Cell Structure & Tumor Microenvironment

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The 2016 Nobel Prize in physiology or medicine goes to Yoshinori Ohsumi of Japan for discoveries about the mechanisms underlying autophagy, a fundamental process for degrading and recycling cellular components.

- He located genes that regulate the cellular "self eating" process known as autophagy.
- Errors in these genes cause disease.
- Dr Ohsumi's work is important because it helps explain what goes wrong in a range of illnesses, from cancer to Parkinson's.

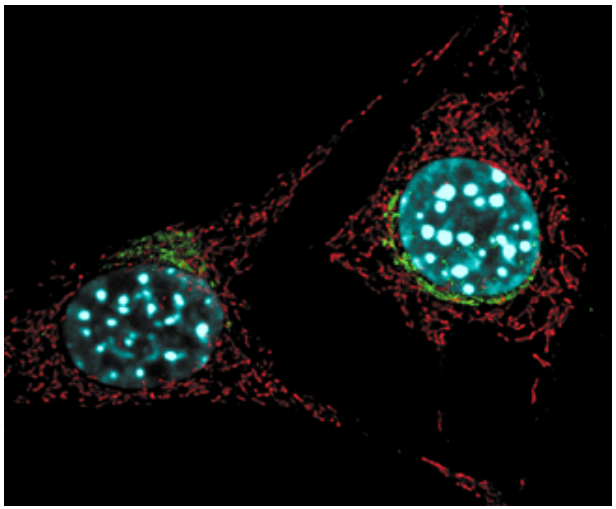
Overview of this lecture

- Cells & cell theory
- Structure and function of specific cell components
- Cancer cells
- Tumor microenvironment

Cell

"is the functional and smallest unit in every organism "

- The **cell** was first discovered by Robert Hooke (in 1665).
- The **cell theory** was developed by *Matthias J. Schleiden*, *Theodor Schwann* and *Rudolph Virchow*



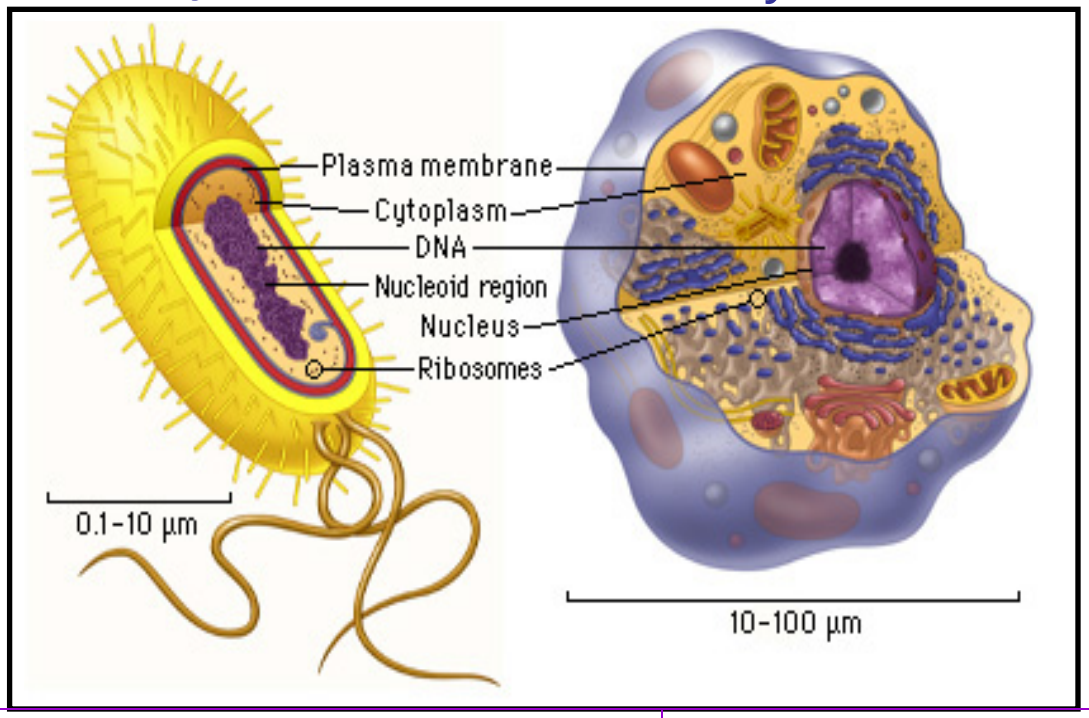
(Ref: *The Molecular Probes® Handbook-11th Edition, 2010, Invitrogen*)

Main principles of the cell theory

- All living organisms are composed of cell(s).
- Cells are the essential unit of structure and function in organisms
- Cells are produced through the division of pre-existing cells *(mitosis)*

Types of Cells

Prokaryotic cell *vs* Eukaryotic cell



Prokaryotes: Archae and bacteria

- do not have nucleus
- do not have membrane-bound organelle
- have cytoskeleton
- have circular-shaped DNA

Eukaryotes: Protists, fungi, plants, and animal (including human)

- contain nucleus and other membrane-bound organelles
- have cytoskeleton,
- have chromatin and chromosome.

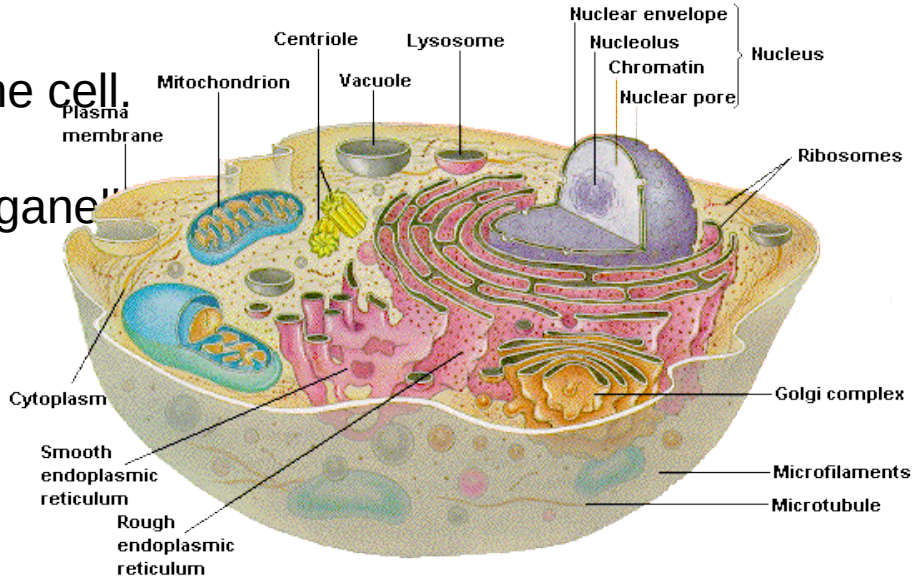
Components of a Cell

The cell is a mass of **Protoplasm** separated from the external environment by a **Plasma Membrane**.

The Protoplasm is made up of two components:

1. Nucleus: that houses the genome of the cell.

2. Cytoplasm: that contains numerous organelles
   - Mitochondria
   - Endoplasmic Reticulum
   - Golgi Apparatus
   - Ribosomes
   - Lysosomes
   - Peroxisomes
   - The cytoskeleton of the Cell: (a) Microfilaments
     (b) Intermediate filaments
     (c) Microtubules
   - Centrosome and centrioles
   - Cytoplasmic Inclusions



Plasma membrane (or *cell membrane*): Structure

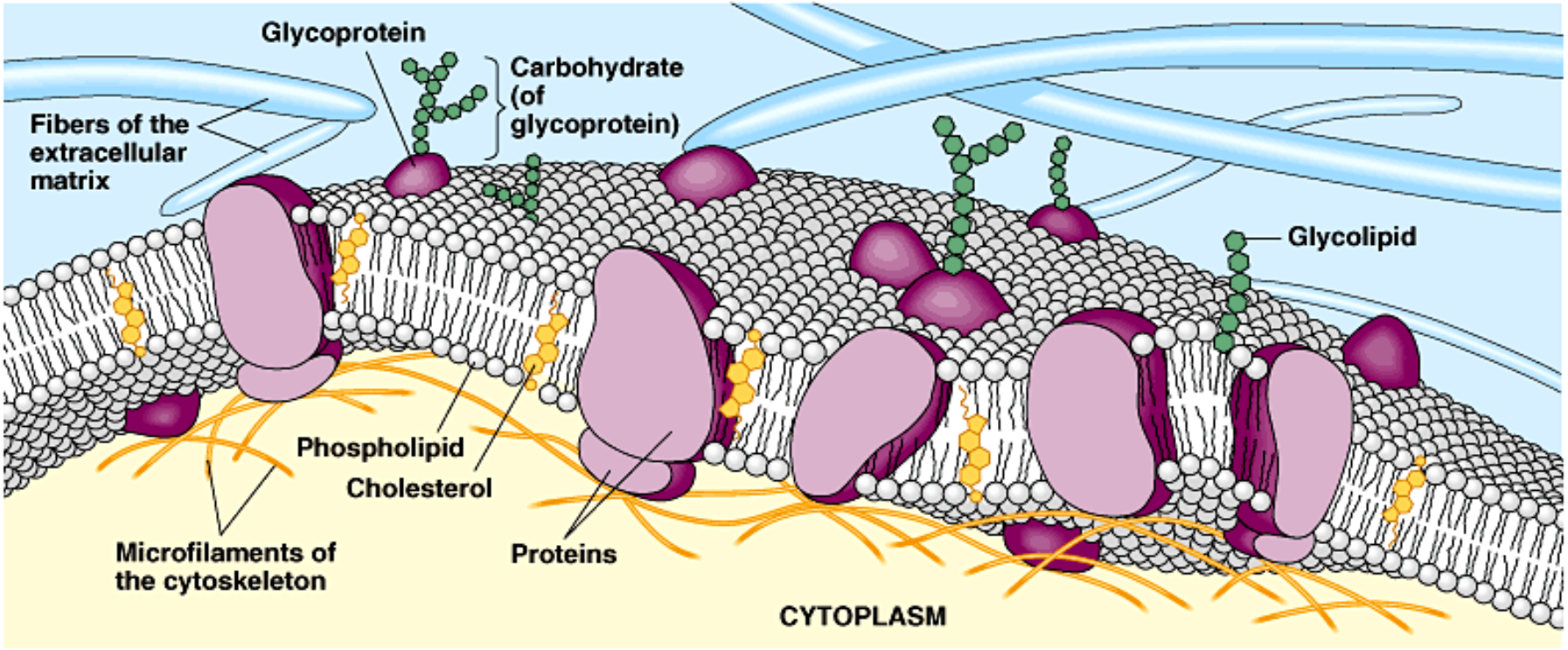
- It is made up of: (1) **Lipid Bilayer**

Phospholipids
Cholesterol
Glycolipids

(2) **Associated Proteins**

Integral /Transmembrane Proteins

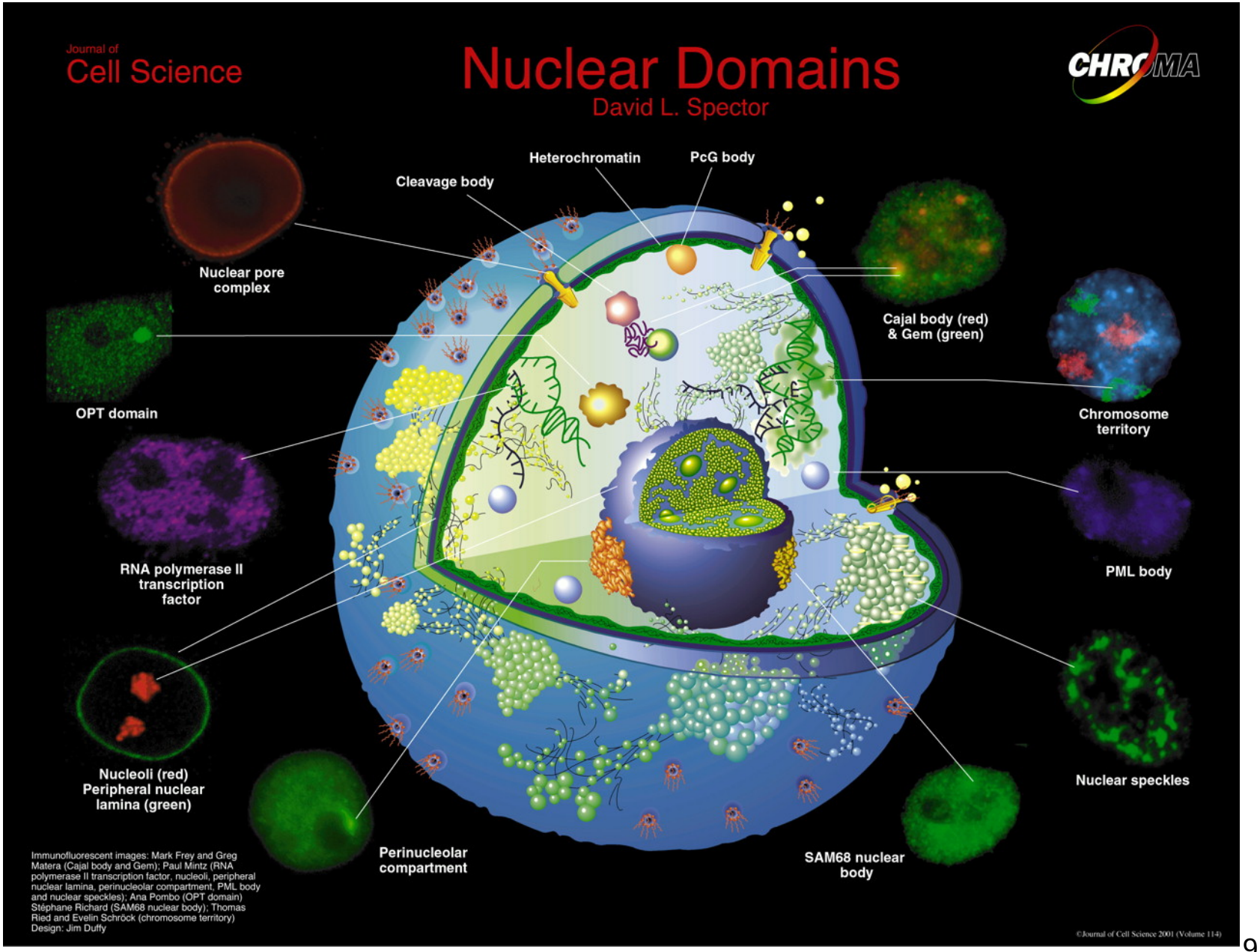
Peripheral Proteins



Plasma membrane (or *cell membrane*): Function

- **Separates** cell contents from the external environment
- Maintains the **shape** of the cell
- Controls the **transport** of molecules in and out of the cell (**selective permeability**), serves as a diffusion barrier

  Regulation of transport by Passive transport includes Diffusion ("Osmosis" and "Dialysis") or Active transport
- Regulates **cell–cell interactions**
- Cell **identity** - It bears receptors that aid in recognizing antigens and foreign cells
- Helps in cell **movement**
- **Transduces** extracellular physical or chemical signals into intracellular events.

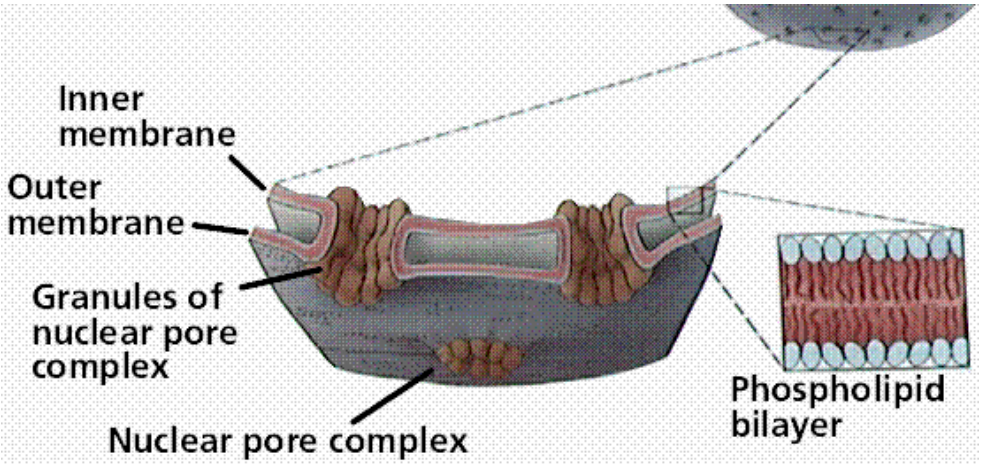

Journal of
Cell Science
Nuclear Domains
David L. Spector
CHROMA
Heterochromatin
PcG body
Cleavage body
Nuclear pore complex
Cajal body (red) & Gem (green)
OPT domain
Chromosome territory
RNA polymerase II transcription factor
PML body
Nucleoli (red) Peripheral nuclear lamina (green)
Nuclear speckles
Perinucleolar compartment
SAM68 nuclear body
Immunofluorescent images: Mark Frey and Greg Matera (Cajal body and Gem); Paul Mintz (RNA polymerase II transcription factor, nucleoli, peripheral nuclear lamina, perinucleolar compartment, PML body and nuclear speckles); Ana Pombo (OPT domain) Stéphane Richard (SAM68 nuclear body); Thomas Ried and Evelin Schröck (chromosome territory)
Design: Jim Duffy
©Journal of Cell Science 2001 (Volume 114)

Nucleus: Structure

I. Nuclear envelope

Composed of inner and outer membrane separated by perinuclear space

Has nuclear pores which connect with ER



II. Nucleoplasm

Fluid of the nucleus

III. Nucleolus

Not separated by membrane

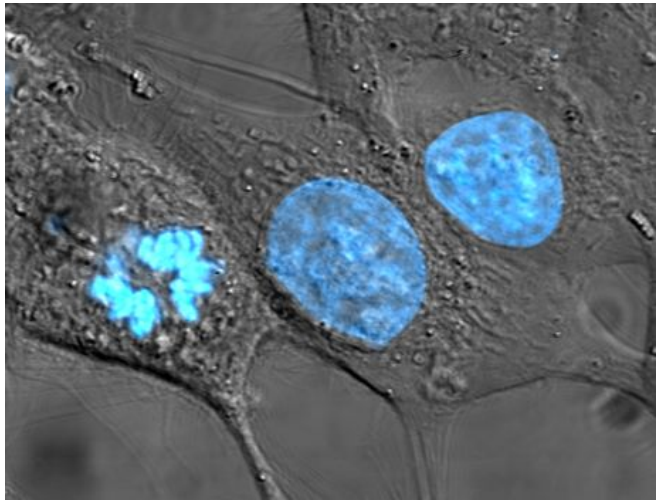
is area of formation of ribosomal RNA,

is area of condensed DNA and chromatin

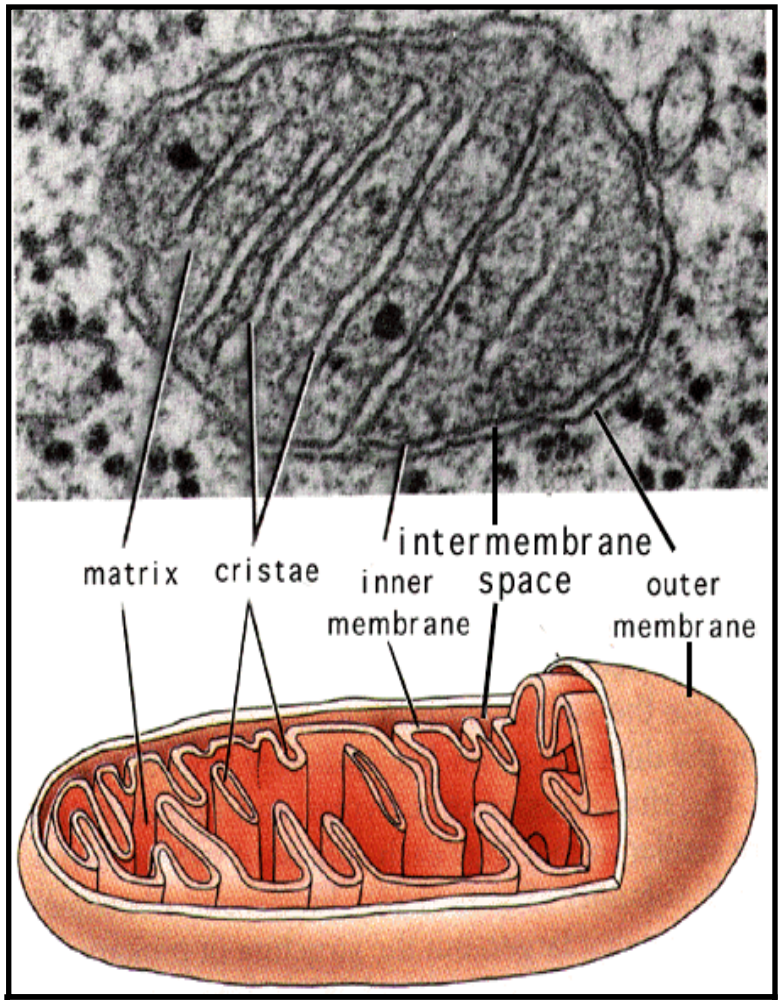
Nucleus: Function

"contains the genetic information of the cell"

- Storage of genetic material (DNA)
- Production of messenger RNA and ribosomes that needed for protein synthesis
- Storage of proteins and RNA in the nucleolus
- During the cell division, chromatins are arranged into chromosomes in the nucleus
- Function in selective transportation of regulatory factors and energy molecules through nuclear pores



