NEOPLASIA 5

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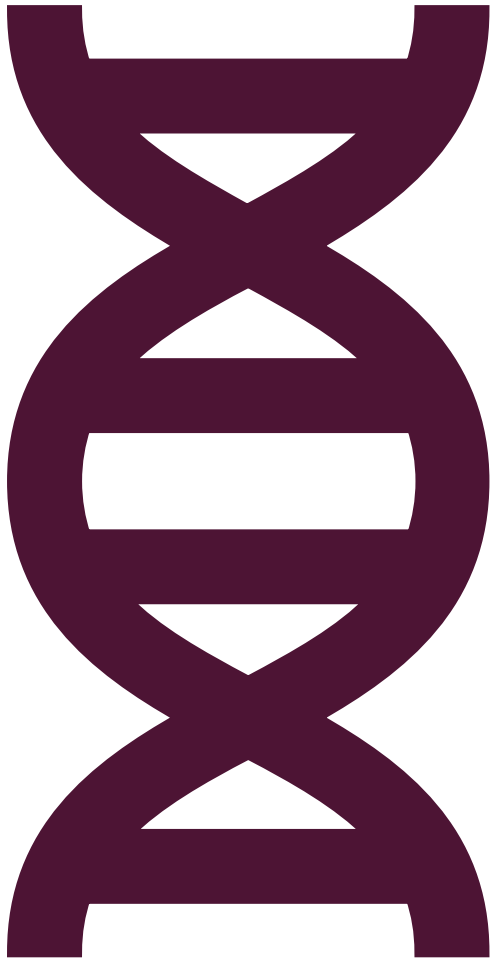
GENERAL PATHOLOGY LECTURES 2023

CONTINUATION OF INSENSITIVITY TO GROWTH INHIBITORS

- RB and TP53 genes which we discussed previously cause growth inhibition by controlling the cell cycle.
- There are other genes that are involved in inhibiting proliferation.
- Inhibitory signals are similar to stimulatory ones regarding their mode of action. –> see next slide



CONTINUATION OF INSENSITIVITY TO GROWTH INHIBITORS

This means there is an inhibitory factor that binds to a receptor which causes transmission of the signal through cytosolic proteins to the nucleus to inhibit transcription factors.

The steps above are well understood in growth factors pathways (which we already discussed) but for growth inhibition the specific molecules involved are not exactly known.

The best-known pathway of growth inhibition is the TGF beta pathway which we will discuss next..

TGF BETA PATHWAY

TGF beta (transforming growth factor beta) is a potent inhibitor of cell proliferation.

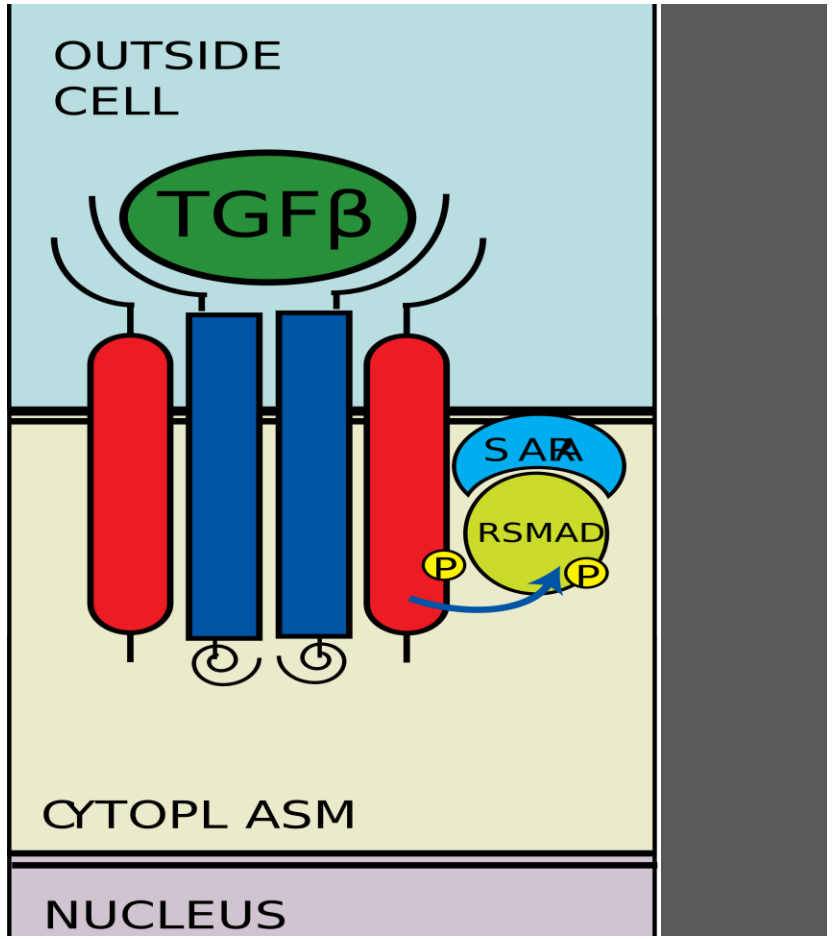
TGF beta binds to receptors… Receptors activated .. Transmit signal through SMAD proteins to the nucleus

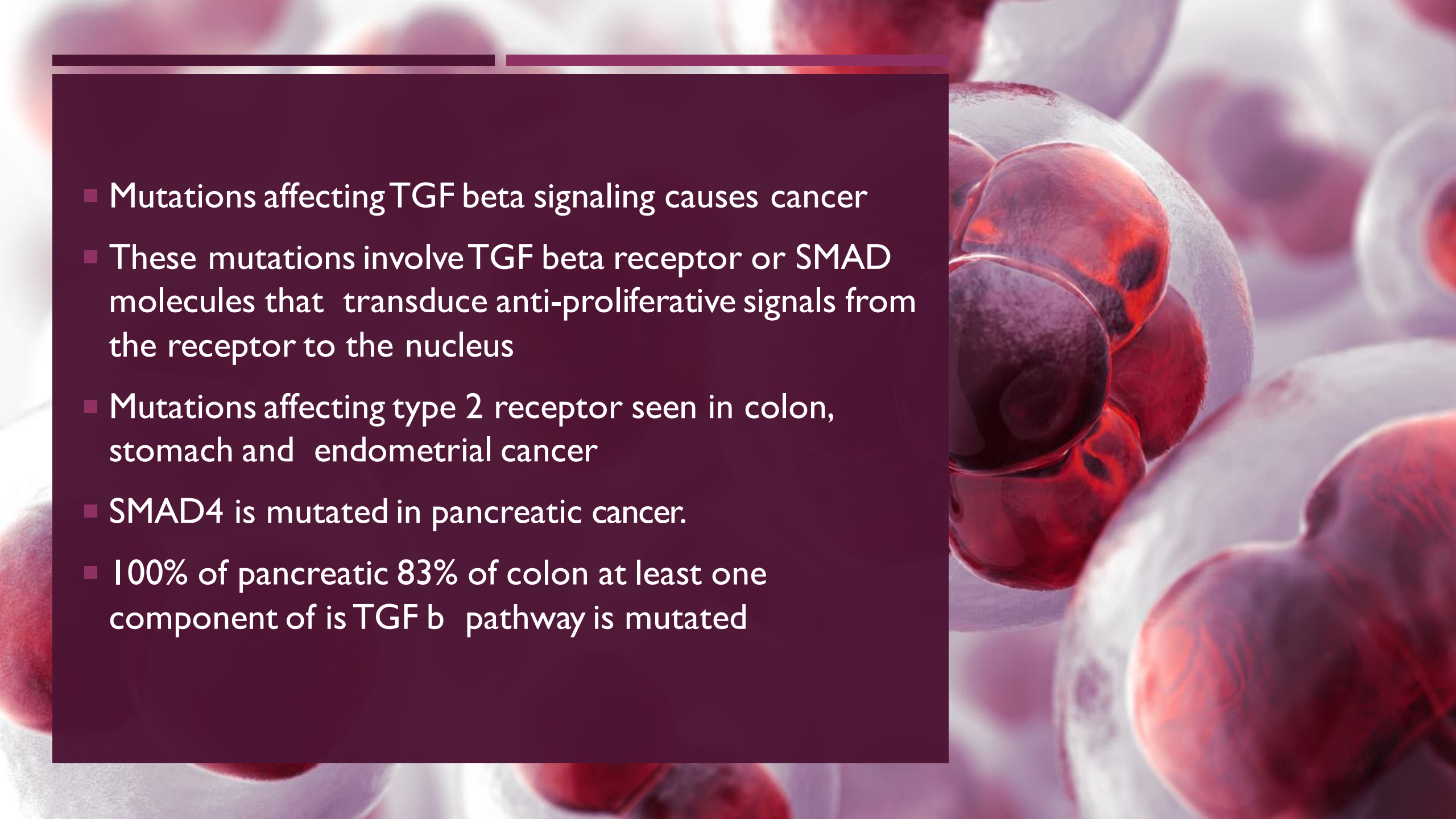
Transmitted signals to the nucleus result in transcriptional activation of CDKIs and repression of MYC and CDK4.

The result is growth inhibition.

TGF BETA

- TGF beta is a negative growth regulator.
- It binds to transmembrane receptors
- This binding stimulated second messengers in the cytosol.. Of the SMAD family
- The message reaches the nucleus: to inhibit growth through upregulation of CDKI and down regulation of CDK4 and MYC.

