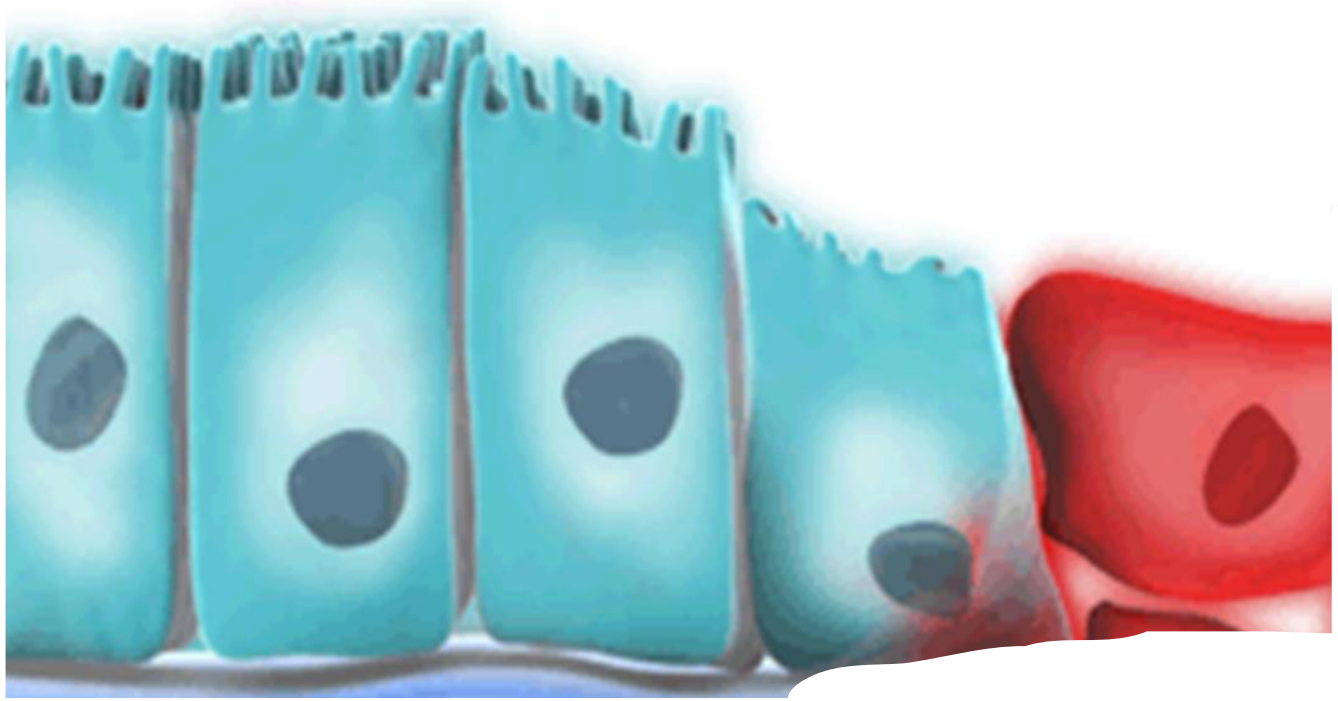
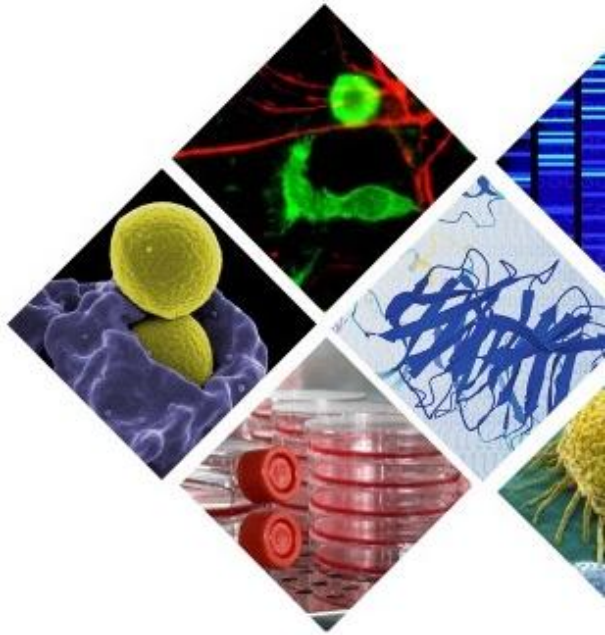


Ciências ULisboa
Faculdade de Ciências da Universidade de Lisboa



MESTRADO
BIOQUÍMICA E BIOMEDICINA

Tumor microenvironment, inflammation and life style factors

Oncobiology

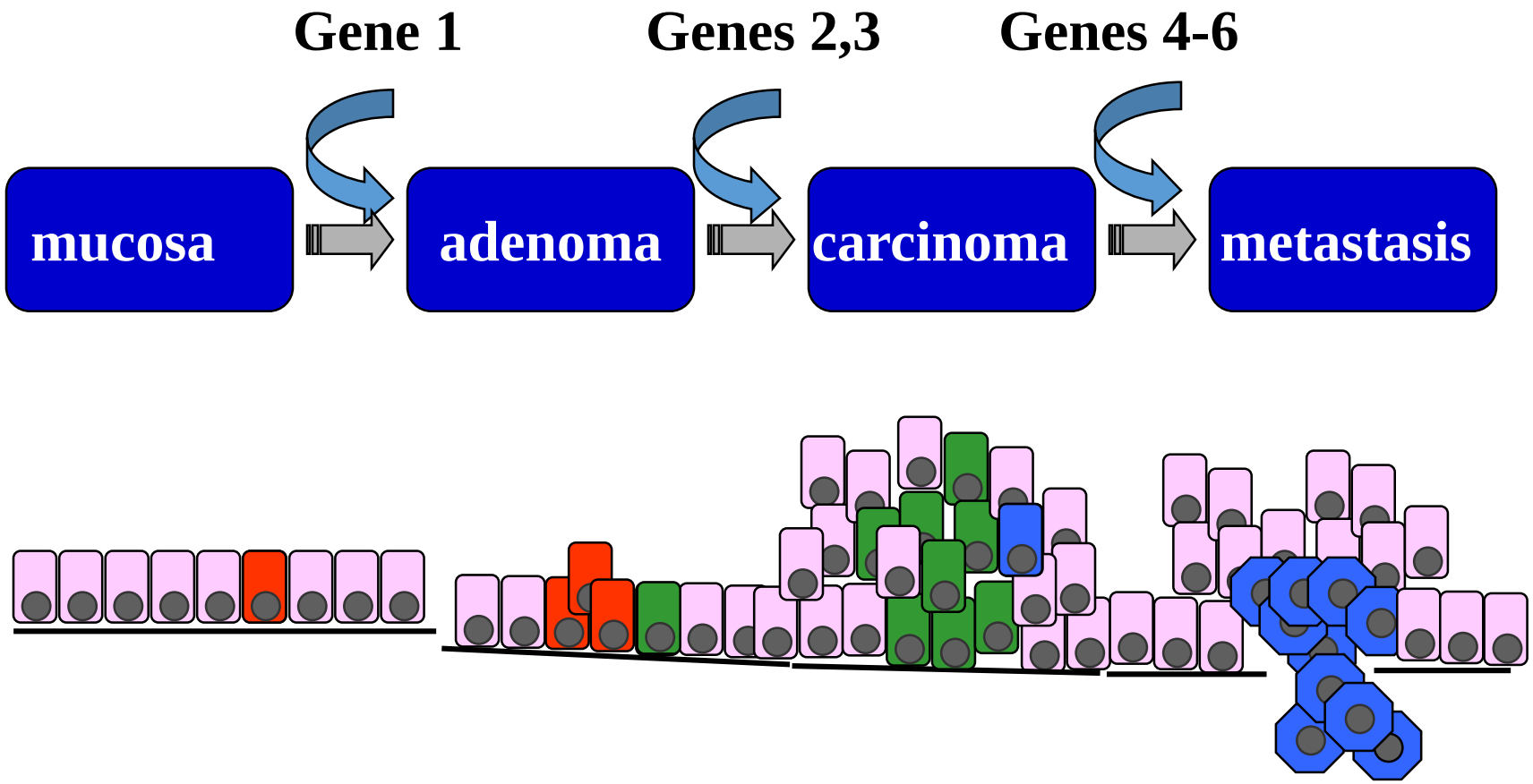
Margarida Gama-Carvalho (DQB/FCUL) and Peter Jordan (INSA)

REPÚBLICA PORTUGUESA
SAÚDE

Instituto_Nacional de Saúde
Doutor Ricardo Jorge

The classical model of *clonal selection:*

a cancer cell-centered reductionist model



Accumulation of genetic changes in a cell

The tumour microenvironment

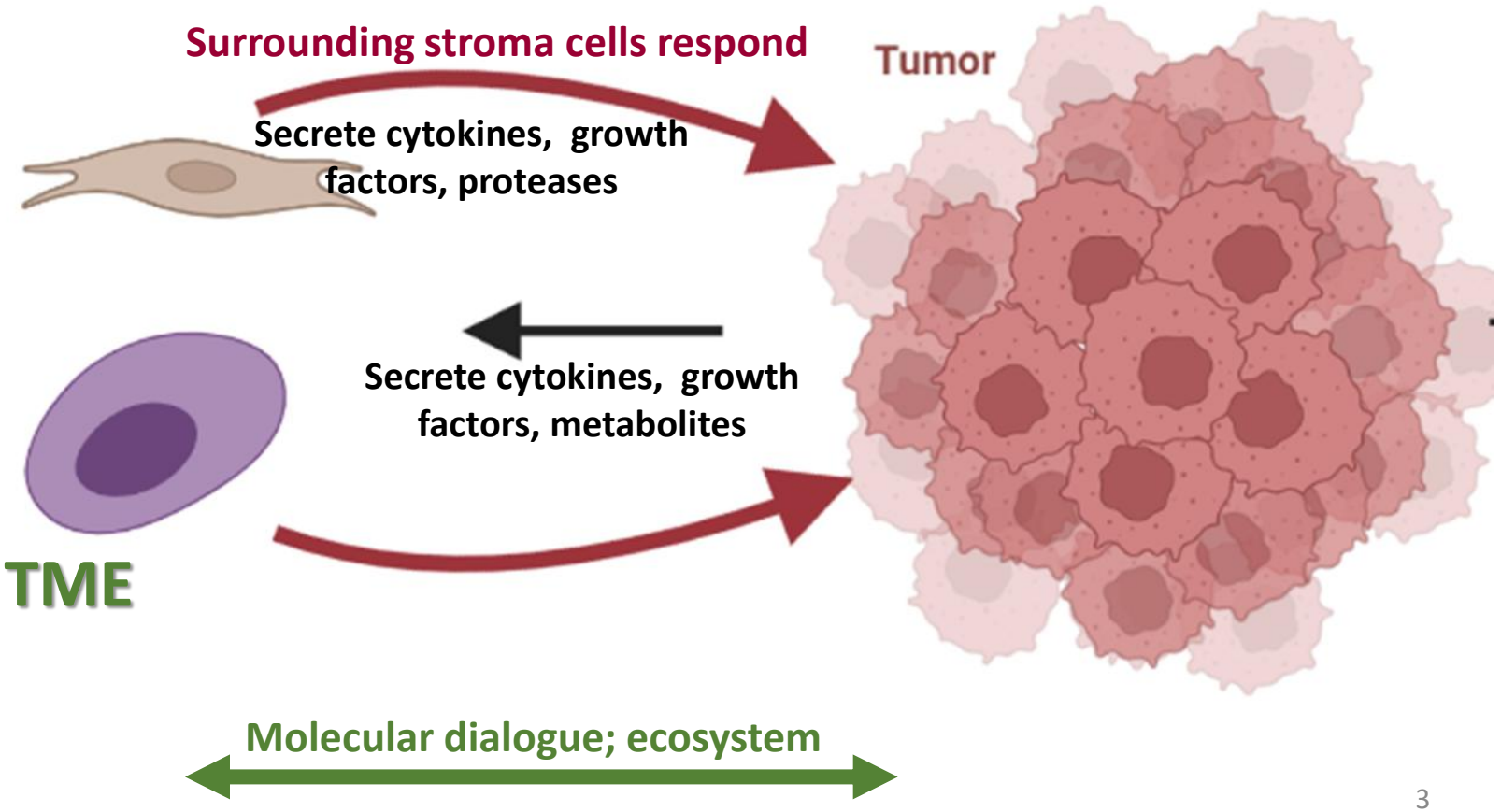
TME is a heterogeneous collection of infiltrating and resident host cells, secreted factors and metabolites, and the extracellular matrix

Updated view:

TME modulates cancer progression

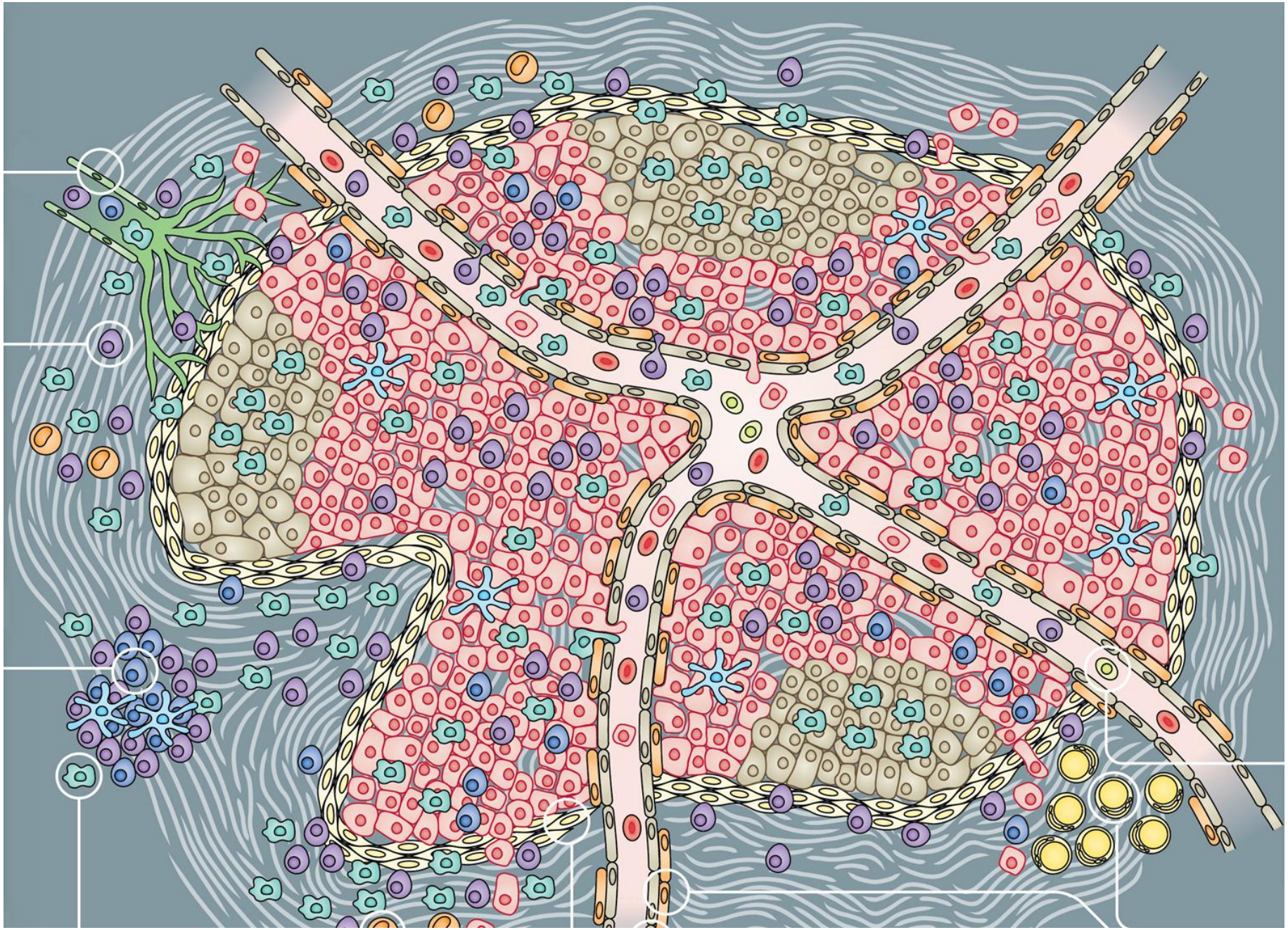
Classic view:

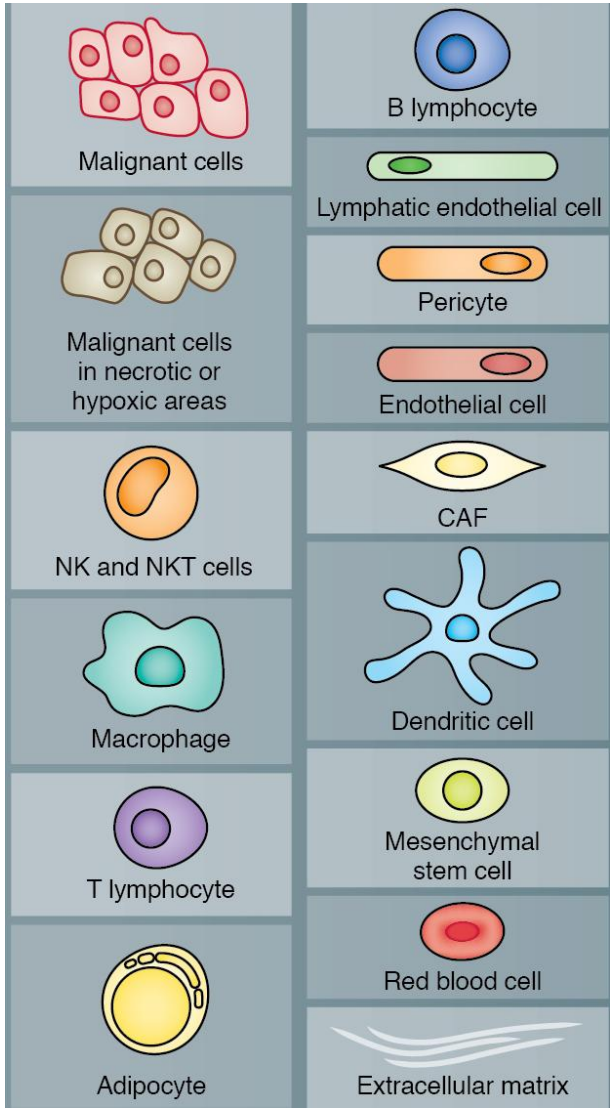
Tumour cells corrupt the TME



Created with BioRender.com

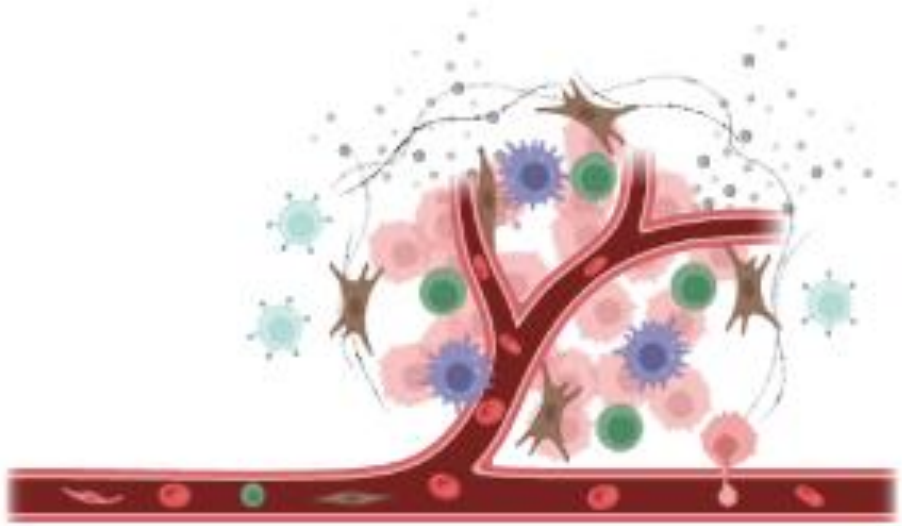
A more realistic view into a tumour





https://doi.org/10.1242/jcs.116392

Stroma and tumour cells in the TME



1. **Endothelial cells**

Normal blood vessels

2. **Tumour cells**

3. **Fibroblasts**

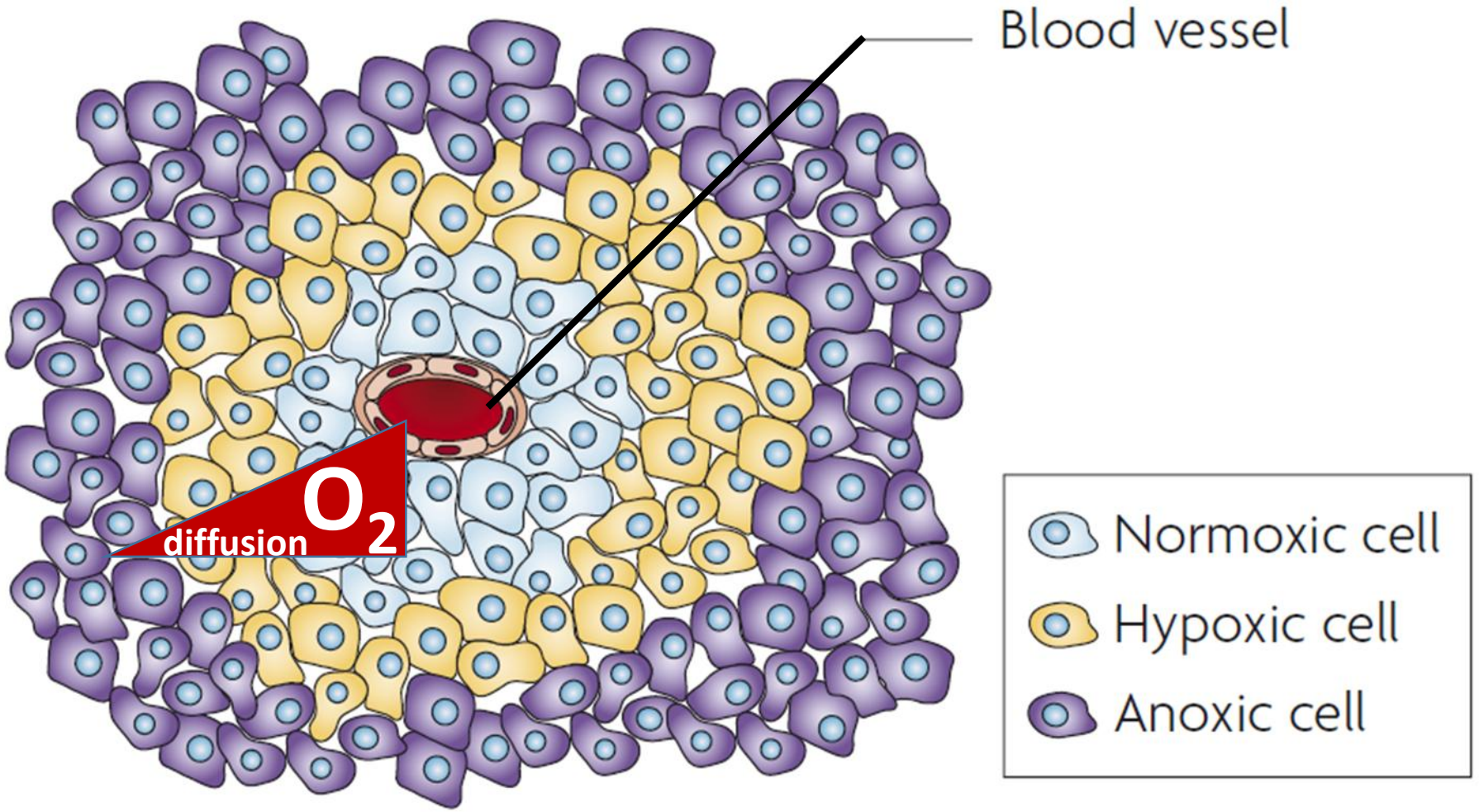
Fibroblast

4. **Immune cells**

Monocyte

T_{reg}

Cell metabolism lecture: hypoxia



Cells respond to hypoxia by a molecular sensor

- Accumulation of the protein **hypoxia inducible factor (HIF-1α)** in hypoxia, a transcription factor