Mitochondria - "the powerhouse of the cell" : Structure



The structure of mitochondria.
Ref:microbewiki.kenyon.edu/index.php/Mitochondria

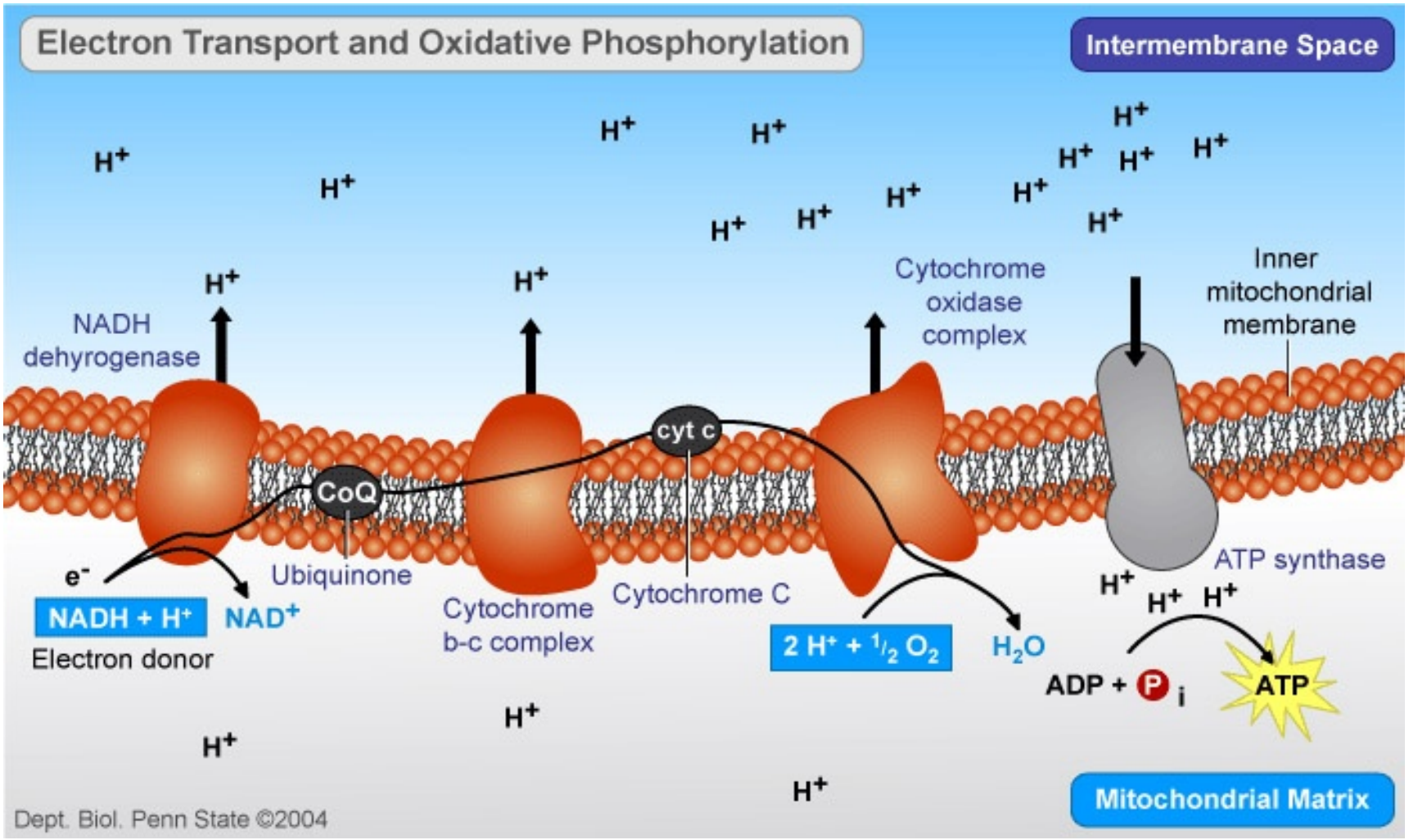
- Surrounded by 2 membranes:
  - **smooth outer membrane** (permeable)
  - folded **inner membrane** (impermeable) with layers called "**cristae**"
- Contain two internal compartments:
  - **mitochondrial matrix** is within the inner membrane (contain ribosomes mitochondrial DNA (**mtDNA**) and enyzmes)
  - **intermembrane space** is located between the two membranes

mtDNA:
- inherited from mother
- not protected by histones

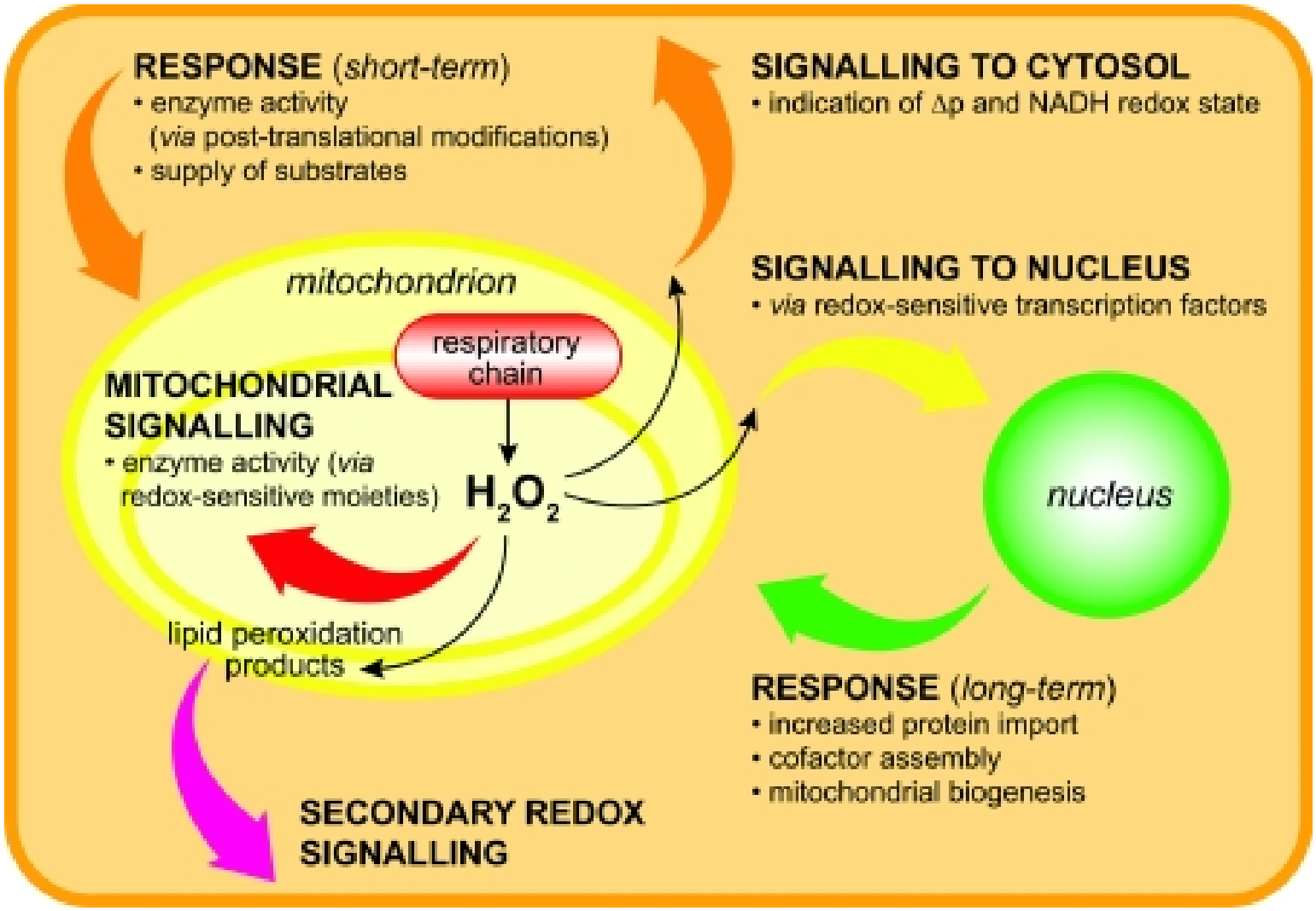
Mitochondria - "the powerhouse of the cell" : Functions

- Supplying cellular energy - ATP synthesis through oxidative phosphorylation – Krebs Cycle
- Signaling
- Cellular differentiation
- Cell death
- Maintaining control of the cell cycle and cell growth

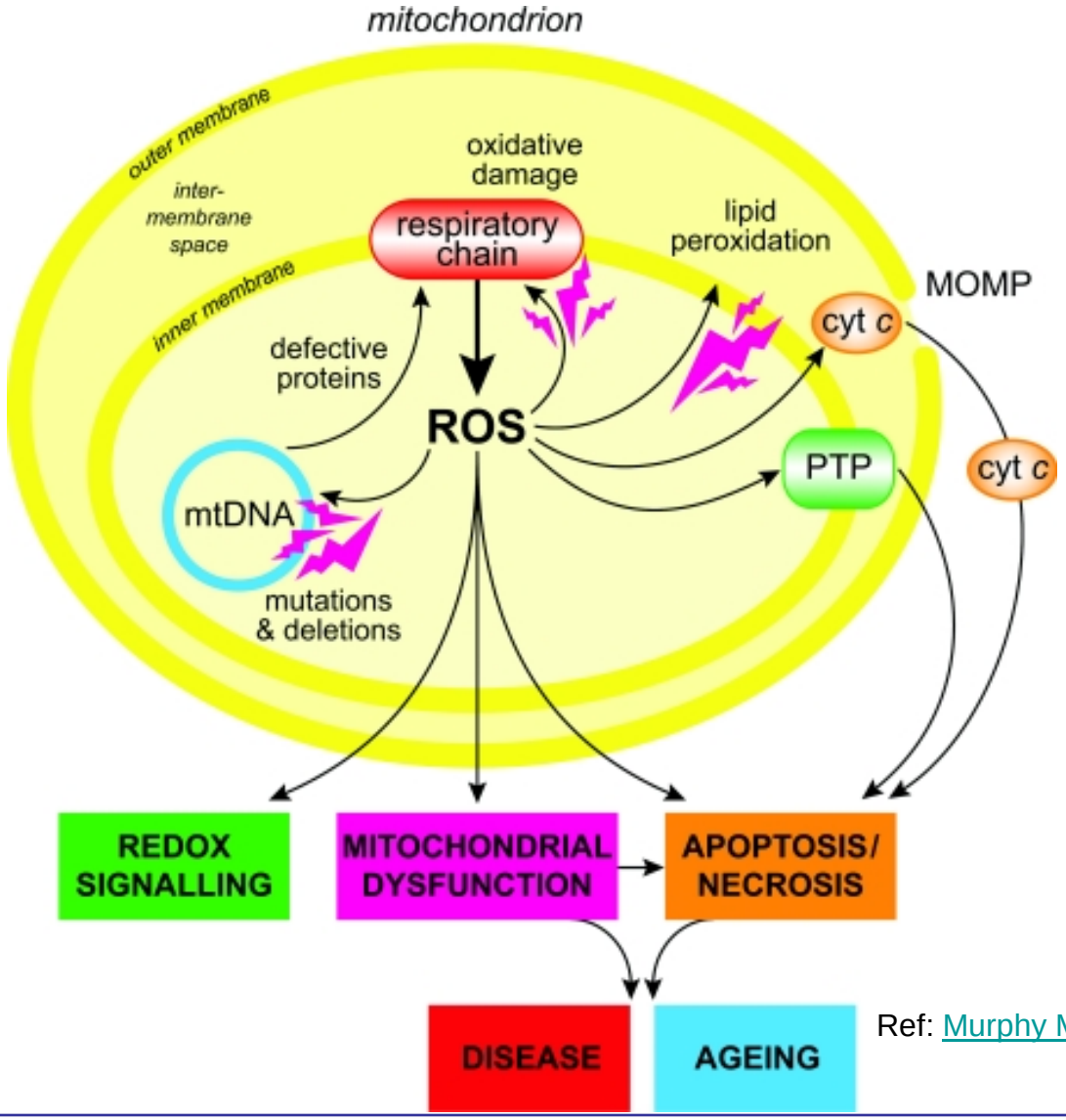
Mitochondria - Function: Oxidative phosphorylation--ATP synthesis



Possible mechanisms of mitochondrial redox signalling

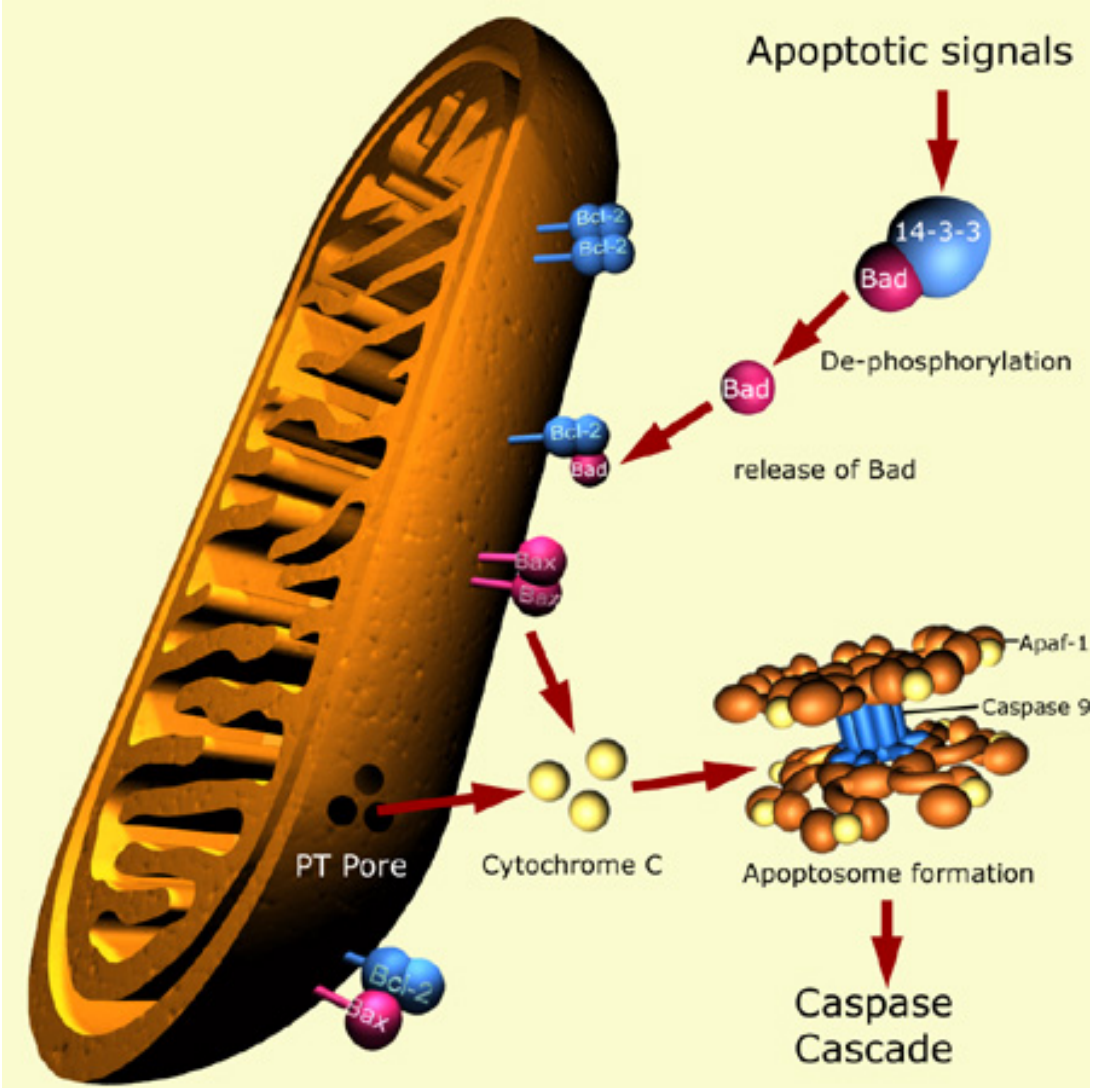


Mitochondria - Function: Reactive Oxygen Species (ROS)/ Free radical generation



Ref: Murphy MP. Biochem J, 2009

Mitochondria - Function: Regulation of apoptotic death and cell growth

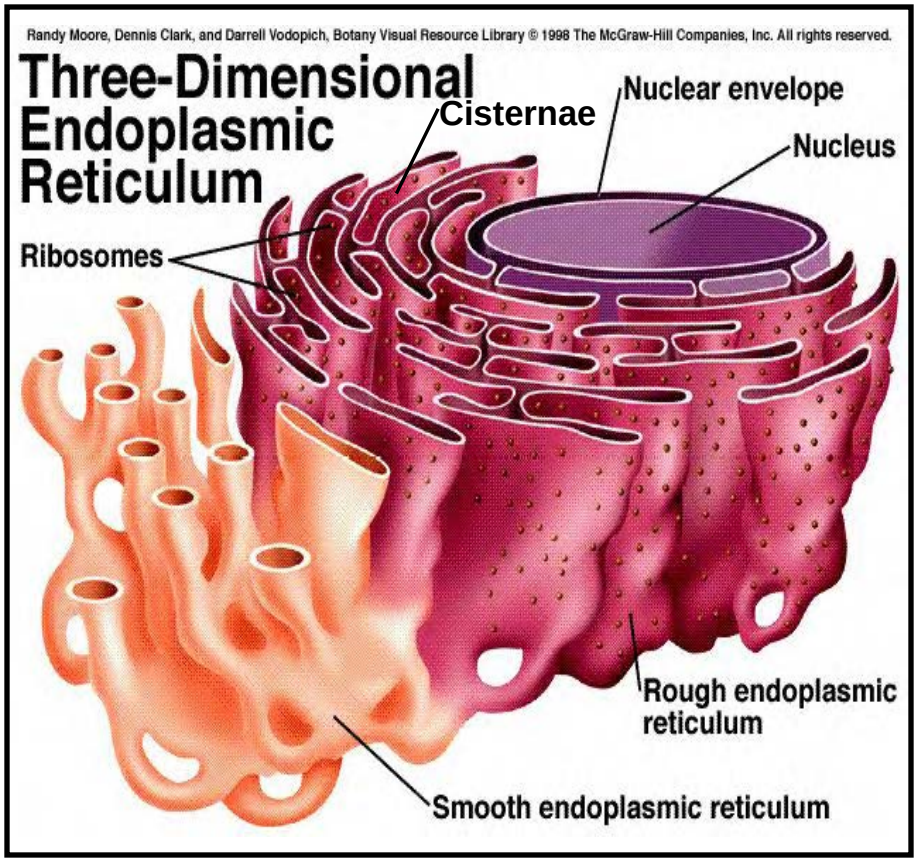


Ref: http://www.reading.ac.uk/nitricoxide/intro/apoptosis/mito.htm

Apaf-1: Apoptotic protease activating factor 1

Endoplasmic reticulum (ER): Structure

"cell's internal membrane system"



• ER membrane is continuous with **nuclear envelope**

• **Cisternae** (sac-like structures)

• **Cisternal space** (or lumen) with soluble proteins and enzymes

➢ Types of ER

• Smooth-type (SER) :

•Ribosome-free

•Contains enzyme for lipid biosynthesis

•Involved in attachment of receptors on cell membrane proteins

• Rough-type (RER) :

•Ribosomes embedded in surface

•Involved in protein synthesis

Endoplasmic reticulum (ER): Function

Rough-type (RER)

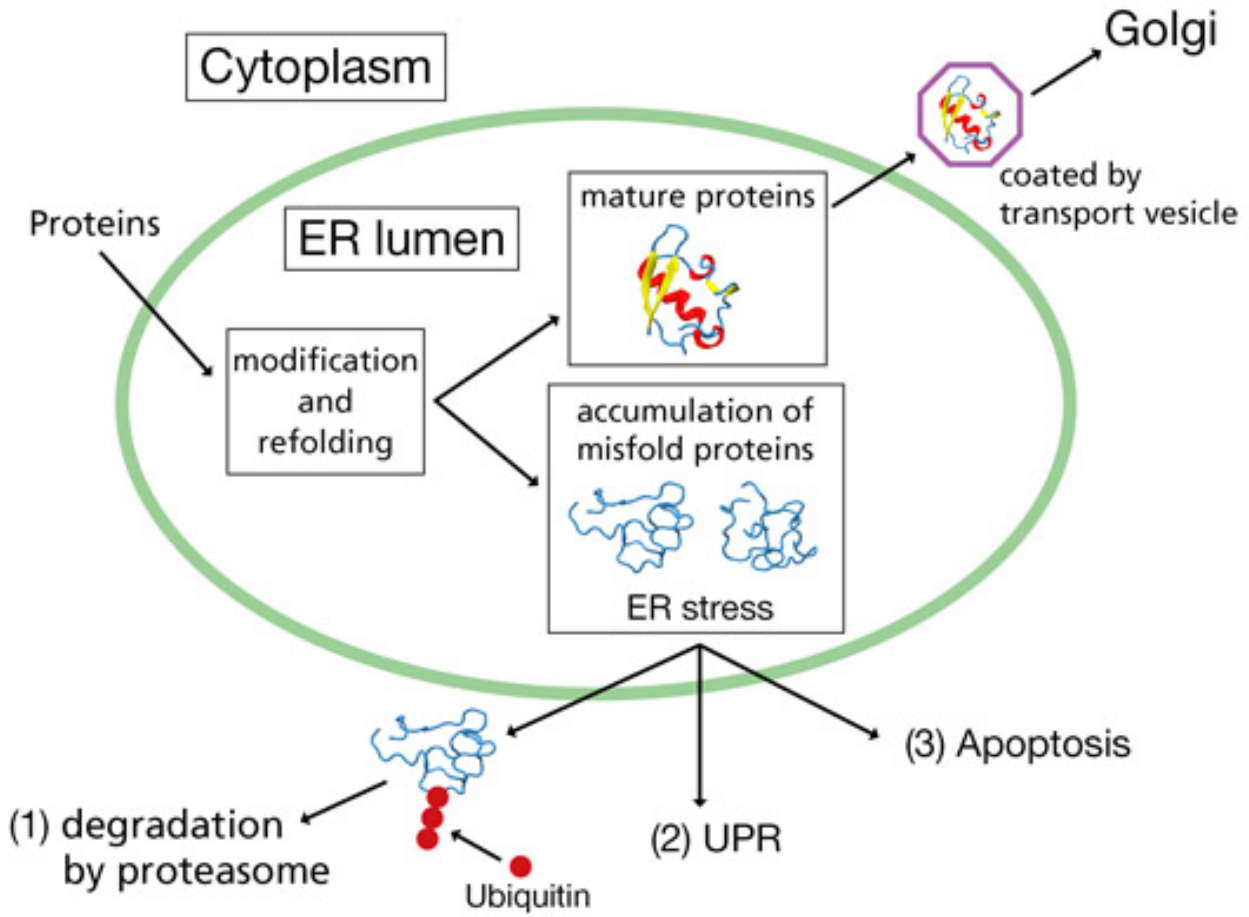
- Protein folding, modification and secretion
- Cellular protein quality control by extracting and degrading unfolded proteins (known as a ER-associated protein degradation-ERAD)
- the transport of synthesized proteins in vesicles to the Golgi apparatus.