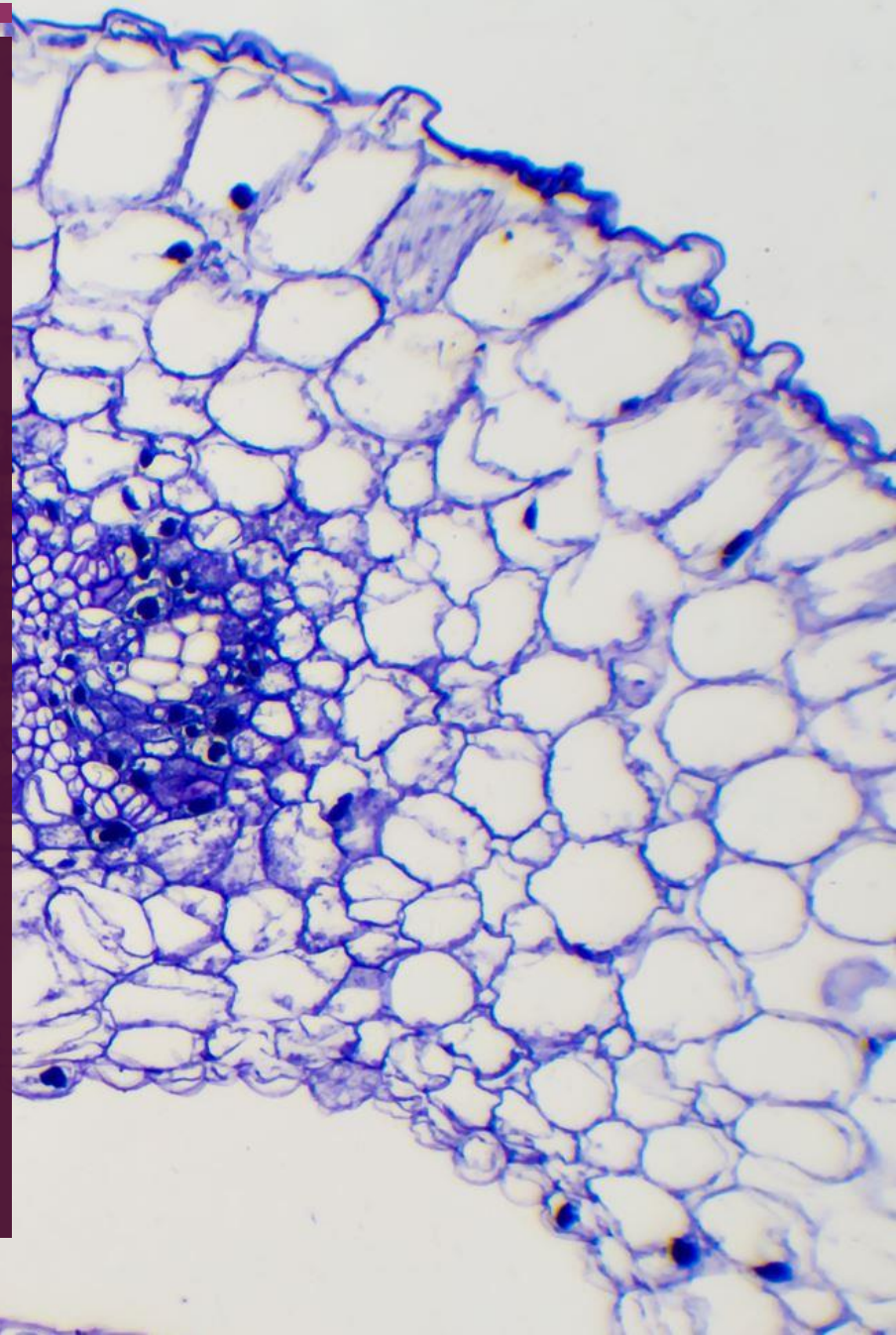




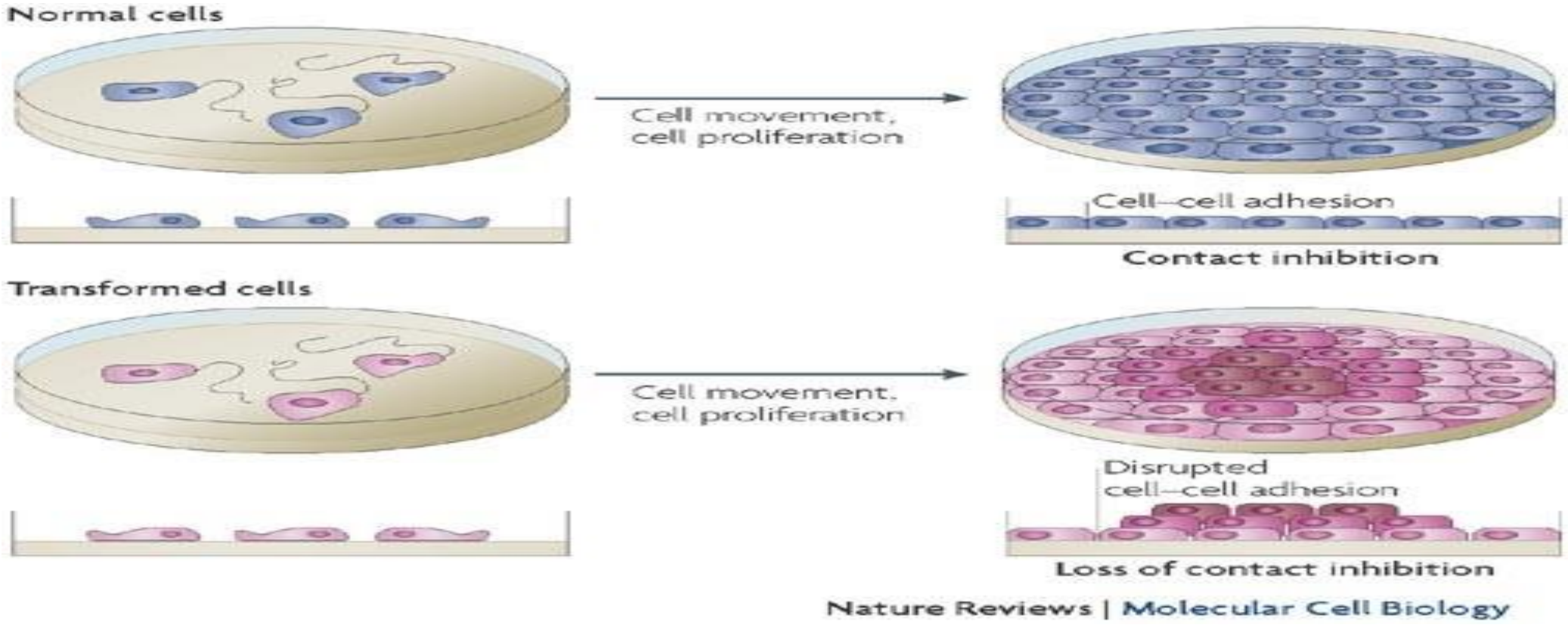
- Mutations affecting TGF beta signaling causes cancer
- These mutations involve TGF beta receptor or SMAD molecules that transduce anti-proliferative signals from the receptor to the nucleus
- Mutations affecting type 2 receptor seen in colon, stomach and endometrial cancer
- SMAD4 is mutated in pancreatic cancer.
- 100% of pancreatic 83% of colon at least one component of is TGF b pathway is mutated

CONTACT INHIBITION

- Normally cells proliferate in organized fashion. Monolayers are formed and contact between adjacent cells inhibits further growth.
- This process is called contact inhibition.
- In cancer cells: contact inhibition is lost.
- Contact inhibition is mediated by cadherin molecules.
- If E cadherin (= epithelial cadherin) is lost: no contact inhibition….. Cells proliferate in an uncontrolled fashion.



CONTACT INHIBITION



E CADHERIN

E cadherin's function is facilitated by NF2 (neurofibromatosis 2) protein

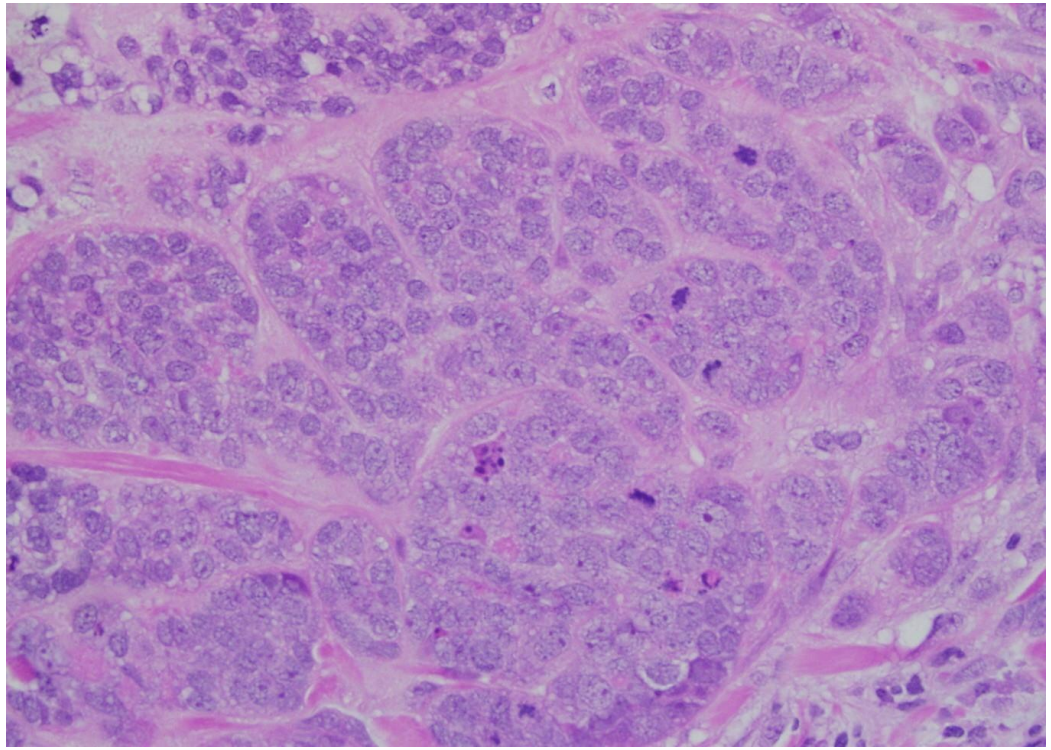
NF2 gene's protein product is neurofibromin 2=merlin which facilitates contact inhibition

Homozygotic loss of NF2 causes neural tumors (neurofibromatosis syndrome)

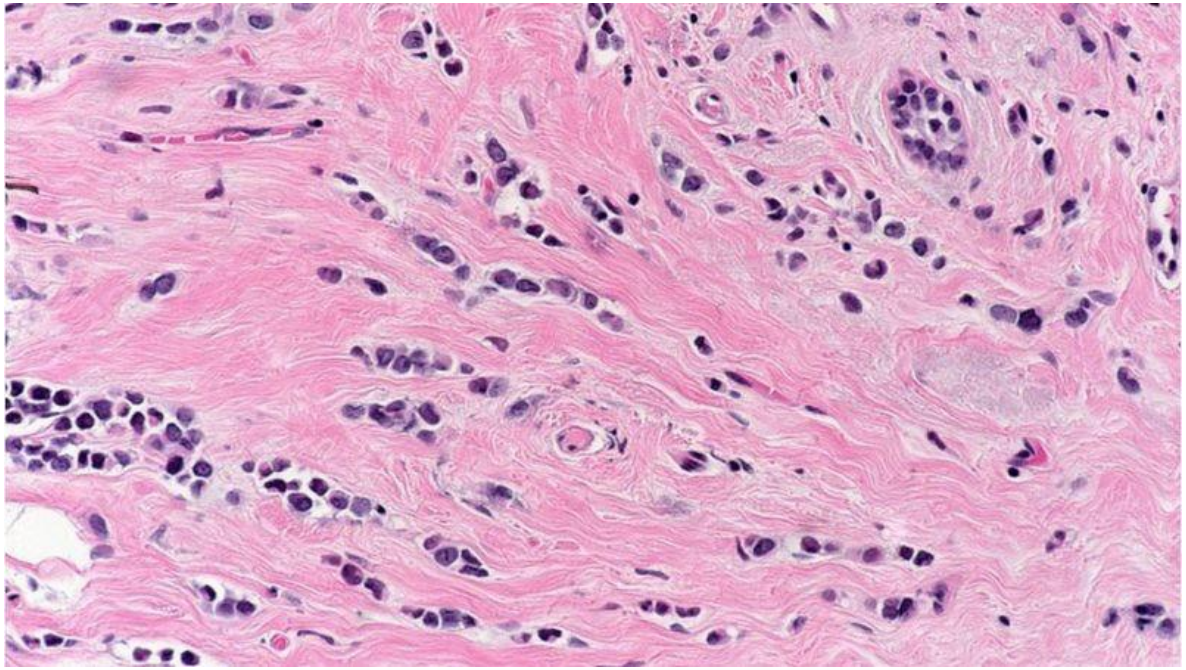
E CADHERIN

- E cadherin is important to "keep cells together"
- Tumors with loss of E cadherin tend to grow in an individual cell fashion : they don't form glandular or other cohesive structures.
- Example: there are two types of breast carcinoma, invasive ductal and invasive lobular.. The tumor cells in the ductal type form glandular structures, whereas in the lobular type, they grow in individual cell pattern. In this lobular pattern E cadherin is lost.. See next slide

Invasive ductal carcinoma, there is cohesion between cancer cells caused by E cadherin.



Invasive lobular carcinoma, E cadherin is lost so there is no cohesion. Tumor cells grow as individual cells.



