenced by different elements that participate in cellular **metabolism**. YAP/TAZ activation is boosted by the synthesis of mevalonic acid induced by SREBP, a transcription factor that activates the mevalonate pathway generating intermediate products necessary for the membrane attachment of **Rho-GTPases**. These proteins in turn **stimulate YAP/TAZ activation through the actin cytoskeleton** (Sorrentino G et al. 2014). In human lung adenocarcinoma, nuclear localization of YAP/TAZ and expression of their target genes requires the activity of SCD1, an enzyme involved in fatty acid biosynthesis (Noto A et al. 2017). YAP/TAZ activity is also positively regulated by **glucose** intake, whose assimilation in the aerobic glycolysis pathway stimulates their transcriptional activity.

Mechanical regulation of YAP/TAZ

The mechanical stimuli coming from the extracellular matrix, can be defined as **mechanical stresses**, and can entail several aspects/features like stretching, change in pressure or fluid flux rate, variation of the available adhesive area, modification of ECM stiffness (Jaalouk DE & Lammerding J 2009). These microenvironmental physical characteristics **impact on the organization and structure of the actin cytoskeleton** (Pancieria T et al. 2017).

The alteration of the actomyosin cytoskeleton directly affects the activity of mechanosensitive regulators of gene transcription, like MRTF, EGR1 and YAP/TAZ, which represent the **ultimate transducers of mechanical cues** (Halder G et al. 2012, Dupont S et al. 2011, Kechagia JZ et al. 2019, Jaalouk DE & Lammerding J 2009).

Indeed, **YAP/TAZ nuclear translocation and activity are regulated by ECM stiffness**: when cells are plated on stiff matrices and acquire a stretched shape, i.e. they are experiencing high mechanical stress, YAP and TAZ are found in the nucleus; on the contrary, when plated on soft matrices, cells present a round shape, and YAP/TAZ are retained in the cytoplasm (Dupont S et al. 2011).