Smooth-type (SER) :

- Lipid and sterol biosynthesis
- Detoxification of drugs
- Core oligosaccharide biosynthesis
- Storage of calcium ions in the ER lumen and their regulated release into the cytosol (calcium homeostasis)
- Apoptosis

Major responses to ER stress

Disturbances in redox regulation, calcium regulation, glucose deprivation, and viral infection or the over-expression of proteins can lead to endoplasmic reticulum stress response



http://www.pdbj.org/eprots/index_en.cgi?PDB%3A2RIO

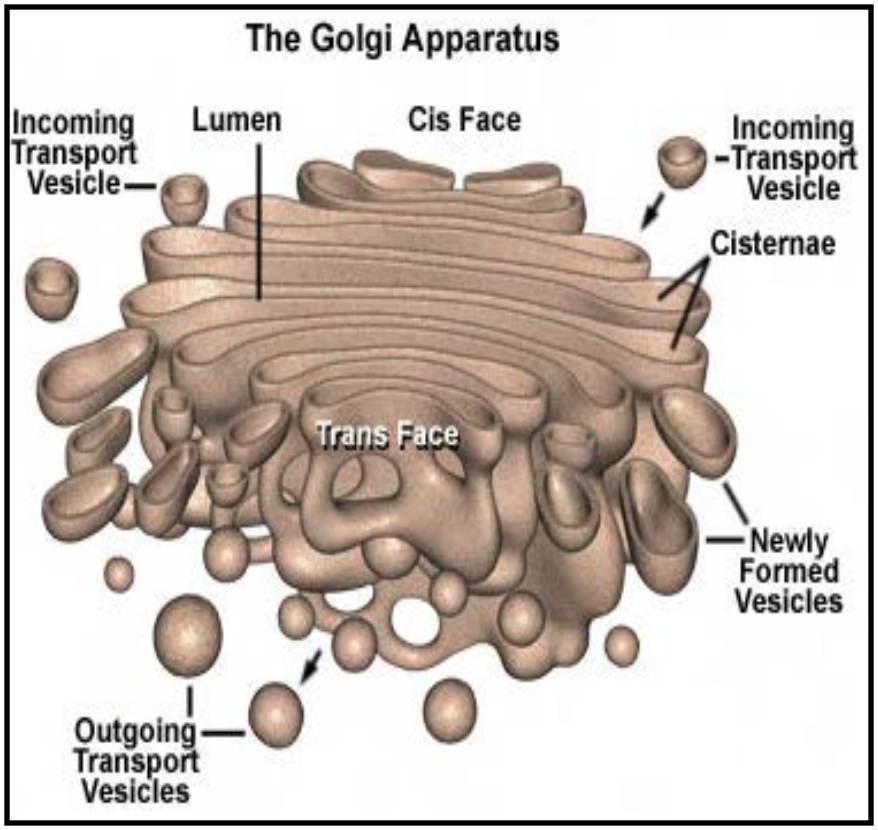
Golgi Apparatus: Structure

• is made up of numerous group of flat membranes called **cisternae** forming a **stac**

Cisternae:

* a complex network of tubules and vesicles are located at the edges of cisternea

•help proteins and cytoplasmic components travel between different parts of the cell



• has three regions:

--- Cis face (near to ER)

Receive transport vesicles from ER

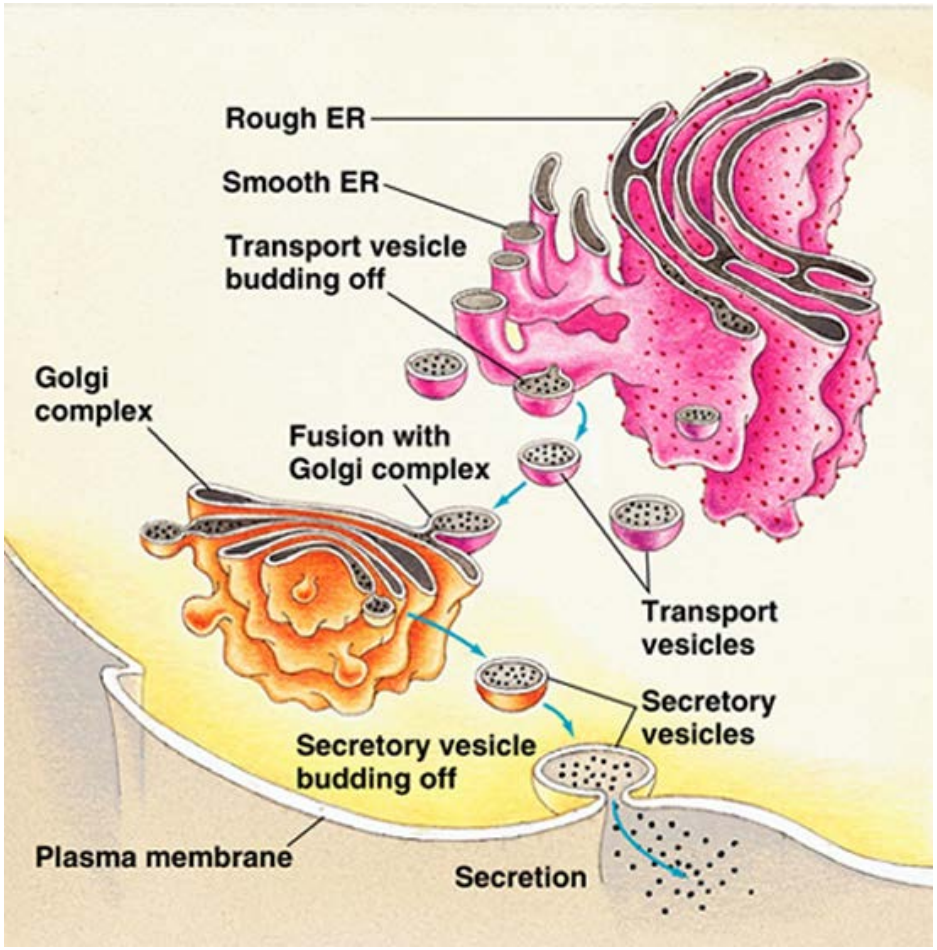
--- Trans face (far away from ER)

Packages the material in vesicles and send outside of Golgi

--- Golgi stack (between these two region)

Main processing area

Golgi Apparatus: Function



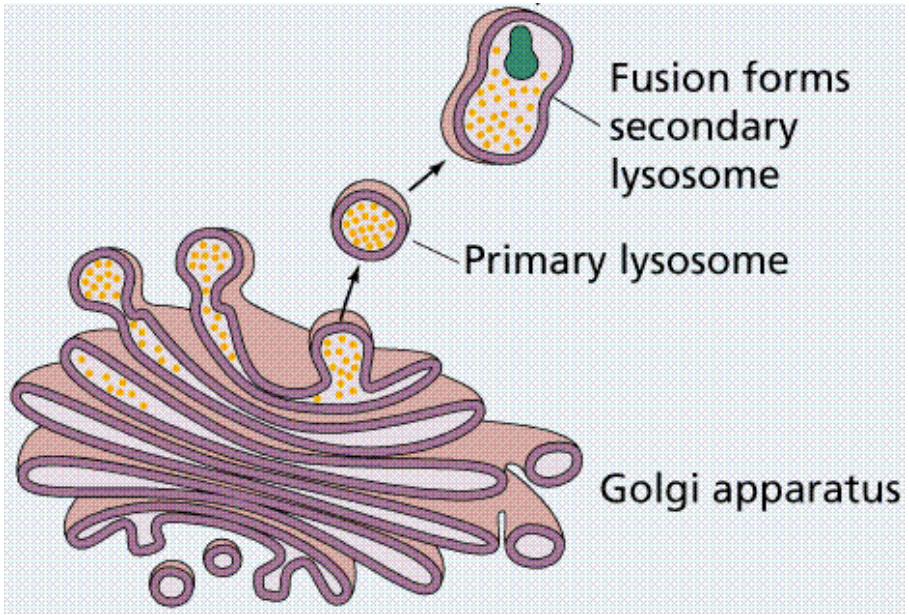
- **Post-translational modification** of proteins
- Receives, sorts, modifies, packs and ships the proteins
- Produce membrane packages called vesicles
- Lipid transport
- Lysosome formation
- ECM building - the formation of proteoglycans

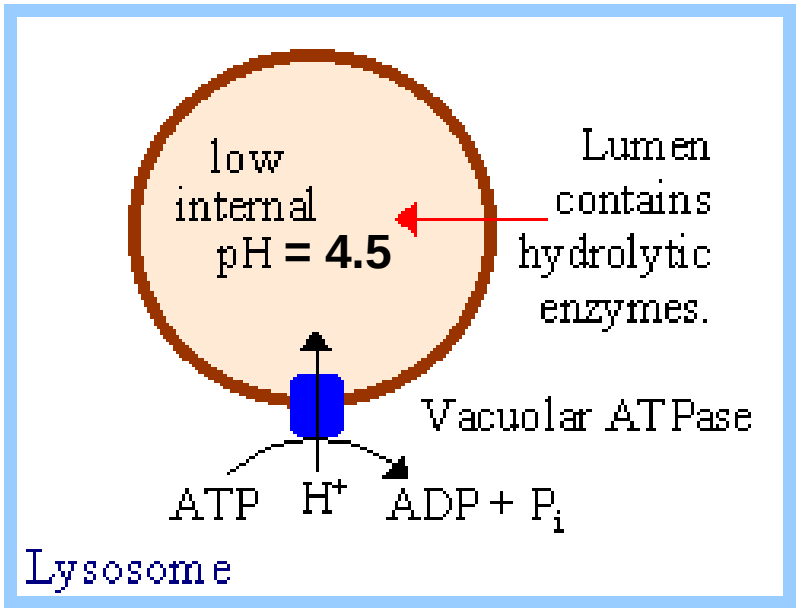
Lysosomes-recycling units of a cell: Structure

- membrane enclosed vesicles
- arise from the Golgi apparatus
- are found in all animal cells
- contain hydrolytic enzymes that digest & destroy macromolecules
- two types of lysosomes:

--- Peroxisomes: **catalases & oxidases**

--- Proteasomes: **proteases- cathepsin**





Lysosomes-recycling units or “suicide bags” of a cell: Function

- Support cellular homeostasis - involvements in secretion, plasma membrane repair, cell signalling and energy metabolism
- Destroy invading bacteria or viruses (**Phagocytosis**)
- Degrade older or damaged organelles (**Autophagy)**
- Degrade macromolecules (**Endocytosis)**

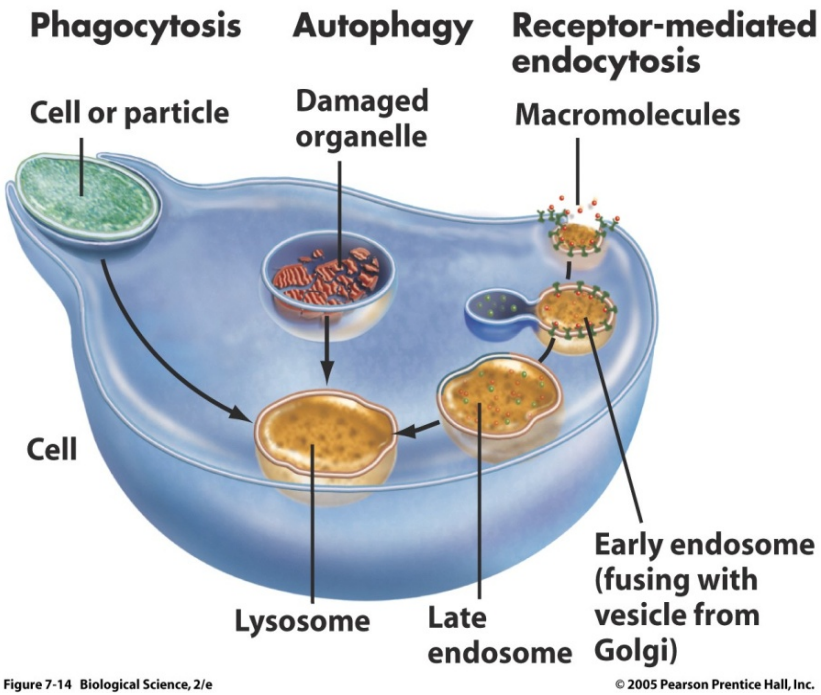
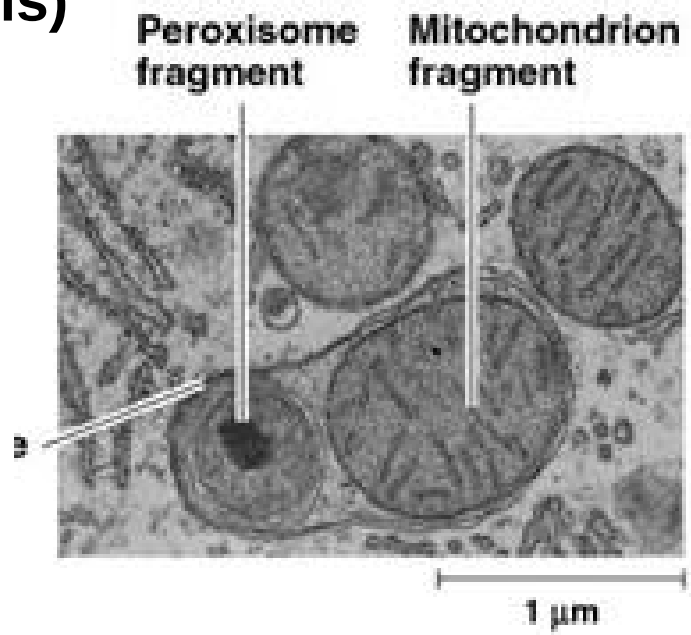


Figure 7-14 Biological Science, 2/e

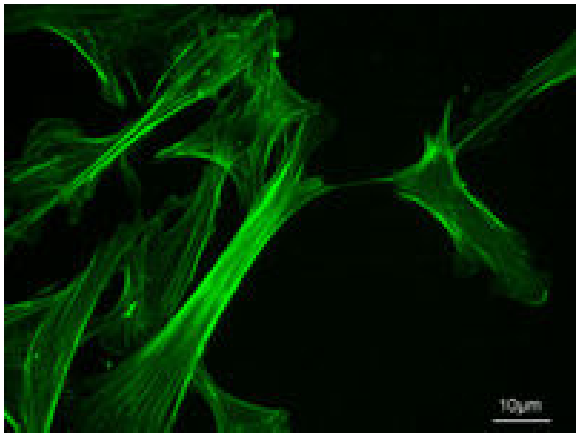


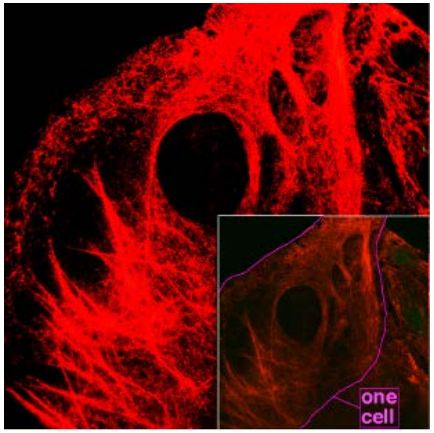
(b) A lysosome in action

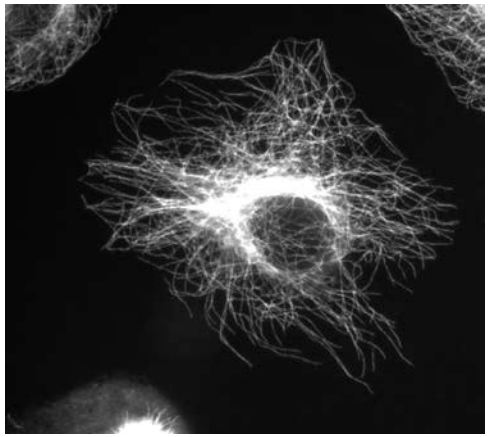
Eukaryotic Cytoskeleton

"the movers and shapers in the cell"

- is found underlying the cell membrane in the cytoplasm
- three main kinds of cytoskeletal filaments:
  - **microfilaments** which are composed of **actin**,
  - **intermediate filaments** which have around 70 different proteins as building blocks,
  - **microtubules** with **tubulin** as the basic subunit.

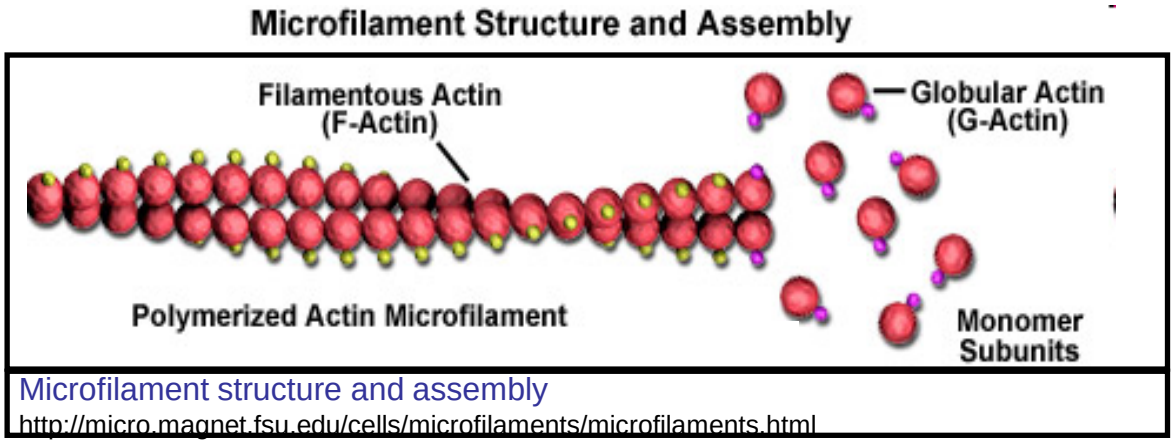






Major Structures of the Cytoskeleton

I. Actin filaments (or Microfilament)

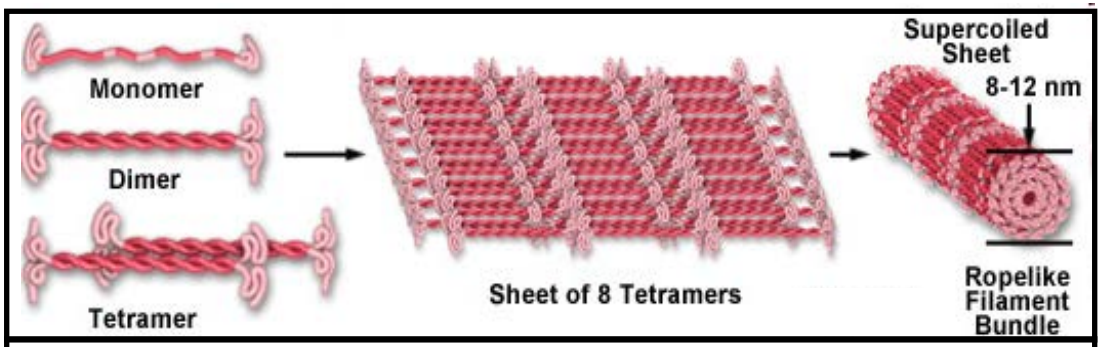


Microfilament structure and assembly
http://micro.magnet.fsu.edu/cells/microfilaments/microfilaments.html

- occur in every cell
- composed of polymerized **actin** proteins
- interact specifically with myosin, helical polymers made of actin flexible, organized into 2D networks and 3D gels
- function in cell movement - cytokinesis, amoeboid movement
- cellular contraction

Major Structures of the Cytoskeleton

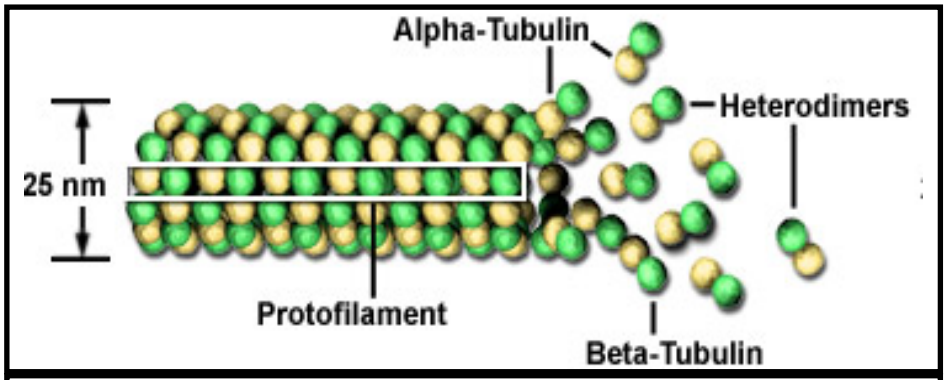
II. Intermediate filaments



Intermediate filament structure
http://micro.magnet.fsu.edu/cells/microfilaments/microfilaments.html

* occur only in animal cells
* composed of polymerized **keratin** proteins (>60)
* rope-like structure
* size: 8 -12 nm
* provide structural stability

III. Microtubules



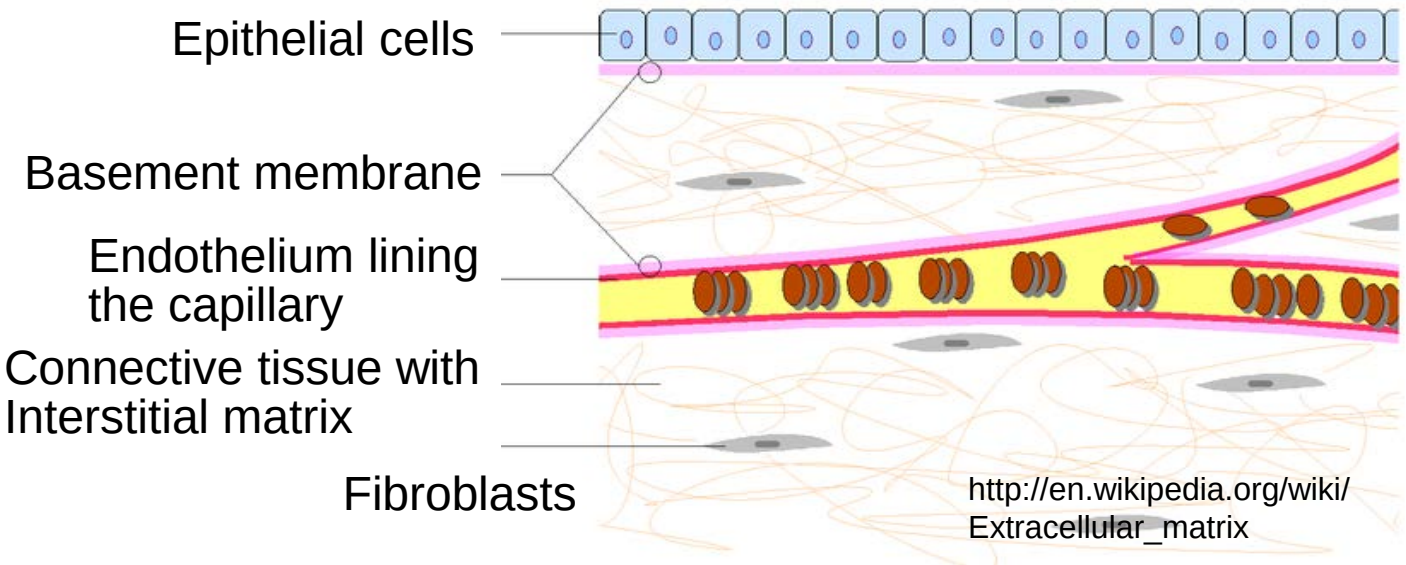
Microtubule helical structure
http://micro.magnet.fsu.edu/cells/microfilaments/microfilaments.html

* composed of polymerized **alpha** and **beta-tubulin**
* rigid, long, straight, holo tube
* length: 200 nm-25 μm
* are used for centrioles
•structural support of Cilia and Flagella
•Involved in the movement of the materials within the cells

Cytoskeleton: Function

- Cell shape,
- Cell polarity
- Cell movement:
  - muscle contraction via actin filaments and myosin proteins
  - microtubules interaction with the motor proteins kinesin and dynein
    - tubulin make up the internal structure of cilia and flagella
- Cell cycle:
  cell division and chromosomal separation (mitosis and meiosis), by actin and tubulin cytoskeletal structures
- Cell adhesion - trigger focal adhesion disassembly, which is necessary for migration
- Provide platforms for intracellular transport - movement of secretory vesicles, organelles, and intracellular macromolecular assemblies
- Phagocytosis (by actin filaments)
- Wound healing

Extracellular matrix (ECM)



- collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells
- includes the **interstitial matrix** and **basement membrane**
- is made up of Adhesive glycoproteins (fibronectin and laminin), Structural proteins (collagen and elastin) and Polysaccharides (glycosaminoglycans, proteoglycans)
- consisting of various cell types (i.e. fibroblasts, epithelial cells) and secreted proteins (cytokines)

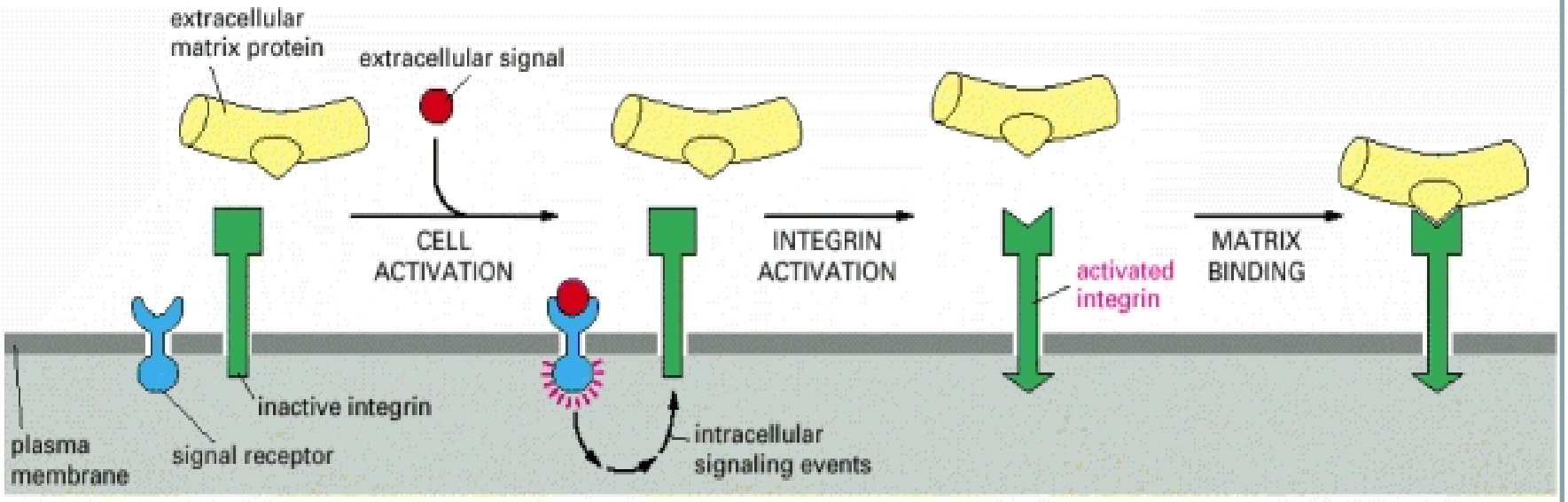
Extracellular matrix (ECM): Function

- Cell shape,
- Cell attachment,
- Adhesion,
- Migration (example: **wound healing**),
- Cell proliferation,
- Polarity,
- Differentiation,
- Survival & apoptosis,
- Motility,
- Management of growth factors,
- Embryonic development.