MERLIN PROTEIN AND CONTACT INHIBITION

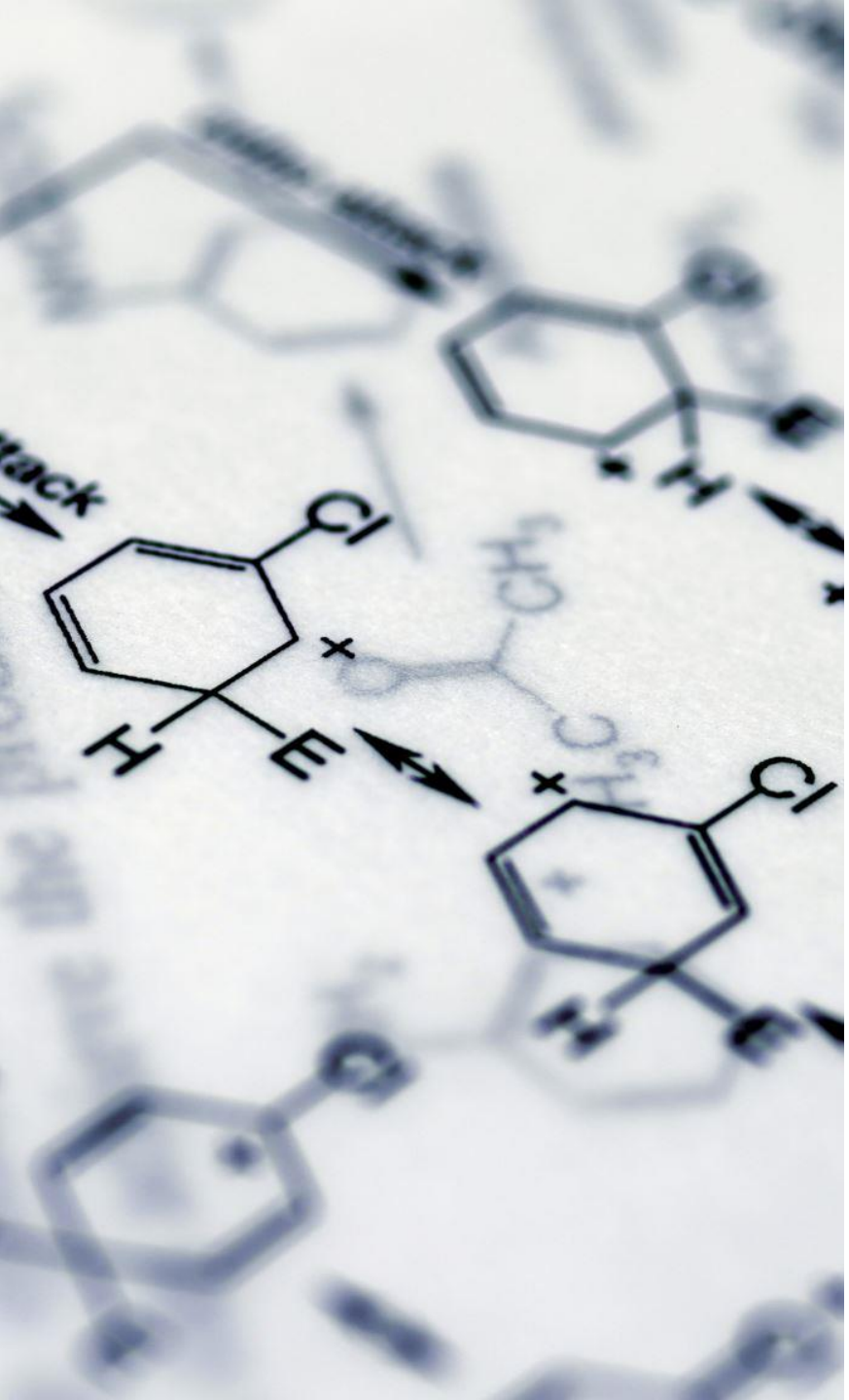
- Please remember that contact inhibition is an important process to limit and regulate cell growth
- If contact inhibition is lost growth can go unchecked
- E cadherin is the most important factor causing contact inhibition
- Merlin protein facilitated contact inhibition
- if merlin is lost then contact inhibition is lost and tumors occur
- Loss of function mutation in merlin protein is the underlying genetic defect in NF2(neurofibromatosis type 2)

APC (ADENOMATOUS POLYPOSIS COLI) GENE

APC gene is a tumor suppressor gene

Suppresses growth by regulating intracellular **beta catenin** level.

Beta catenin is a protein that stimulates growth… APC protein acts as a tumor suppressor through inhibiting beta catenin function.

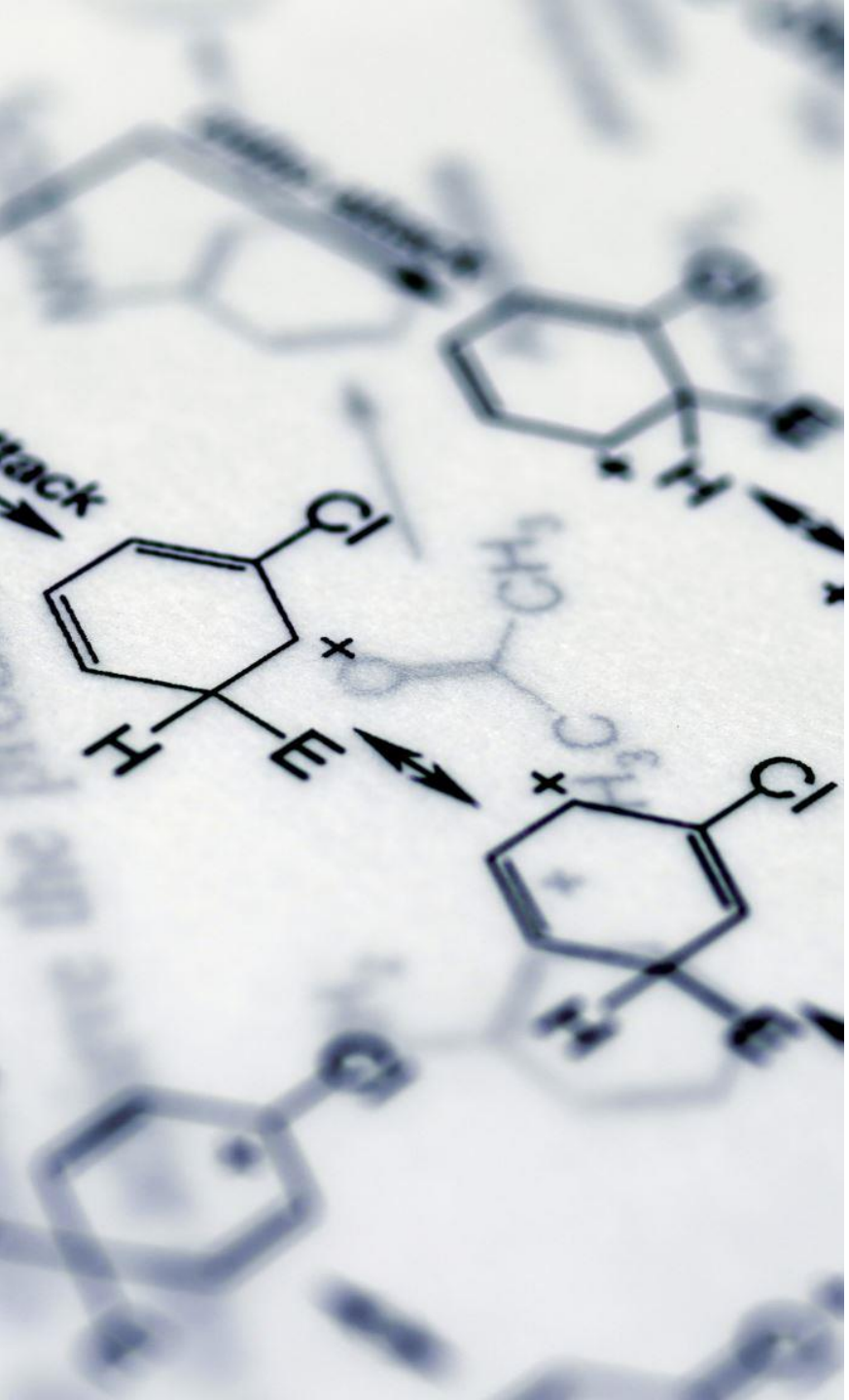


FUNCTIONS OF BETA CATENIN

- **Beta catenin stimulates growth by two ways:**
- Inhibits contact inhibition by stimulating TWIST and SLUG transcription regulators that decrease cadherin expression
- Stimulates growth by increasing transcription of growth promoting genes like cyclin D1 and MYC .

BETA CATENIN AND CONTACT INHIBITION: EXPLANATION:

- Note that beta catenin stimulates transcription of SLUG and SNAIL that inhibit E cadherin.. By doing this beta catenin decreases contact inhibition and hence stimulates growth.
- In normal situations, beta catenin binds to E cadherin and stabilizes it. So if beta catenin is in the cytoplasm and there is no growth signal (through WNT pathway) then beta catenin acts as a stabilizer of E cadherin, hence helps contact inhibition and reduces growth.
- But when beta catenin is translocated to the nucleus it increases SNAIL/SLUG and decrease E cadherin, decrease contact inhibition and causes increased growth.



- APC suppresses growth by being part of a complex that destructs the beta catenin.
- Beta catenin is an important component of WNT signaling
- WNT is a soluble factor that induces cell proliferation by binding to a receptor and transmit signals that prevent degradation of beta catenin.
- Undegrared beta catenin moves to the nucleus where it acts as a transcription activator

RECAP ^^

- In quiescent cells not exposed to WNT, cytoplasmic beta catenin is degraded by destruction complex (of which APC is a main component)
- Loss of APC means that B catenin is not degraded and WNT pathway activated without the WNT
- This leads to transcription of growth promoting genes cyclin D1 ,MYC and transcription regulators: TWIST AND SLUG that repress E cadherin and thus reduce contact inhibition

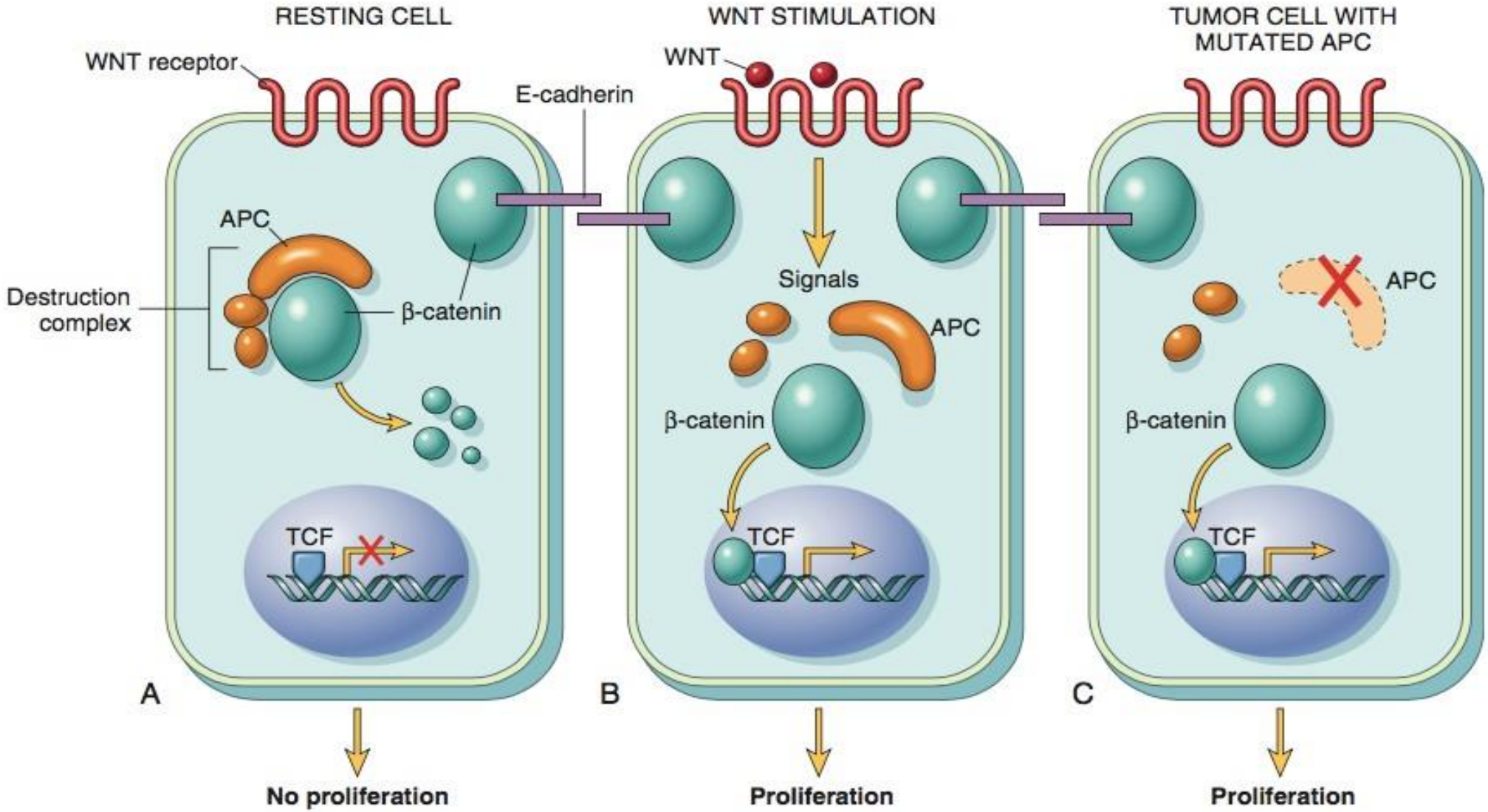
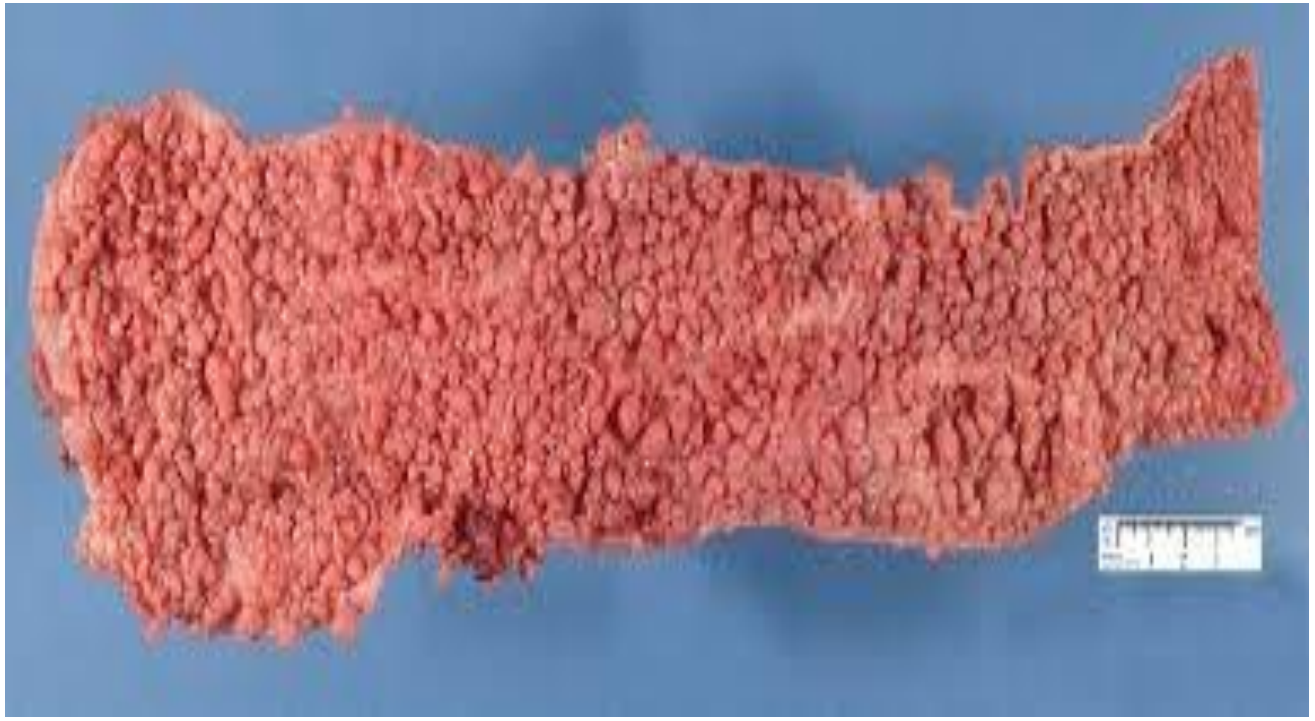