- Normoxia: constitutive degradation of HIF-1α following **hydroxylation of prolines** 402 and 564 (mediated by the *von-Hippel-Lindau* protein E3-ubiquitin ligase complex)

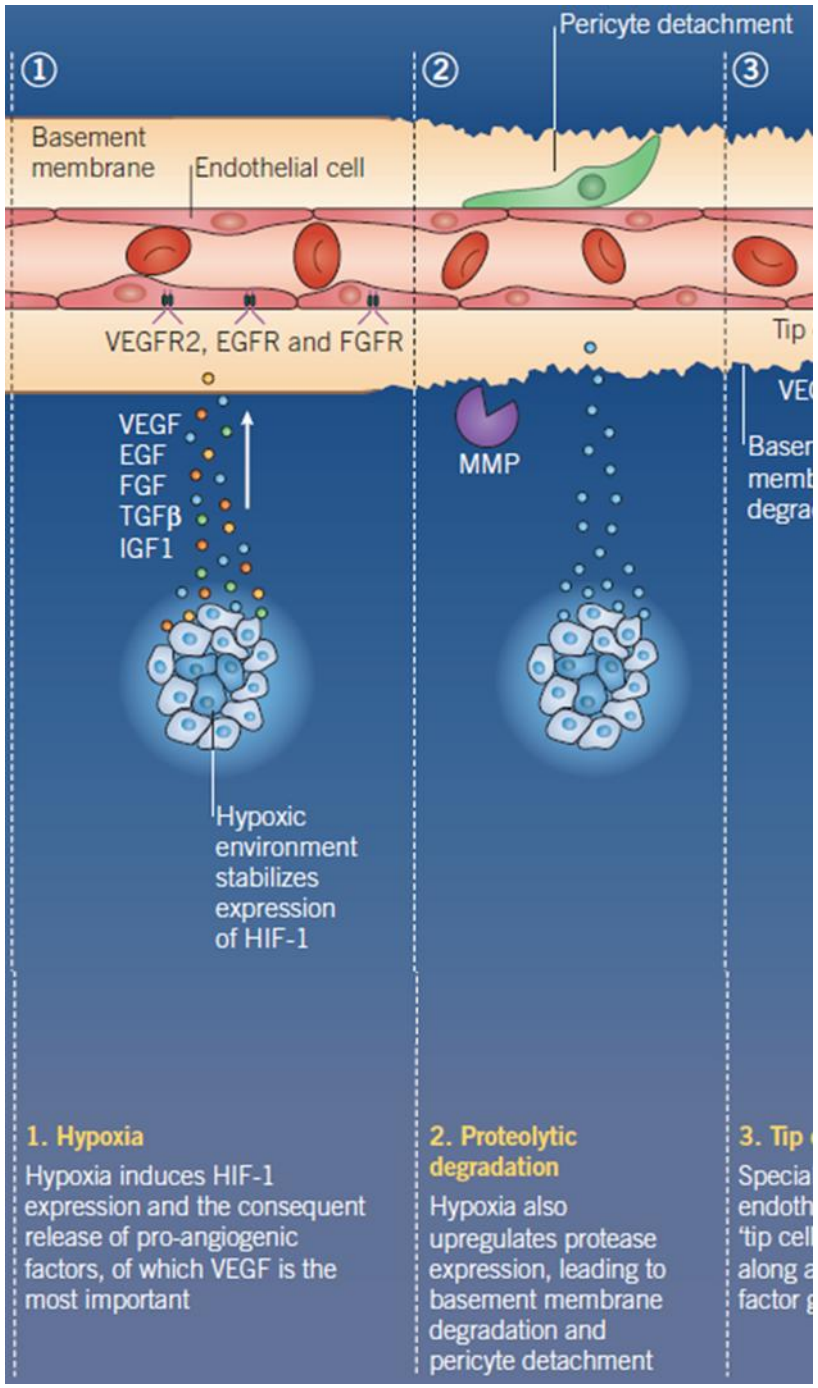
- Prolyl hydroxylases have **low-affinity** to $O_{2;}$ therefore in hypoxia HIF-1α is no longer hydroxylated and thus not degraded

HIF-1 target genes

- regulate glucose metabolism
- **induce angiogenesis in the TME**

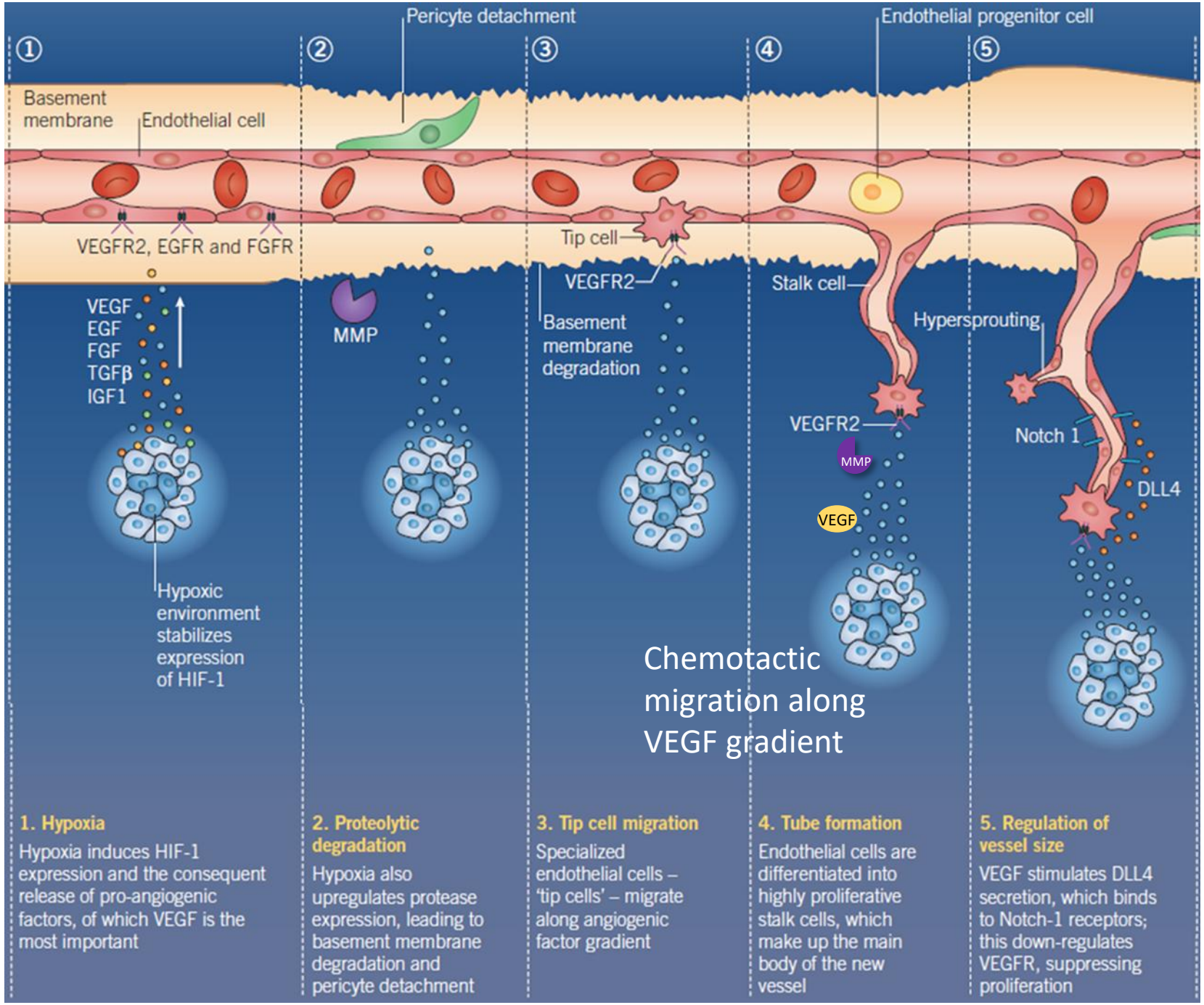
Product(s) of HIF1 target gene(s)	Metabolic function
Glucose transporters GLUT1 and GLUT3	Glucose entry into the cell
Hexokinase2	1st glucose phosphorylation step
PGI, PFK1, aldolase, TPI, GAPDH, PGK, PGM, enolase, PK, PFKFB1	Glycolysis
LDHA	Conversion of pyruvate to lactate
MCT4	Removal of lactate from the cell
PDK1, MXI1	Decreased mitochondrial activity and ROS
VEGF	angiogenesis

doi:10.1038/nrc2468



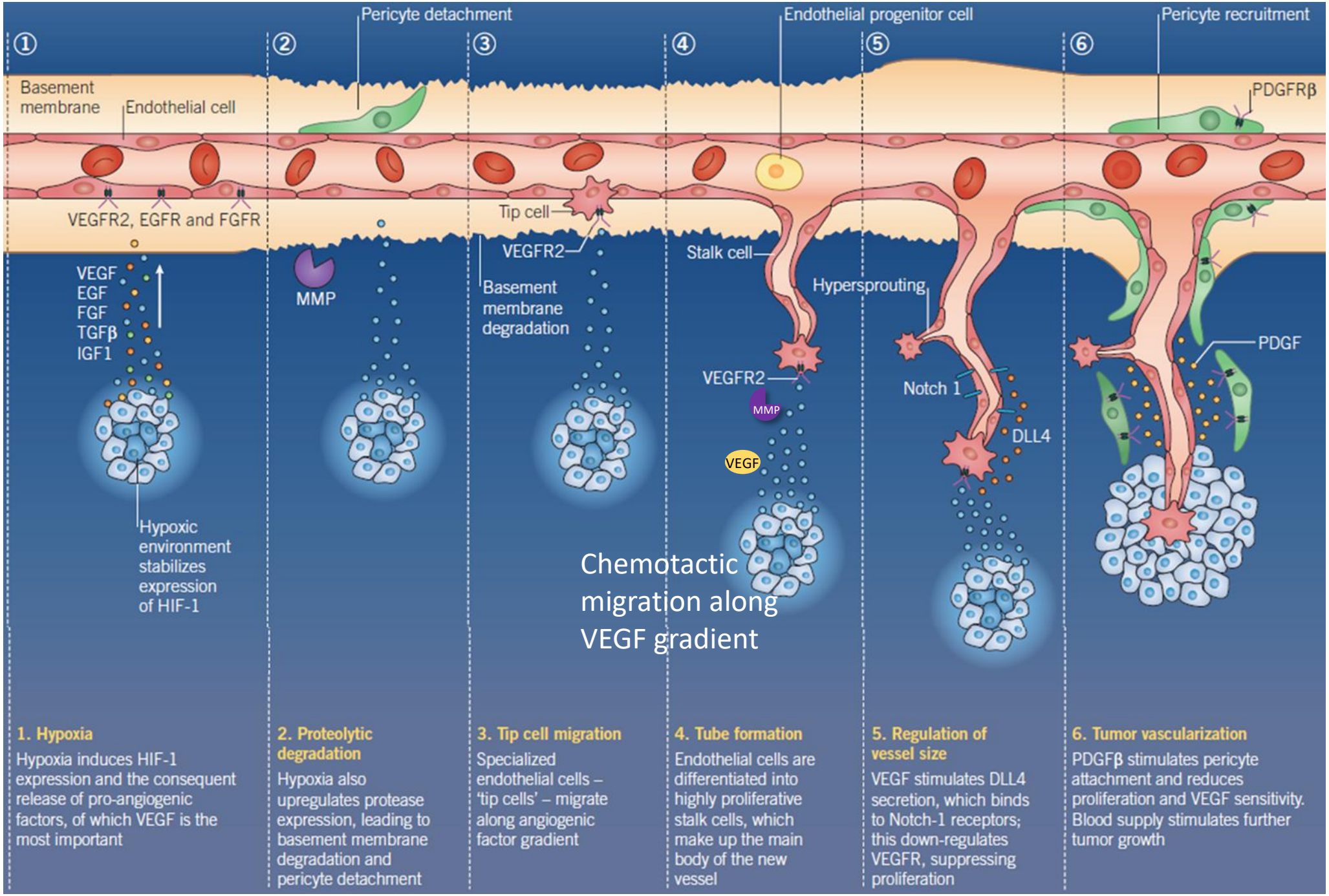
1. – Hypoxic cells secrete VEGF that is perceived by endothelial cell receptors

2. - The capillary wall is made of an endothelial cell layer surrounded by a basement membrane and is supported by pericytes.



2. - The capillary wall is made of an endothelial cell layer surrounded by a basement membrane and is supported by pericytes.

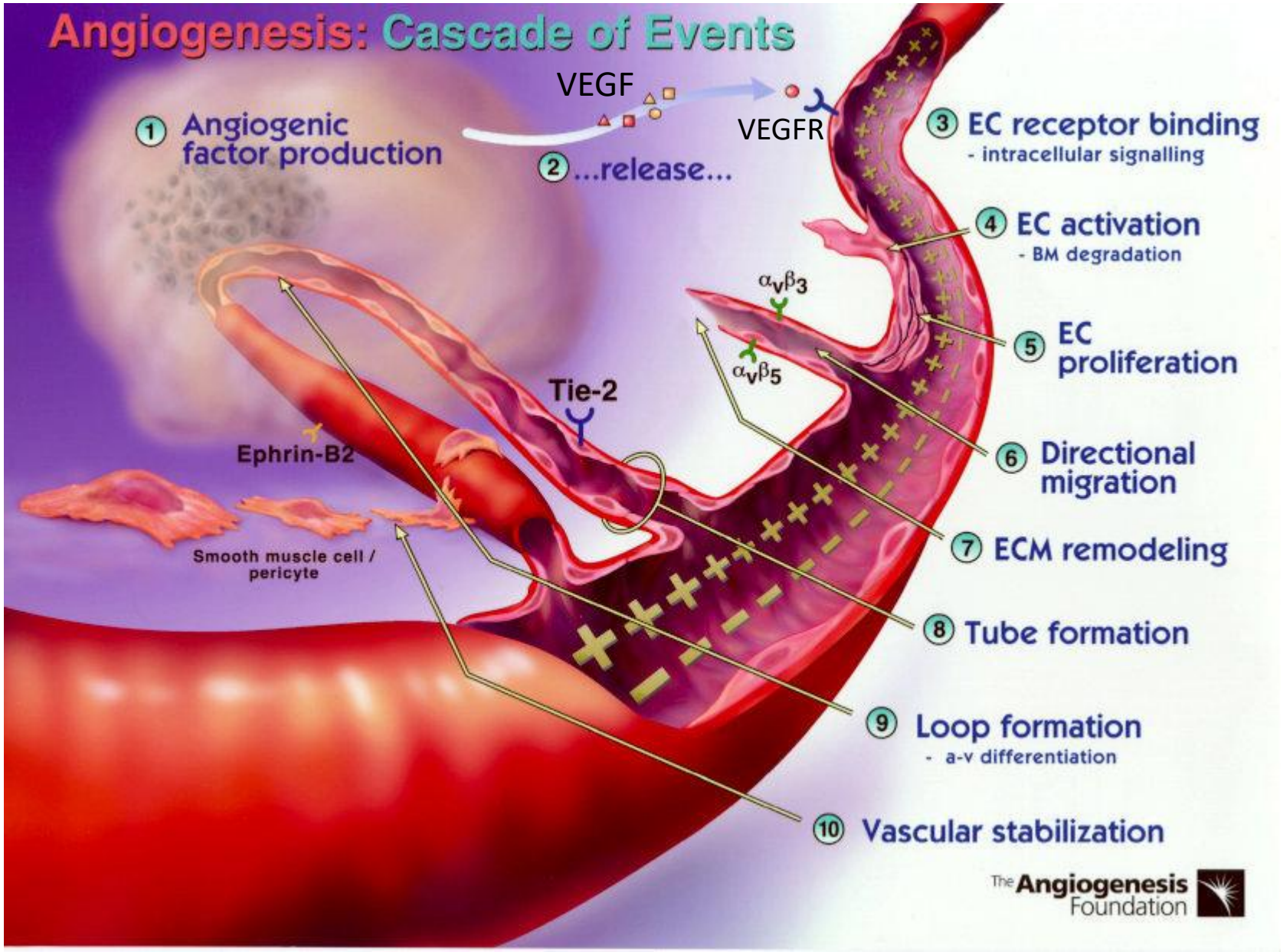
3.-5.- One cell becomes a chemotactic **tip cell,** proliferation of stalk cells, DLL4/Notch signaling coordinates neighboring endothelial cells



2. - The capillary wall is made of an endothelial cell layer surrounded by a basement membrane and is supported by pericytes.

3.-5.- DLL4 secreted by tip cells activates Notch signaling in the neighboring endothelial cells, suppressing tip cell formation

6. Tumor vessels are less-well organized and more permeable



Sprouting angiogenesis:

the growth of new capillary vessels out of preexisting ones.