Cell-ECM adhesion

Cell adhesion molecules-"Integrins"

- transmembrane heterodimeric cell-surface molecules
- receptors for ECM
- concentrated in cell at specific zone called Focal adhesions
- important for **adhesion** to ECM and transmembrane **signaling**
- function as a link between ECM and the actin cytoskeleton
- important for cell growth



The regulation of the extracellular binding activity of a cell's integrins Ref: Molecular Biology of the Cell, 4th edition

Cell-cell adhesion

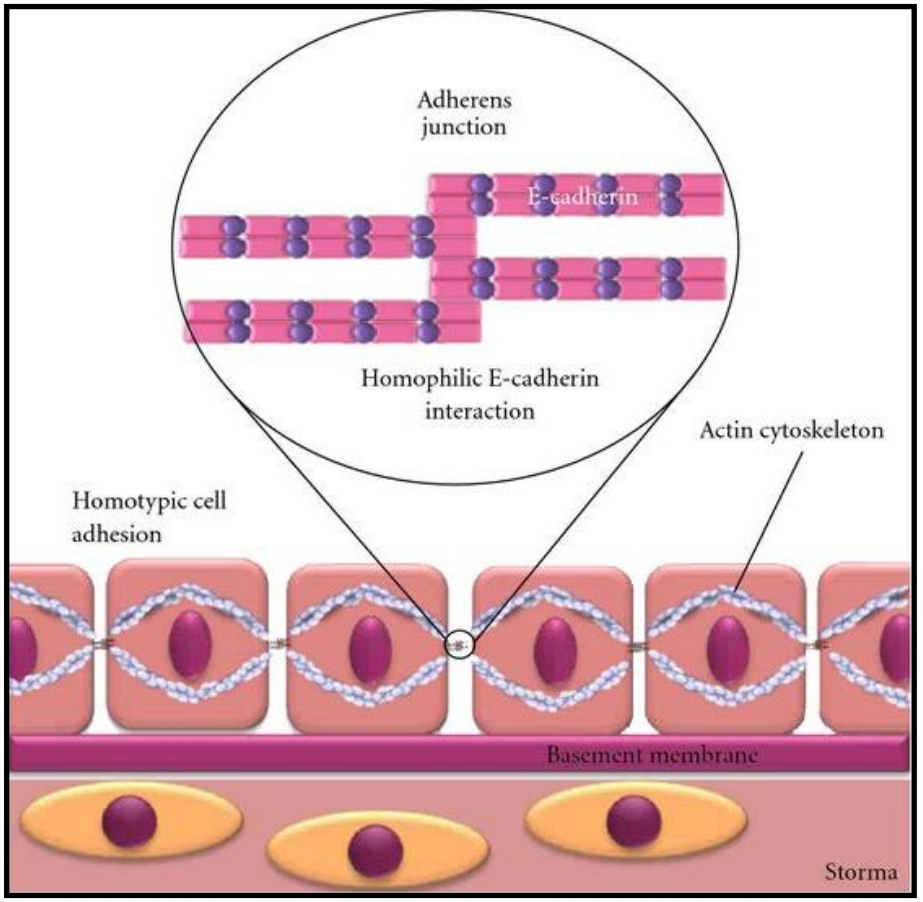
Cadherins

- are transmembrane proteins
- mediate Ca+2-dependent cell-cell adhesion
- interact in a zipper-like fashion
- stabilized by catenin complex
- are cell type specific

Several types of cadherins:

* E-cadherin (epithelial)
* P-cadherin (placental)
* N-cadherin (neuronal)

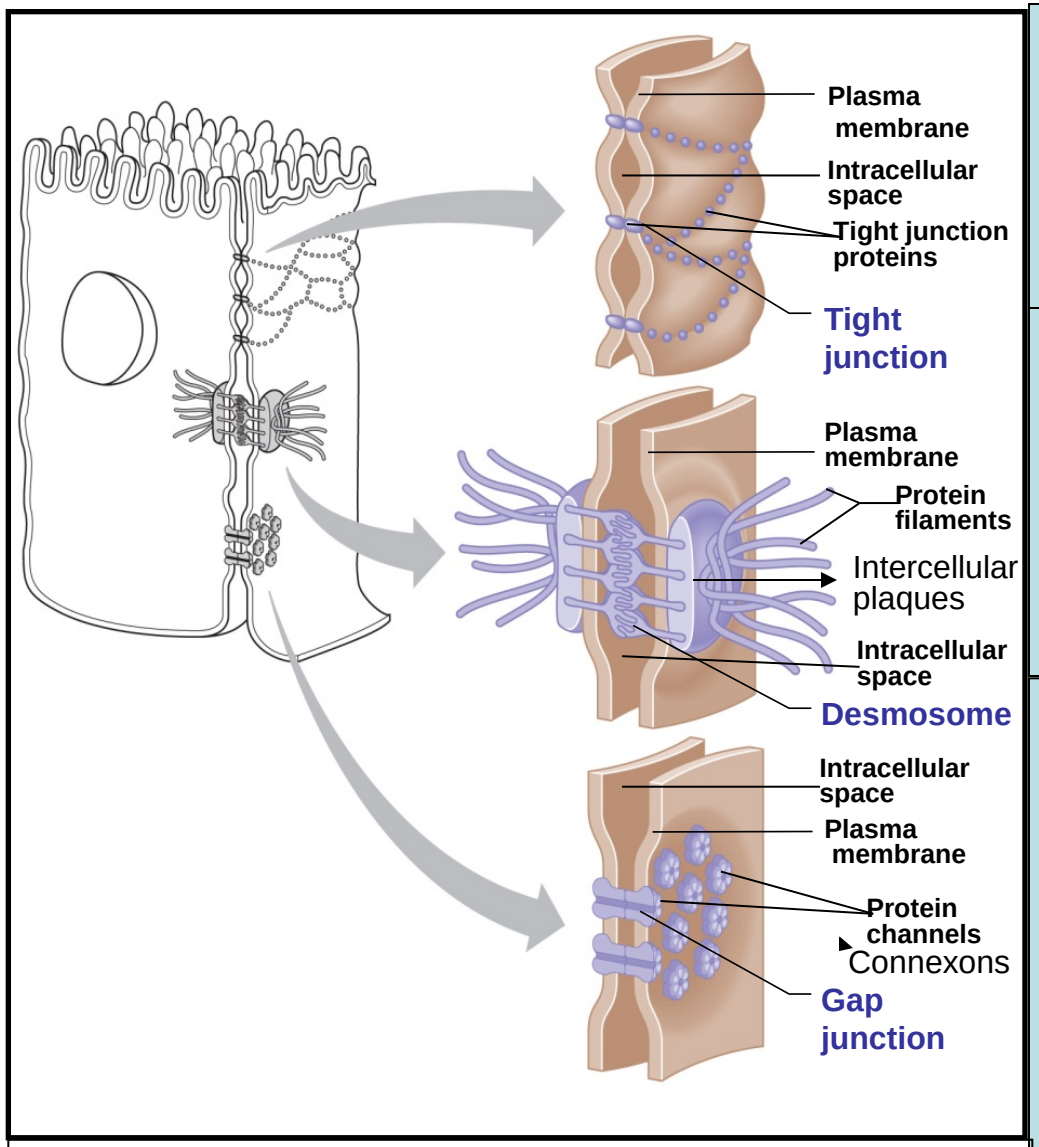
Homotypic cell adhesion by E-cadherin within the epithelium.



(Ref: Mohamet et al., Journal of Oncology, 2011)

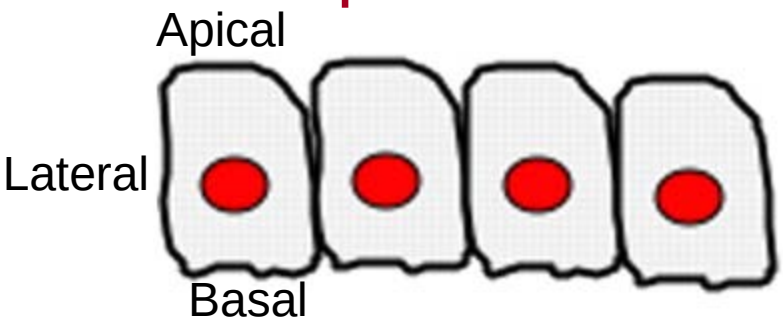
Cell-cell adhesion

"intercellular junctions"

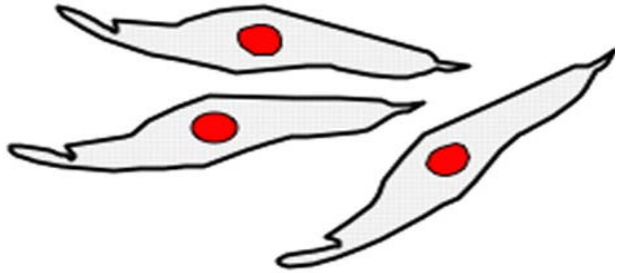


- **Tight junction:**
  - fused membranes of adjacent cells
  - form a continuous belt around cells
  - impermeable
  - ex: intestine, kidneys, bladder, epithelium of skin
- **Desmosomes**
  - fastening adjacent cells together by connecting cell membrane to cytoskeleton proteins
  - binding spots between cells with cadherins
  - ex: skin, heart
- **Gap junction**
  - Transmembrane proteins called connexons joint together to make a channel.
  - allow small molecules to pass directly from cell to cell
  - ex: heart muscle, animal embryos. (in heart muscle, the flow of ions through gap junctions coordinates the contraction)

Epithelial Cells & Mesenchymal cells



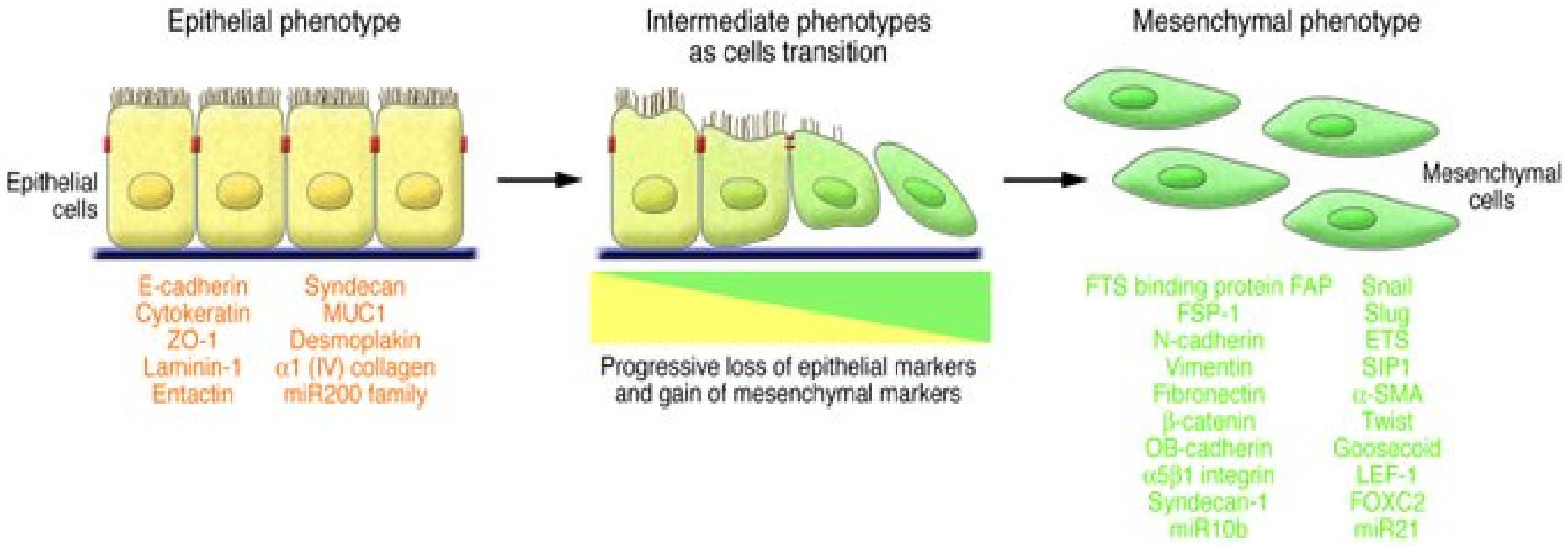
- are polygonal in shape
- have three membrane domains: **apical**, **lateral** and **basal**,
- have adherens junctions
- have tight junctions between **apical** and **lateral** domains,
- express cell-cell adhesion markers such as E-cadherin,
- are bound by a basal lamina at their basal surface - lack of mobility.

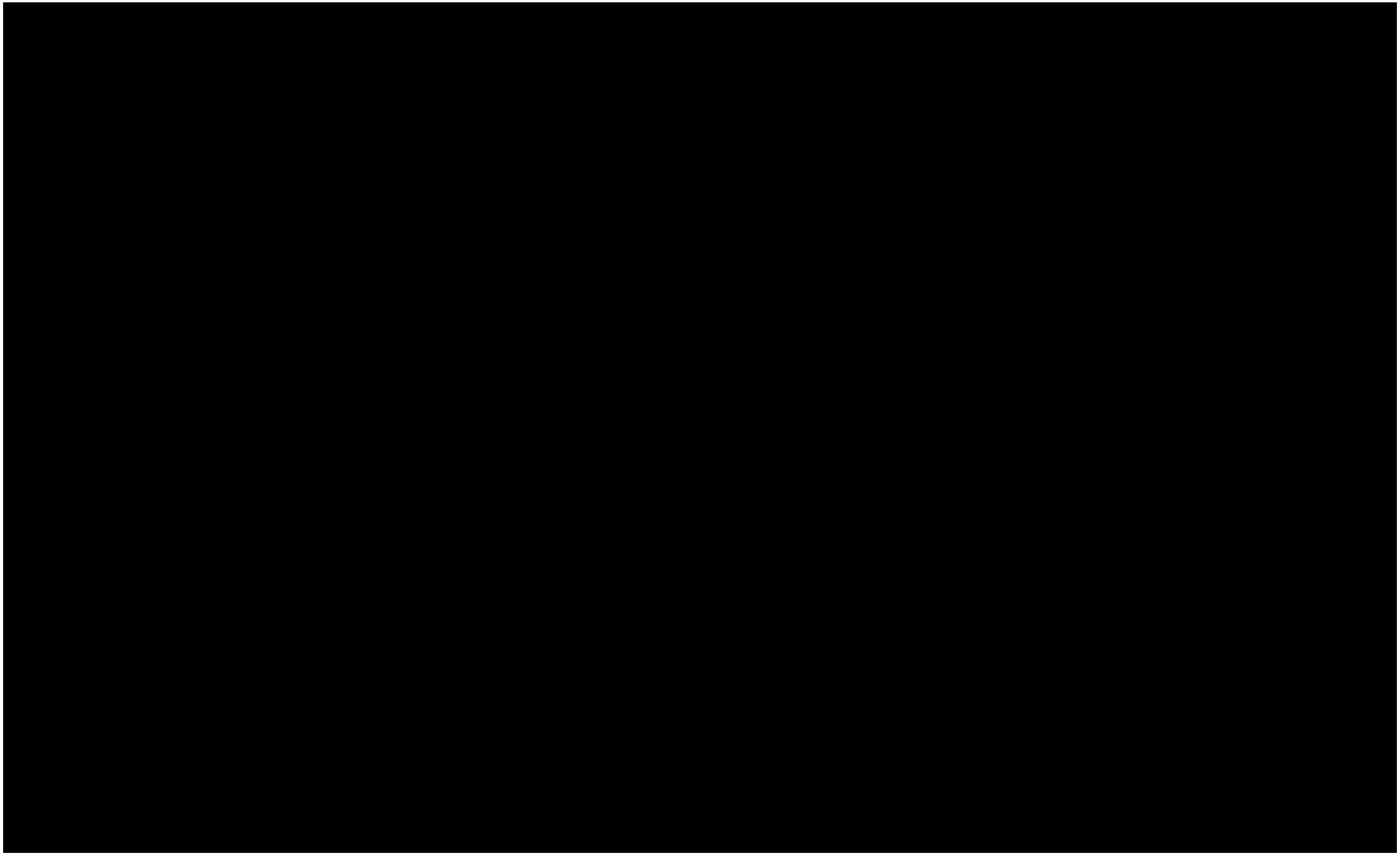


- are fairly uniform small spindle-shaped cells,
- do not make mature cell-cell contacts, and can invade through the ECM,
- are connected to other cells within a 3D- cellular network,
- bipolar
- express markers such as N-cadherin, fibronectin, vimentin, Twist, Snail,
- can migrate easily

Epithelial-Mesenchymal transition (EMT)

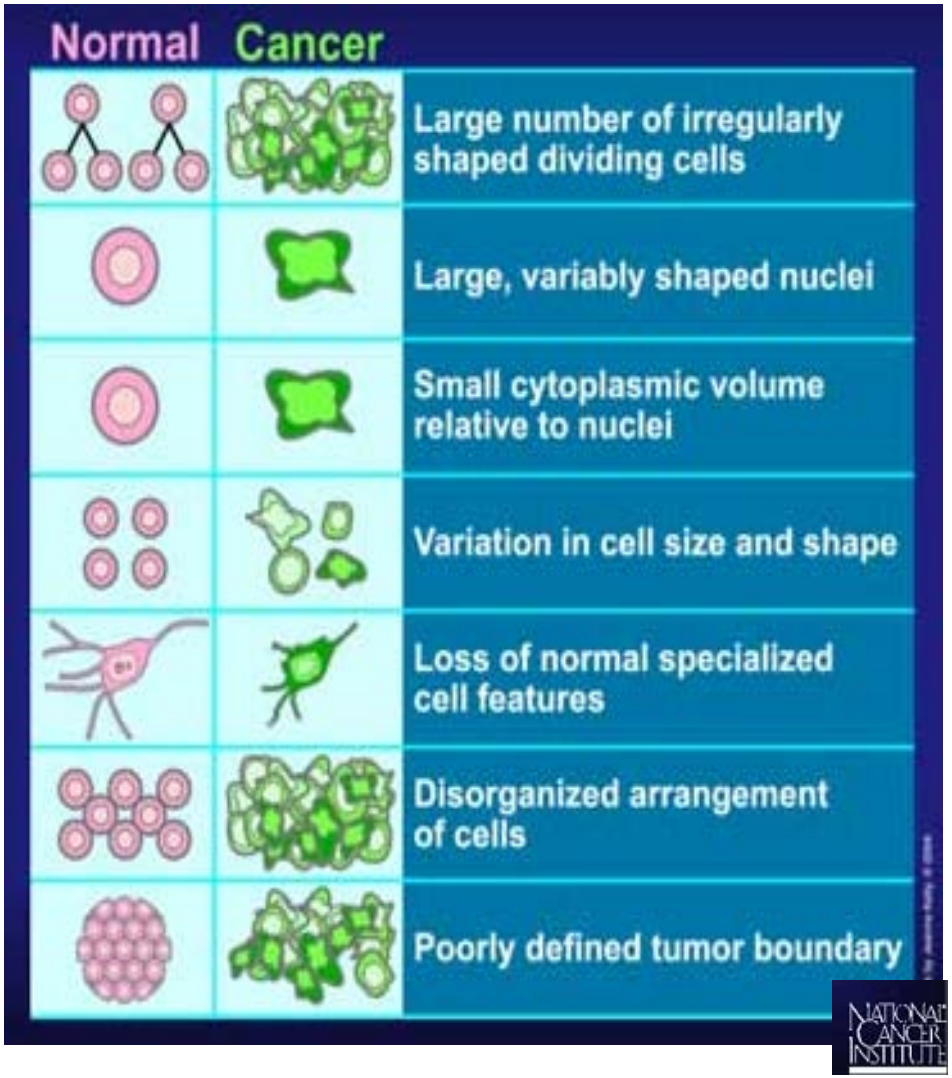
- is characterized by loss of E-cadherin, disruption of cell adhesion, and induction of cell motility and invasion to convert a mesenchymal phenotype
- function in embryonic development, organ formation, tissue regeneration, wound healing, organ fibrosis and cancer progression and metastasis.