Fig. 6.22 The role of APC in regulating the stability and function of β-catenin. APC and β-catenin are components of the WNT signaling pathway. (A) In resting cells (not exposed to WNT), β-catenin forms a macromolecular complex containing the APC protein. This complex leads to the destruction of β-catenin, and intracellular levels of β-catenin are low. (B) When cells are stimulated by secreted WNT molecules, the destruction complex is deactivated, β-catenin degradation does not occur, and cytoplasmic levels increase. β-Catenin translocates to the nucleus, where it binds to TCF, a transcription factor that activates several genes involved in the cell cycle. (C) When APC is mutated or absent, the destruction of β-catenin cannot occur. β-Catenin translocates to the nucleus and coactivates genes that promote the cell cycle, and cells behave as if they are under constant stimulation by the WNT pathway.

APC ADENOMATOUS POLYPOSIS COLI

- APC syndrome is similar to inherited retinoblastoma, both are inherited in an autosomal dominant fashion, but in both the gene responsible for the syndrome is a recessive, tumor suppressor gene.
- In APC syndrome :one APC allele lost in germ line. Patients with this single loss develop intestinal polyps (adenomatous polyps= adenoma).. Hundreds of adenomas.
- These patients acquire a second mutation in the other APC gene, and this homozygous loss results in colonic adenocarcinoma.
- Patients have 100% risk of malignancy, so prophylactic total colectomy is performed 70-80% of sporadic colon cancers have APC mutation
- Colonic cancers with normal APC have mutated beta catenin making them undegradable by APC

FAP SYNDROME: COLON FULL OF ADENOMAS!



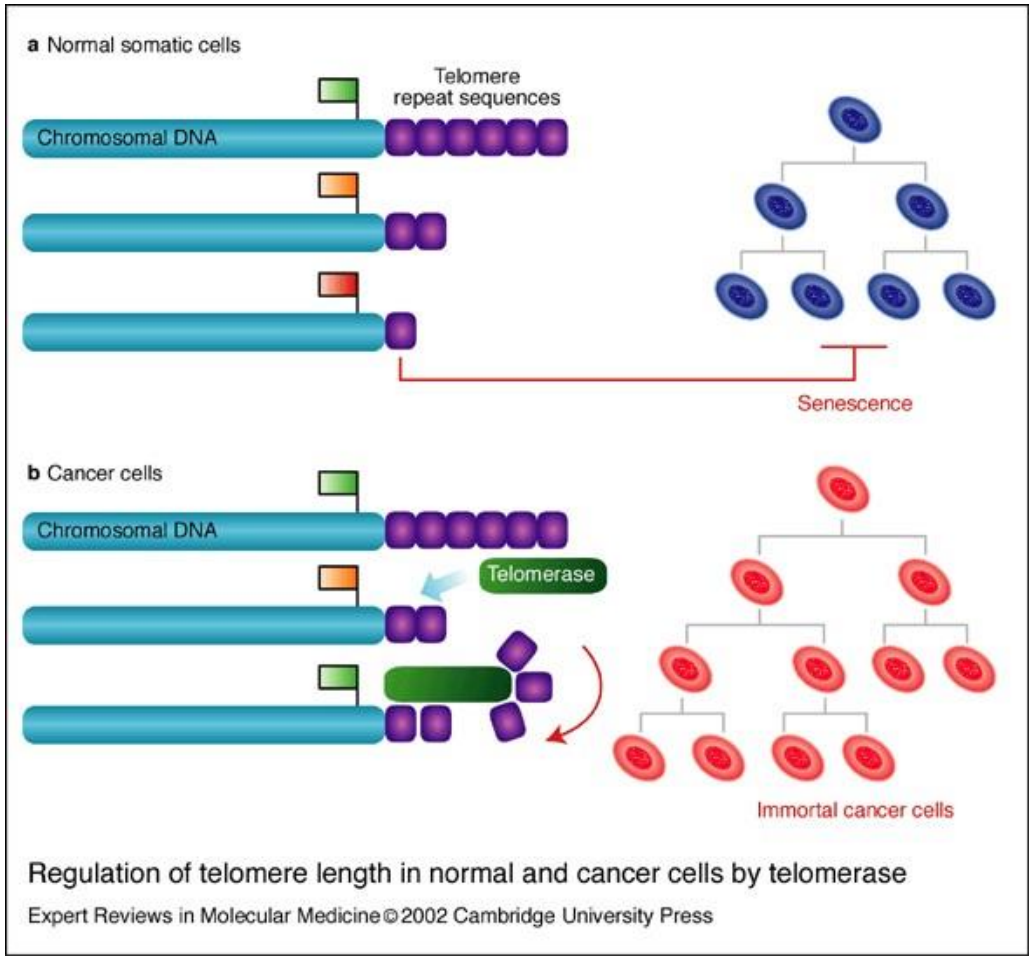
THIRD HALLMARK: LIMITLESS REPLICATIVE POTENTIAL

- Normal cells: limited capacity to duplicate (usually 60 -70 doublings)
- After these doublings cells lose capacity to replicate and become senescent
- This is because of progressive shortening of telomeres