DOI:10.1160/TH07-03-0225

Bevacizumab or Avastin

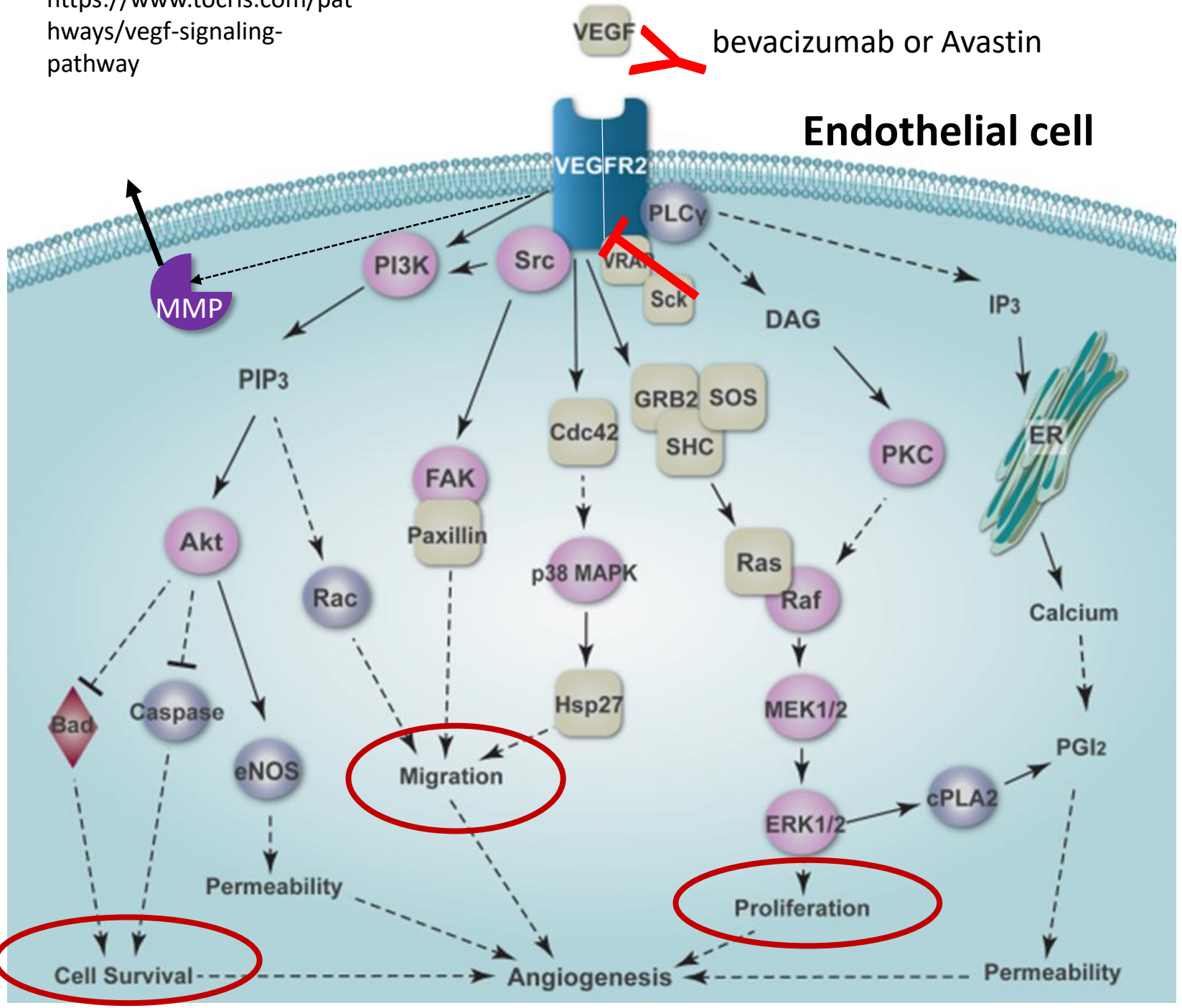
Anti-angiogenic therapy

- Neutralizing antibodies targeting anti-vascular endothelial growth factor (VEGF);
- Tyrosine kinase inhibitors to block VEGFR signaling

provides short-term relief from tumour growth before resistance occurs, e.g:

- activation of signaling pathways independent of VEGF;
- recruitment of myeloid cells with pro-angiogenic chemokines

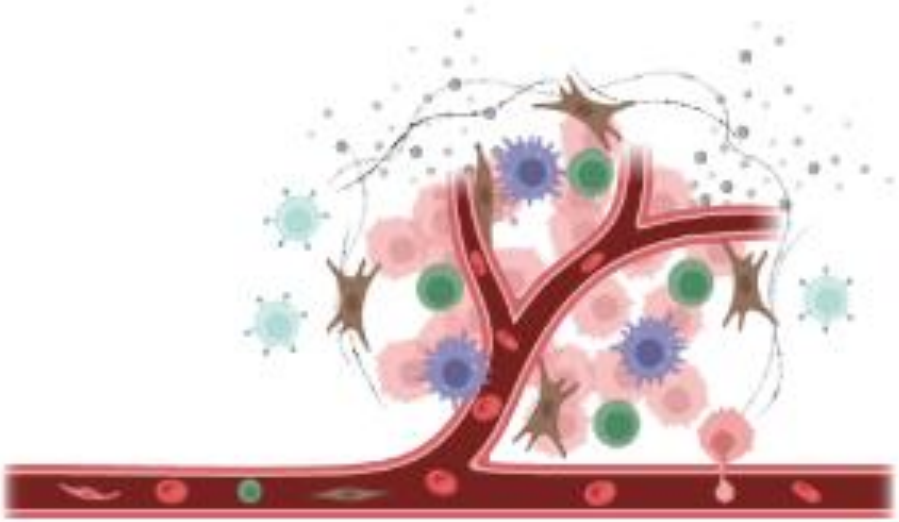
https://www.tocris.com/pathways/vegf-signaling-pathway



Anti-angiogenic therapy

Complexity of VEGFR signalling events

Stroma and tumour cells in the TME



1. **Endothelial cells**

Normal blood vessels

2. **Tumour cells**

Metabolic symbiosis

3. **Fibroblasts**

Fibroblast

4. **Immune cells**

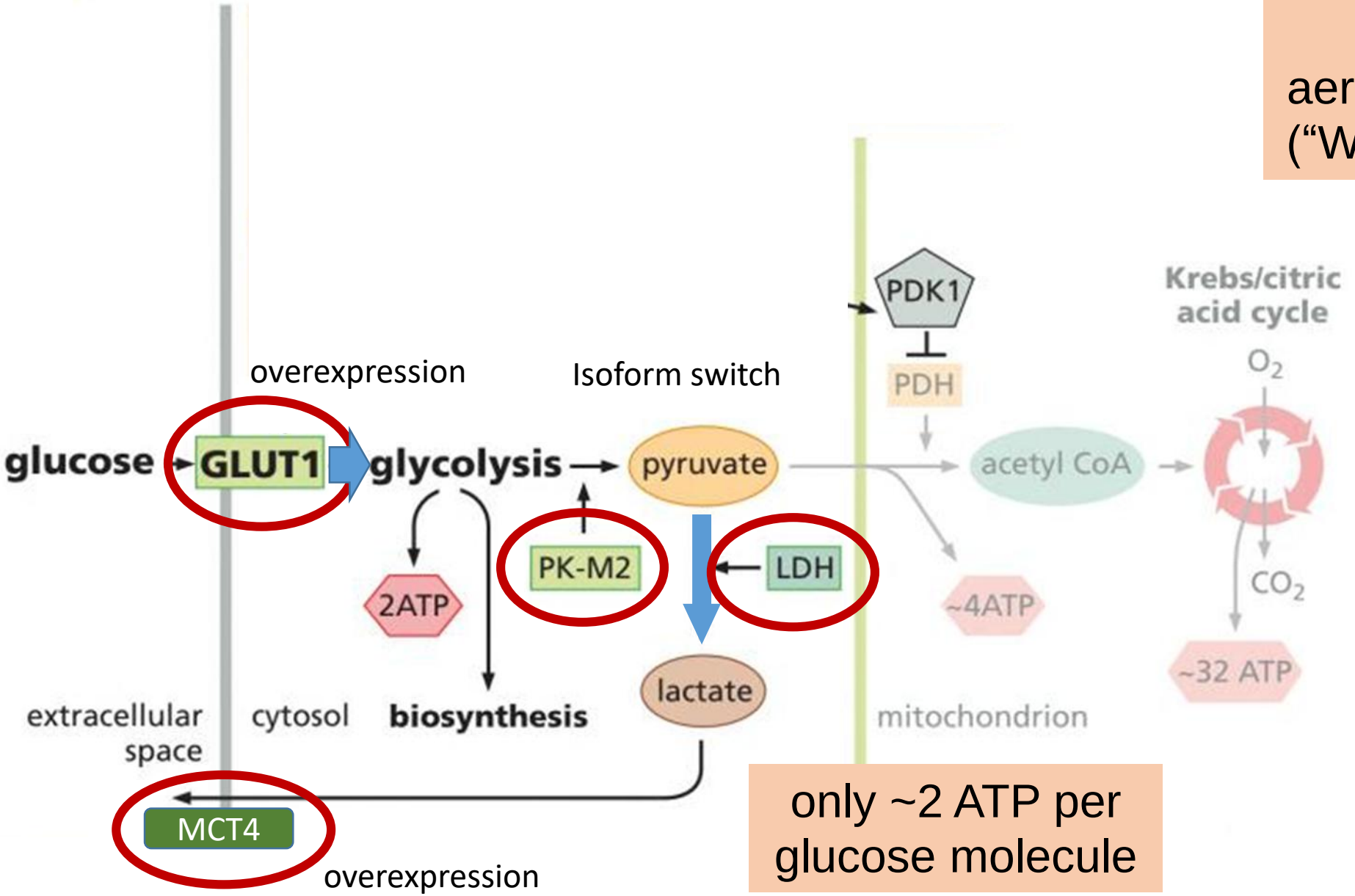
Monocyte

T_{reg}

Cancer cells

Cell metabolism lecture

cancer cells favour aerobic glycolysis ("Warburg effect")



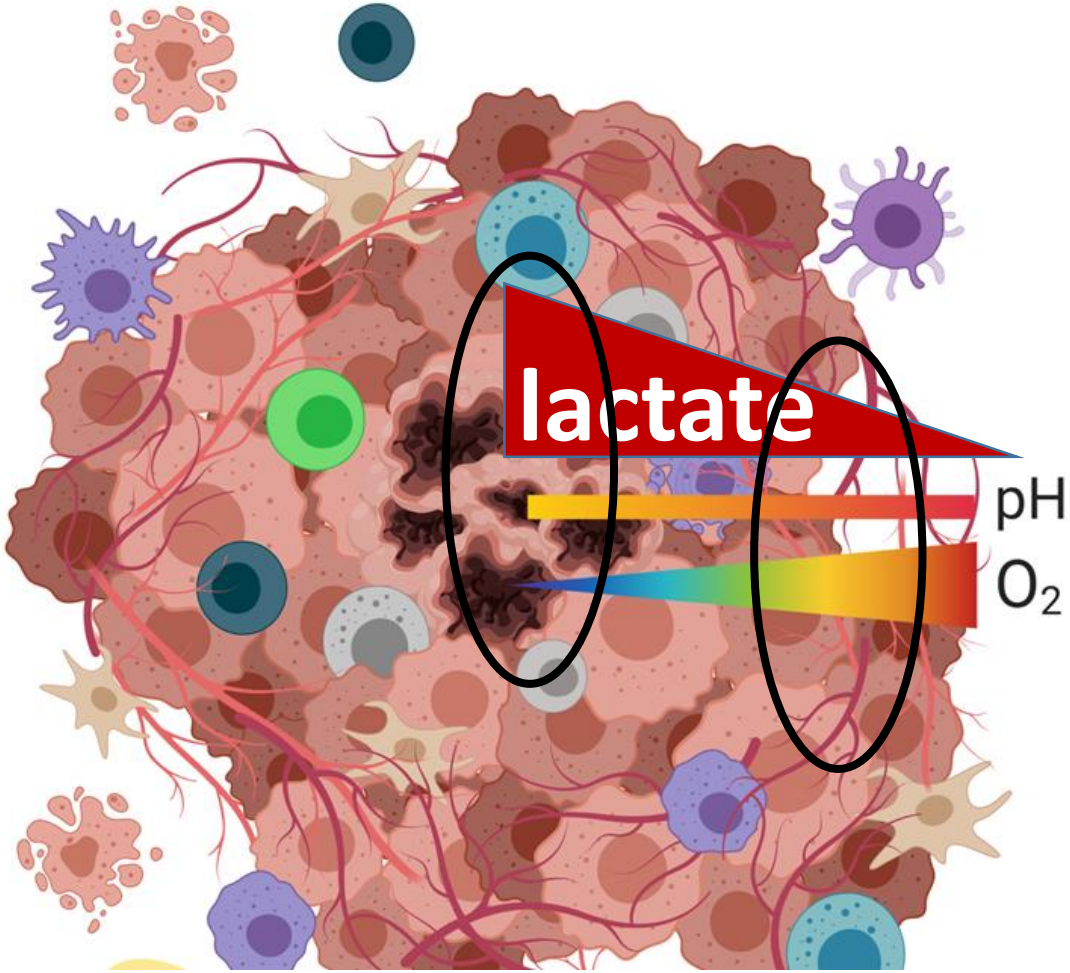
only ~2 ATP per glucose molecule

Metabolic symbiosis:

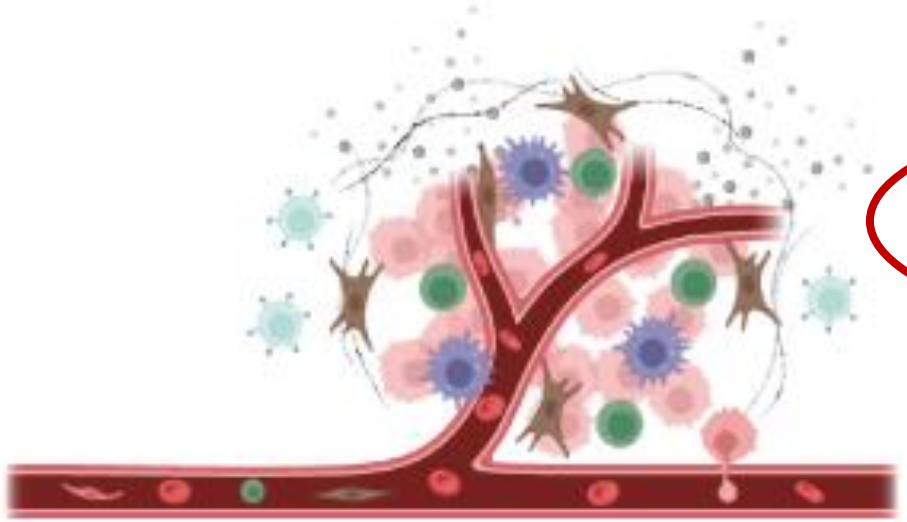
- Cells in hypoxic regions are highly glycolytic and generate high amounts of lactate

- Cells in normoxic regions take up the excess of lactate and metabolize it via pyruvate by aerobic mitochondrial respiration.

Leads to increased tumour tolerance against anti-angiogenic therapies and hypoxia



Stroma and tumour cells in the TME



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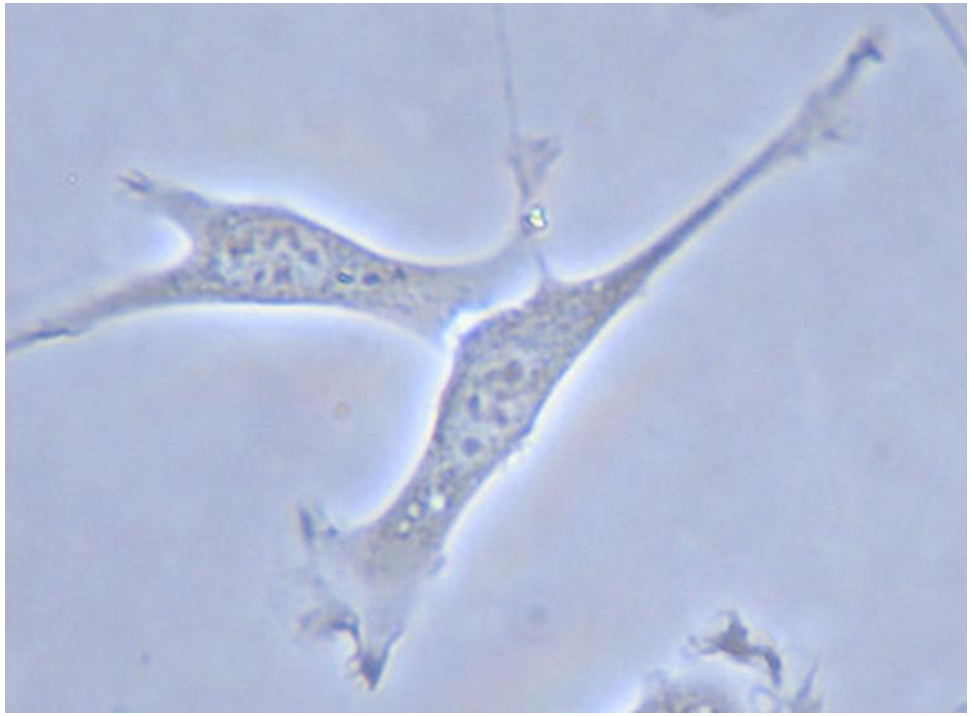
Fibroblasts



- non-epithelial, non-immune cells of mesenchymal origin;
- single cells present in the interstitial space of the connective tissue

Tissue-resident fibroblasts are usually quiescent, but become activated by TGF-β:

- during wound healing response to regulate epithelial cell proliferation and differentiation;

- or by the presence of tumour cells= **Cancer-associated fibroblasts** (CAFs)
  (CAFs can also differentiate from recruited mesenchymal or endothelial cells or adipocytes)

Biomarker analysis and single-cell sequencing identified up to 18 CAF subtypes:

- **myofibroblastic CAFs** (myCAF) express high levels of SMA; are tumour-suppressing
- **inflammatory CAFs** (iCAFs) express low levels of SMA but high levels of cytokines; have tumour-promoting effects

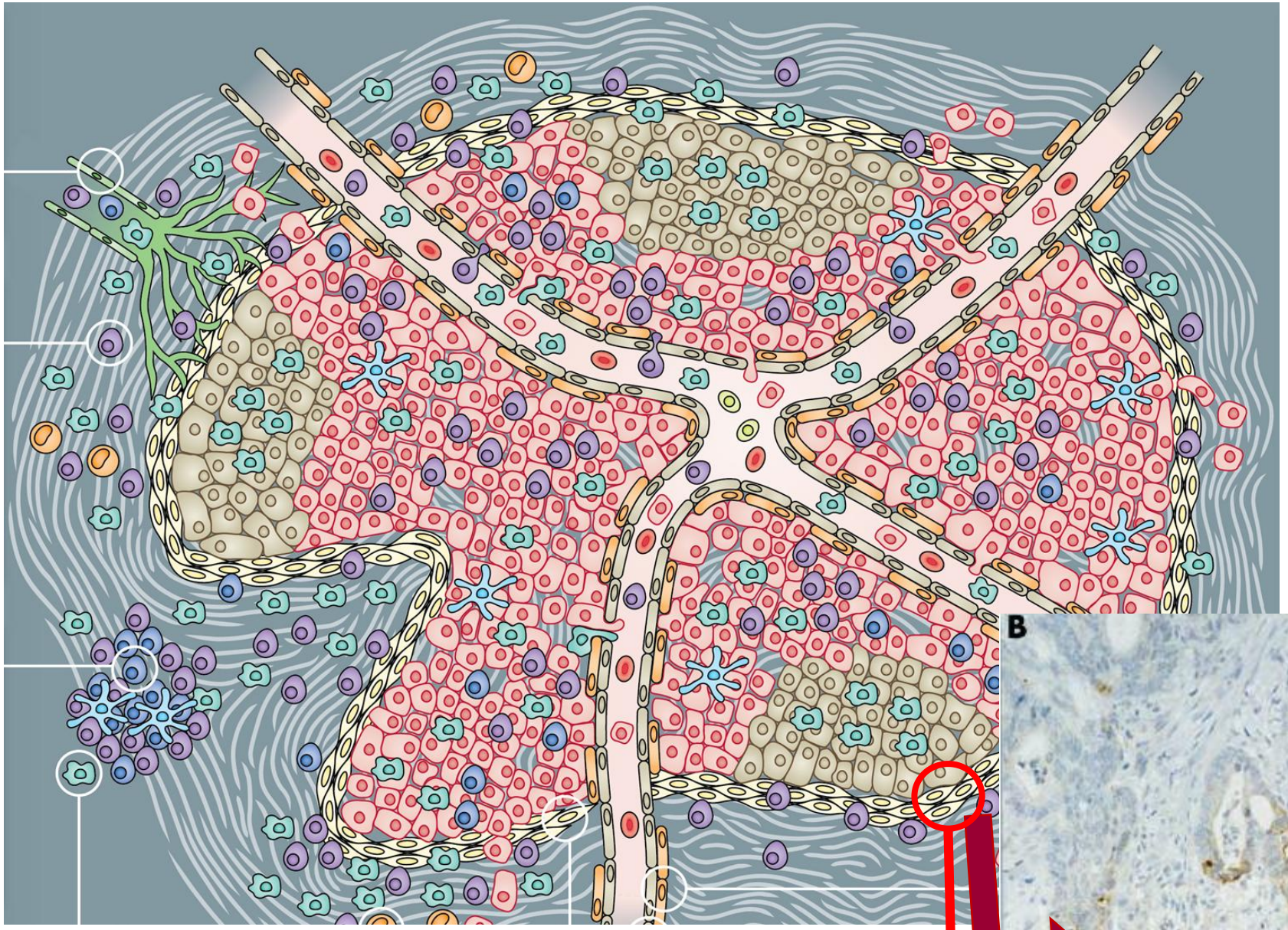
SMA= smooth muscle actin

Main impact of CAFs on cancer progression

- remodel extra-cellular matrix proteins with proteases

Matrix metalloproteinase (MMPs)

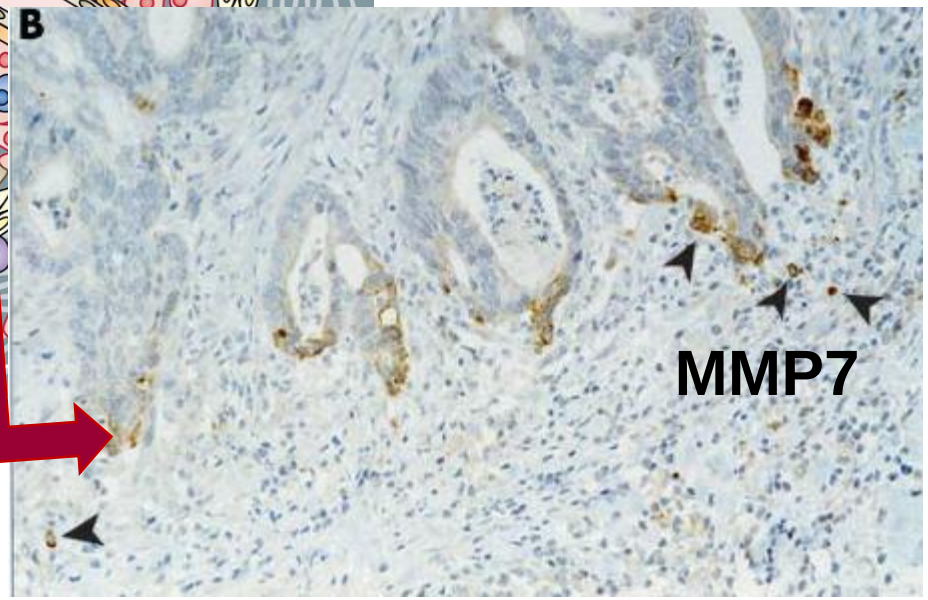
- 24 genes identified
- involved in ECM degradation:
  - Collagenases (MMP-1, -8 and -13)
  - Gelatinases (MMP-2 and MMP-9)
  - Stromelysins (MMP-3, -10 and -11)
  - Matrilysin (MMP-7 and MMP-26)
  - Membrane-type (MT)-MMPs (MMP-14, -15, -16, -17, -24 and -25)
  - regulated by endogenous inhibitors:
    - α2-macroglobulin
    - TIMPs 1-4= tissue inhibitors of metalloproteases.