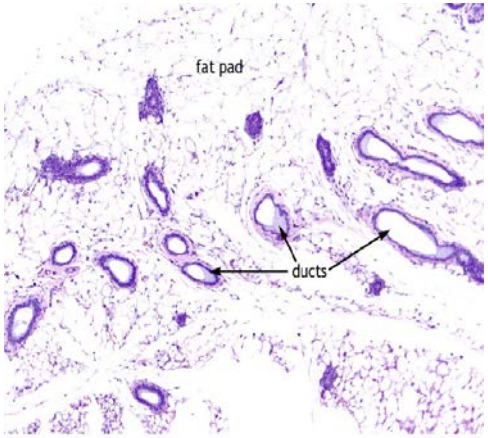


Normal Cells *vs* Cancer Cells

Microscopic appearance of cancer cells

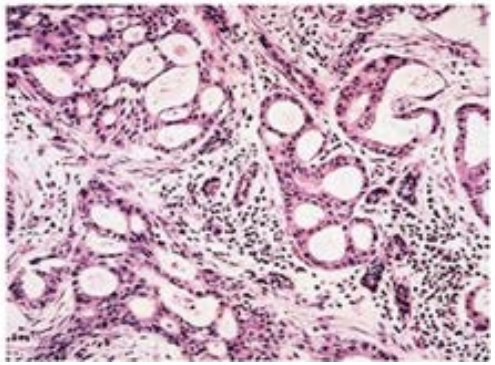


Mammary gland



http://www.gfmer.ch/selected_images_v2/detail_list.php?cat1=2&cat2=8&cat3=0&cat4=3&stype=n

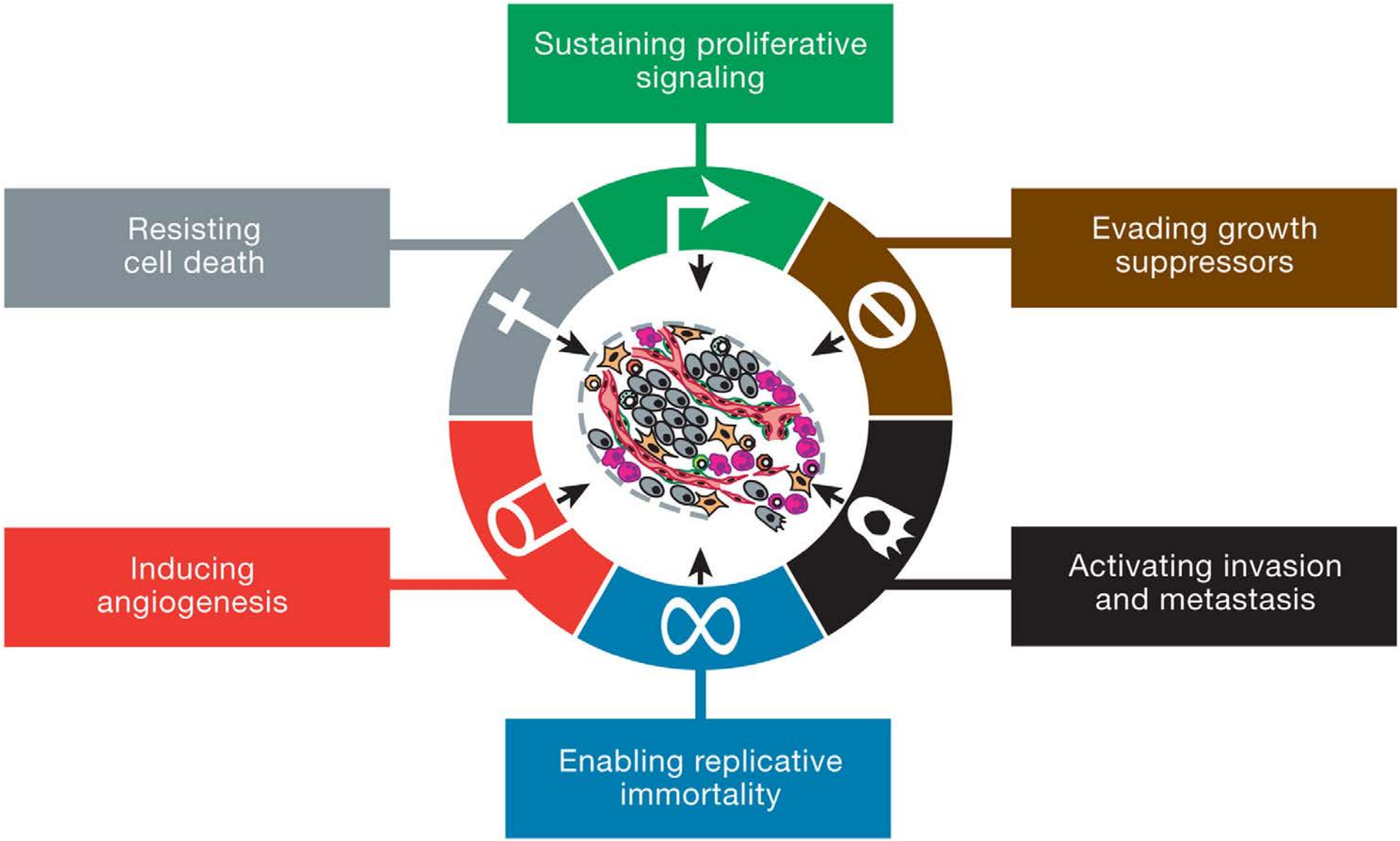
Mammary carcinoma



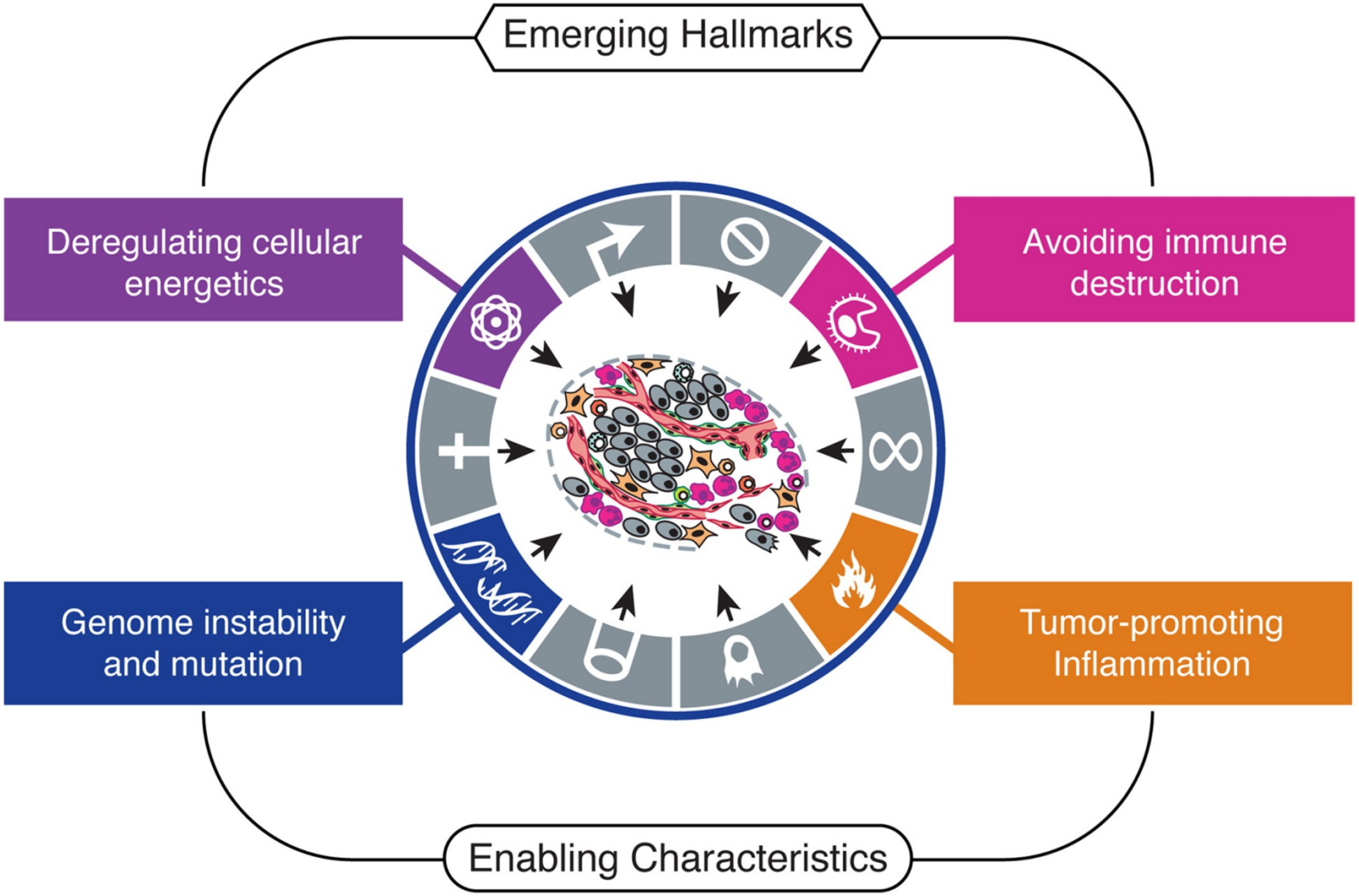
http://www.hindawi.com/journals/ijol/2011/187623/fig1/

Common characteristics of cancer cells

- Uncontrolled cellular proliferation
- Unbalanced cell division
- Loss of apoptosis
- Loss of special function (abnormal organelles and cell components and high rate of differentiation)
- Abnormal angiogenesis and vessel wall structure
- Tissue invasion & metastasis
- Tumor-associated angiogenesis
- High glycolytic rate
- Hypoxia



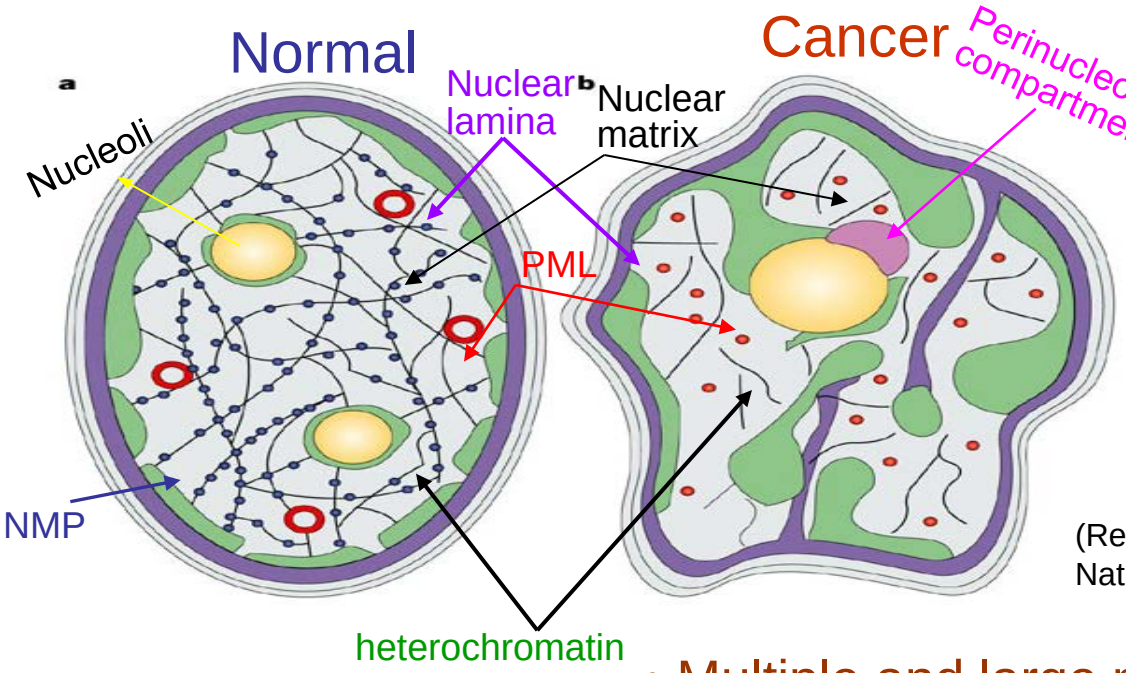
Hanahan D. and Weinberg R. Hallmarks of Cancer: The Next Generation.
***Cell* 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)**


Emerging Hallmarks
Deregulating cellular energetics
Avoiding immune destruction
Genome instability and mutation
Tumor-promoting Inflammation
Enabling Characteristics

GENOME INSTABILITY AND MUTATIONS

Develop an aberrant DNA or gene structure or acquire abnormal numbers of chromosomes.

Nuclear structure in cancer cells



PML: Promyelocytic leukaemia

NMP: nuclear matrix proteins

(Ref: Zink et al., Nature reviews, 2004)

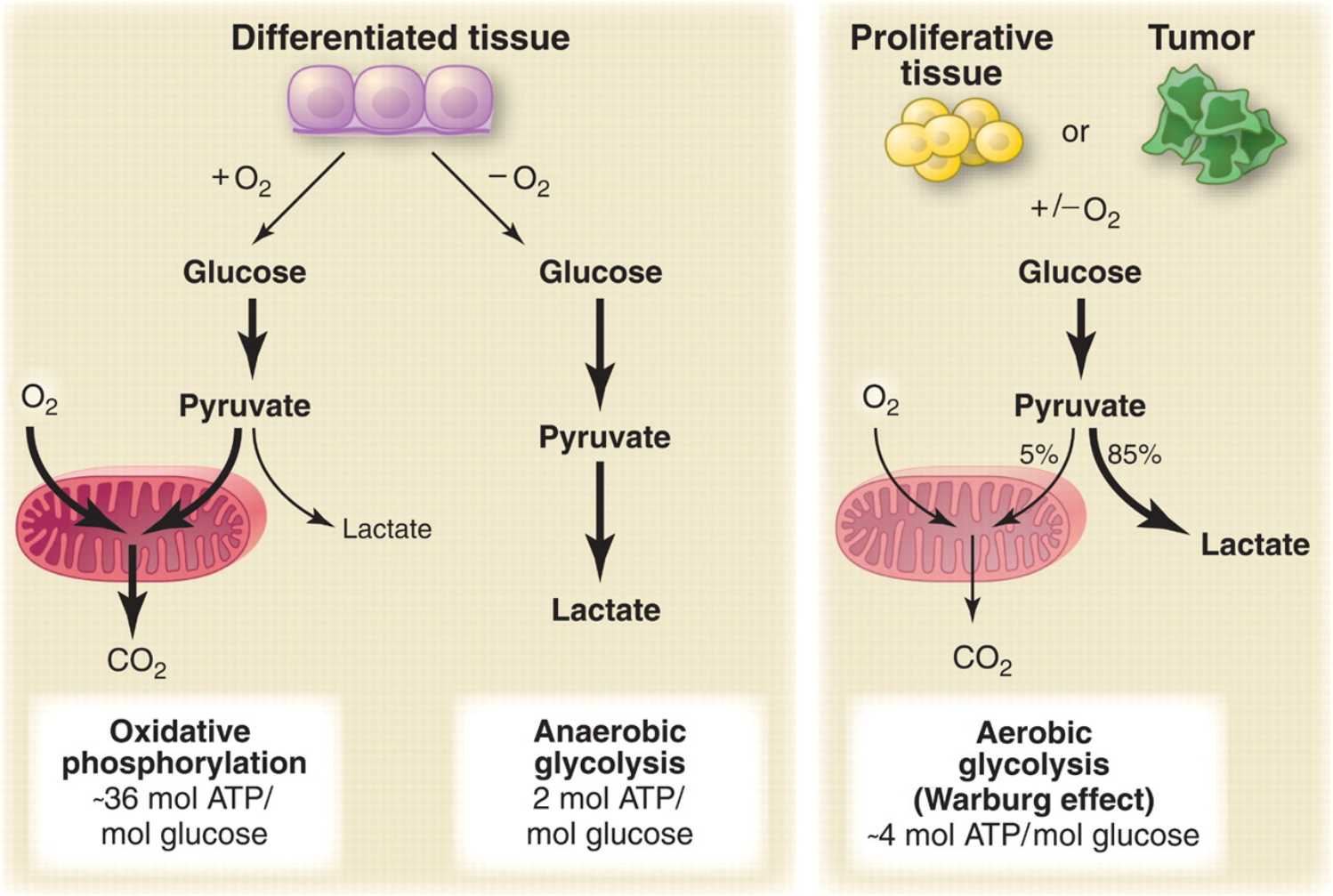
- Single and small nucleus & nucleolus
- Fine chromatin granules in the nucleus
- Clear nuclein stain
- The smooth nuclear border

- Multiple and large nucleolus & nuclei
- Large chromatin clumps in the nucleus
- The dark staining of the nucleus and irregular nuclear border.
- Nuclei can become irregular and begin to fold
- PML bodies can mislocalized.
- Specific NMPs are absent
- Perinuclear compartment is present

DEREGULATING CELLULAR ENERGETICS

- Exhibit a defective Krebs cycle and derive little or no energy from it
- Derive almost all their energy from glycolysis
- Derive most of their energy in the absence of oxygen

Schematic representation of the differences between oxidative phosphorylation, anaerobic glycolysis, and aerobic glycolysis (Warburg effect).



Matthew G. Vander Heiden et al. Science 2009;324:1029-1033

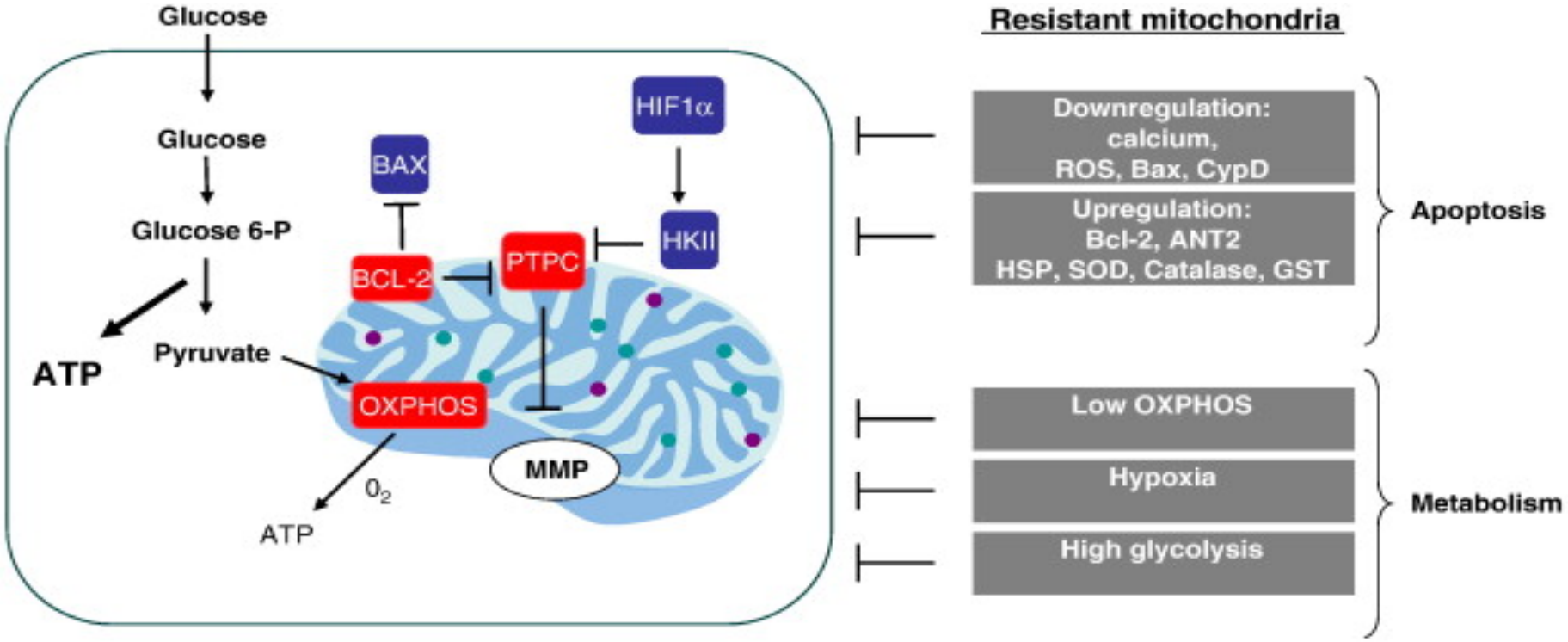
RESISTING CELL DEATH

- Resistance to mitochondrial membrane permeabilization (MMP) - the release of pro-apoptotic proteins from the mitochondrial intermembrane space is inhibited.
- down regulation of pro-apoptotic factors and up-regulation of anti-apoptotic proteins

Mitochondria in cancer cells

• mtDNA mutations inhibit oxidative phosphorylation: Increase ROS level and tumor cell proliferation

Apoptosis-resistant mitochondria and cancer



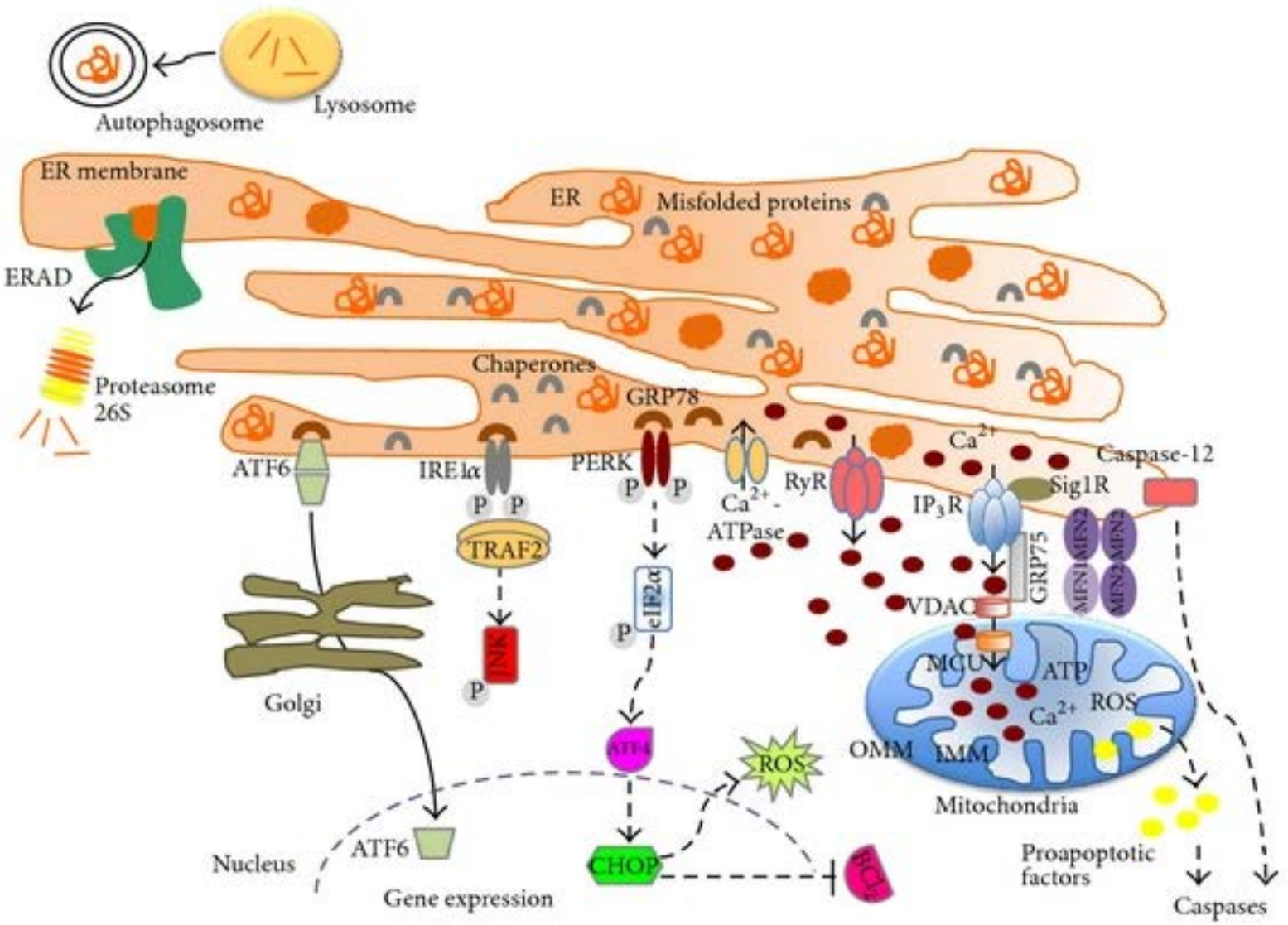
mitochondrial membrane permeabilization (MMP)

permeability transition pore complex (PTPC)