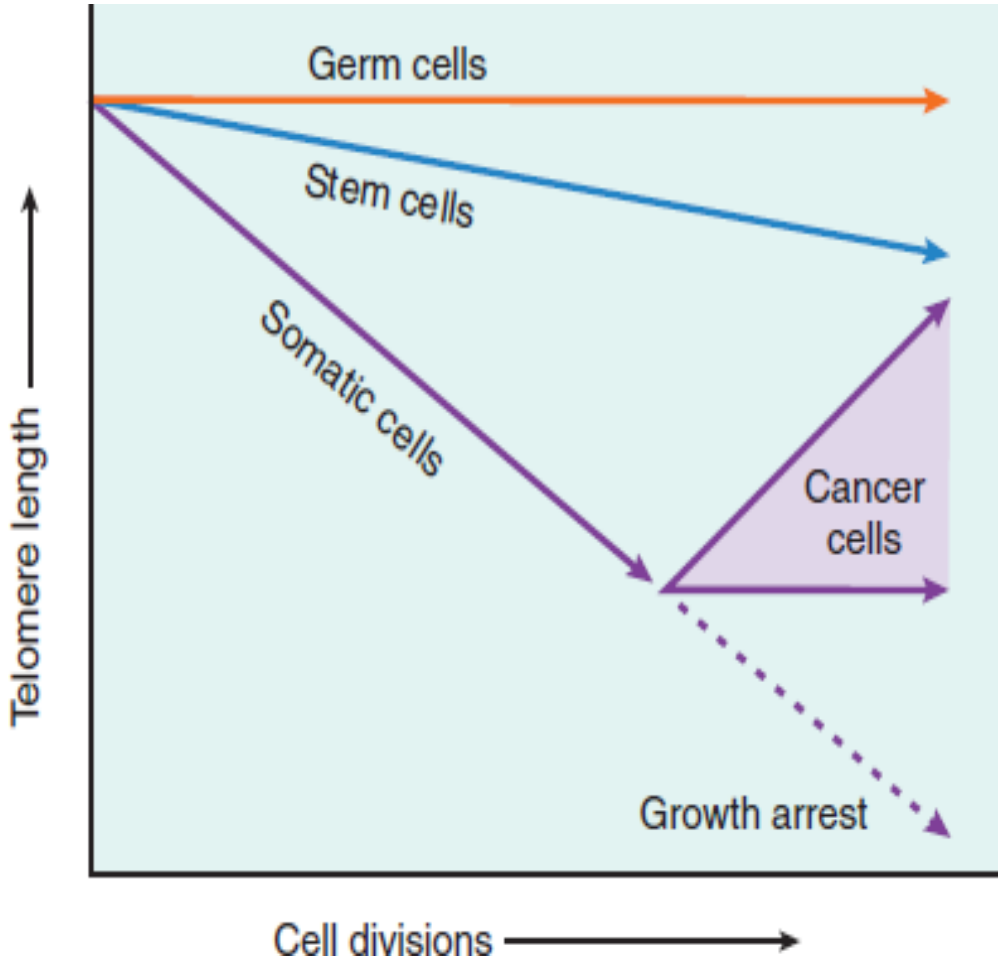
TELOMERES

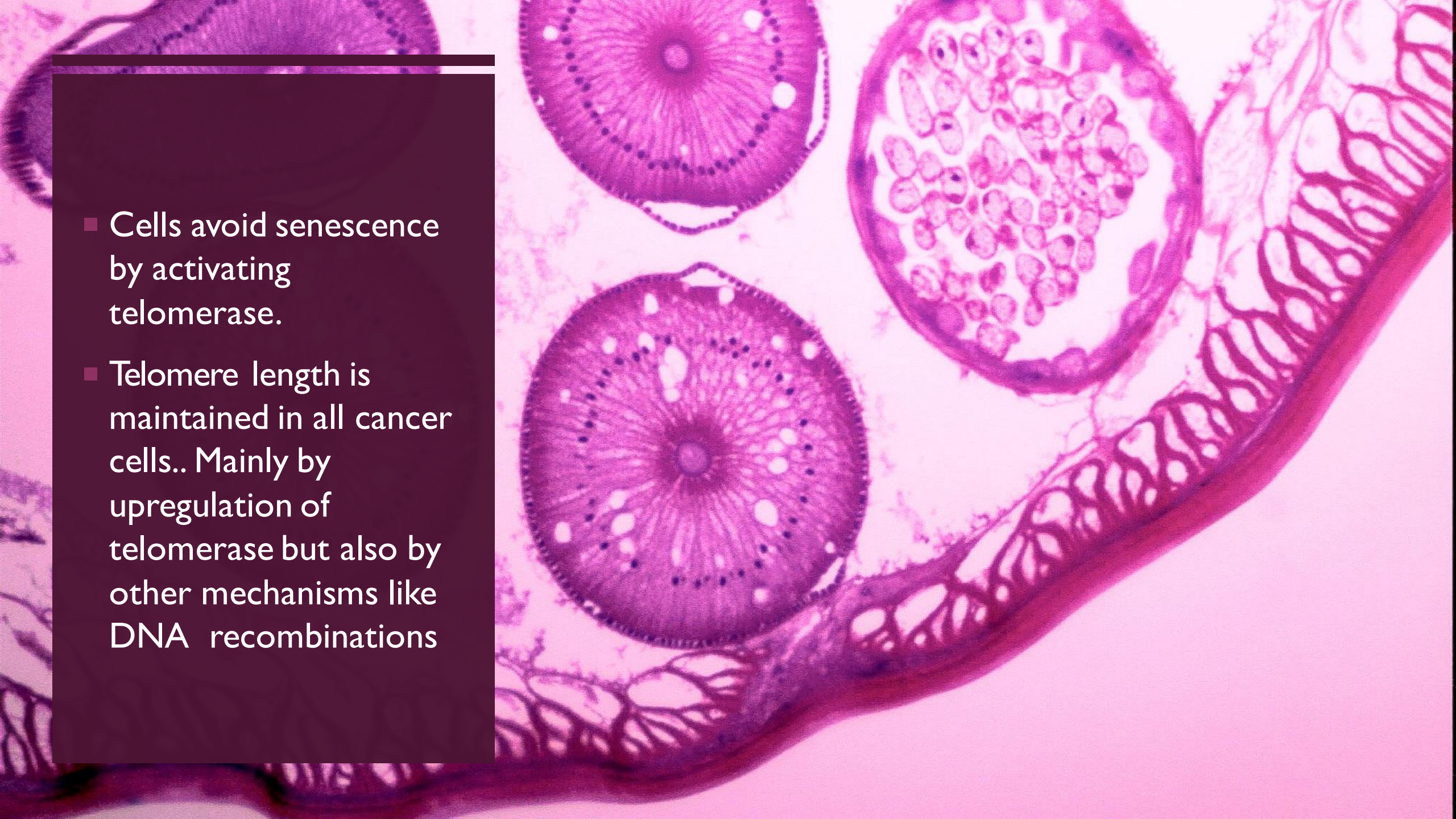
- Each cell has a limited replicative potential.
- This is because chromosomes have repeated nucleotide sequences at the ends of each chromosome.
- With each cell replication, telomeres shorten.. Till they become too short and the chromosomal ends fuse together which causes cell death by apoptosis.
- Stem cells have limitless replicative potential because they have telomerase enzyme which uses its RNA nucleotide sequence to replace the lost telomeres.
- Cancer cells upregulate telomerase transcription and become immortal.

CELL SENESCENCE & TELOMERES

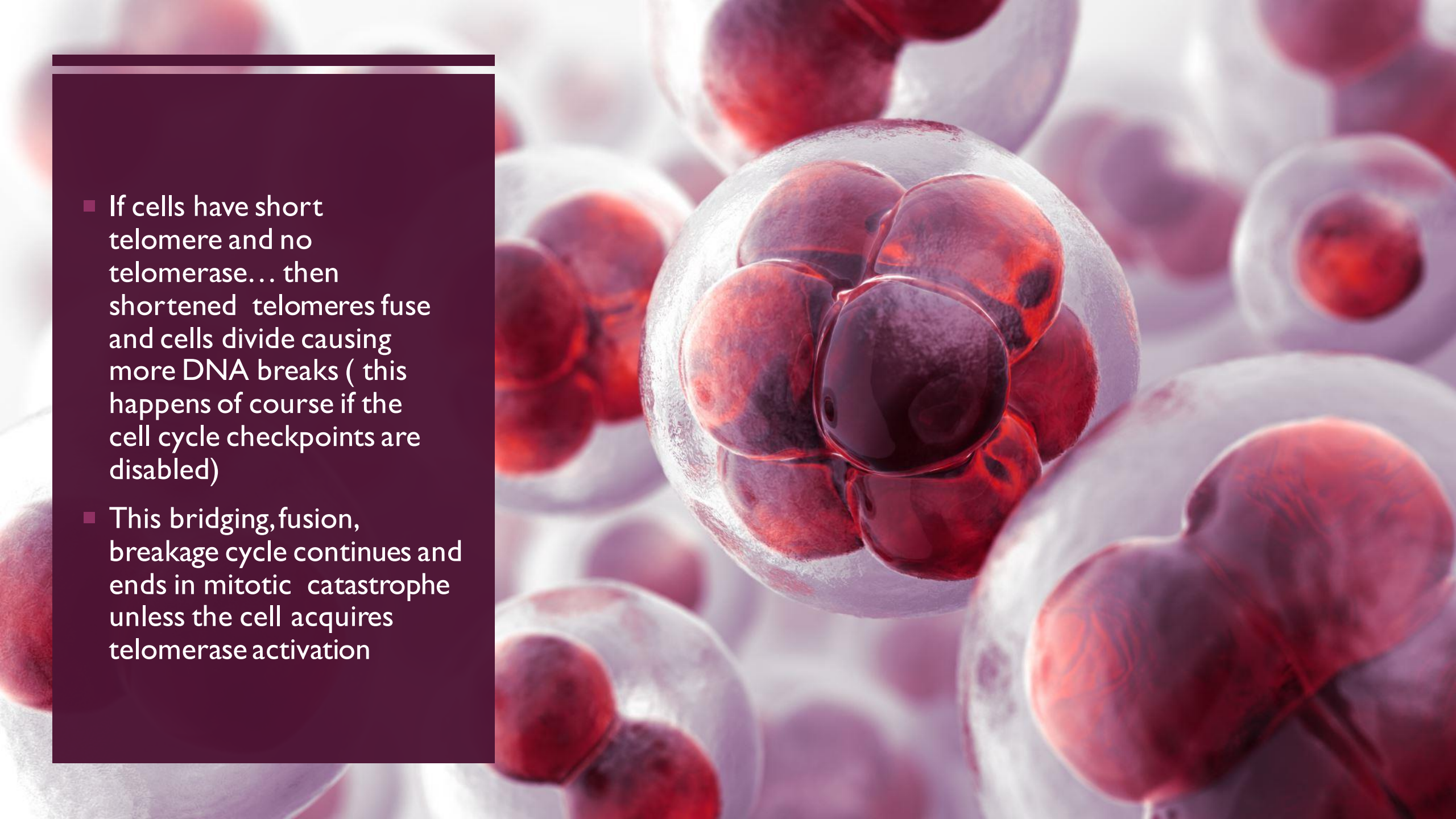


Regulation of telomere length in normal and cancer cells by telomerase



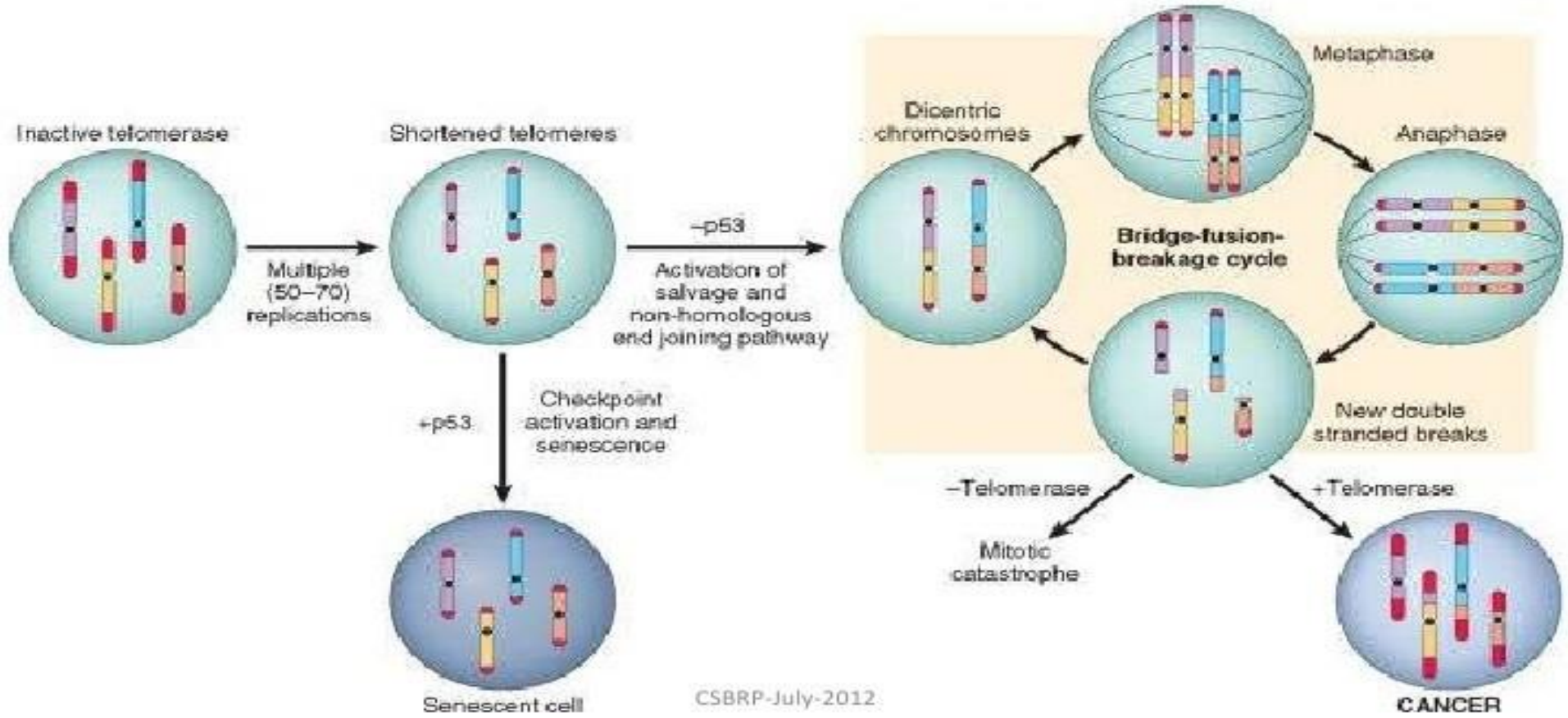


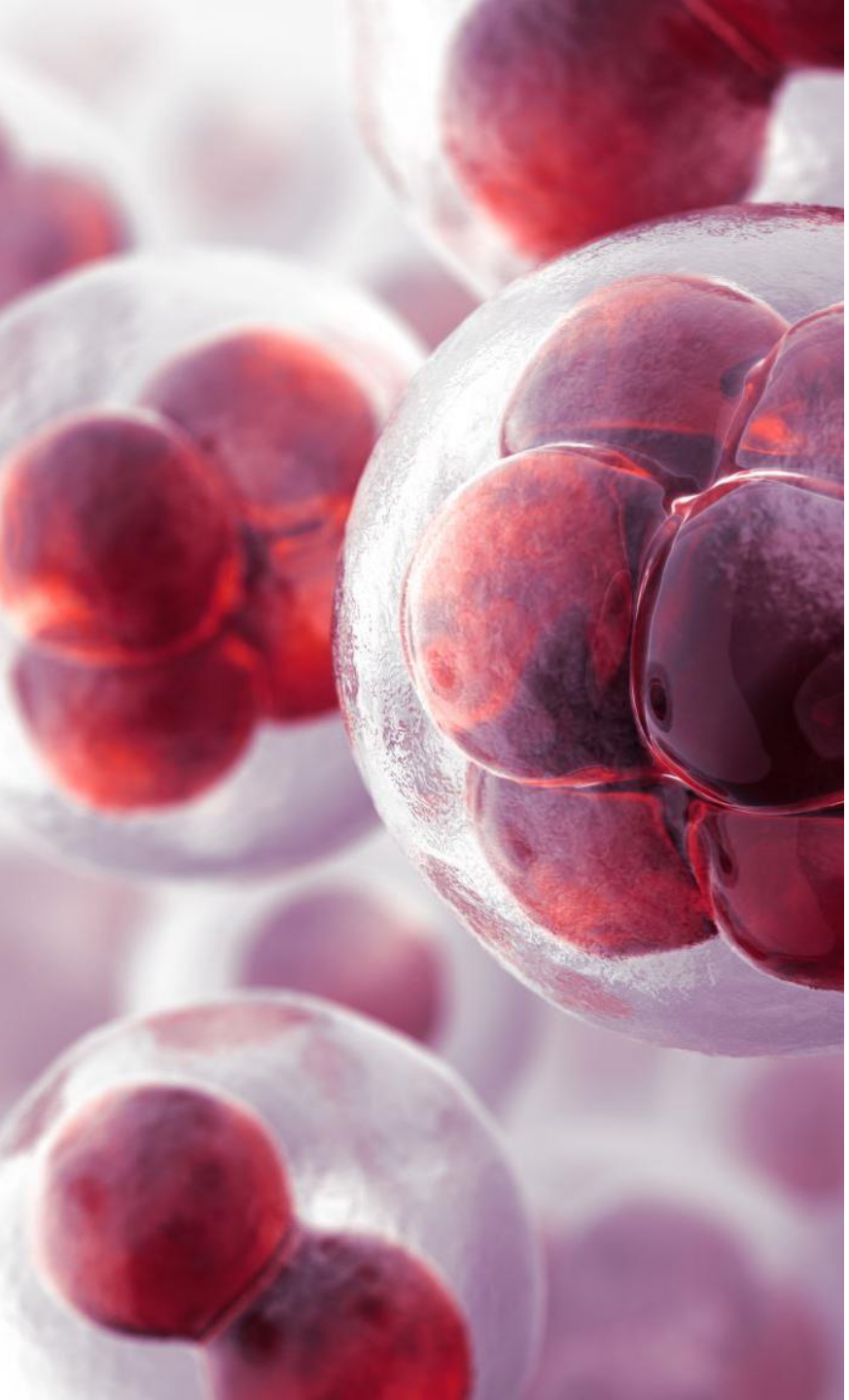
- Cells avoid senescence by activating telomerase.
- Telomere length is maintained in all cancer cells.. Mainly by upregulation of telomerase but also by other mechanisms like DNA recombinations