CAFs

CAFs can remodel the extracellular matrix and facilitate cancer cell invasion

- active at the invasive front of a tumour



Main impact of CAFs on cancer progression

- remodel extra-cellular matrix proteins with proteases

- secrete extracellular matrix components
- secrete growth factors that can stimulate cancer cell proliferation
- secrete cytokines to recruit inflammatory cells

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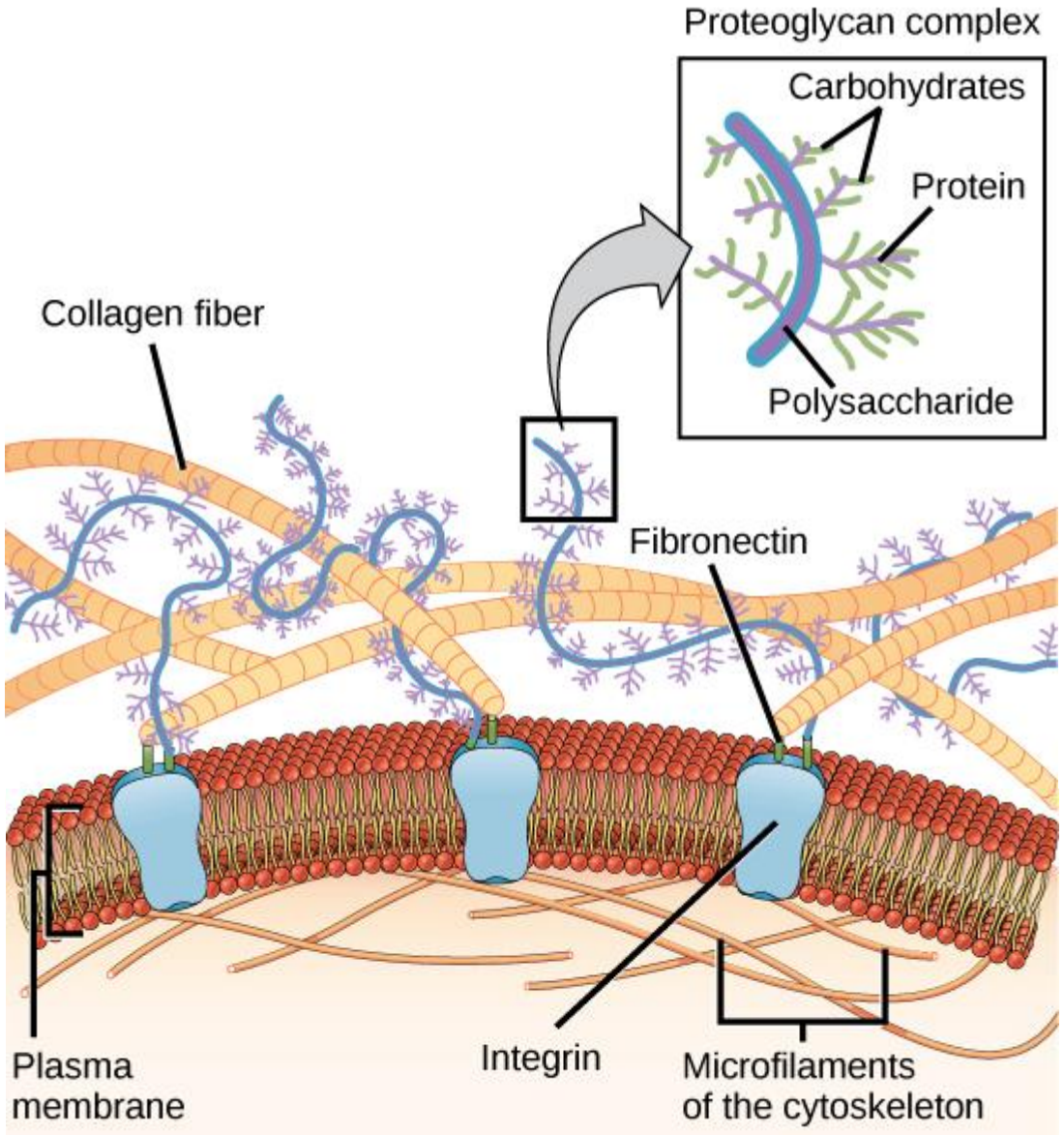
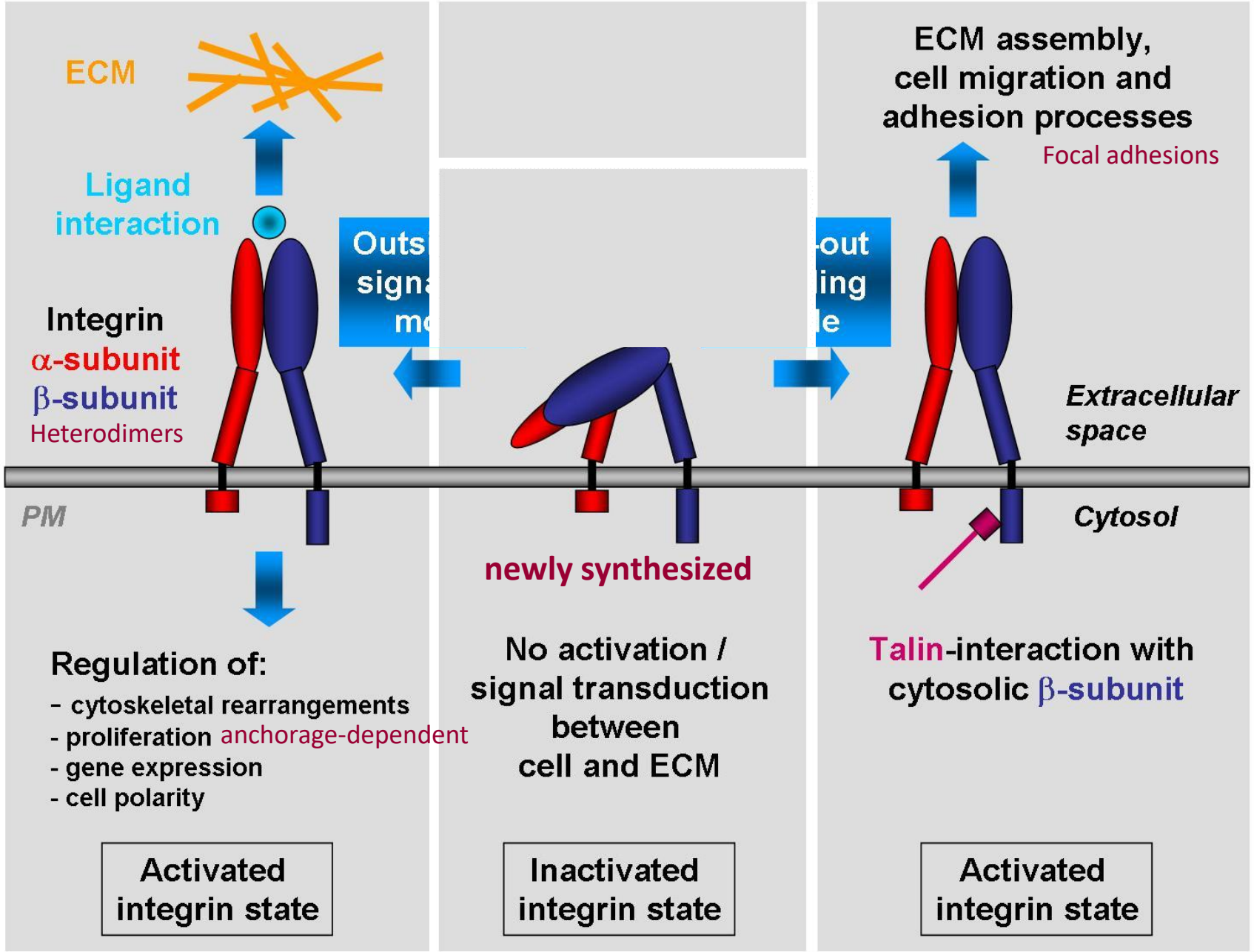
  - ➢ regulated by endogenous inhibitors:
    - α2-macroglobulin
    - TIMPs 1-4= tissue inhibitors of metalloproteases.

The extracellular matrix (ECM)

- Is a 3D network of protein fibers (collagen, elastin), connecting glycoproteins (fibronectin, laminins), and glycans (hyaluronic acid);
- Provides mechanical support for cells, but also for cell migration;
- ECM proteins serve as ligands for cell surface receptors such as **integrins**;





18 α and 8 β integrin subunits are known in humans, generating 24 cell type specific heterodimers

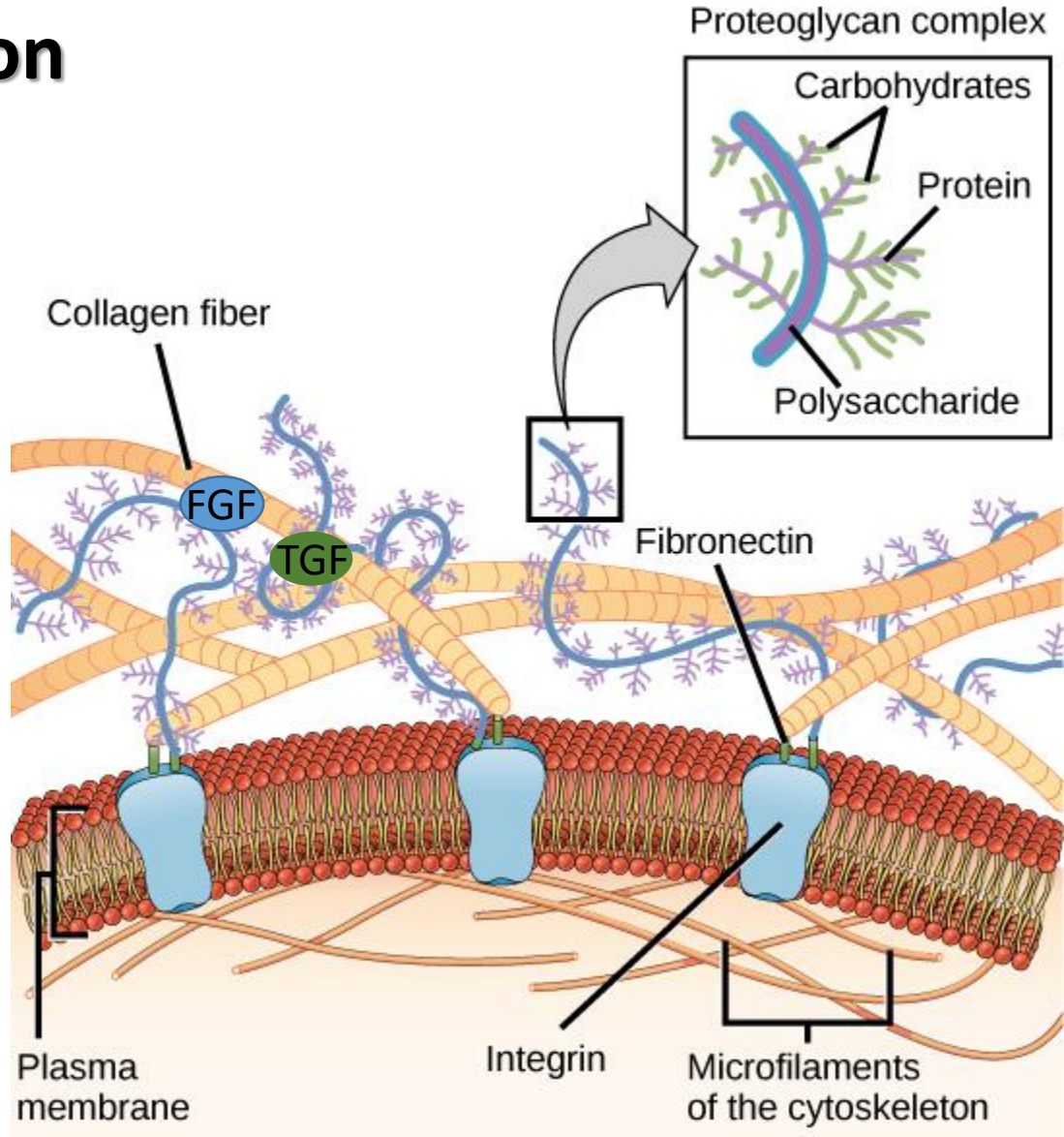
The integrin family of cell adhesion receptors mediates bi-directional signaling:

By '**outside-in**' signalling a cell responds to ligand binding to integrins

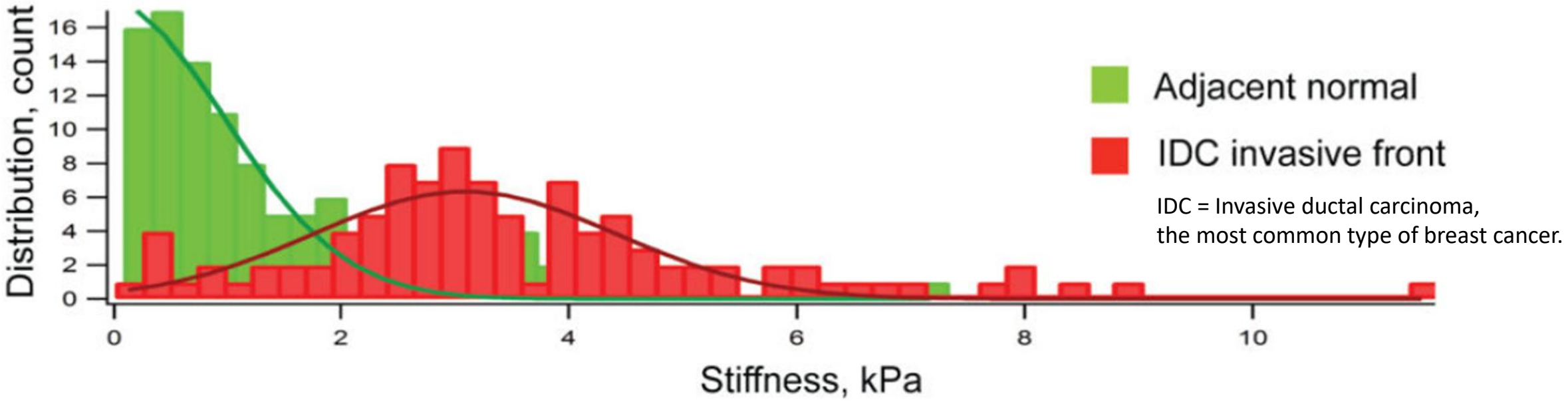
By '**inside-out**' signalling, a cell activates the ligand binding function of integrins

Impact of ECM on cancer progression

- contains deposits of cell-secreted growth factors such as VEGF, FGF, PDGF, TGF-β, that get released during remodeling or wound healing;
- abnormal ECM deposition leads to fibrosis and scaring (stiffness);
- CAFs shape a tumour-associated ECM with more collagen fibers, being stiffer than normal stroma, which promotes cell migration;
- Dense ECM restricts access of immune cells



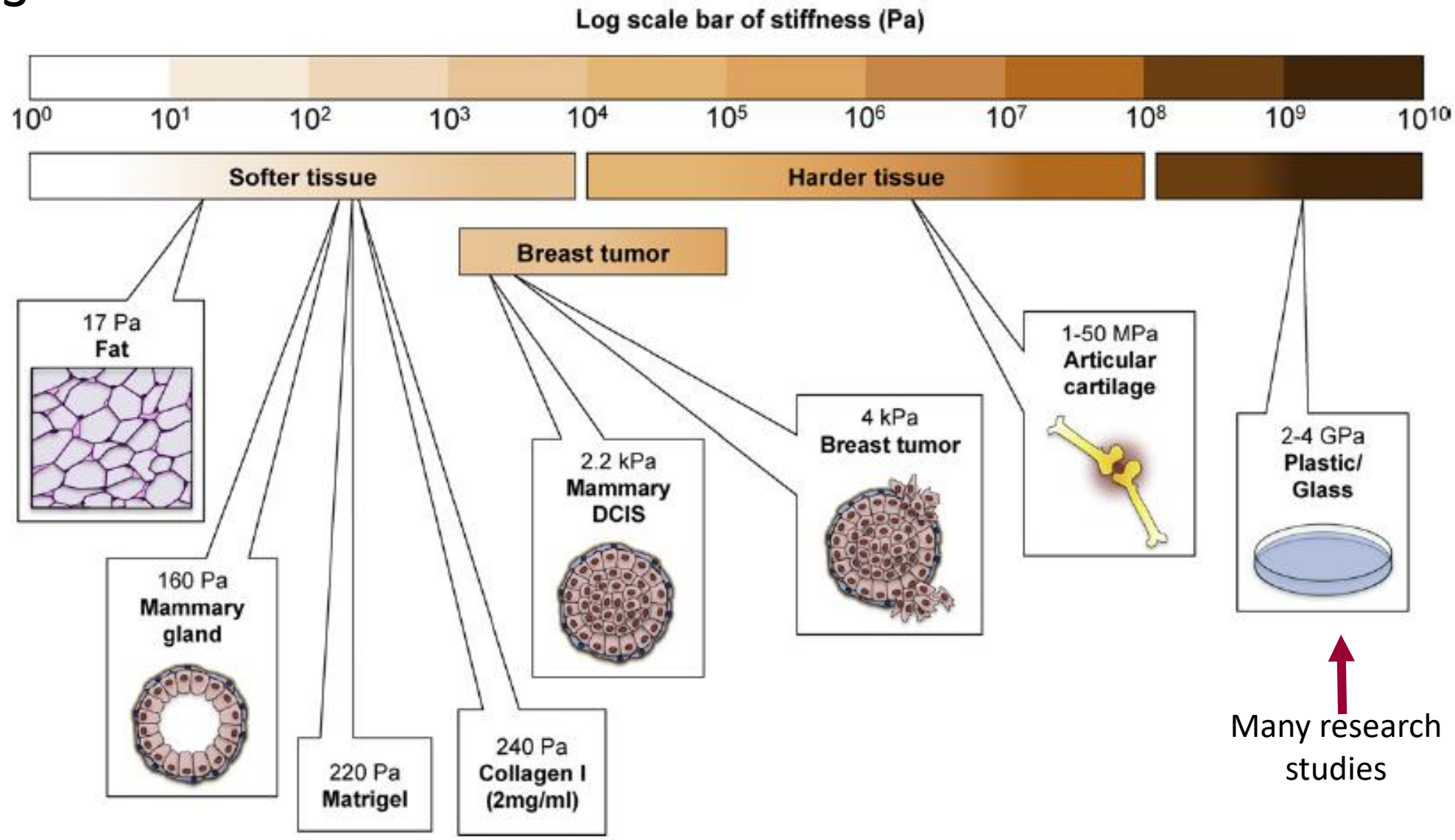
ECM stiffness measured in breast tumour tissue



IDC = Invasive ductal carcinoma,
the most common type of breast cancer.