HKII: hexokinase II

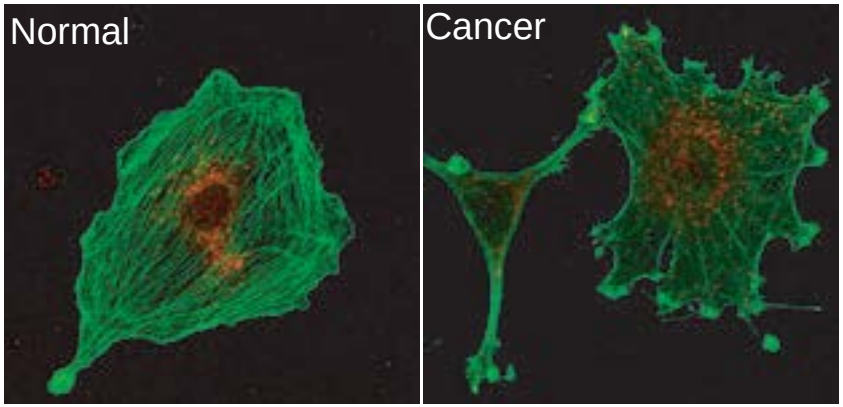
Ref: Indran et al., BBA, 2011

UPR & ER stress in cancer cells



http://www.hindawi.com/isrn/cell.biology/2013/256404/fig1/

Lysosome alterations & Autophagy in cancer cells

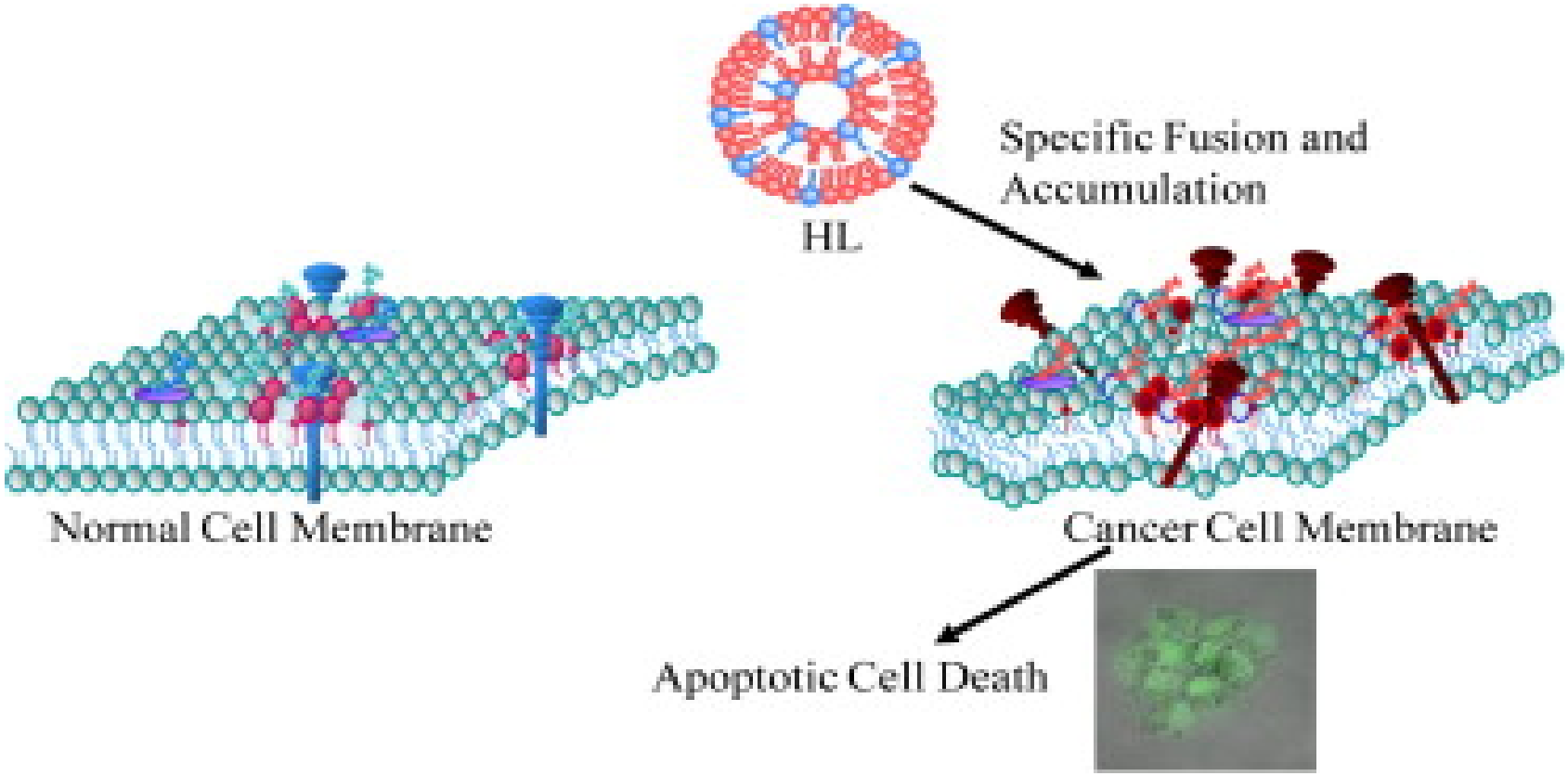


Lysosomes in normal versus cancer cells. Visualization of the **lysosomal compartment** (using lysosome-associated membrane protein 1) monoclonal antibodies, **red**) and the **actin cytoskeleton** (using anti-β-actin monoclonal antibodies, **green**) in murine embryonic fibroblasts.
Note the perinuclear and peripheral localization of lysosomes in control and transformed cells, respectively.
Ref: Kroemer and Jäättelä nature Reviews, 2005, 5: 886-897

Defects in lysosome increase expression and altered trafficking of lysosomal enzymes participates in tissue invasion, angiogenesis and lysosomal death pathway.

Autophagy act as both a "**tumor suppressor**" by preventing the accumulation of damaged proteins and organelles and as a **"mechanism of cell survival"** that can promote the growth of established tumors.

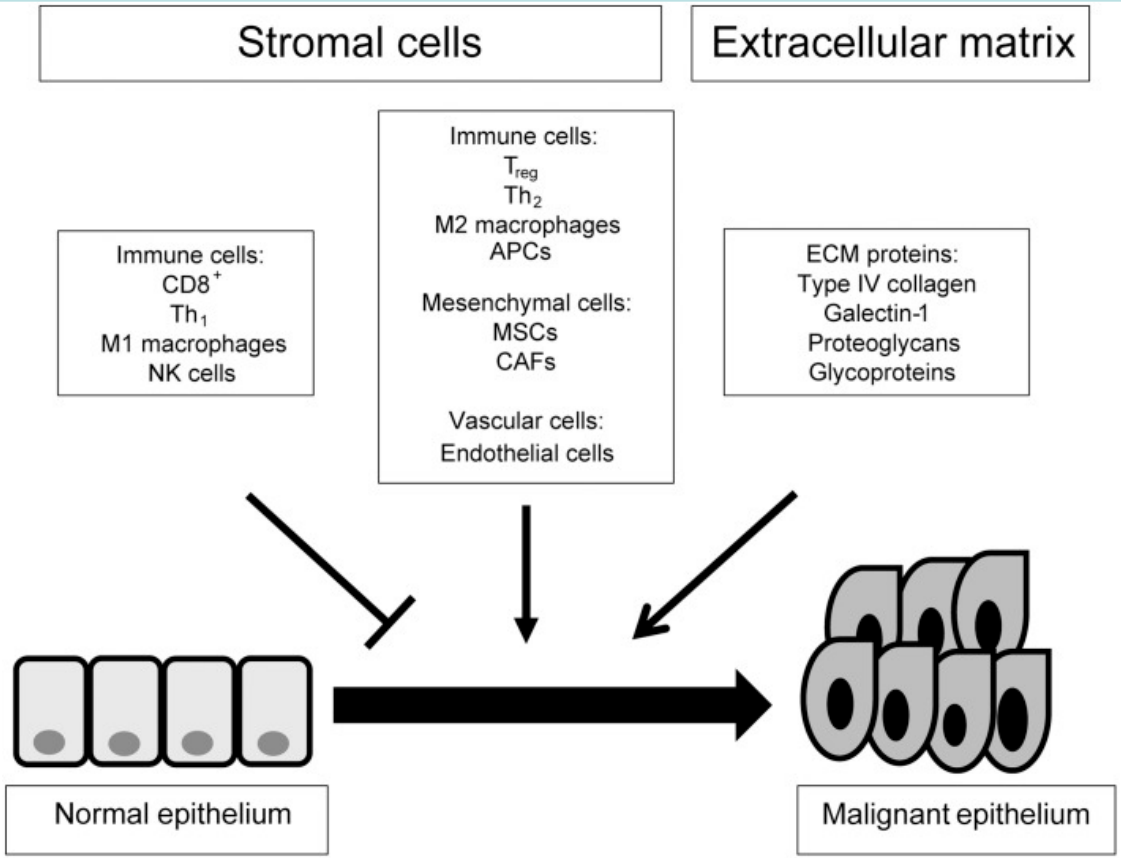
Plasma membrane structure in cancer cells



Changes in the plasma membrane fluidity of tumor cells may affect antigens and receptors as well as the cancer cell motility and capacity to infiltrate the basement membrane and the deformability potential of metastatic cells.

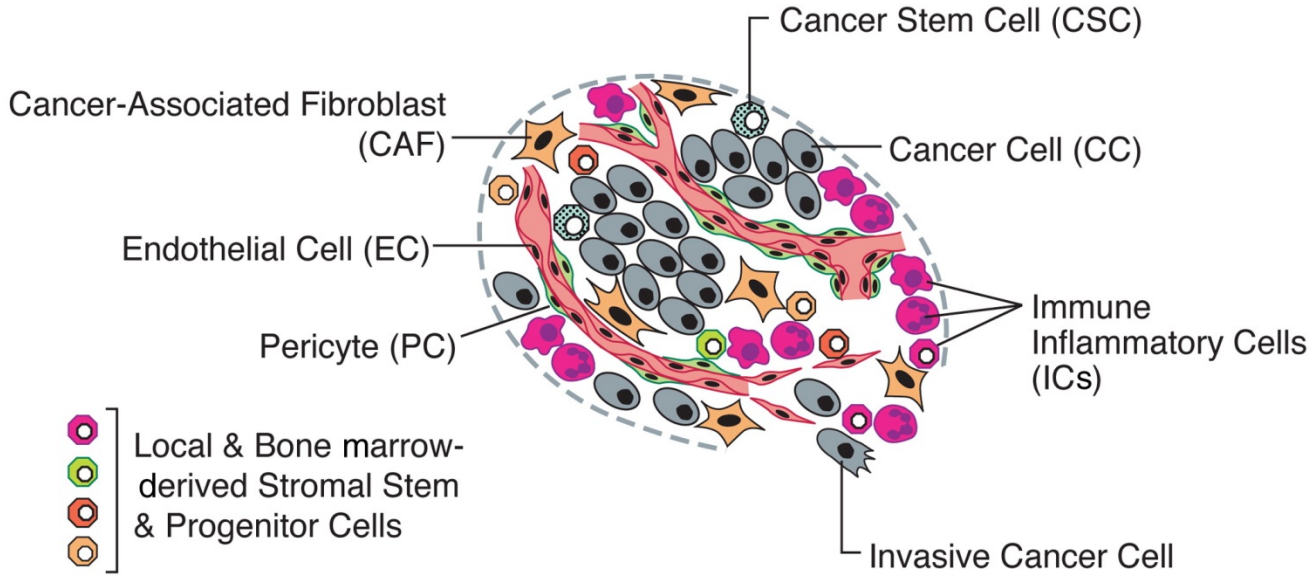
The tumor microenvironment TME

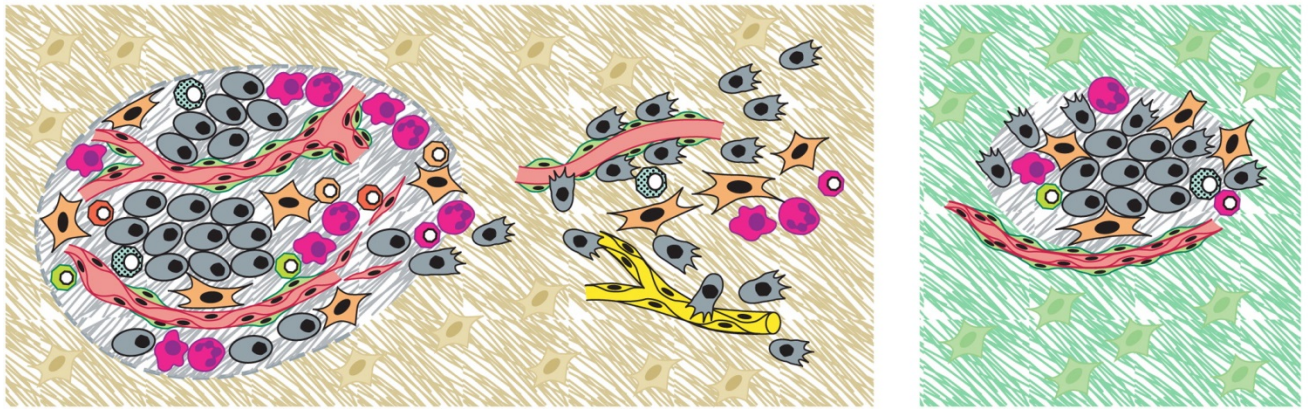
- The **TME** is a functional ecosystem of tumor and stromal elements that interact through signaling molecules
- The **stroma** is a histological unit consisting of peri-tumoral cells within an extracellular scaffold
- **Stromal cells -**mesenchymal, vascular, and immune.



Front Cell Dev Biol. 2015; 3: 33.

The Cells of the Tumor Microenvironment





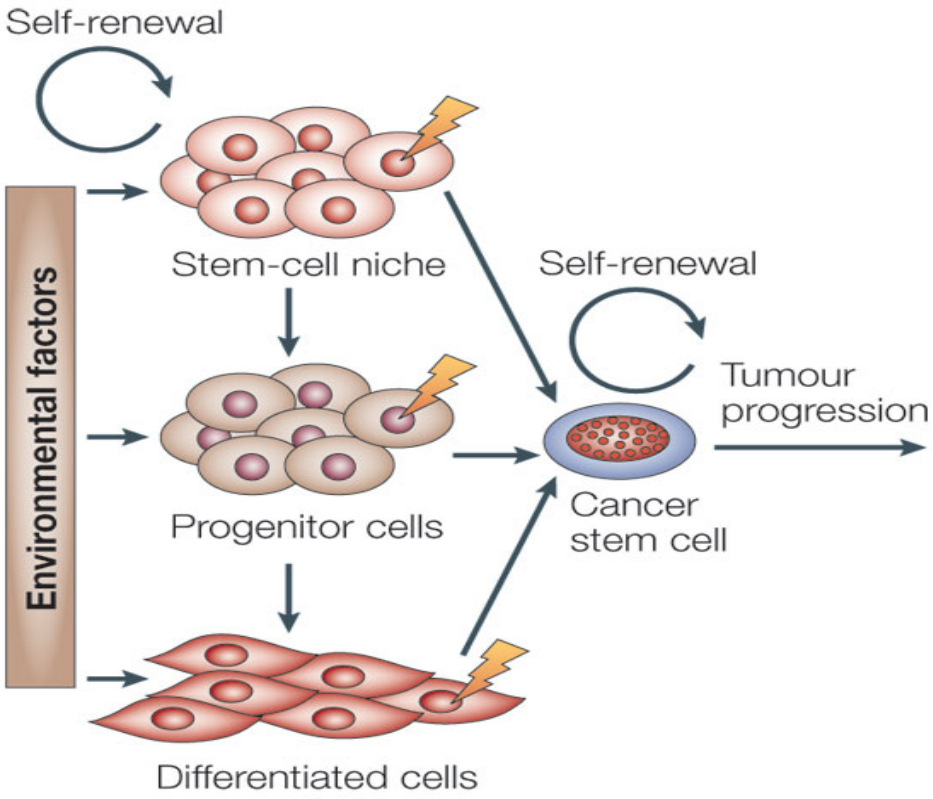
Cell 2011 144, 646-674DOI: (10.1016/j.cell.2011.02.013)

The tumor microenvironment

- **Neoplastic cells**: i.e. cancer stem cells, cancer associated fibroblast,
- **Infiltrating cells:** i.e. lymphocyte, macrophage,
- **Resident cells:** i.e. fibroblasts, endothelial cells,
- **Secreted soluble factors**
  - Cytokines (i.e. CXCR-4 and CXCL-12, TNF-α),
  - Matrix-altering enzymes" (i.e. matrix metalloproteinases (MMPs))
  - Growth factors
    - VEGF: Vascular endothelial growth factor,
    - FDG: Fibroblast growth factors
    - PDGF: The platelet derived growth factor
    - TGF-β: Transforming growth factor beta
- **The extracellular matrix**
- **Hypoxia** (low oxygen levels)
- **Acidic conditions** (low pH level)
- **Hypoglycemia** (low glucose level)
- **Massive cell death**
- **Abnormal properties of surrounding cells**

Mesenchymal Stem Cells MSC

- adherence properties
- ability to differentiate into different cell types
- surface markers (CD73, CD90, and CD105)
- 20% CAFs originate from MSCs and recruitment is dependent on TGF-β and SDF-1

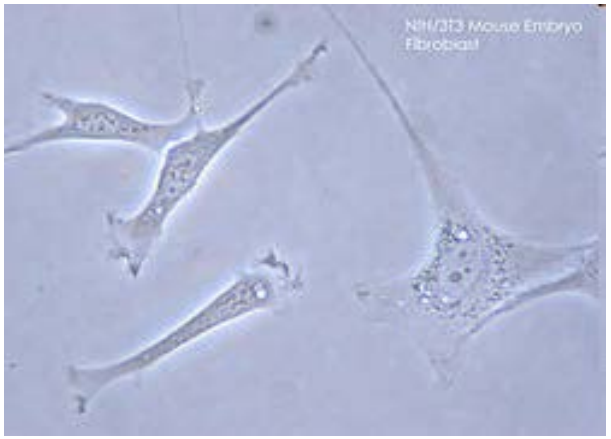


Cancer-associated Fibroblasts

- the predominant cell type in the stroma
- responsible for the structural architecture of the ECM
- synthesize ECM proteins: collagen, periostin and tenascin-C
- α-SMA (smooth muscle actin) positive fibroblasts

remain persistently activated, facilitating cancer progression

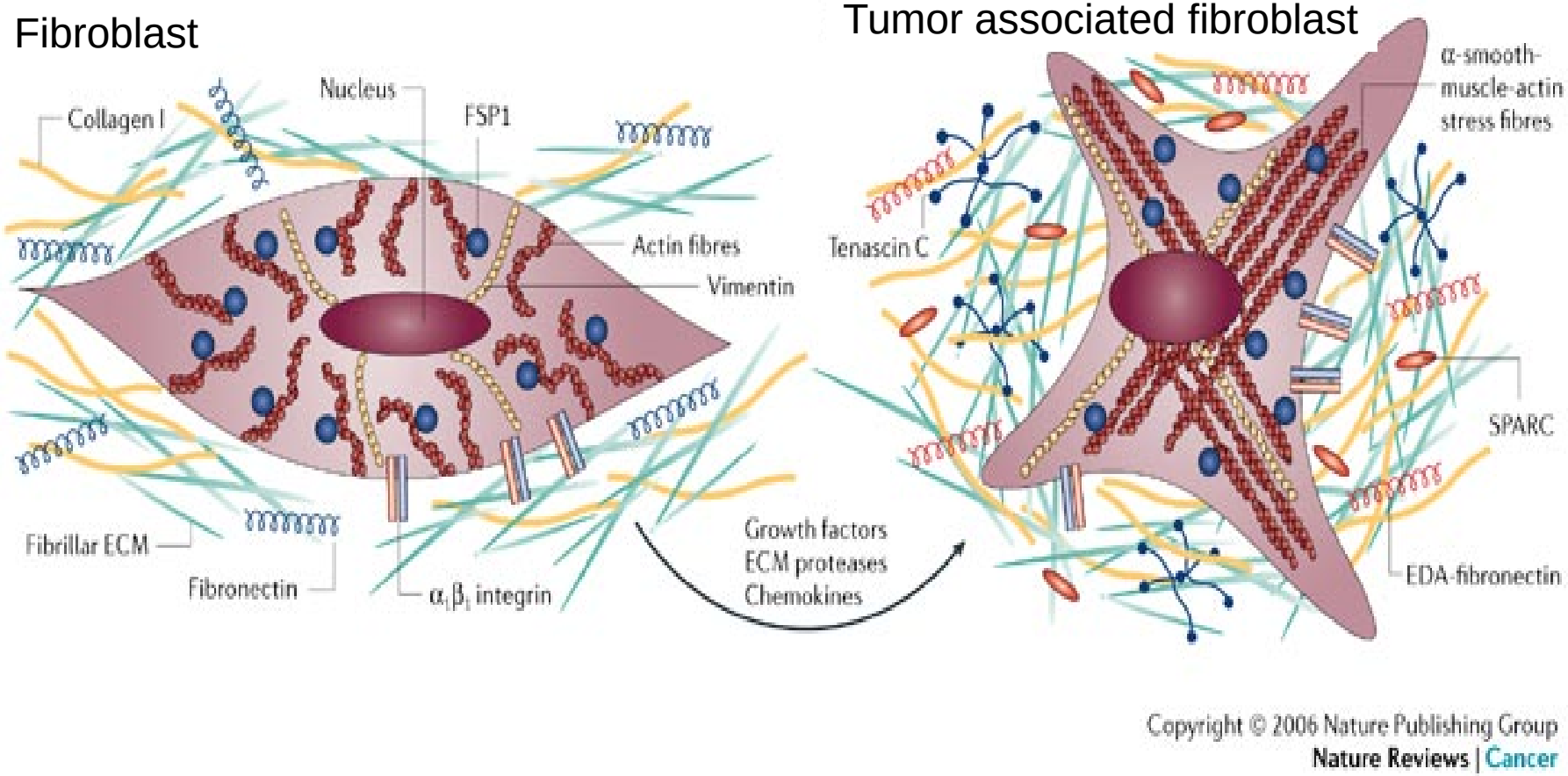
- provide potentially oncogenic signals such as TGF-β and hepatocyte growth factor (HGF) to resident epithelia
- stimulate cancer-cell proliferation and invasion by secreting growth factors such as TGF-β and stromal-cell-derived factor 1 (SDF1)
- play an important role in angiogenesis by secreting FGF2 and SDF1
- there is growing evidence suggesting that CAFs induce invasiveness and metastatic capability of cancer cells – promote EMT



Cancer Associated Fibroblasts

Fibroblast

Tumor associated fibroblast



AVOIDING IMMUNE DISTRUCTION

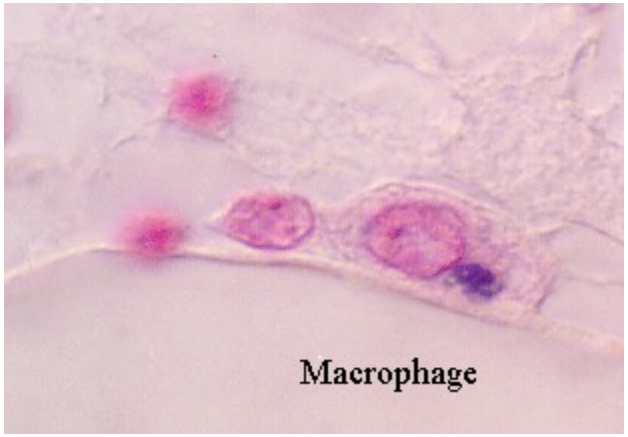
Immune cells

- Immune cells in the TME can have pro- or anti-tumor effects.
- anti-tumor effects - the immune response produced by M1 macrophages, T helper-1 cells, cytotoxic T cells, antigen presenting cells (APCs), and natural killer (NK) cells supports tumor rejection
- pro-tumor effects - M2 macrophages, regulatory T cells, and T helper-2 cells support tumor progression
- In addition to fully differentiated immune cells present in tumor stroma, a variety of partially differentiated myeloid progenitors have been identified in tumors and have demonstrable tumor-promoting activity