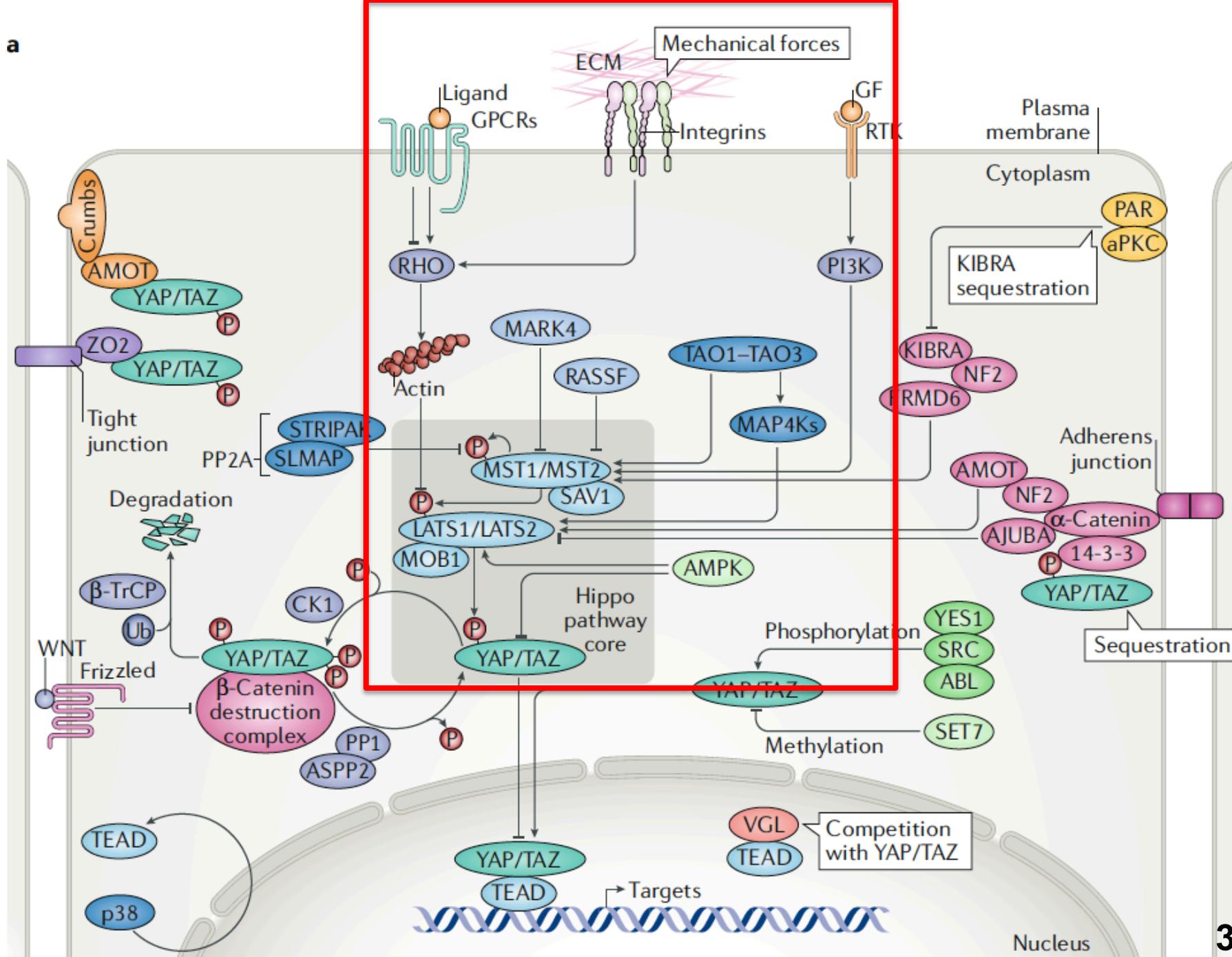
Modifications of ECM stiffness are for example due to the **alteration of fibronectin and collagen deposition** (Vogel V & Sheetz M 2006), whose increase has been shown to activate YAP/TAZ through **focal adhesion formation** (Kim NG et al. 2015, Aragona M et al. 2013). YAP and TAZ react also to the **available adhesive area**, that depends on the **tissue architecture**. Most epithelia are formed by cells that arrange themselves into sheets packed together, which is a structural organization that provokes contact-dependent inhibition of proliferation (CIP).

Mechanical regulation of YAP/TAZ ...

CIP involves the mechanical regulation of YAP/TAZ, indeed spatial arrangement of cells influences ECM rigidity, the shape of adjacent cells and the attachment to the adhesive area. The mechanical regulation of YAP/TAZ that depends on these physical features is Hippo-independent and converges in the remodeling of actin cytoskeleton (Aragona M et al. 2013).