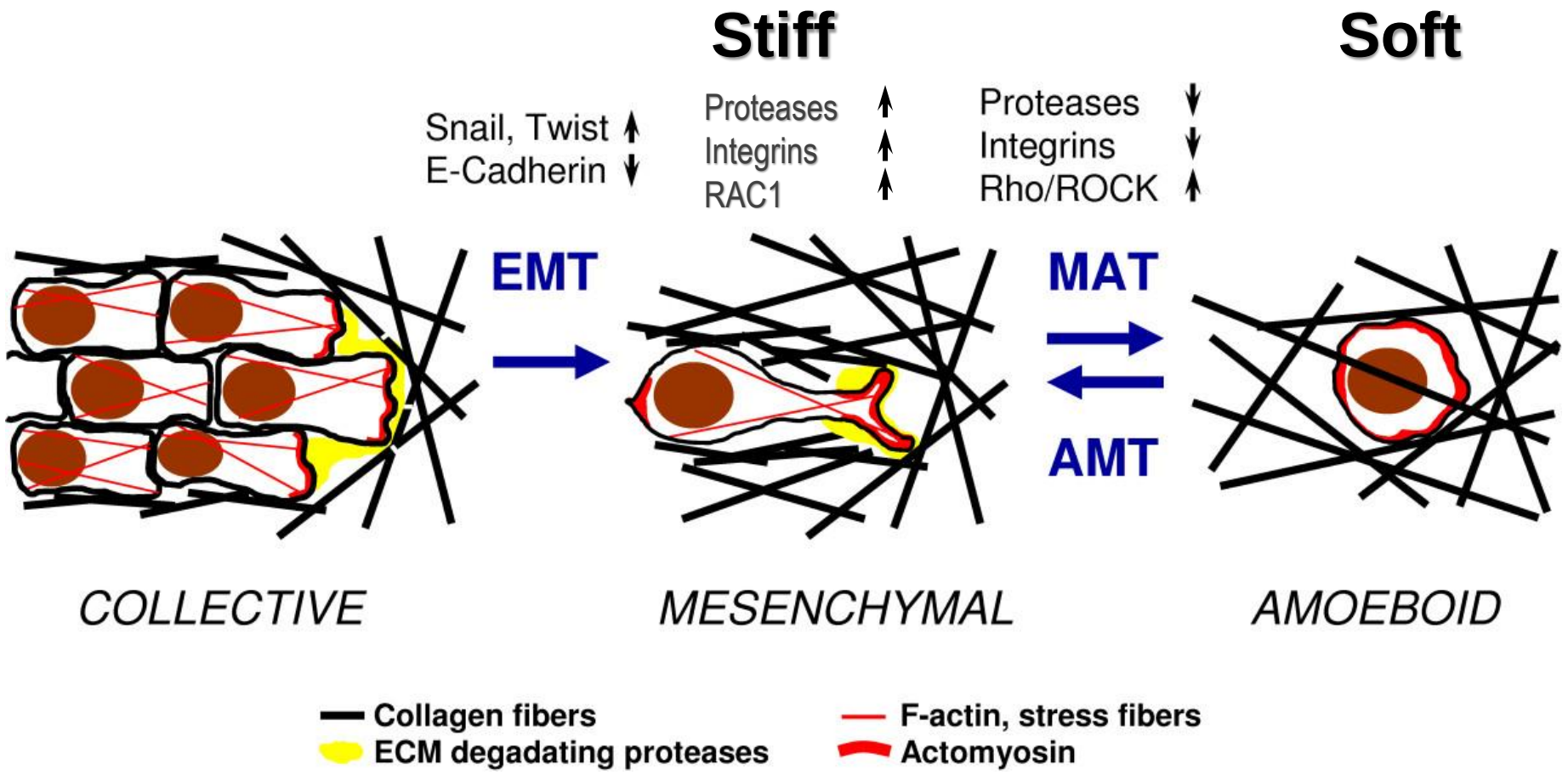
Degree of collagen deposition, with fiber linearization and thickening
DOI:10.1039/C5IB00040H

ECM stiffness

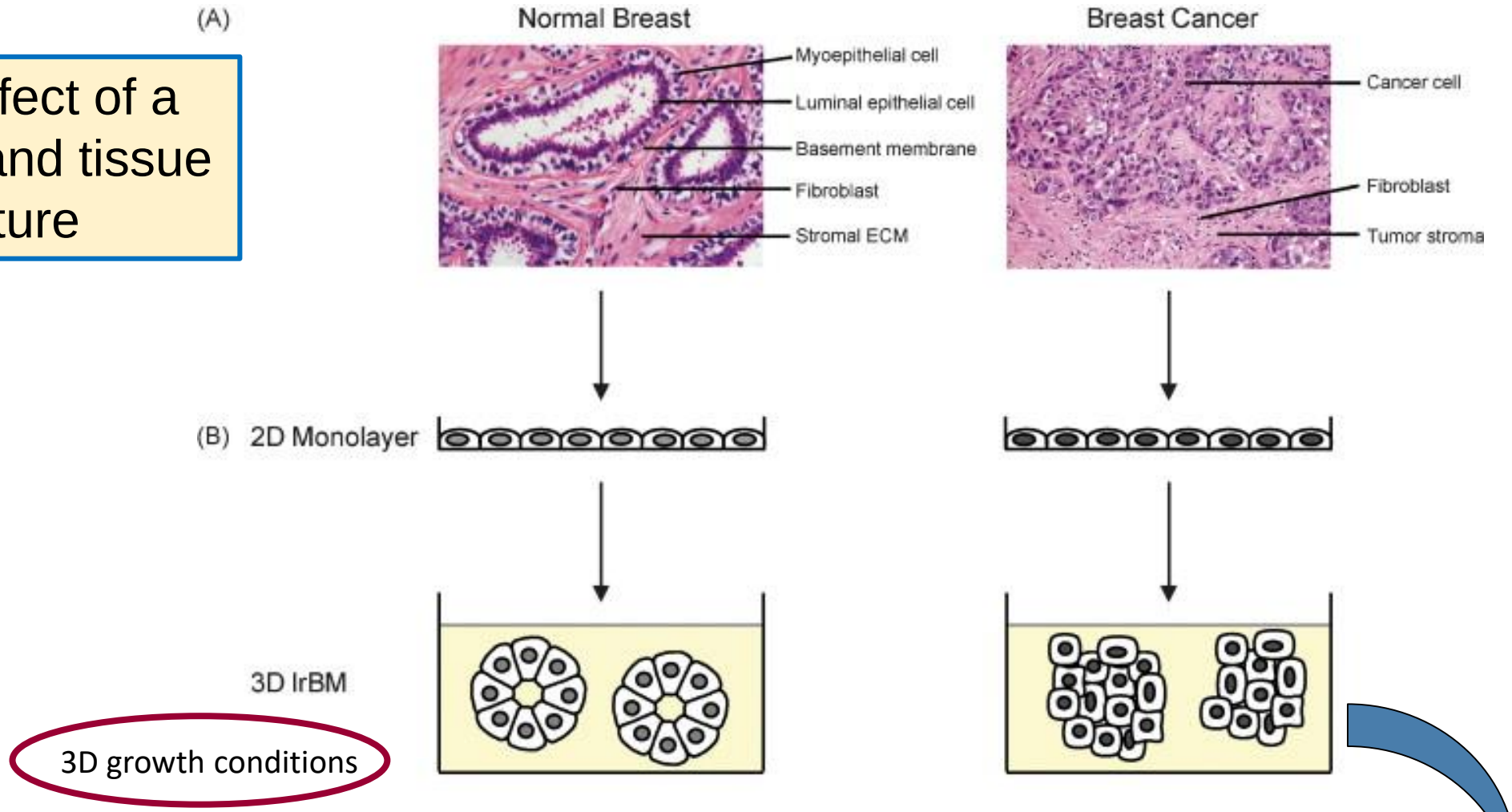


Correia AL, Bissell MJ, (2012) The tumor microenvironment is a dominant force in multidrug resistance. **Drug Resist Updates** 15, 39-49.

Different modes of cell migration depend on ECM stiffness



Protective effect of a normal ECM and tissue architecture



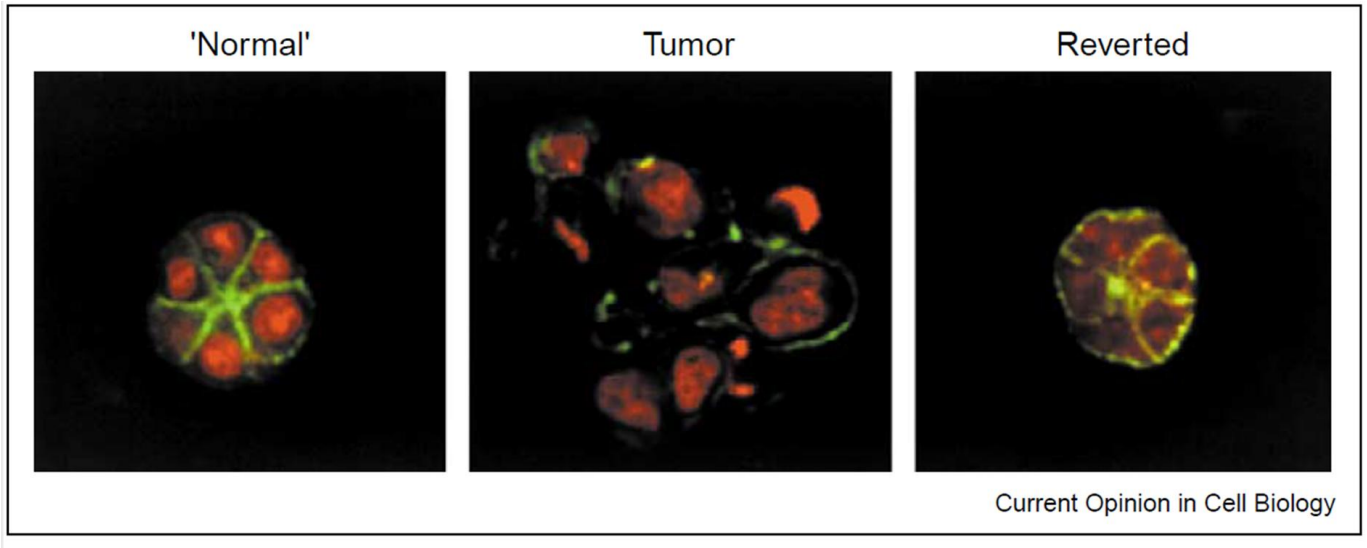
+ anti-β1 integrin
(inhibits adhesion to collagen fibers, 12 d)

Reversion

Despite their mutations..

..cells stop proliferating and differentiate:

(reexpress E-cadherin, reduced EGFR and MAPK activation, decreased cyclin D and increased $p21^{WAF}$expression)



Mina Bissell Doi: 10.1146/annurev.cellbio.22.010305.104315; 10.1016/j.ceb.2003.10.01

The tissue architecture information can be dominant over the malignant cell genotype

"The microenvironment influences gene expression so that the behavior of a cell is largely determined by its interactions with the extracellular matrix, neighboring cells, and soluble local and systemic cues."

What leads to perturbation of normal ECM and tissue architecture?

Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression

Mina J Bissell & William C Hines, doi:10.1038/nm.2328, Nature Medicine 2011

Autopsies on car accident victims (age 40) show the presence of small tumor foci (<1 mm) in:

Thyroide- ~100 % (cancer rate = 0,1 %)

Breast- 39 % at age 40 (rate= 1 %)

Prostate- 34 % at age 40 (rate= 1 %)

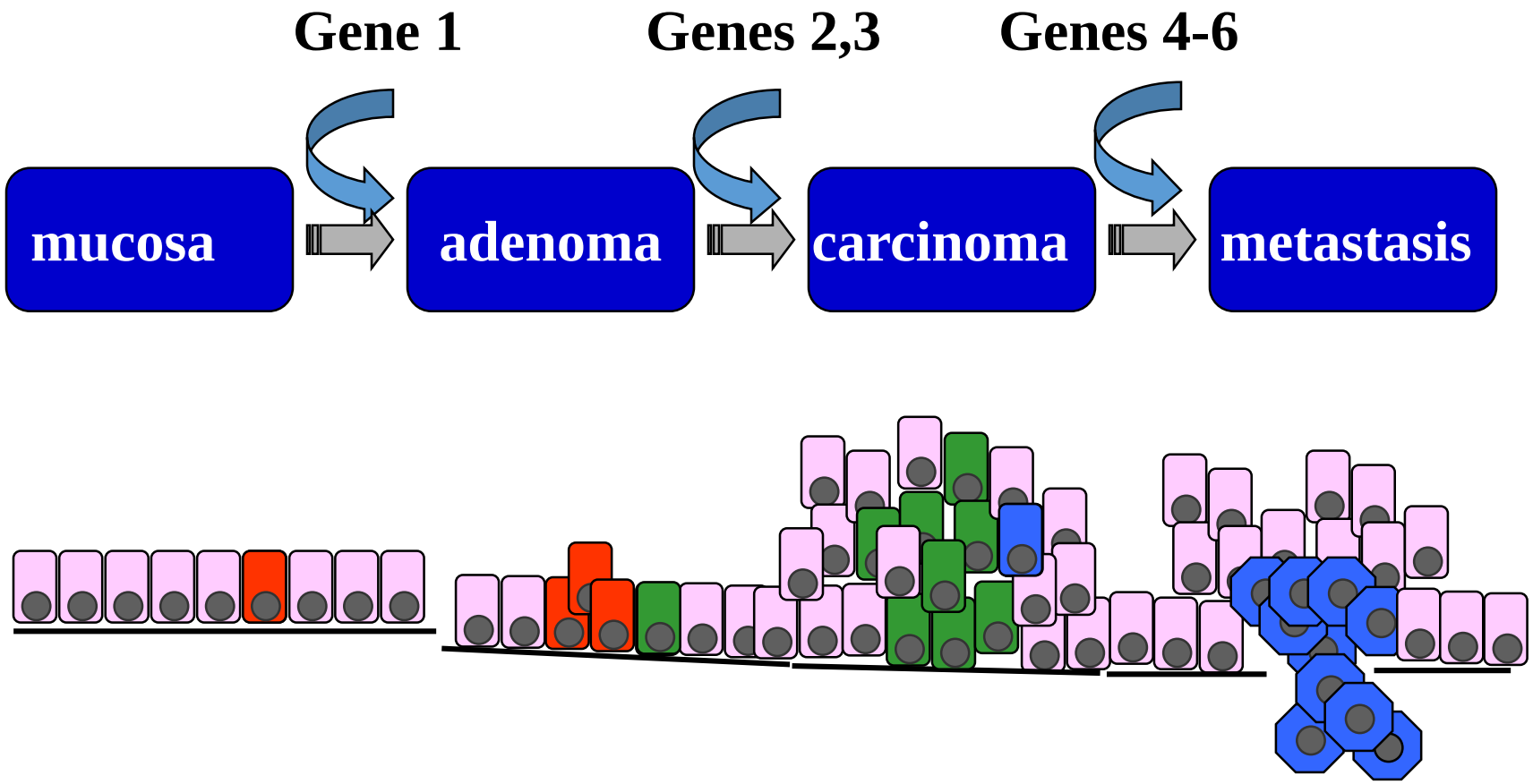
The majority of healthy individuals carry small pre-cancerous foci ("in situ tumors") ……but only a fraction progress to cancer; **Why**???

TME

- Chance and timing of accumulated mutations?
- Angiogenesis inhibition or immune cell fitness?
- Unperturbed tissue architecture (fibrosis, chronic inflammation)?

The classical model of *clonal selection:*

a cancer cell-centered reductionism



Accumulation of genetic changes in a cell

Stroma and tumour cells in the TME

1. **Endothelial cells**

Normal blood vessels

2. **Tumour cells**

3. **Fibroblasts**

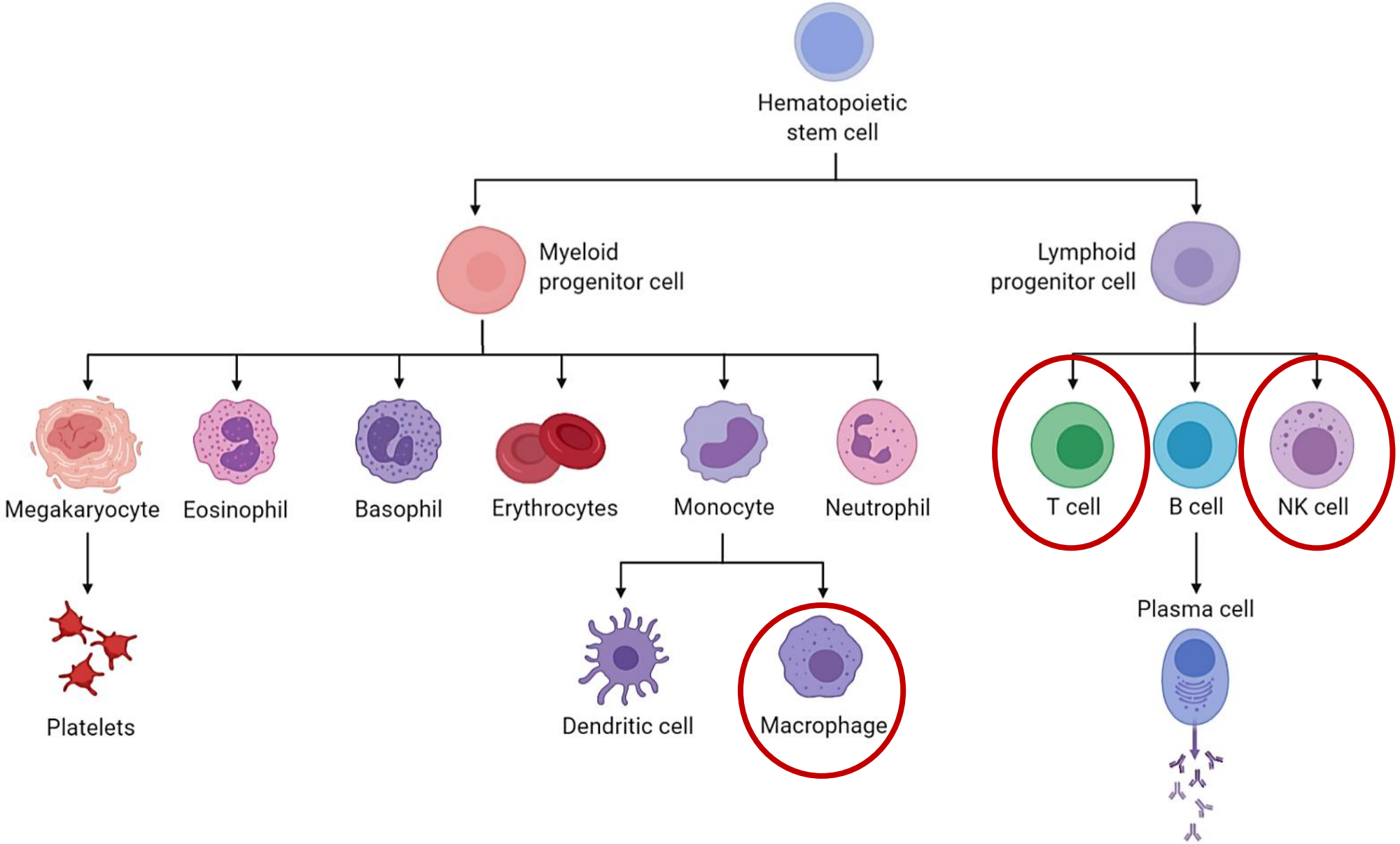
Fibroblast

4. **Immune cells and inflammation**

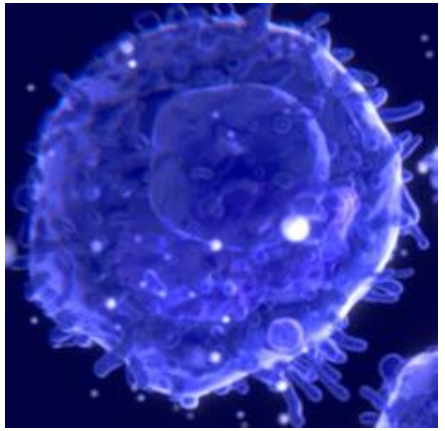
Monocyte

T_{reg}

Diagram of the different lineages of hematopoietic cell differentiation that give rise to the immune cells found in the tumor microenvironment



Main types of tumour-infiltrating immune cells



1) T-lymphocytes

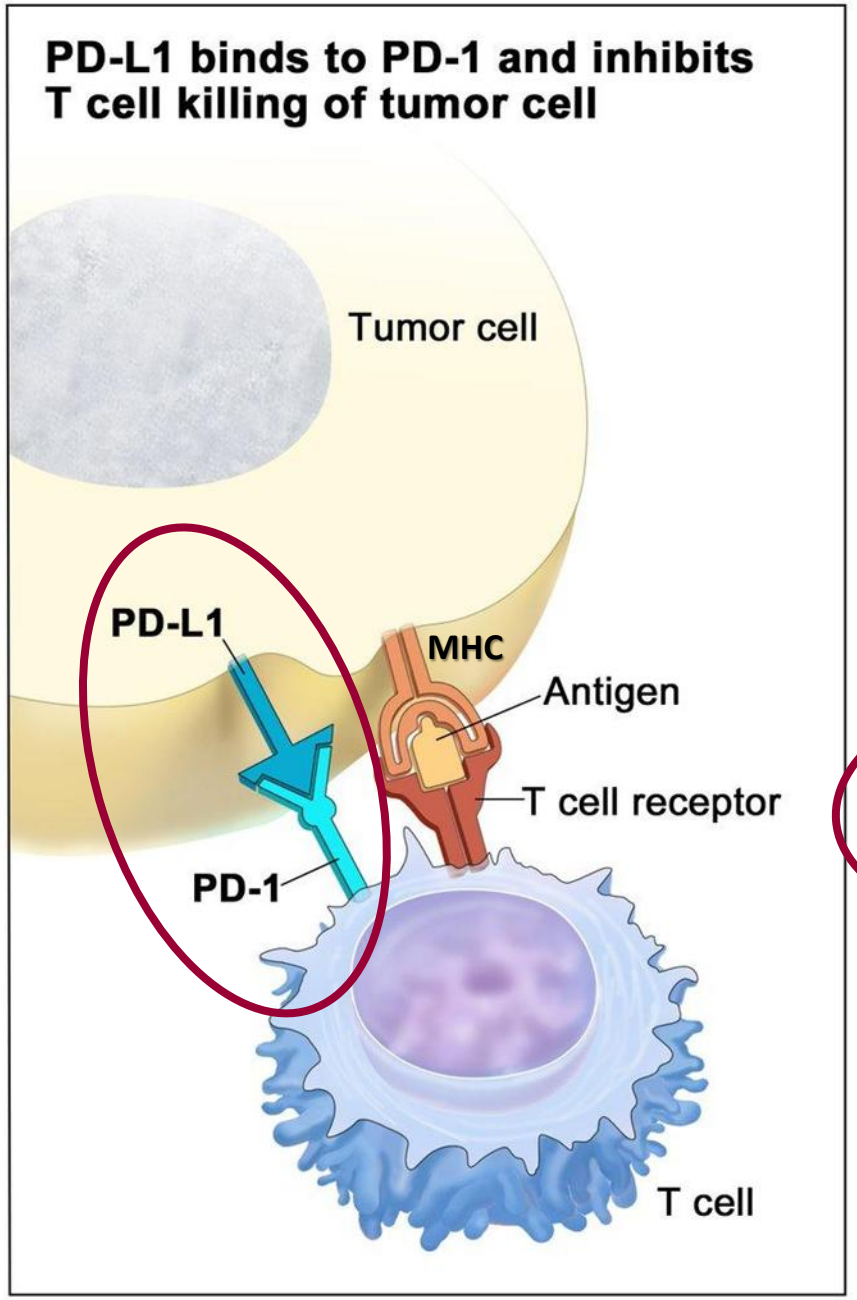
- Lymphoid-derived lineage; non-adherent cells
- **CD8+** T cells: cytotoxic cells that induce cell death in virus-infected or tumour cells, either by granular exocytosis or apoptosis induction; TCR recognizes non-self peptides bound to MHC molecules on target cell surface;
- **CD4+** T helper cells (Th cells) act in adaptive immune response, aiding the activity of other immune cells by releasing cytokines;
- T regulatory cells (**Treg**) modulate the activity of other immune cells, promote tolerance to self-antigens (peripheral tolerance) and prevent excessive immune reaction;