- If cells have short telomere and no telomerase… then shortened telomeres fuse and cells divide causing more DNA breaks (this happens of course if the cell cycle checkpoints are disabled)
- This bridging, fusion, breakage cycle continues and ends in mitotic catastrophe unless the cell acquires telomerase activation

Sequence of events in the development of limitless replicative potential





TEST YOUR UNDERSTANDING: READ THIS PATHOLOGY REPORT AND SPOT THE MISTAKE THERE

- Sections taken from the breast mass show a tumor forming glandular structures lined by atypical cells with a high mitotic rate. The tumor cells are negative for E cadherin stain. The features are those of an invasive lobular carcinoma.

ANSWER

- This is a strange report that doesn't make sense!
- If the cells form glands then this is a ductal not lobular carcinoma.
- Negative E cadherin means that the cells lost the protein that glues them together, so they should grow in an individual cell pattern rather than in glandular structures.

- **Note: if the E cadherin is really negative then the tumor is a lobular**

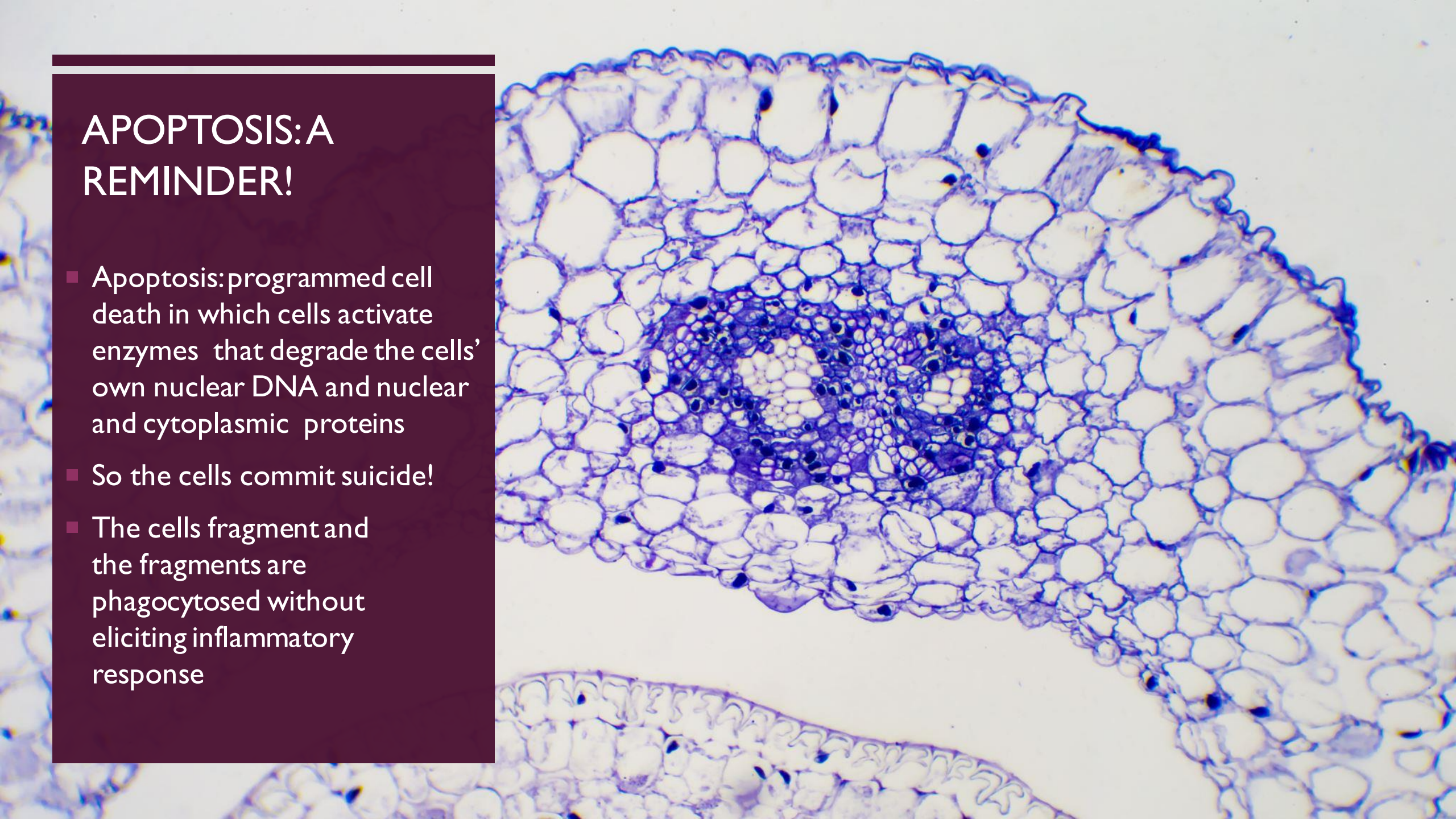
- TAKE HOME MESSAG: always read histopathology reports carefully. Phone your pathologist if you need an explanation of any point or to question any findings or results… being able to correctly interpret these reports and to keep good communication with the pathologist is important to give your patients the correct management.

FOURTH HALLMARK

- Evasion of cell death by evading apoptosis.

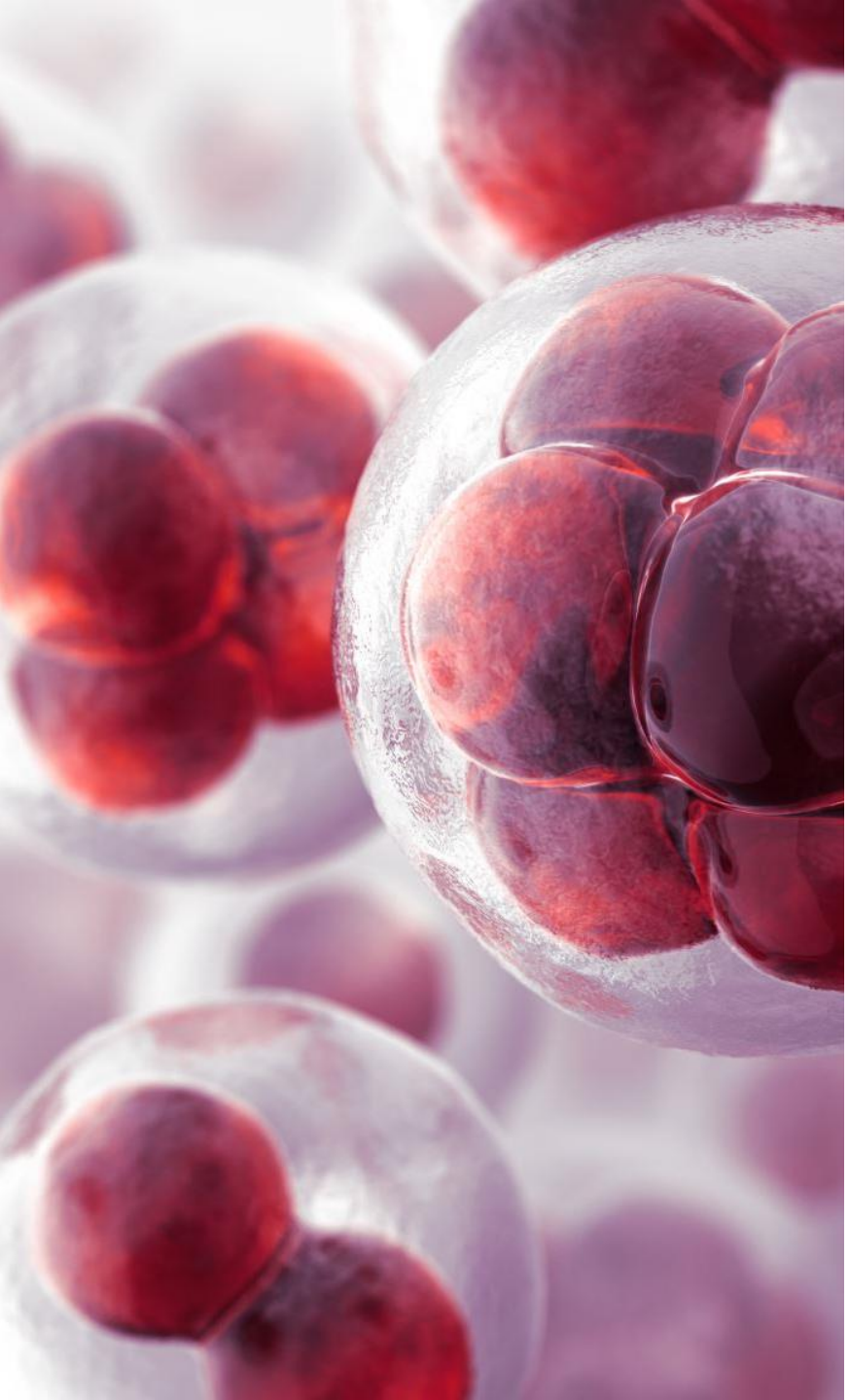
APOPTOSIS: A REMINDER!