Immune cells

Macrophages

• are white blood cells
• are phagocytic cells that play a critical role in innate and adaptive immunity
• are crucial member of tumor stromal cells :
inflammatory M1 take part in immunosurveillance (IL-I and TNFa)
anti-inflammatory M2 release immunosuppressive cytokines such as interleukin-10 and allow tumor progression= tumor associated macrophages (TAMs)



http://legacy.owensboro.kctcs.edu/gcaplan/anat/histology/api%20histo%20connective.htm

Tumor associated macrophages

- induce tumor growth (colon, renal)
- promote angiogenesis (melanoma)
- enhance of tumor cell migration and invasion,
- promote metastasis

Immune cells

T- lymphocytes

CD8+ cytotoxic T cells induce growth arrest, necrosis, and apoptosis in tumor cells by the release of various cytokines including interferon gamma (IFN-γ)

↓

apoptosis

↓

phagocytosed by antigen presenting cells APCs and exposed to maturing lymphocytes in lymphoid organs

↓

Tumor suppression

Regulatory T cells (Tregs) promote immune tolerance by expressing a cytokine profile that attenuates the proliferation of CD8+ cells, inhibits APCs and macrophages and reduces the lytic activity of NK cells

↓

Tumor progression

Immune cells

Antigen-presenting Cells (APCs)

APCs process and display antigens with MHC proteins to naïve T cells

MHC I-expressing cells stimulate CD8+ T cells

MHC II-expressing cells stimulate CD4+ cells

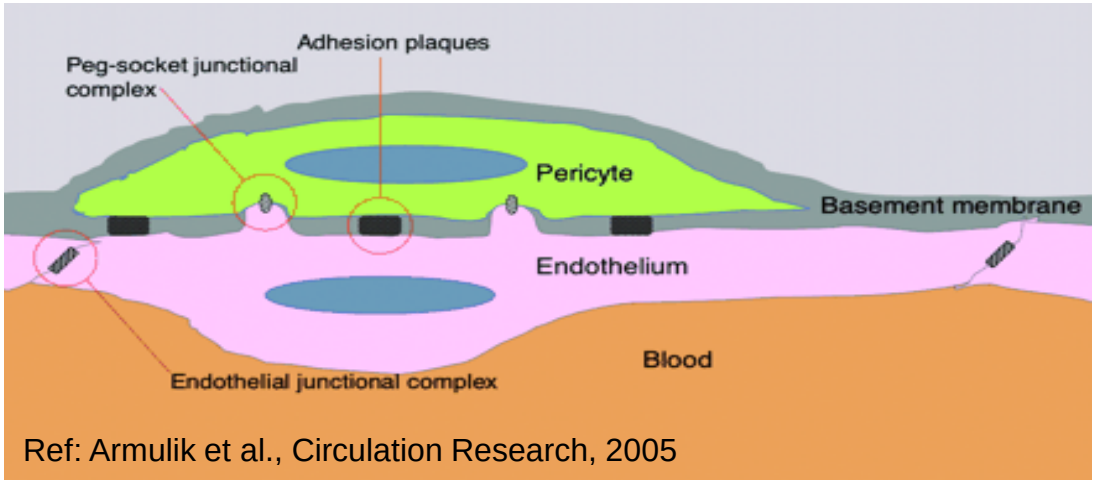
Natural Killer Cells (NK cells)

- NK cells are innate immune cells
- important in halting tumor progression
- NK cells destroy tumor cells in animal models of several human cancers by detecting cell surface changes such as reduced MHC I

INDUCING ANGIOGENESIS

Endothelial cells & Pericytes

Pericytes are adjacent to endothelial cells and embedded within the vascular basement membrane of normal blood microvessels



Ref: Armulik et al., Circulation Research, 2005

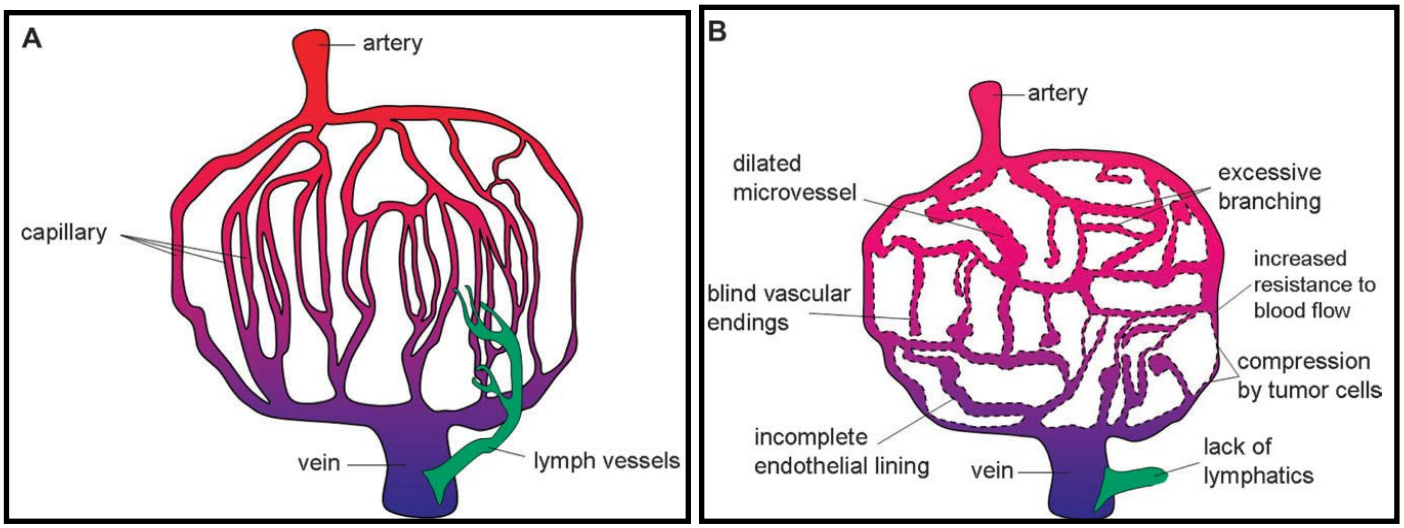
ECs in the TME lack a pericytes covering, have leaky tight junctions and exhibit sprouting

Pericytes - Endothelial cells signaling network can contribute to tumor development and metastasis:

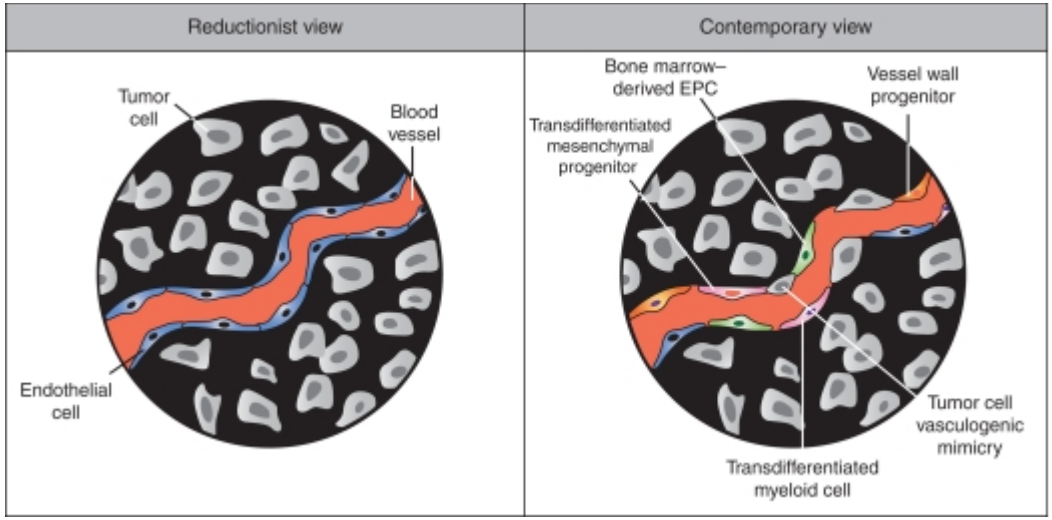
Stromal tissue hypoxia triggers the release of VEGF from pericytes → activating VEGF-2 receptors on adjacent ECs →

ECs become “tip” cells and migrate toward hypoxic tissue that has the highest VEGF concentration

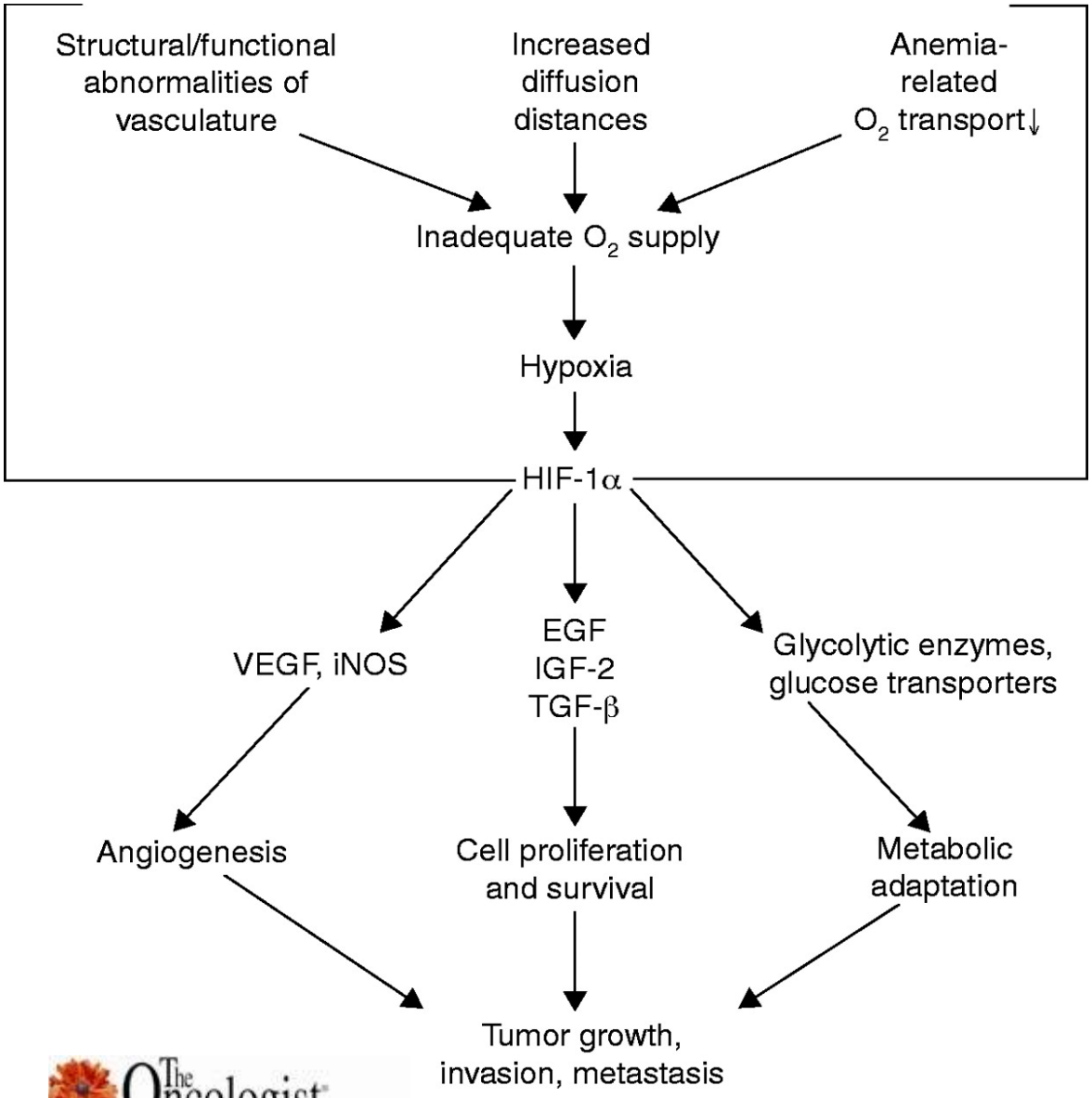
Blood vessels & flow in cancer cells



Diagrammatic representation of the vascular system. **A.** Normal tissue. **B.** Solid tumor. **Red** represents well-oxygenated arterial blood, **blue** represents poorly oxygenated venous blood, and **green** represents lymphatic vessels. (Ref: Tredan et al., JNCI, 2007, 99:1441-1454)



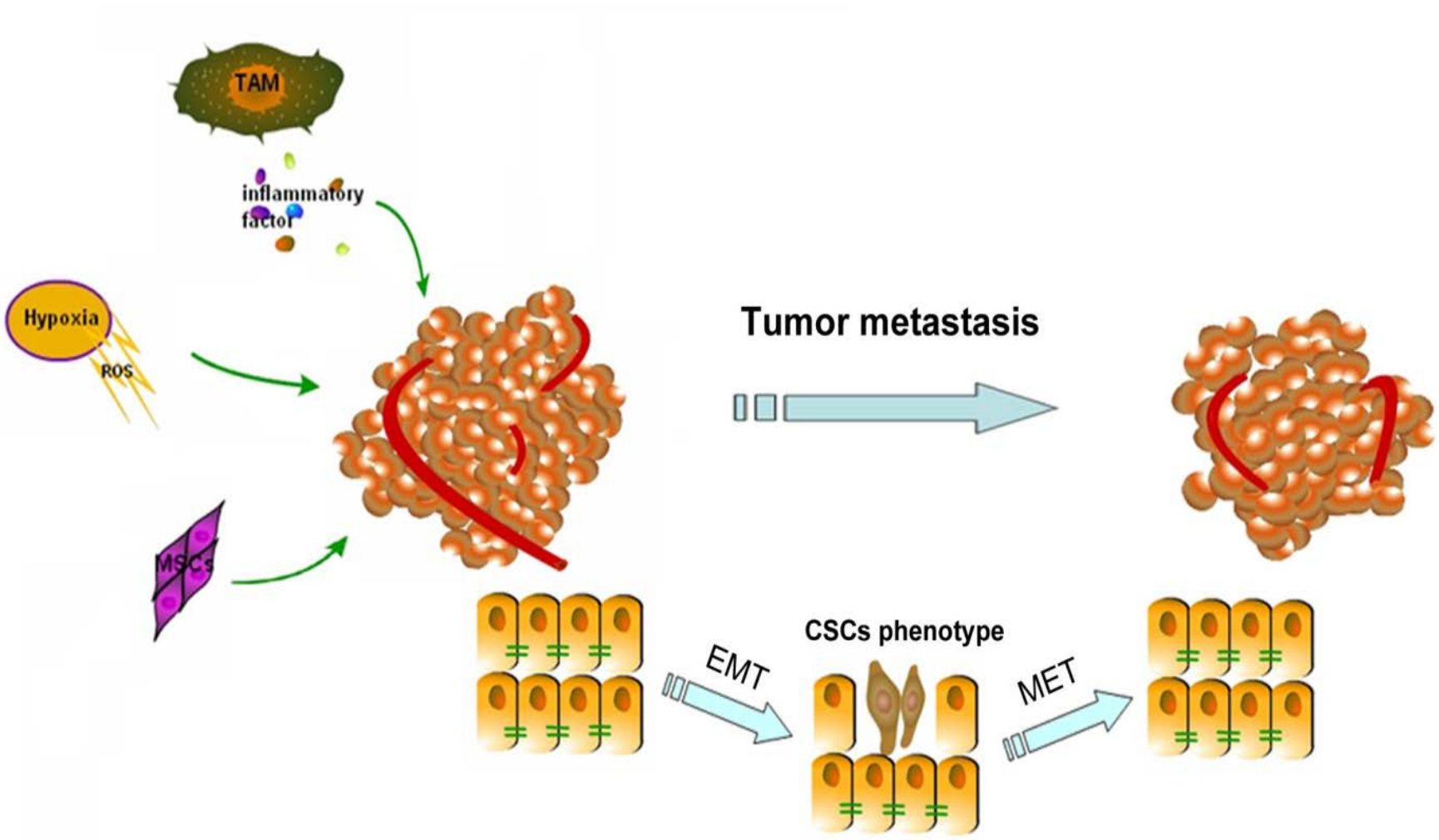
Hypoxia in solid tumor



The Oncologist

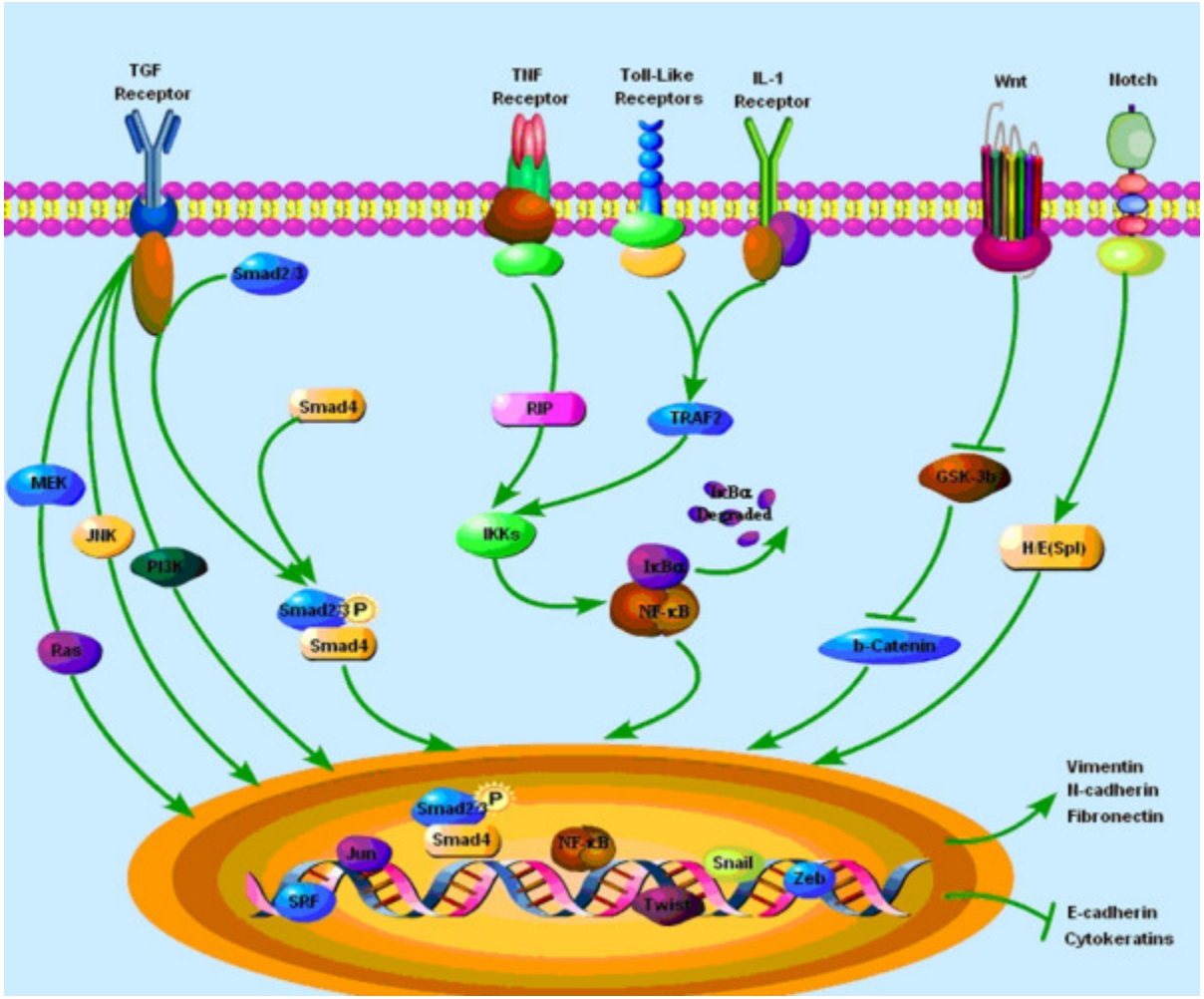
Imbalance between oxygen supply and oxygen consumption in tumors

Hypoxia-induced EMT in tumor microenvironment



Various factors that induce cancer cell EMT in tumor microenvironment. (Ref: Jing et al., Cell & Bioscience 2011, 1:29)

Signaling pathways that regulate Epithelial-Mesenchymal Transition (EMT) in tumor microenvironment



Jing et al., Cell & Bioscience 2011, 1:29

TME - Extracellular matrix (ECM)

- ECM constitutes the cellular scaffold of the TME providing structural support to tumor epithelium and stromal cells
- is produced by mesenchymal cell types including fibroblasts, chondrocytes, and osteoblasts and consists of various components including collagens, galectins, proteoglycans, and glycoproteins
- has the capacity to both initiate and channel signaling cascades within the TME
- biomechanical properties determine the dynamics of ECM turnover - invasion
- ECM may provide a “cancer stem-cell” niche
- is implicated in angiogenesis and inflammation pathways - pro-metastatic TME

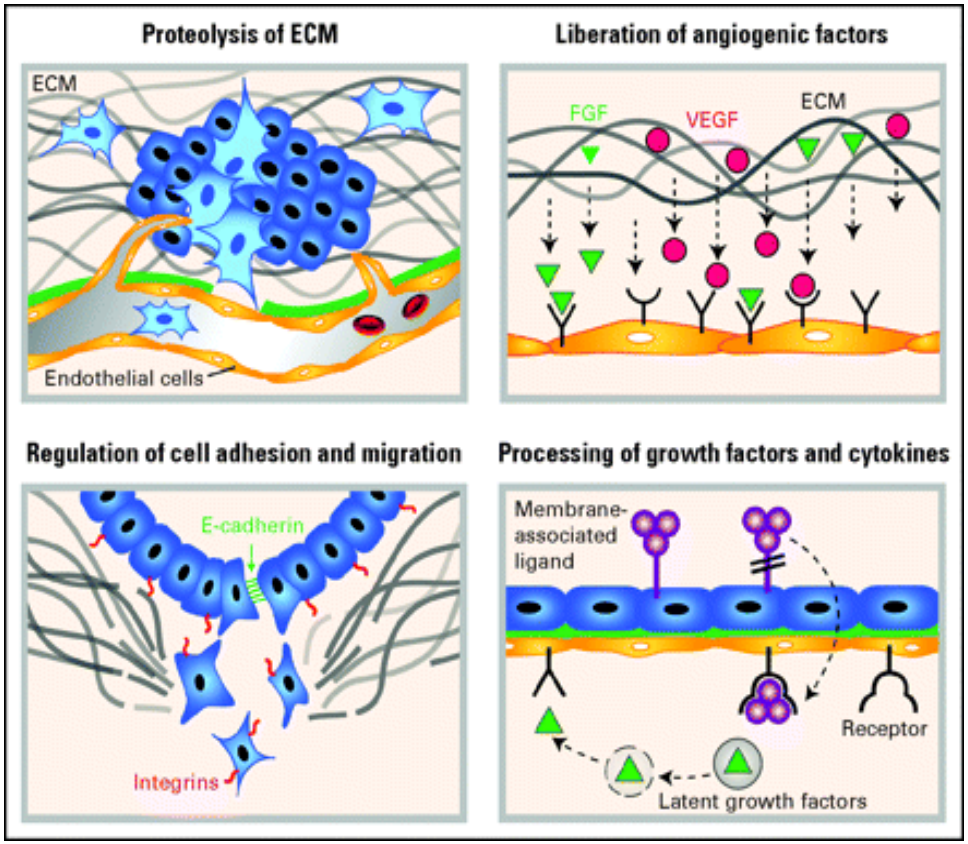
TME - Extracellular matrix (ECM)