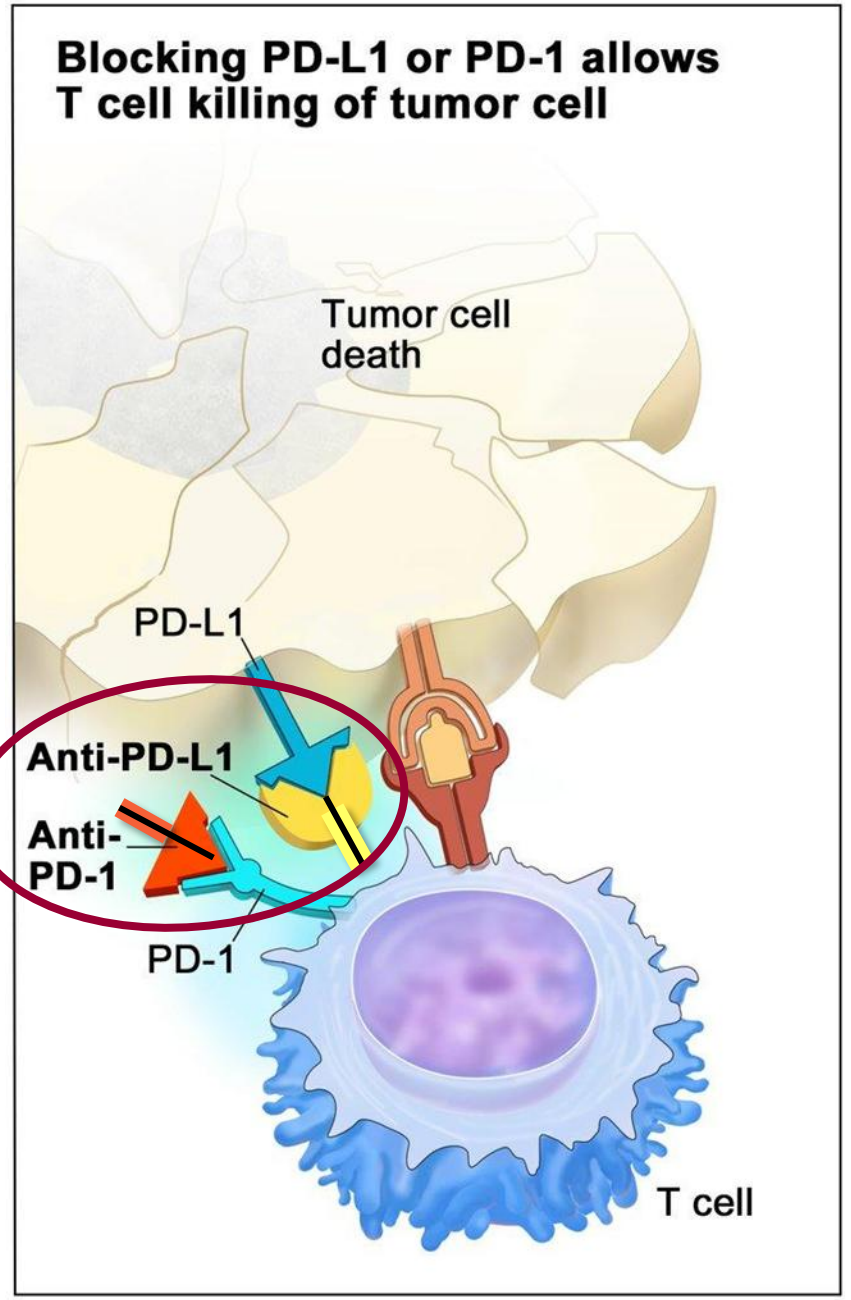
Main impact on cancer development

- Successful activation of CD8+ T cells can lead to tumour cell killing;
- Treg cells can suppress anti-cancer immunity;
- Cancer cells express ligands for immunosuppressive T cell surface proteins, such as CTLA-4 or PD1, that inhibit CD8+-T cell activation (**immune checkpoint molecules**)

Immune checkpoint inhibitor therapy

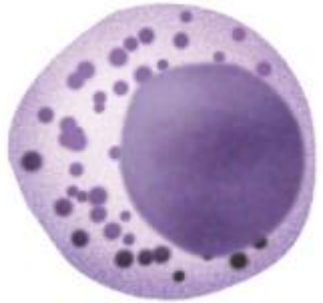
Immune escape





PD-1= programmed death-1
CTLA-4= cytotoxic T-lymphocyte-associated protein-4

Main types of tumour-infiltrating immune cells



2) NK cells

- Innate lymphoid-derived lineage; specialized immune effector cells for MHC-independent immunosurveillance;
- **cytotoxic cells** that induce cell death by exocytosis of perforin and granzymes granules, or extrinsic apoptosis-inducing death ligands (TNFα, FasL, TRAIL);
- recognize target cells through reduced MHC expression or stress;
- relatively low abundance;
- cytokine and chemokine production to promote maturation and activation of macrophages and T cells

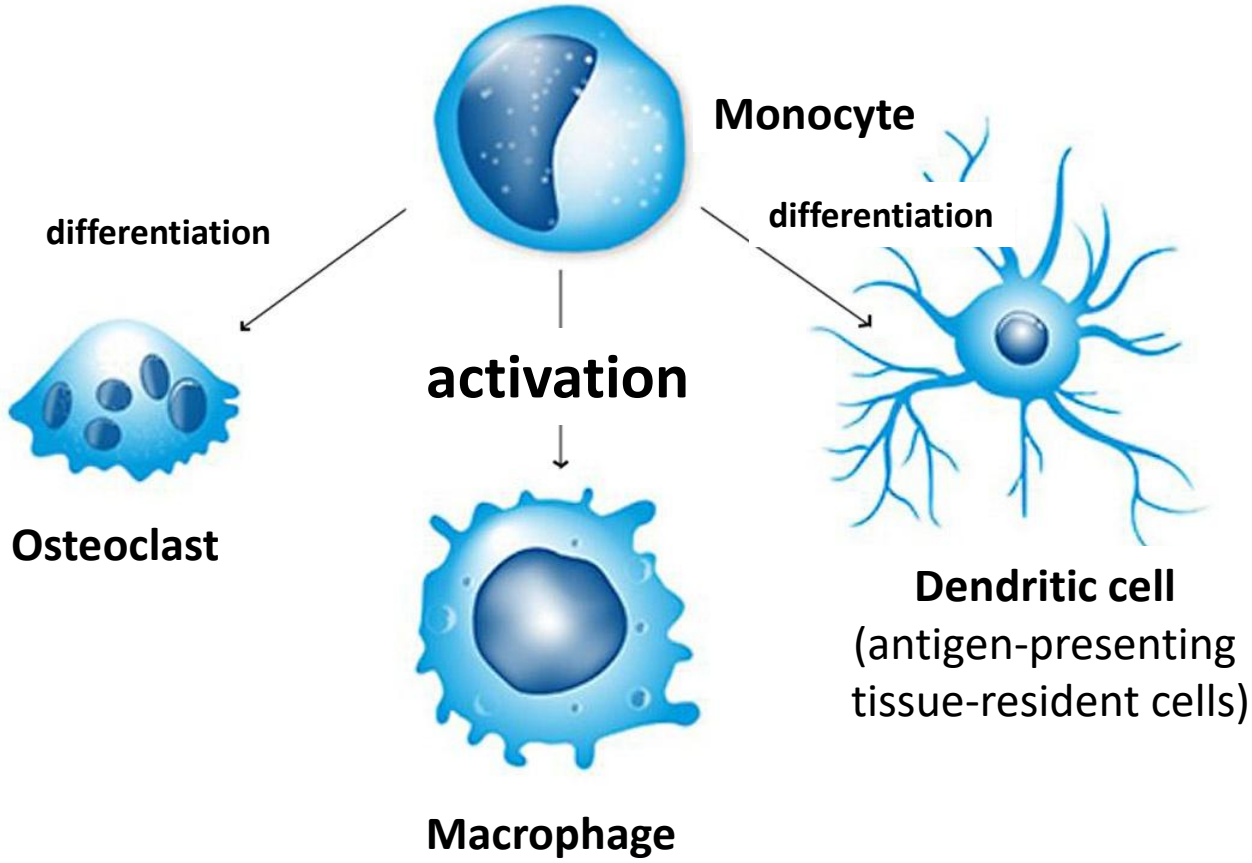
Main impact on cancer development

- Successful activation of NK cells would lead to tumour cell killing;
- Function suppressed owing to multiple immunosuppressive factors in the tumor microenvironment;

Main types of tumour-infiltrating immune cells

3) Macrophages

- Myeloid progenitor-derived tissue sentinels that maintain tissue integrity;
- Phagocytotic cells that eliminate bacteria, or damaged cells or matrix components during tissue repair
- Activated by cytokines, bacterial lipopolysaccharide, extracellular matrix debris, or by tumour cells



Main types of tumour-infiltrating immune cells

Macrophages

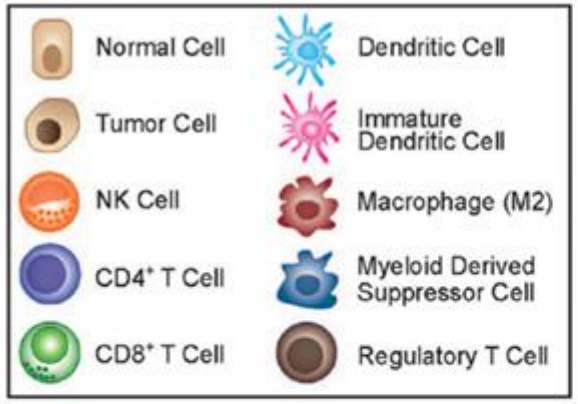
Tumour-Associated Macrophages are of two main subtypes:

- **M1** or classical macrophages; activated by LPS and/or interferon gamma; have a pro-inflammatory phenotype with pathogen-killing abilities; produce cytokines such as IL-6 and tumor necrosis factor;
- **M2** or alternative macrophage; are immunosuppressive via IL-10 secretion. activated by IL-4 and lactate; promote cell proliferation and produce TGF-β to start tissue repair (EMT);

Main impact on cancer development

- M1 create a pro-inflammatory TME that promotes tumour cell survival, but can produce anti-tumorigenic tumor necrosis factor;
- M2 are immunosuppressive and thus promote escape of tumour cells and malignant progression

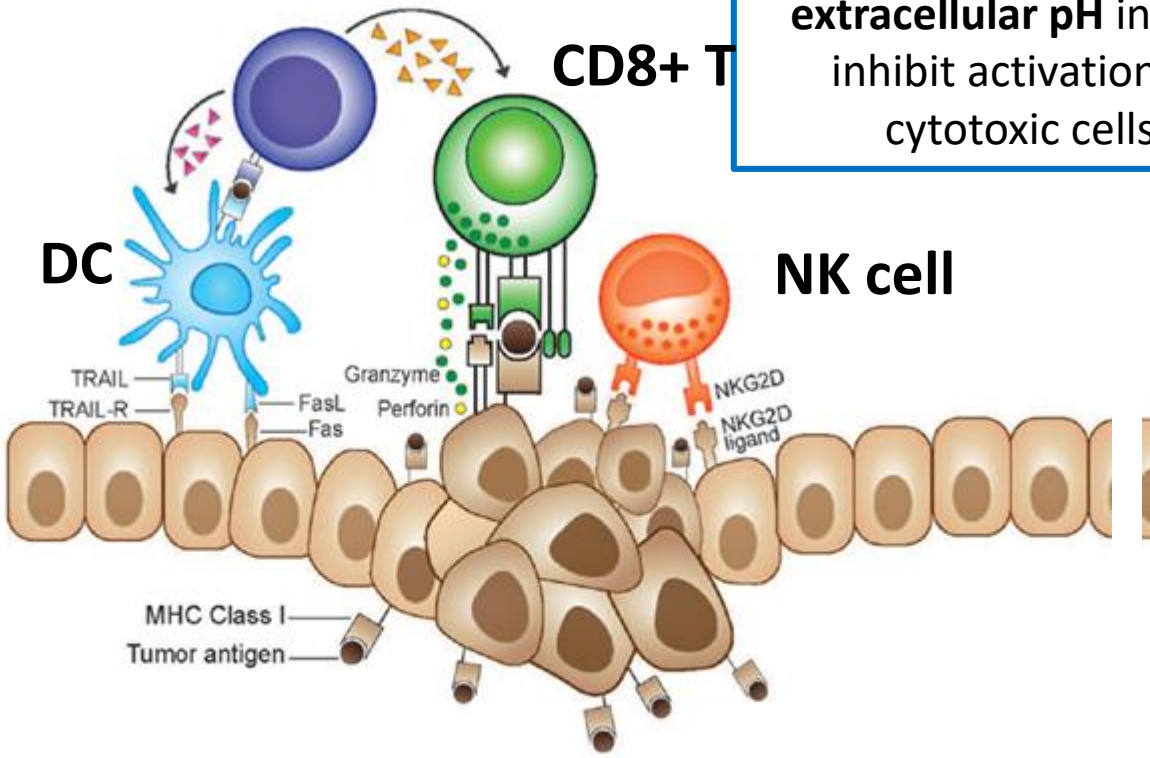
Immune tumour microenvironment

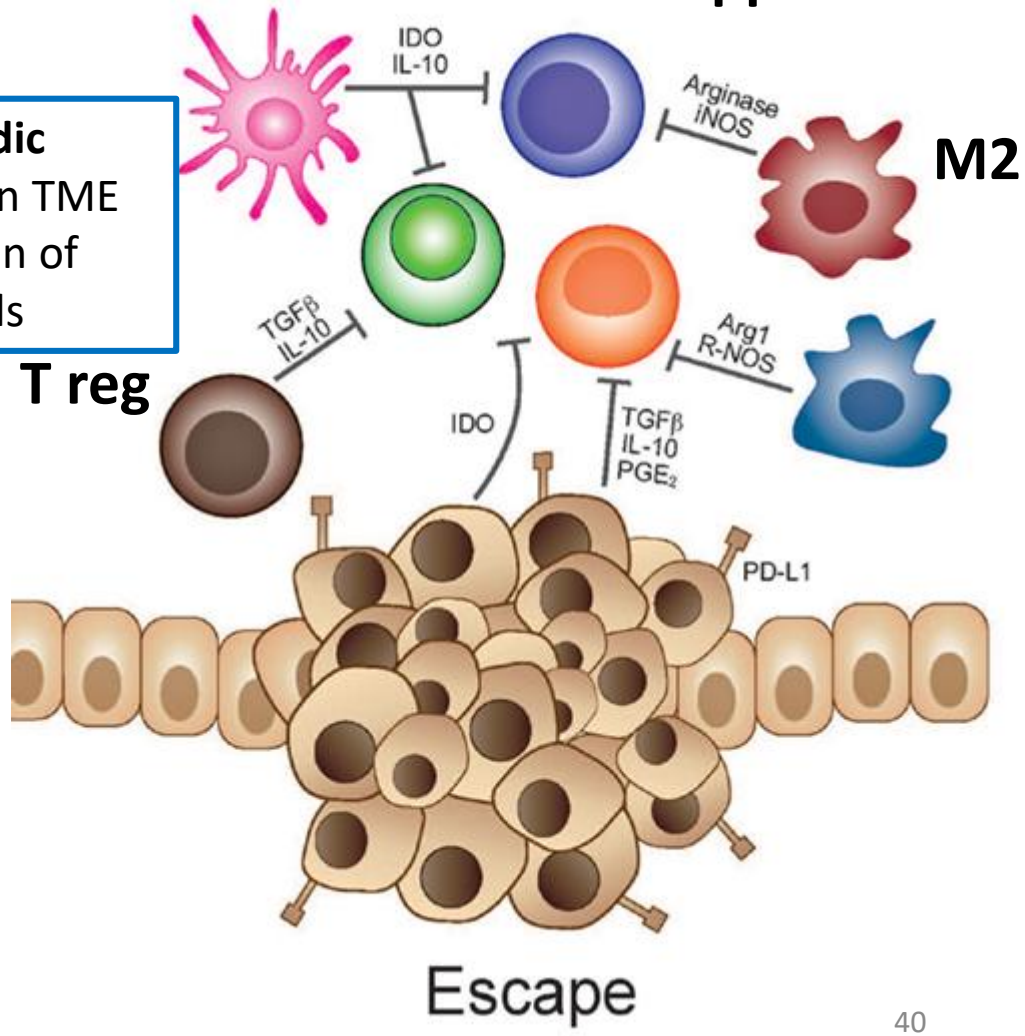


Immune competent

Immune suppressive

Lactate & acidic extracellular pH in TME inhibit activation of cytotoxic cells





Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression

The majority of healthy individuals carry small pre-cancerous foci ("in situ tumors")
……but only a fraction progress to cancer; **Why**???

TME

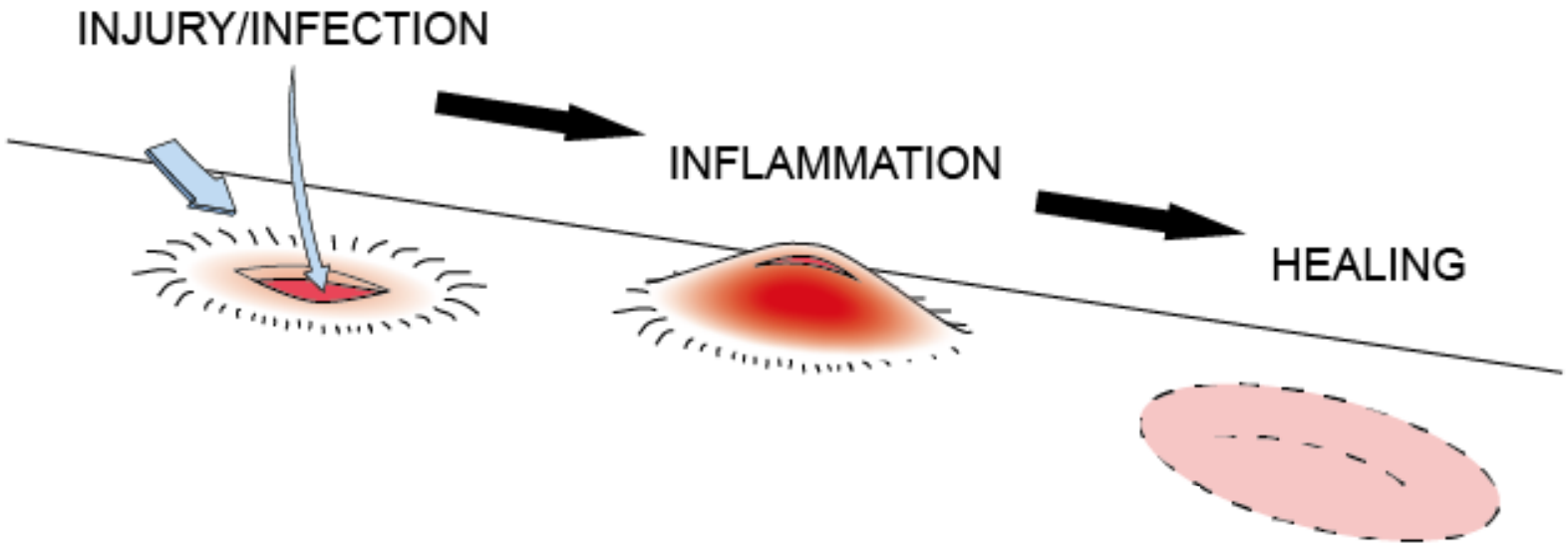
- Chance and timing of accumulated mutations?
- Angiogenesis inhibition or immune cell fitness?
- Unperturbed tissue architecture (fibrosis?, chronic inflammation)?

What is the effect of inflammation on ECM and tissue architecture ??