- Apoptosis: programmed cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins
- So the cells commit suicide!
- The cells fragment and the fragments are phagocytosed without eliciting inflammatory response



EXTRINSIC PATHWAY

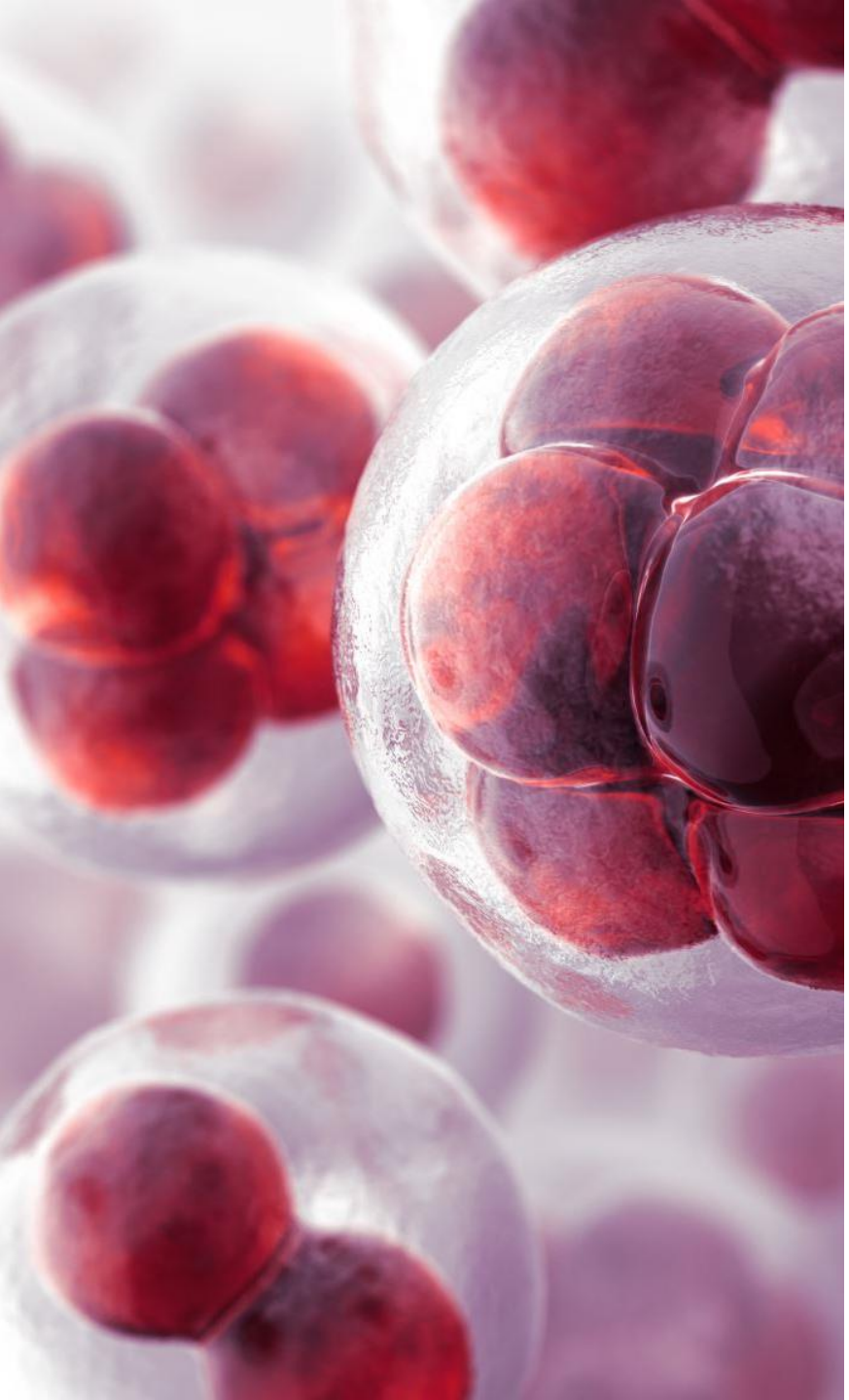
- Trigger that starts apoptosis is outside the cells.
- The pathway starts when **Fas** ligand binds to **Fas** receptor
- Upon this the receptor is activated; it trimerizes and its cytoplasmic part (death domain) is activated.
- Activation of the receptor attracts a cytoplasmic protein= FADD
- FADD recruits procaspase 8
- Procaspase cleaved to active caspase 8 (initiation caspase)
- Caspase 8 activates caspase 3 (executioner) which cleaves DNA and cellular protein



EXTRINSIC PATHWAY

- Fas ligand
- Fas receptor
- FADD
- Caspase 8
- Caspase 3

- Decrease any of the above….. Evasion of cell death



EXTRINSIC PATHWAY

- FLIP is a caspase 8 antagonist
- So if FLIP is increased cells can evade apoptosis

- FLIP-similar proteins are produced by some viruses.. Helping them to keep infected cells alive.

INTRINSIC PATHWAY = MITOCHONDRIAL PATHWAY

- This pathway is stimulated if there is DNA damage secondary to stress, radiation, chemicals or due to withdrawal of survival factors
- This pathway is intrinsic.. So not initiated by membrane receptors… instead it is initiated by increased mitochondrial permeability
- When mitochondrial permeability increases ..cytochrome c leaks out and initiates apoptosis
- Now cytochrome c is in the cytosol.. So it binds APAF 1
- This binding activates caspase 9
- Caspase 9 activates caspase 3

INTRINSIC PATHWAY

- Internal stresses within cells Increase mitochondrial permeability
- Cytochrome c leaks outside the mitochondria Cytochrome c binds to APAF1
- Caspase 9 activated
- Caspase 3 activated
- Again: decrease any of these and the cell can avoid apoptosis

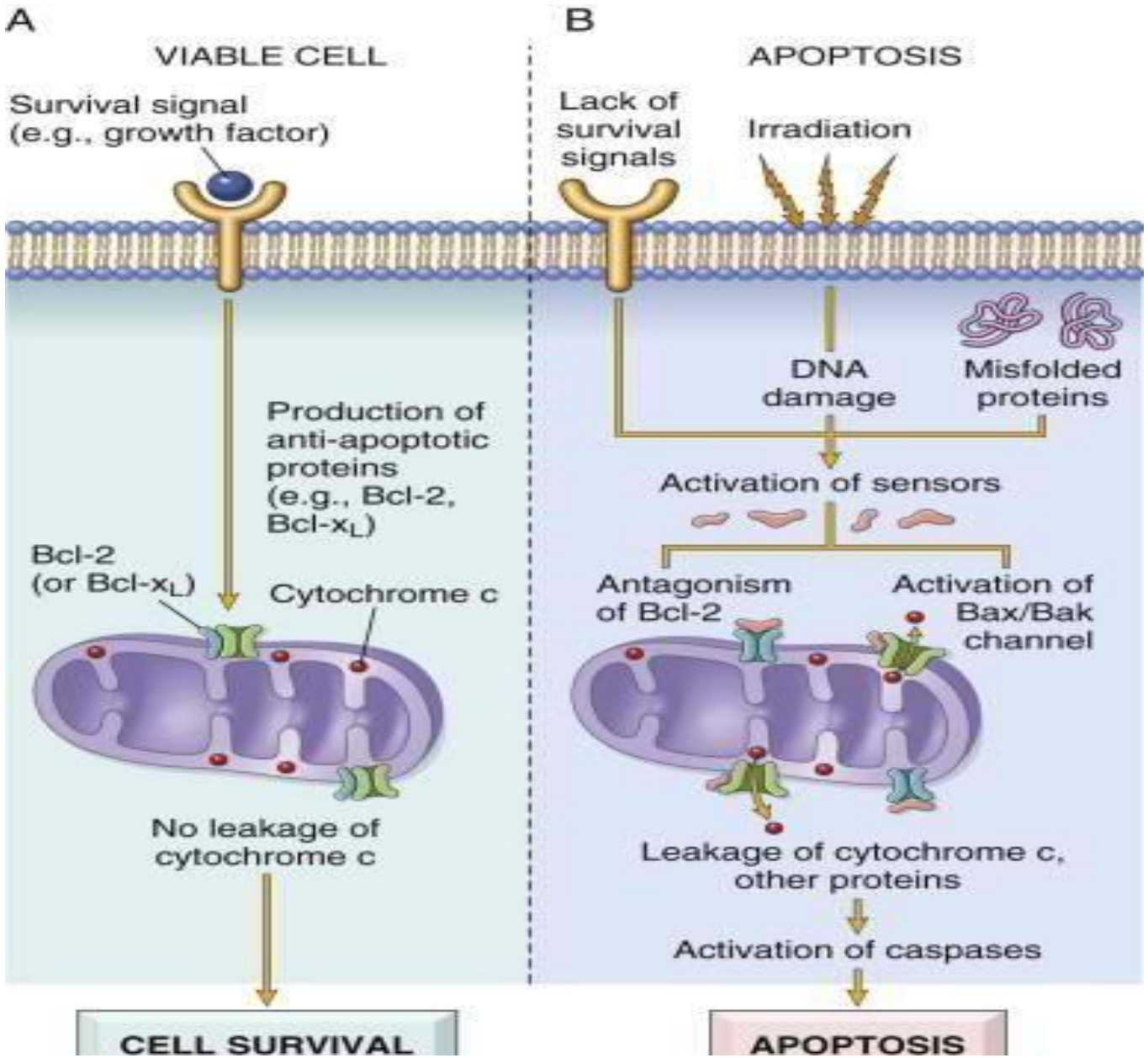
MITOCHONDRIAL PERMEABILITY

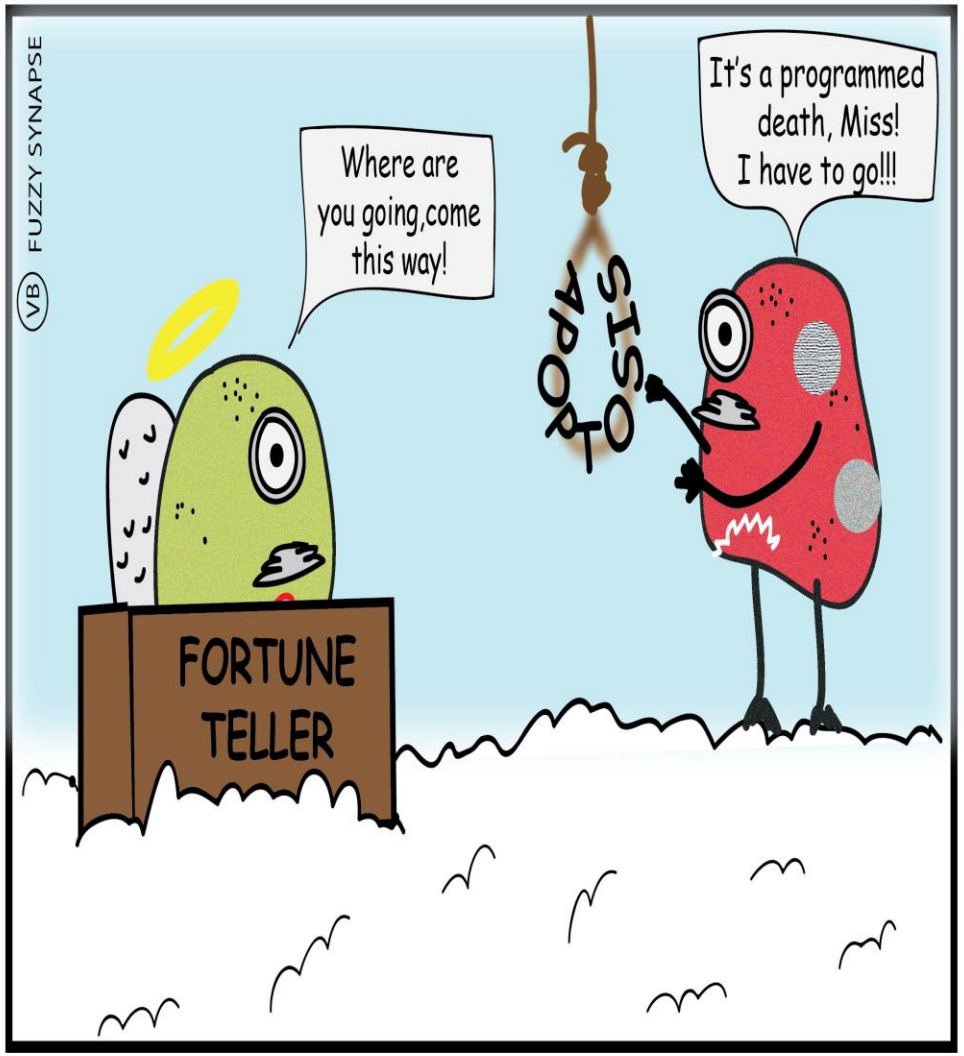
- Mitochondrial permeability is controlled by BH 3 proteins (BAD, BID, PUMA)
- When BH3 proteins sense internal stress.. Stimulate proapoptotic proteins and inhibit antiapoptotic ones
- Proapoptotic: BAX, BAK
- Antiapoptotic: BCL2, BCL- XI

- So decrease BAD, BID, PUMA, BAX, BAK… NO APOPTOSIS
- Increase BCL2 AND BCL-XI…. No apoptosis

NOTE

- IAP= inhibitor of apoptotic protein , inhibits caspase 9
- So increase IAP and apoptosis can be avoided.