- **Type IV collagen** is the major component of the basement membrane, the most important protein in the ECM
  binds to integrin receptors on cancer cells, promoting their survival
- **Galectin-1** is a carbohydrate binding protein important for: adhesion to the ECM, increased migration and stromal immune suppression
- **Proteoglycans** such as heparan sulfate maintain the physical connections between different ECM components
- **Glycoproteins** - fibronectin and laminin-1 are ligands for β-integrins, cellular proteins which mediate cell-ECM signaling. ECM expression of fibronectin and laminin-1 correlates with poor prognostic features in breast cancer

ECM Turnover

Matrix Metalloproteinases (MMPs) and Tissue Inhibitors of Metalloproteinases (TIMPs)

- MMPs - zinc-dependent endopeptidases, capable of degrading almost all ECM proteins
- Increased MMP expression is associated with most tumors
- MMPs are secreted by both tumor and stromal cells
- important in other aspects of cancer progression such as angiogenesis, adhesion, migration and processing of growth factors and cytokines
- TIMPs - negatively regulate MMP activity. TIMP-3 is inactivated in cancer

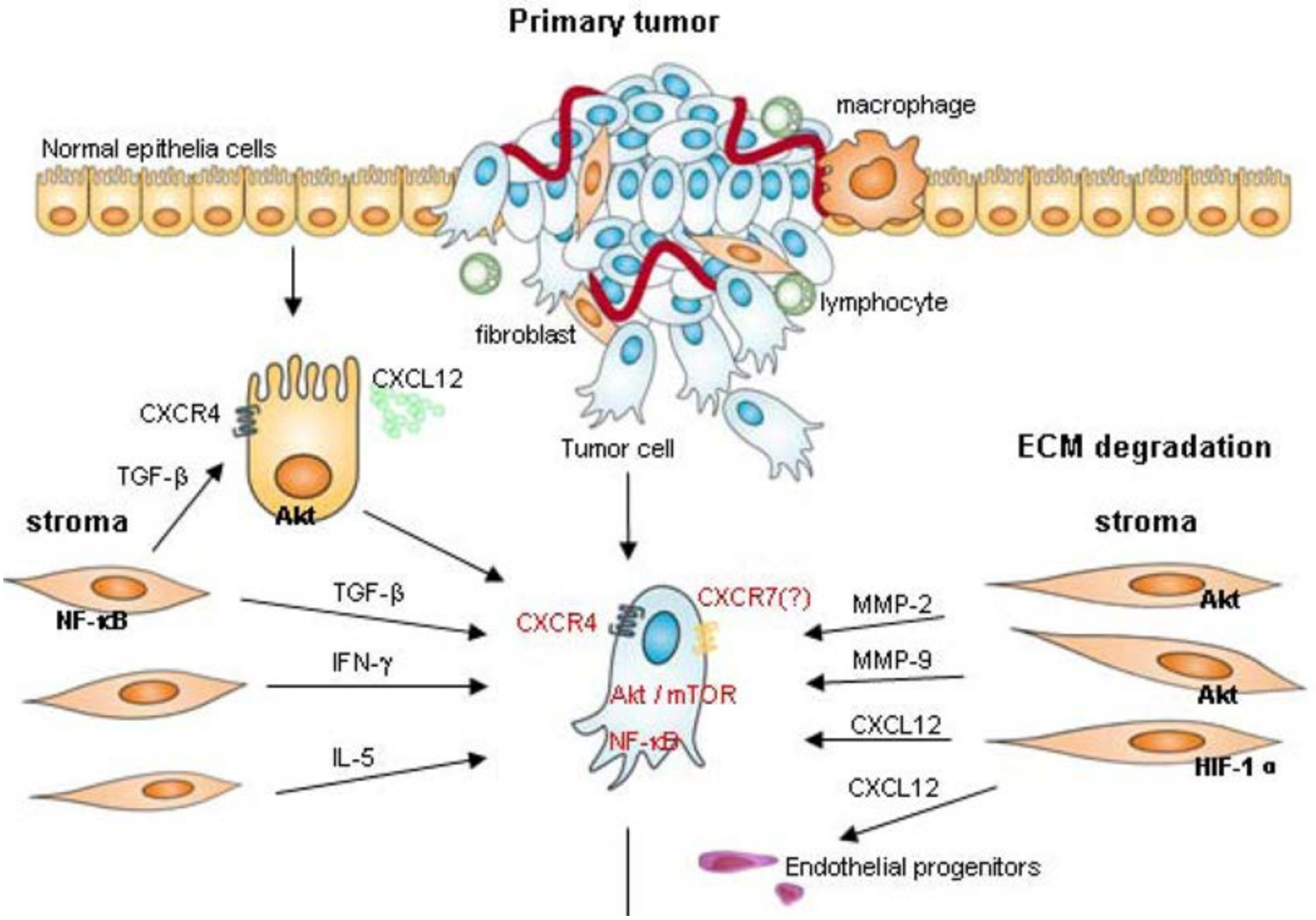


Ref: Roy et al., JCO, 2009

New Players in Stroma-Cancer Cell Interaction: microRNAs and Exosomes

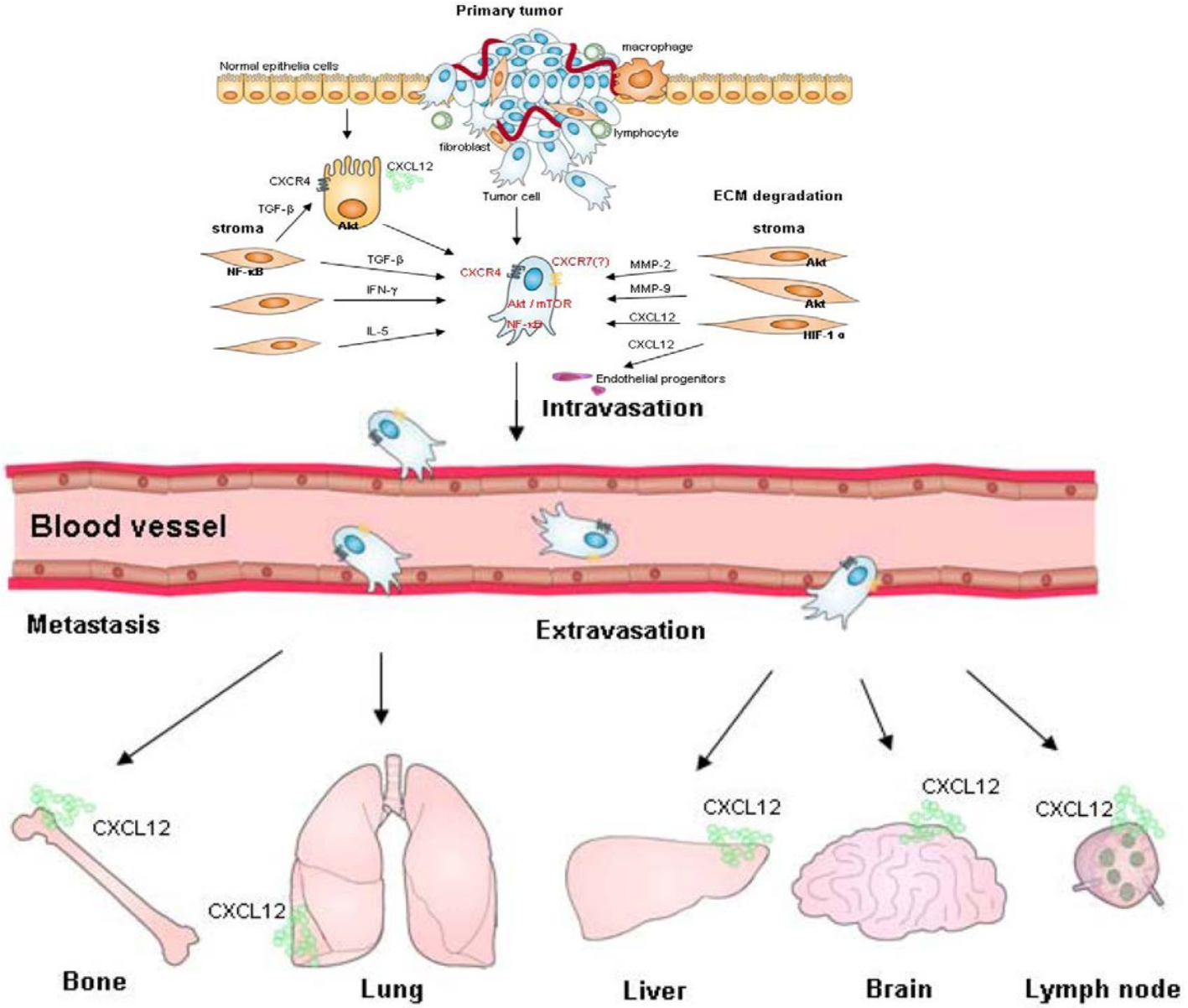
- exosomes, are secreted from both cancer and stromal cells and influence gene expression of cells in the vicinity
- deliver their RNA and protein cargo and alter gene expression in the recipient
- miRNAs stand out as major players because they are relatively stable compared to mRNA and proteins
- miRNAs are non-coding RNAs that are approximately 20 nucleotides long. They undergo enzymatic activation in the cytoplasm, where they bind to the 3′ untranslated region of coding mRNAs to prevent protein translation
- miRNAs regulate a variety of cellular processes such as proliferation, differentiation, and apoptosis. Aberrant miRNAs fail to properly regulate these processes, leading to malignant transformation
- miR-21 is an oncomir and associated with aggressive colorectal cancer ; it is produced by stromal fibroblasts

Communication of tumor cells with tumor microenvironment



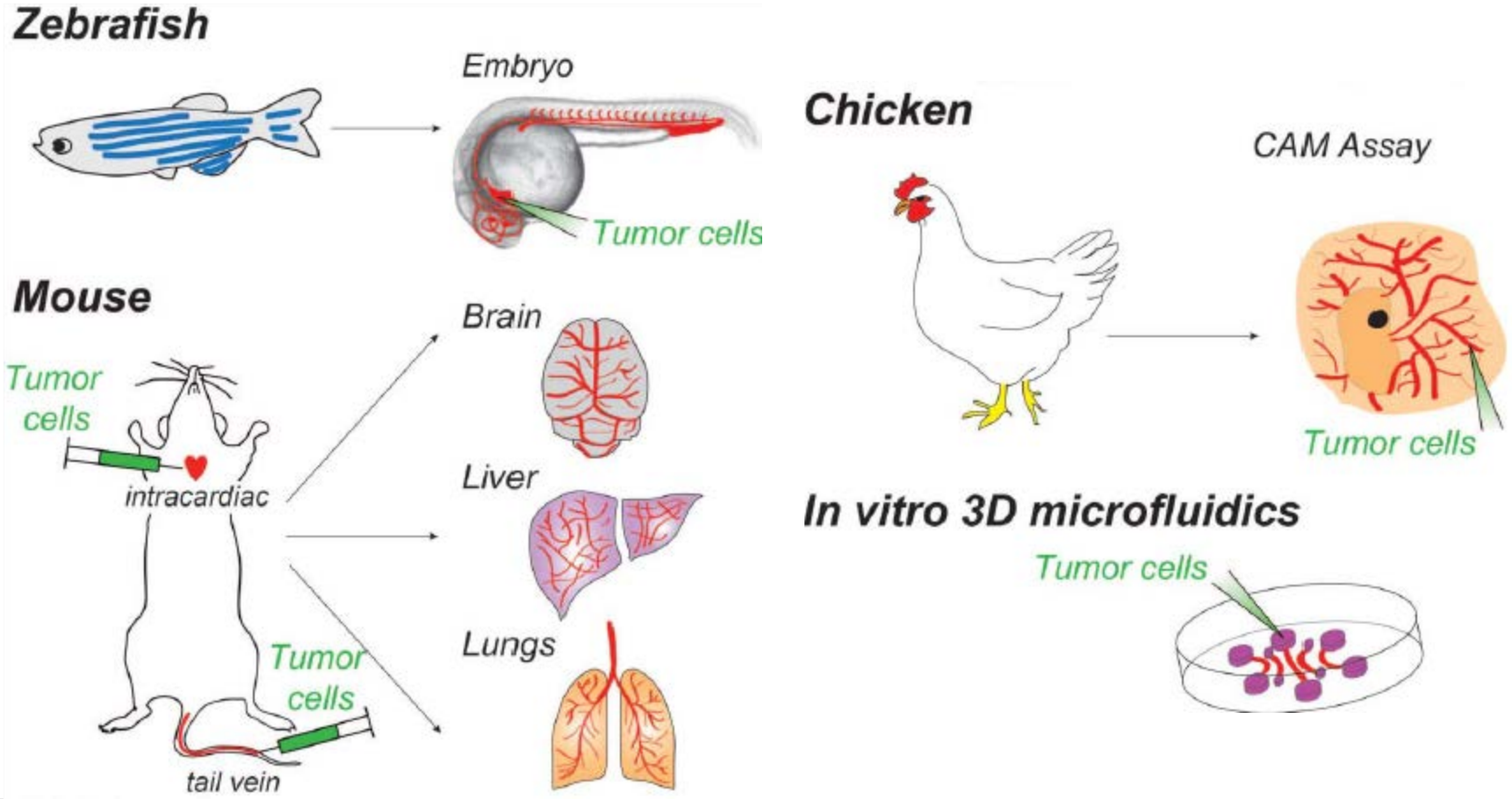
Ref: Sun et al., Cancer Metastatis Rev. 2010

Organ-specific metastasis & tumor microenvironment



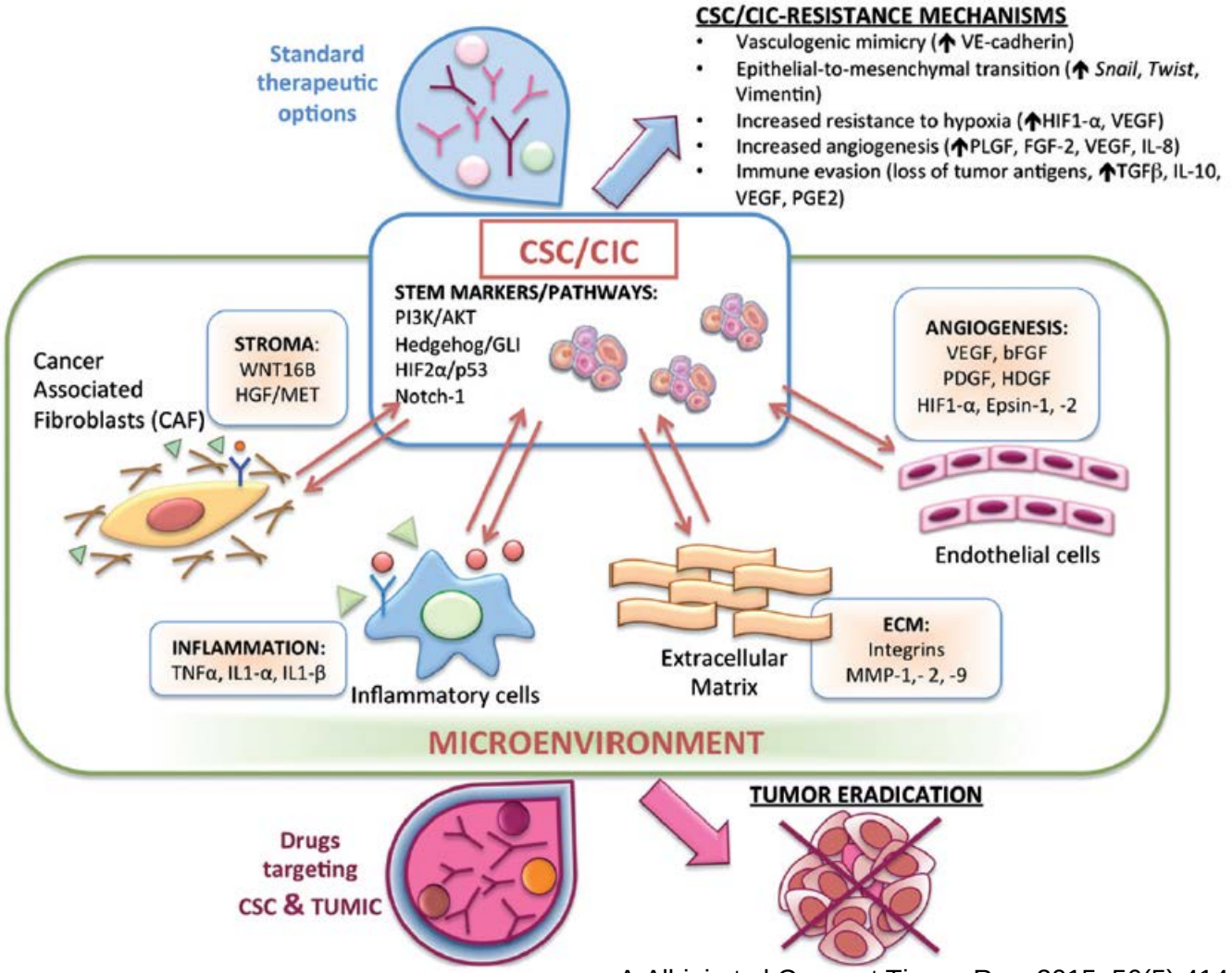
Ref: Sun et al., Cancer Metastatis Rev. 2010

Relevant models to study tumor cell extravasation and hemodynamics



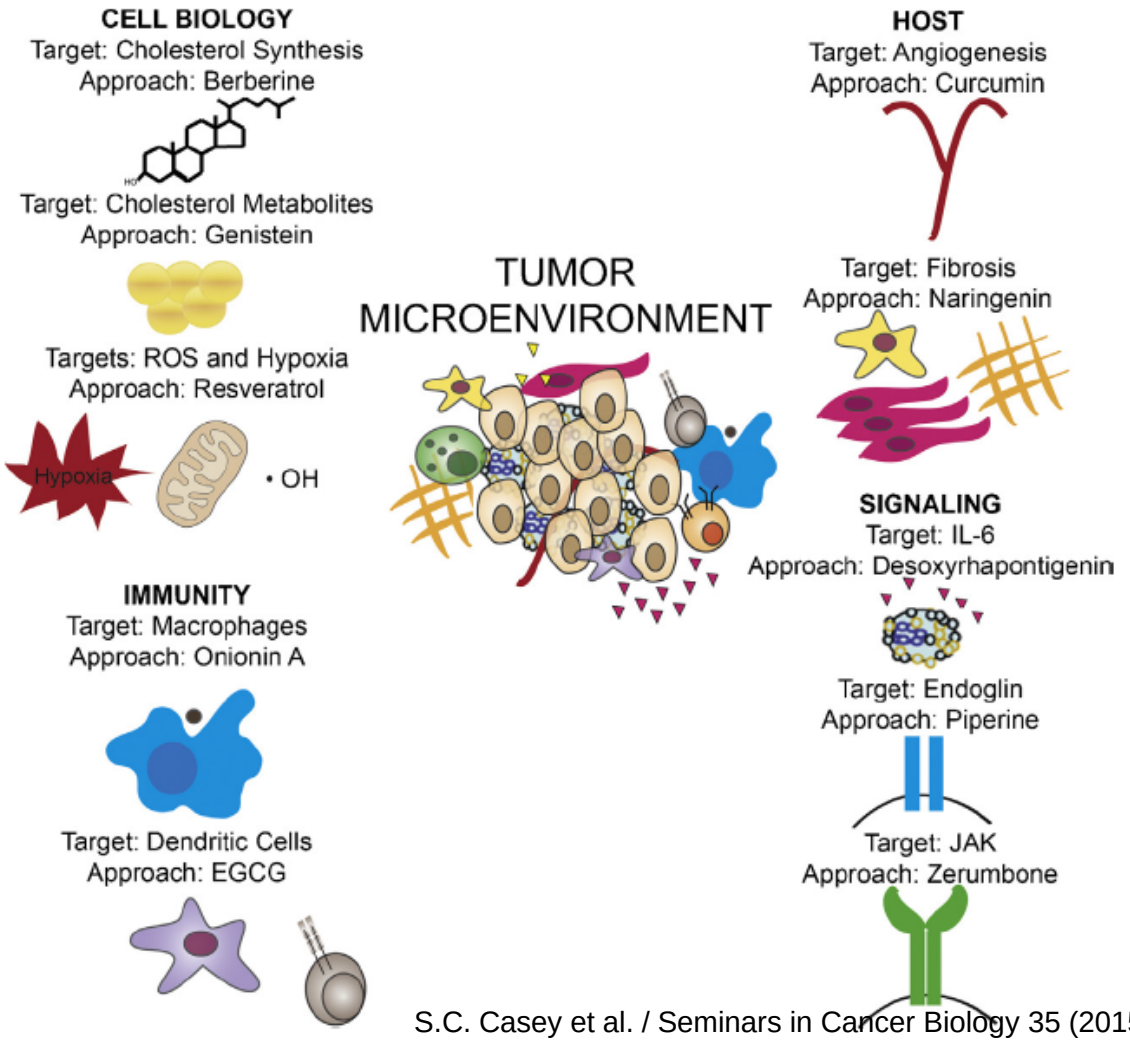
Cell Adhesion & Migration 9:5,345--356; 2015

Novel therapeutic treatments targeting both the CSC/CIC compartment and the TUMIC with the potential to eradicate the tumor needed



A.Albini et al.Connect Tissue Res, 2015; 56(5):414–425

Targets and approaches identified that could modulate the tumor microenvironment to prevent or treat cancer



S.C. Casey et al. / Seminars in Cancer Biology 35 (2015) S199–S223

Sample Questions

1. Match the following

a-Metalloproteinases	1. is a part of endomembrane system
b-Mitochondria	2. express *alpha* smooth muscle actin
c-VEGF	3. cause extracelullar matrix degradation
d-Integrin	4. contains own DNA that is not protected by histons
e-Myofibroblasts	5. stimulates angiogenesis
f-Golgi apparatus	6-helps cell-extracellular matrix adhesion

2. Which of the following(s) is/are true?

A. Pericytes acquire an “activated” phenotype within the tumor characterized by expression of α-SMA and increasing proliferation and motility.

B. Adipocytes act as a energy source for the cancer cells

C. EMT is characterized by loss of integrin and disruption of cell adhesion

3. List the common characteristics of cancer cells

Reading

Book

- **Chapter 13:** "*The Biology of Cancer*" (2nd Edition). Robert Weinberg, Garland Science, 2013
- "*Molecular Biology of the Cell*" (5th Edition). Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, Peter Walter; 2008
- "*The Emperor of All Maladies: A Biography of Cancer*" Siddhartha Mukherjee; Scribner, 2010

Review

- Hanahan & Weinberg, *Hallmarks of Cancer: the next generation*. Cell. 2011. 144: 646-674
- Bhome et al. *A top-down view of the tumor microenvironment: structure, cells and signaling*. Frontiers in Cell and Developmental Biology. 2015. 3: 33

Reading

Review

- Azevedo et al. *Metastasis of circulating tumor cells: favorable soil or suitable biomechanics, or both?* Cell Adhesion & Migration. 2015. 9:5,345--356
- Casey et al. *Cancer prevention and therapy through the modulation of the tumor microenvironment*. Seminars in Cancer Biology. 2015. 35:S199–S223
- Justus at al. *Molecular Connections between Cancer Cell Metabolism and the Tumor Microenvironment*. Int. J. Mol. Sci. 2015, 16, 11055-11086

- Bhome et al. *A top-down view of the tumor microenvironment: structure, cells and signaling*. Frontiers in Cell and Developmental Biology. 2015. 3: 33