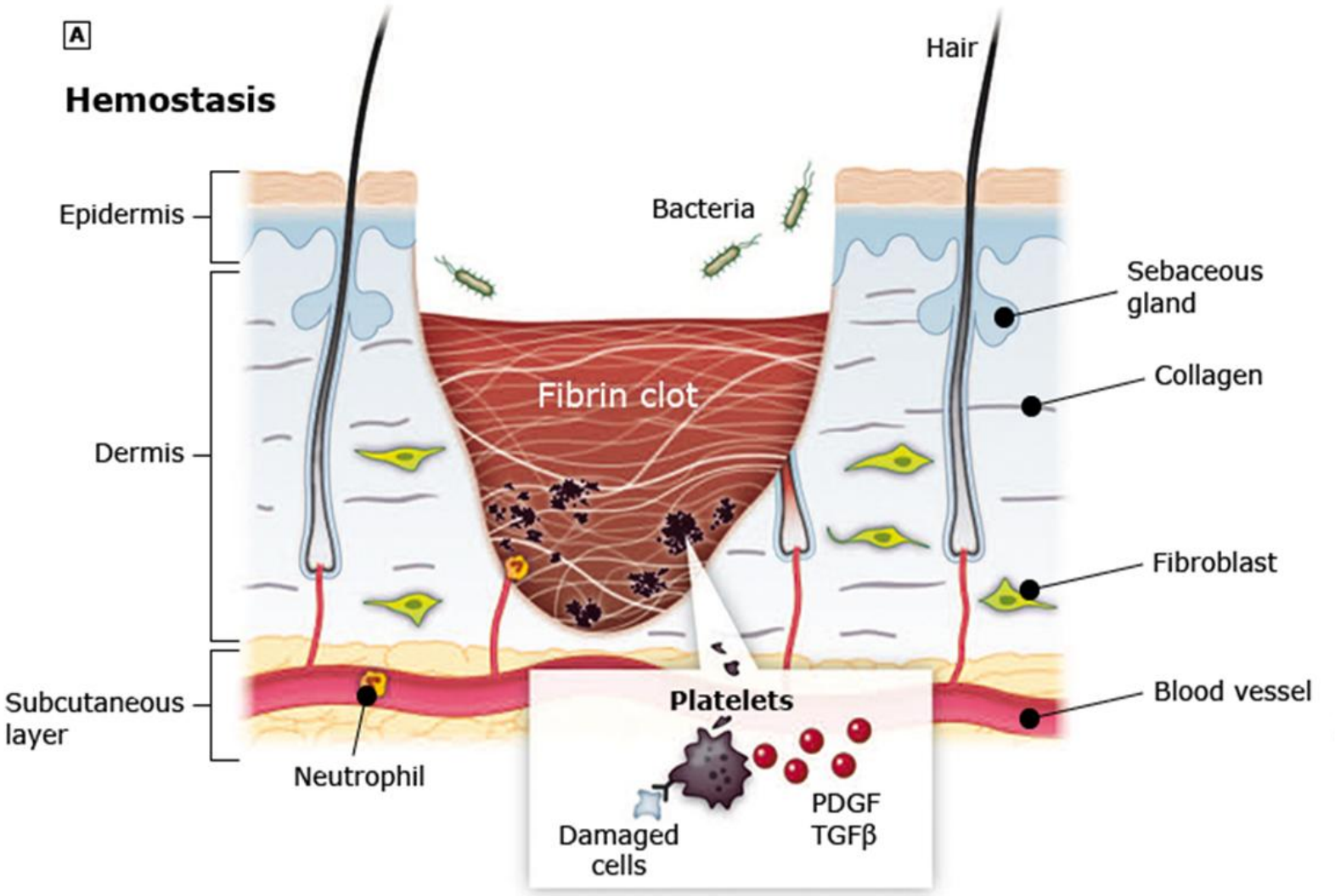
Cancer and inflammation

Tumours are *wounds that do not heal*

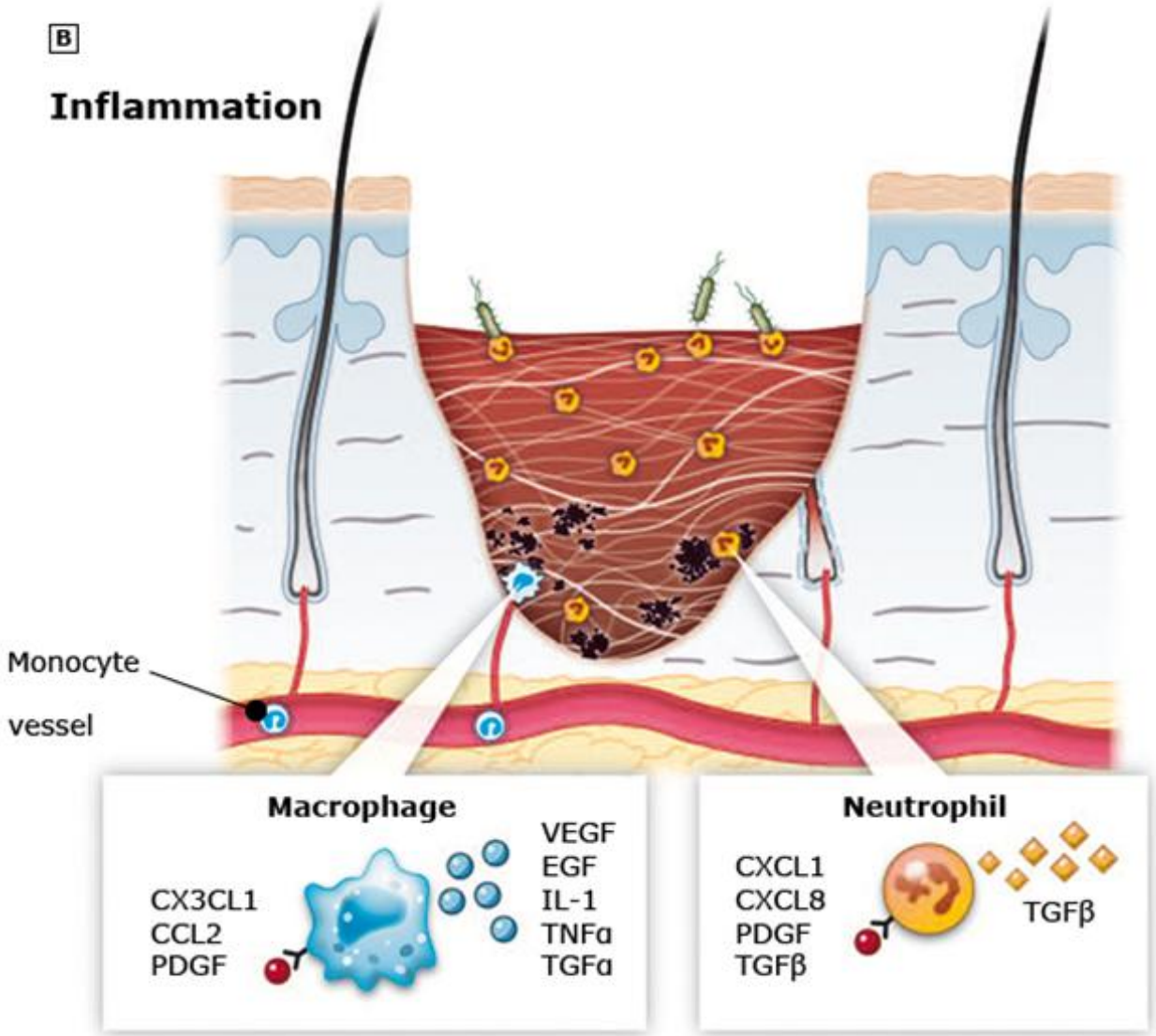
Virchow 1863



The different phases of normal inflammation



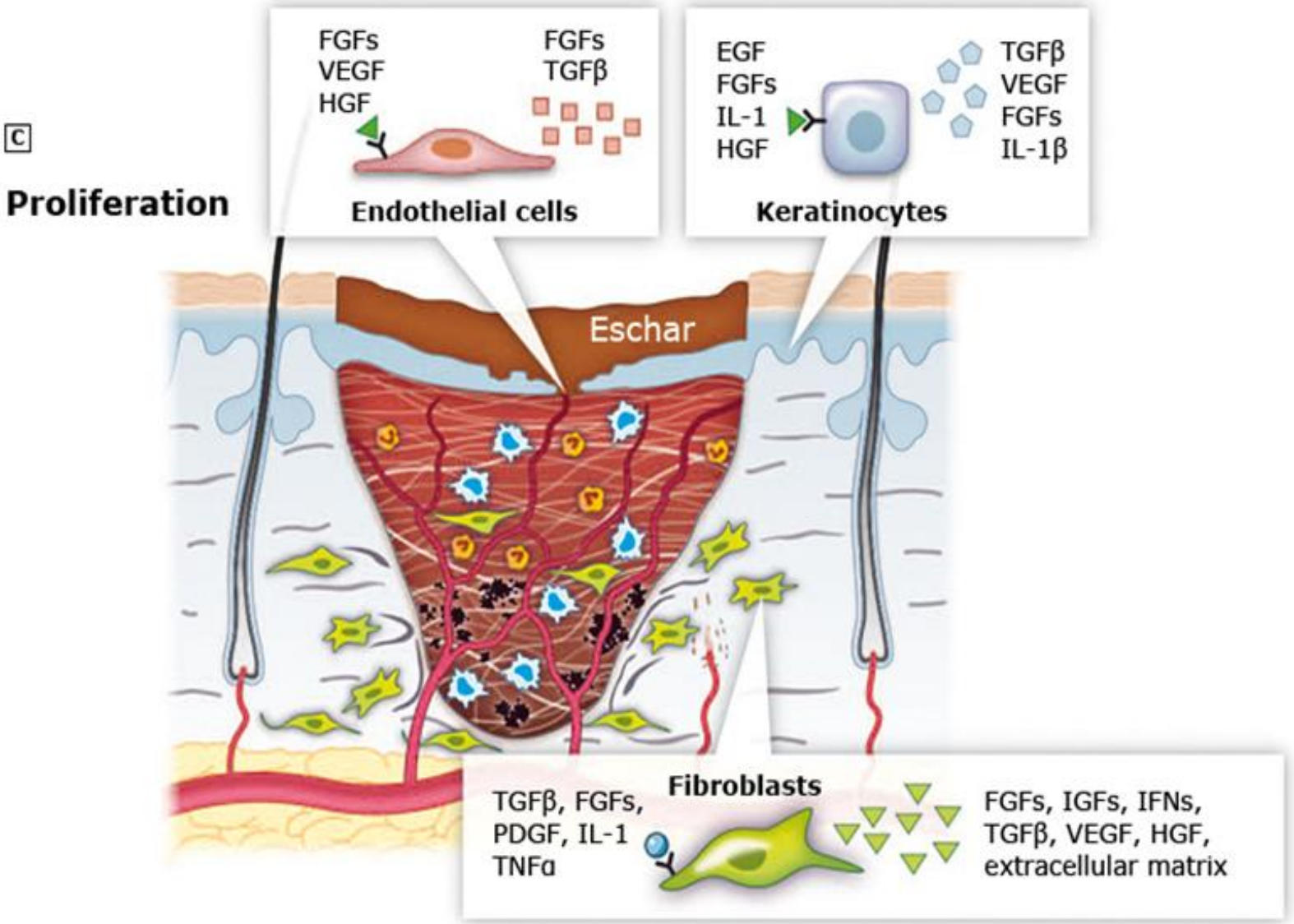
- Wounded tissue releases pro-inflammatory growth factors from the matrix and platelets (TGF-β, PDGF, FGF);
- Once bleeding is controlled by the fibrin clot, neutrophils, macrophages, and lymphocytes migrate into the wound by chemotaxis;



- Macrophages clear debris;
- Macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes;
- Neutrophils clear invading microbes;
- Hypoxia stimulates macrophages to secrete VEGF;
- Increased permeability of adjacent blood vessels;

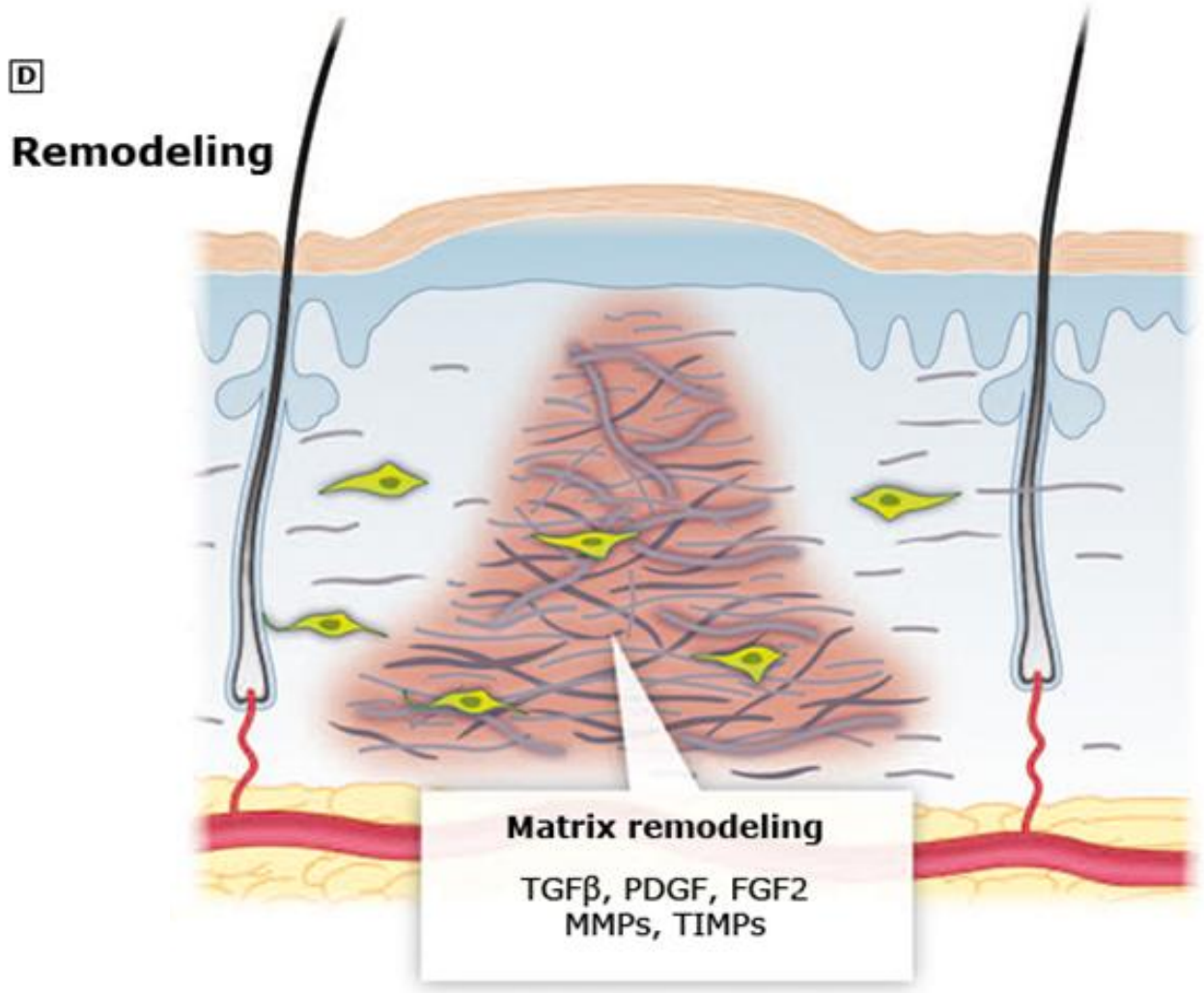
<u>Characteristics of inflammation:</u>

- Rubor: redness
- Tumor: swelling
- Calor: heat
- Dolor: pain
- Functio laesa: loss of function



- Overlaps with the inflammation phase;
- Promotes angiogenesis for re-vascularization;
- Fibroblasts produce MMPs, then start producing collagen and major components of the extracellular matrix (ECM) (provisional matrix);

- Secreted growth factors stimulate epithelial cell proliferation, EMT and migration;
- Downregulation of inflammatory cells and transition to an immuno-suppressive environment



- ECM remodeling to restore an architecture that approaches that of the normal tissue;
- Regression of many of the newly formed capillaries and consolidation of vasculature;
- Contractile myo-fibroblasts help stabilize the remodeled wound area;

Final outcome:

1. Regeneration (complete reconstitution of lost or damaged tissue and cells)
2. Repair (restores function but original structure is incomplete (scar formation; fibrosis)

Summary: Normal Wound-healing Process

Phase	Cellular and Bio-physiologic Events
Hemostasis	1. vascular constriction 2. platelet aggregation, degranulation, and fibrin formation (thrombus)
Inflammation	1. neutrophil infiltration 2. monocyte infiltration and differentiation to macrophage 3. lymphocyte infiltration
Proliferation	1. re-epithelialization 2. angiogenesis 3. collagen synthesis 4. ECM formation
Remodeling	1. collagen remodeling 2. vascular maturation and regression

tumour-promoting phases

growth and survival factors,
angiogenesis,
ECM remodelling

Inflammation: Acute, chronic, subclinical

Acute= wounds, infection

Chronic conditions:

Agent/induction process	**Type of Cancer**
Helicobacter pylori (bacteria)	gastric mucosa
Inflammatory bowel disease	colon
Prostatitis	prostate
Viral hepatitis B and C	liver
Acid reflux, alcohol	esophagus
Tobacco, asbestos	lung
Chronic Pancreatitis	pancreas

General consequences:

- Constant cell proliferation,
- Loss of tissue architecture,
- Oxidative stress

Inflammation as a tumour promoter

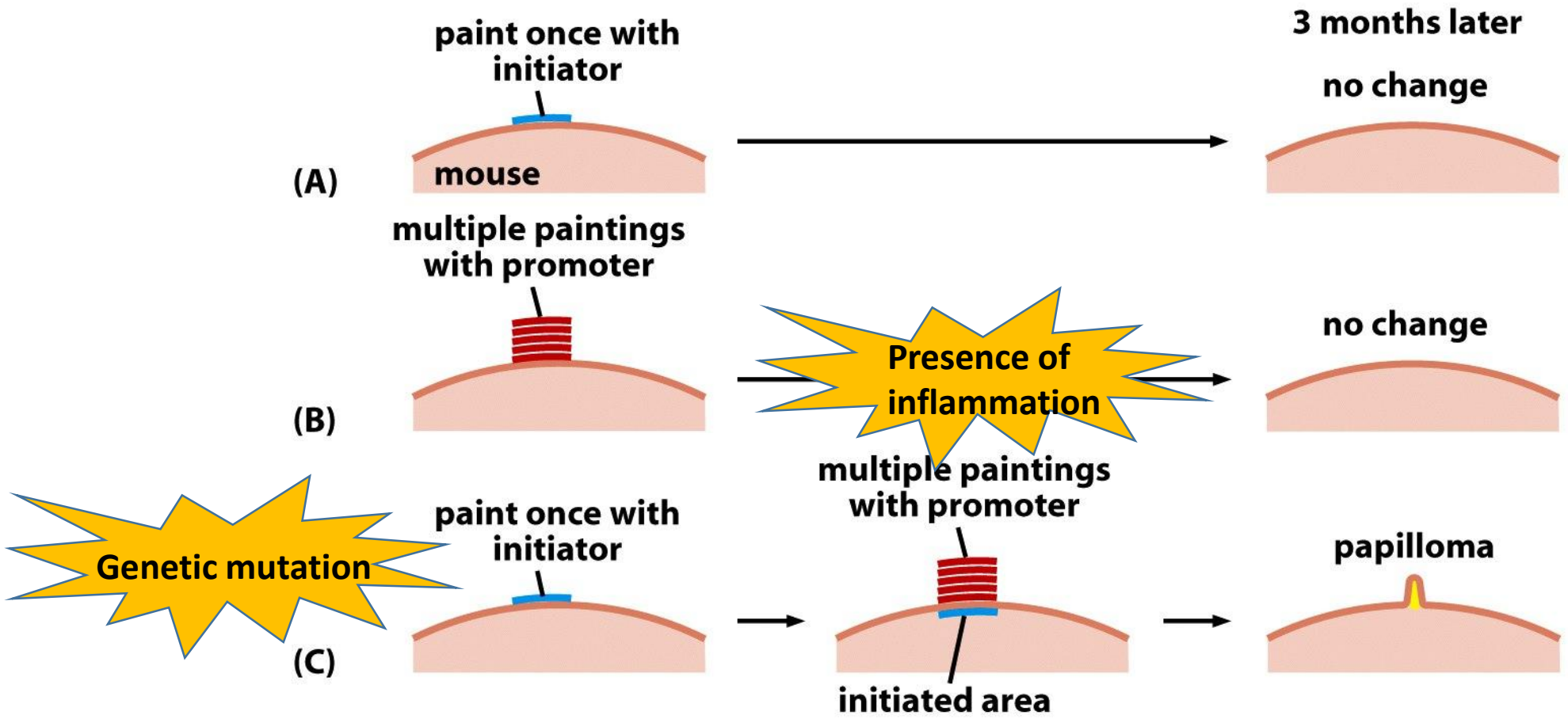
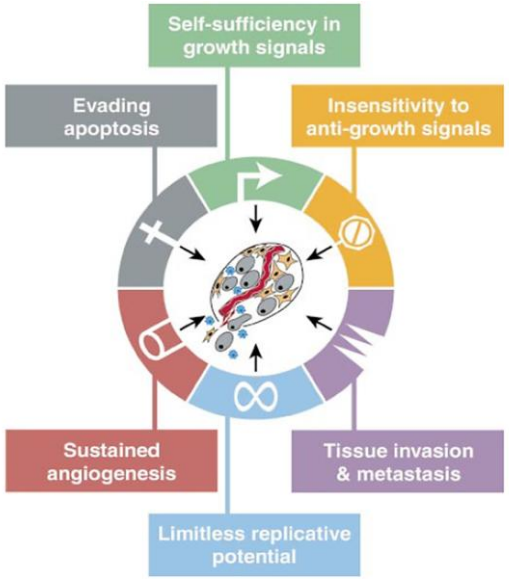
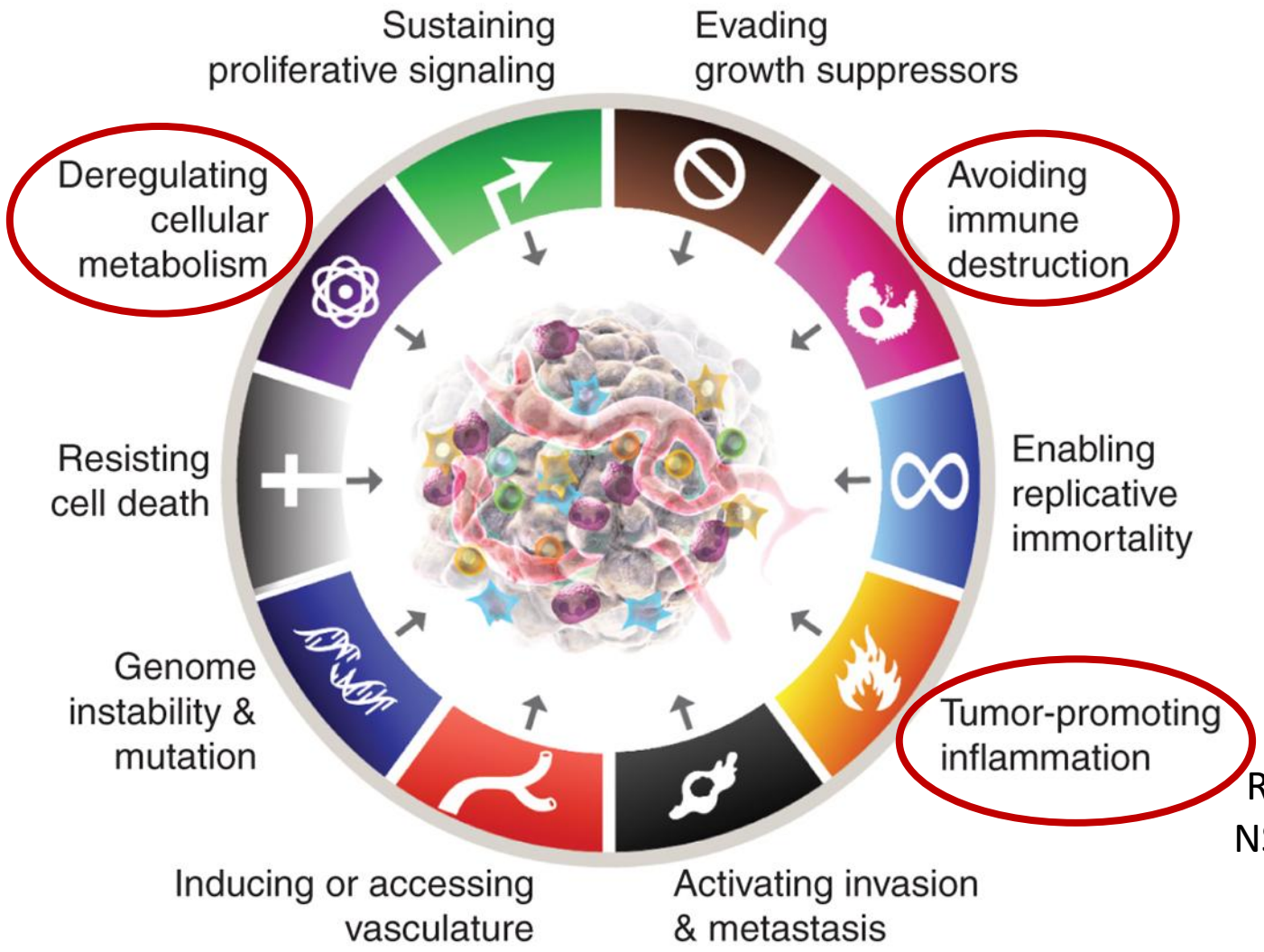


Figure 11.28 part 1 of 2 *The Biology of Cancer* (© Garland Science 2007)



Hanahan D, Weinberg RA (**2000**) , Cell 100, 57–70

Revised *Hallmarks of Cancer*



Regular, long-term NSAID consumption reduces cancer incidence

Hanahan D, Weinberg RA (**2011**), Cell 144, 646-674.