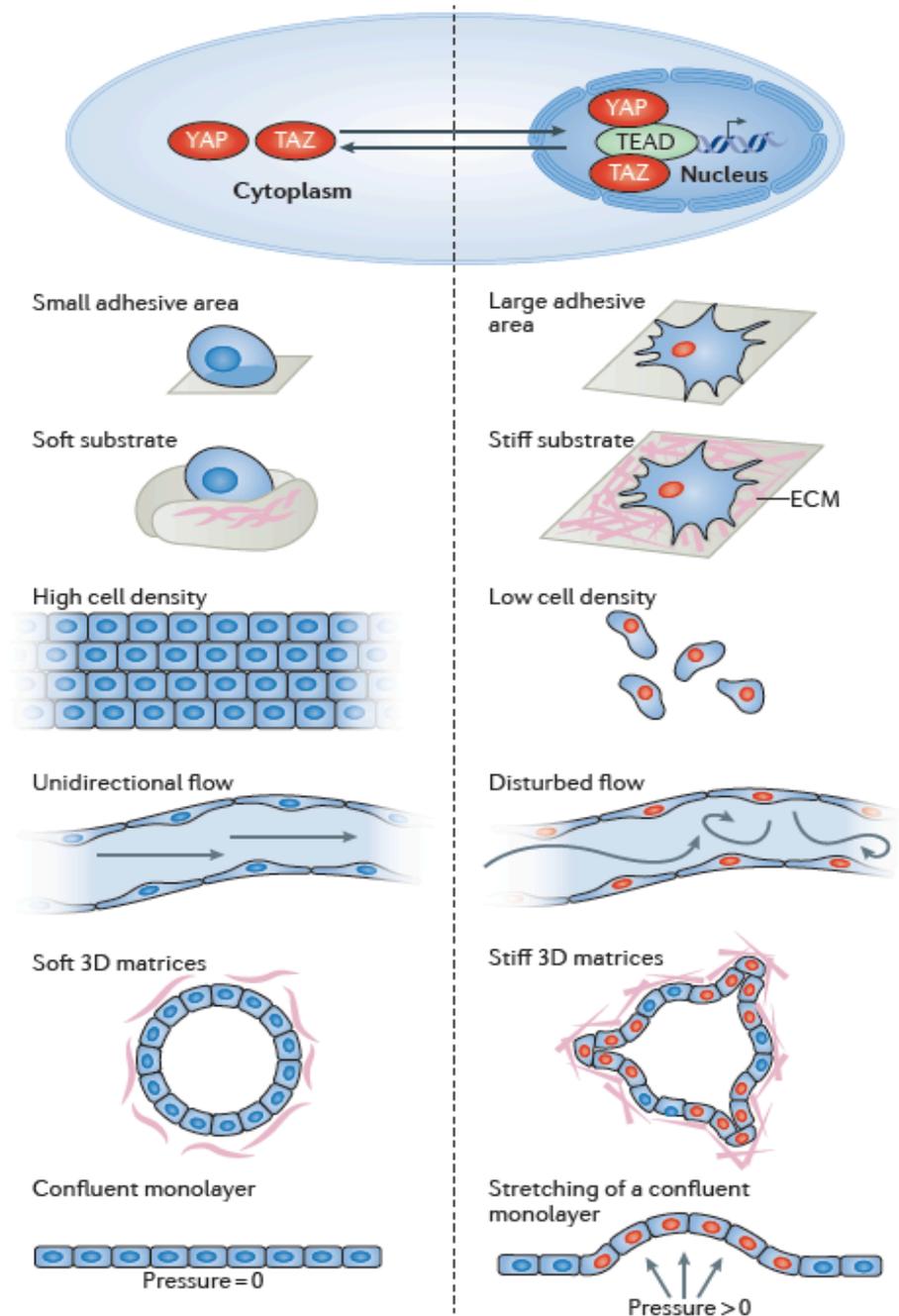
In particular, YAP/TAZ function is not influenced by the ratio of globular versus filamentous actin, but **relies on the organization of the actin cytoskeleton in terms of formation of stress fibers, actin bundle and tensile actomyosin structures** (Dupont S et al. 2011, Aragona M et al. 2013). For example, Piccolo's group demonstrated that **YAP/TAZ nuclear localization is regulated by the activation of Rho-GTPases that promote the assembly of non-muscle myosin II with F-actin**. Furthermore, this group has also shown the role of CAPZ and Cofilin, potent inhibitors of stress fiber formation, in determining YAP/TAZ nuclear exclusion when cells are cultured at high density (Dupont S et al. 2011, Aragona M et al. 2013).

Interestingly, YAP and TAZ have been reported to act themselves as mediators of cytoskeleton remodeling. YAP/TAZ nuclear translocation triggers a **transcriptional program** that promotes the formation of actomyosin structures, thus creating a feed-forward loop that further enhances their activation. For example, it was observed that YAP/TAZ induce the expression of focal adhesion components in mesenchymal stem cells and of modulators of stress fiber contraction in cancer associated fibroblasts (CAFs) (Totaro A et al. 2018, Calvo F et al. 2013).

a

Mechanical stimuli coming from the extracellular matrix = **mechanical stresses**, include **stretching, change in pressure or fluid flux rate, variation of the available adhesive area, modification of ECM stiffness.**

These physical characteristics **impact on the organization and structure of the actin cytoskeleton.**



La rigidità della ECM ingaggia il citoscheletro actinico ad attivare YAP/TAZ