Life style factors

…a cause for subclinical inflammation

Recent review (moodle pdf):
Metabolic interplays between the tumour and the host shape the tumour macroenvironment.
Nat Rev Cancer 25, 274–292 (2025). https://doi.org/10.1038/s41568-024-00786-4

Life style factors and inflammation

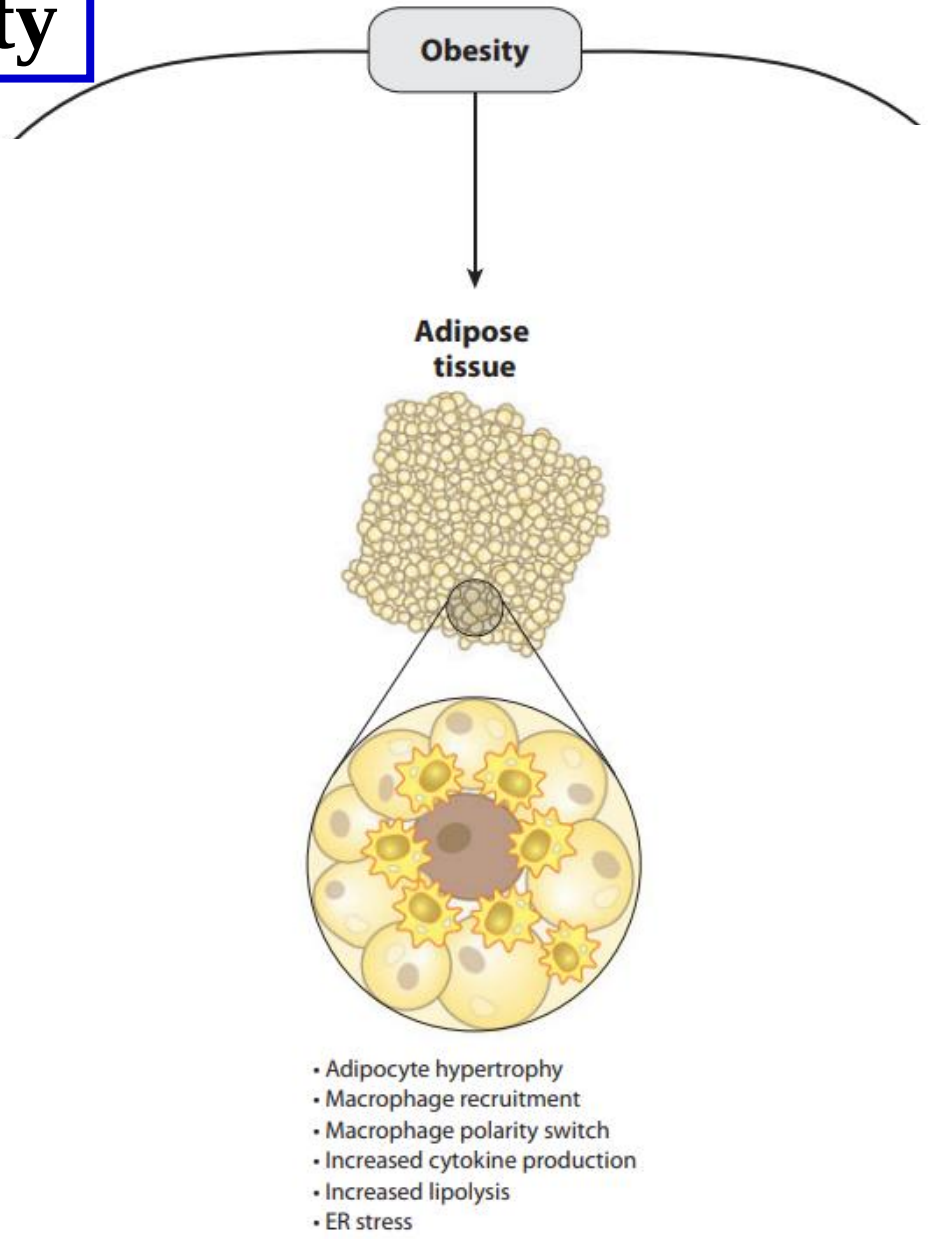
Low-level, subclinical inflammatory conditions accompany many diseases of industrialized countries: obesity, metabolic syndrome, type 2 diabetes, atherosclerosis, neurodegenerative diseases, and **cancer**:

- Tobacco= lung inflammation (besides systemic effect through oxidative stress and DNA adducts from alkylating carcinogens)
- Environment= asbestos and lung mesothelioma

➢ Obesity

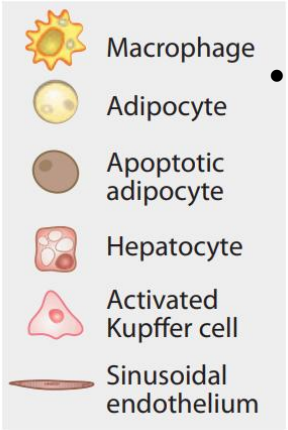
➢ Diet (fat, protein, fibers; *H. pylori* infection)

Obesity



10.1146/annurev-physiol-021909-135846

- Adipocyte hypertrophy facilitates physical cell rupture, which evokes an inflammatory reaction;
- Adipose tissue from obese mice and humans is infiltrated with increased numbers of M1-type macrophages as a major source of pro-inflammatory cytokines;



- Fat deposition in other organs due to inability of adipose tissue to engulf all incoming fat:

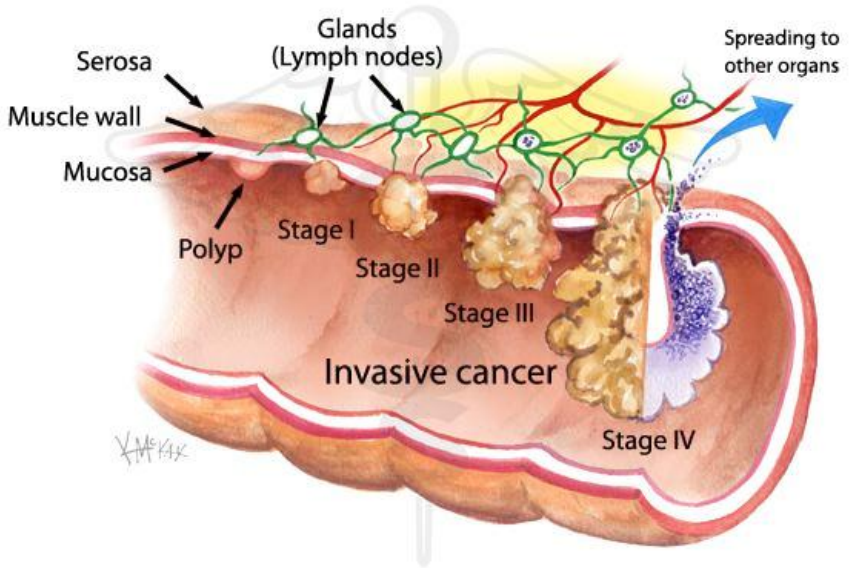
- liver: increasing insulin resistance and leading to increased blood glucose levels that favour tumor cell metabolism

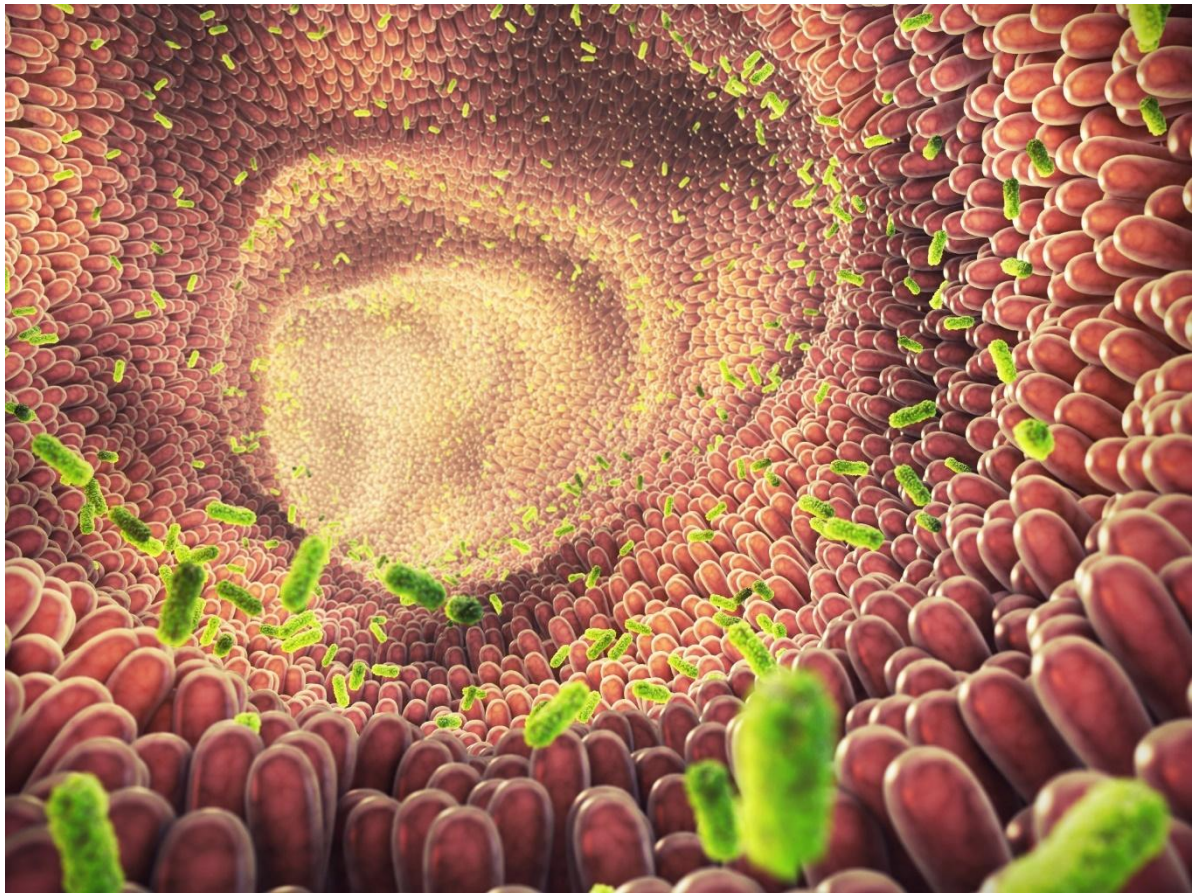
Cancer-promotimg local inflammation

- visceral fat surrounding intestines;

- subcutaneous fat in skin or breast tissue

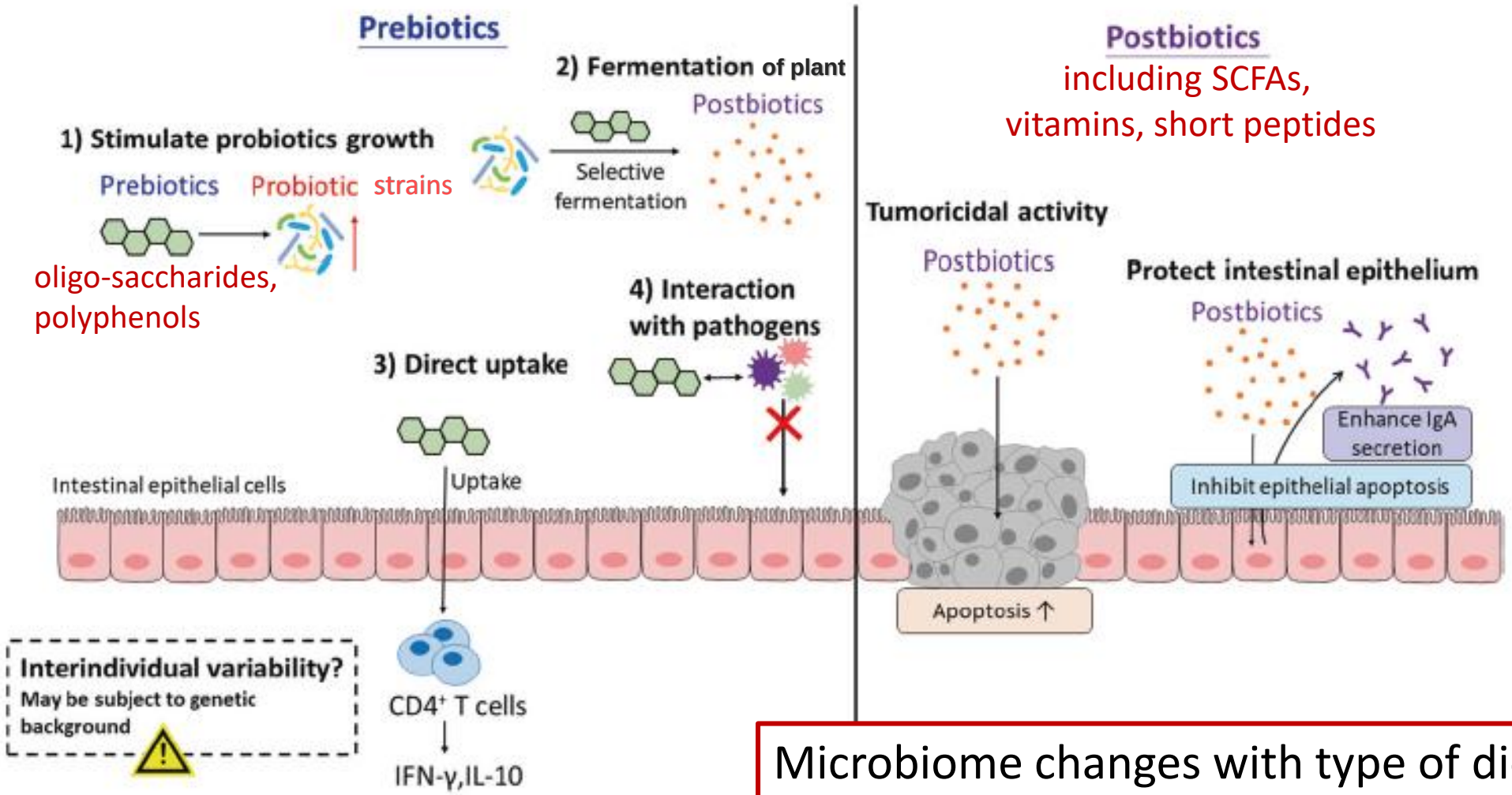
Diet, the gut … and its inhabitants





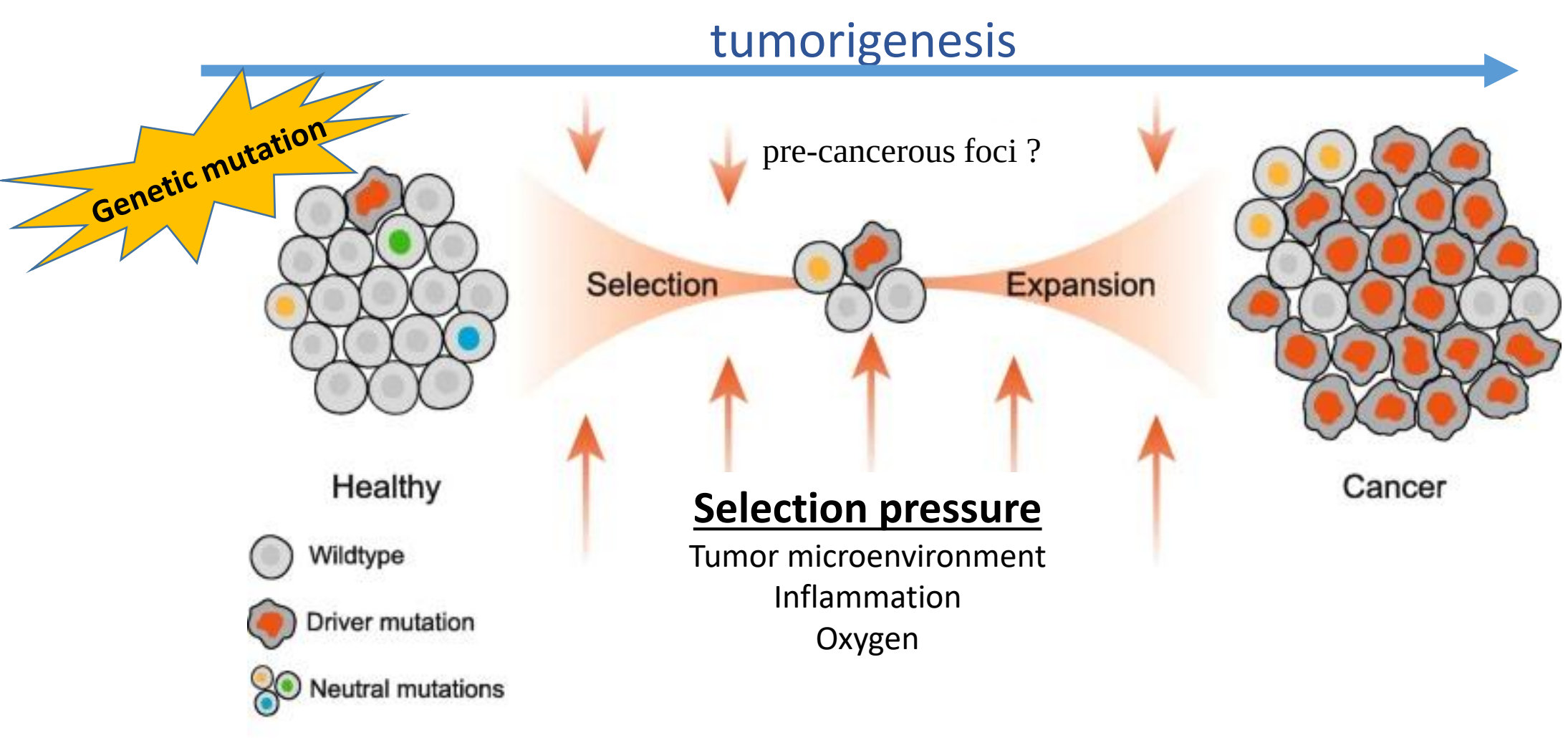
- Populated by microbiome
  (recente NGS metagenomic data)
- The collective genome of the gut microbiome exceeds over **100 times** the amount of human DNA in the body
- Can be pathogenic or probiotic;
- When probiotic, produce e.g. short-chained fatty acids (SCFAs) by fermentation of plant fibers

Gut microbiota modulation: a novel strategy for prevention and treatment of colorectal cancer



Microbiome changes with type of diet (fat, protein, fibers)
-> “you are what you eat”

Oncogene (2020) 39:4925–4943,
Doi: 10.1038/s41388-020-1341-1



Tumour formation is the echo of a selection process shaped by the tumour microenvironment

Doi: 10.1186/s13059-021-02501-0, Genome Biol 2021

Lecture 13- Some take-home concepts

- **Although cancer cells accumulate mutations, tumour development also depends on the tumor microenvironment (TME) and its signal exchange with the tumour cells;**
- **The TME consist of stromal cells (fibroblasts, immune cells, endothelial cells), the extracellular matrix and secreted growth factors or metabolites;**
- **Tumour cells secrete VEGF to stimulate endothelial cells for sprouting angiogenesis towards the tumour;**
- **Fibroblasts secrete extracellular matrix proteins and remodel it by metallo-proteinases during wound repair or when activated by tumour cells; a stiff matrix promotes tumour cell invasion;**
- **CD4+ cytotoxic T cells can recognize and kill tumour cells, except when in an immunosuppressive TME;**
- **Macrophages can differentiate into pro-inflammatory M1 and immunosuppressive M2 types;**
- **Chronic inflammation is a tumour-promoting condition that provides survival signals to tumour cells.**