A
VIABLE CELL
Survival signal
(e.g., growth factor)
Production of
anti-apoptotic
proteins
(e.g., Bcl-2,
Bcl-xL)
Bcl-2
(or Bcl-xL)
Cytochrome c
No leakage of
cytochrome c
CELL SURVIVAL
B
APOPTOSIS
Lack of
survival
signals
Irradiation
DNA
damage
Misfolded
proteins
Activation of sensors
Antagonism
of Bcl-2
Activation of
Bax/Bak
channel
Leakage of cytochrome c,
other proteins
Activation of caspases
APOPTOSIS


VB FUZZY SYNAPSE
Where are
you going,come
this way!
It's a programmed
death, Miss!
I have to go!!!
APOPTOSIS
FORTUNE
TELLER

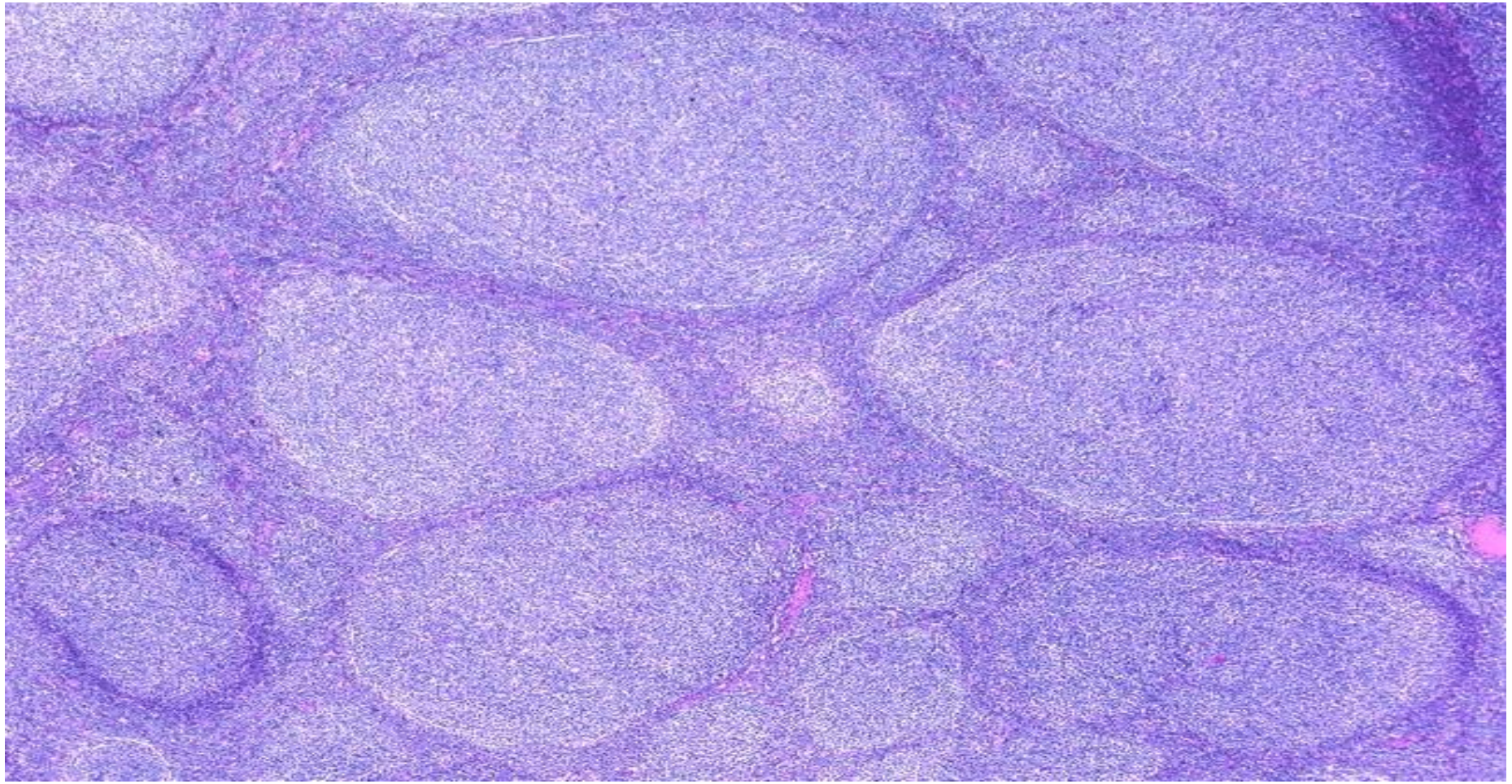
P53 AND APOPTOSIS

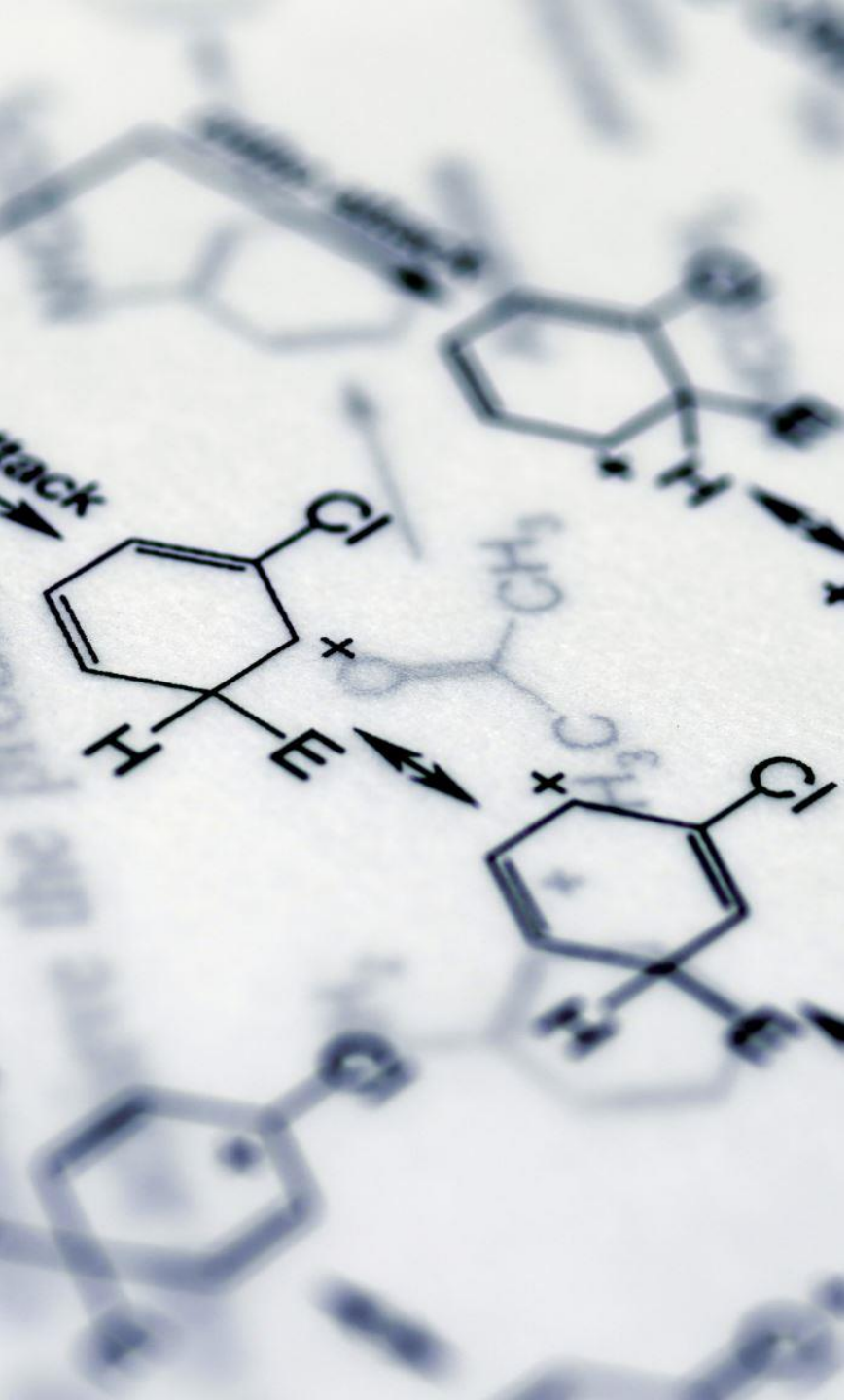
- DNA damage causes accumulation of p53 in cells
- It arrests cells in G1 phase of cell cycle to give the cell a chance to repair itself
- If no repair, p53 triggers apoptosis by stimulating bax and bak
- *P53 can be mutated in cancer cells.. If mutated it cannot initiate apoptosis, so the cell survives even if its DNA is damaged.. Longer survival of a cell with damaged DNA increases the chances of accumulating more mutations.. So this cell can become malignant*

BCL2

- Follicular lymphomas are slow growing (indolent) tumors that have a translocation causing increased bcl2
- T (14;18) …. Bcl2 translocated and overexpressed
- In lymphocytes having this mutation… apoptosis is decreased
- These lymphocytes live longer rather than being transformed… that's why this type of lymphoma (follicular lymphoma) is indolent

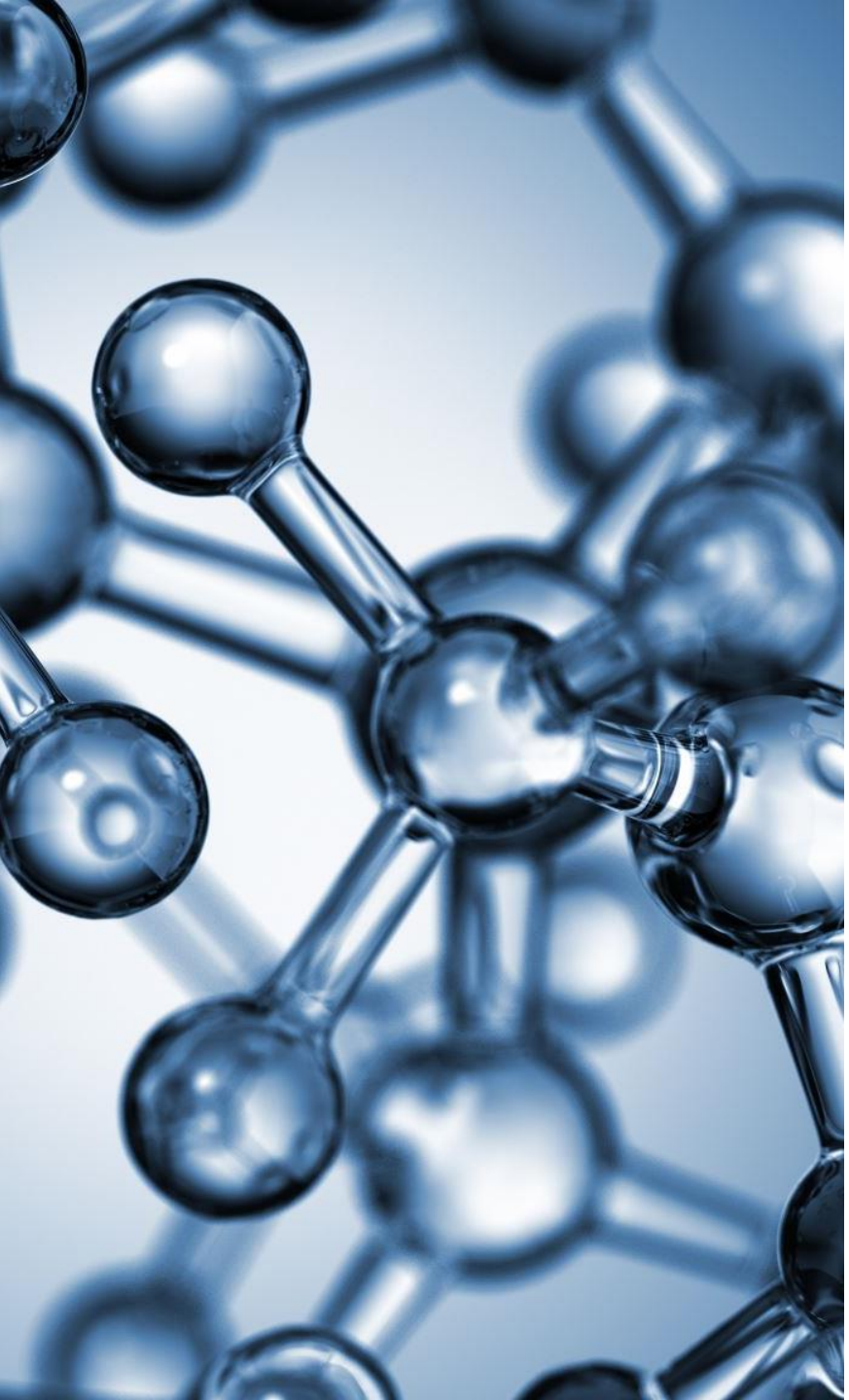
FOLLICULAR LYMPHOMA/ NOTE THE FORMATION OF FOLLICLES





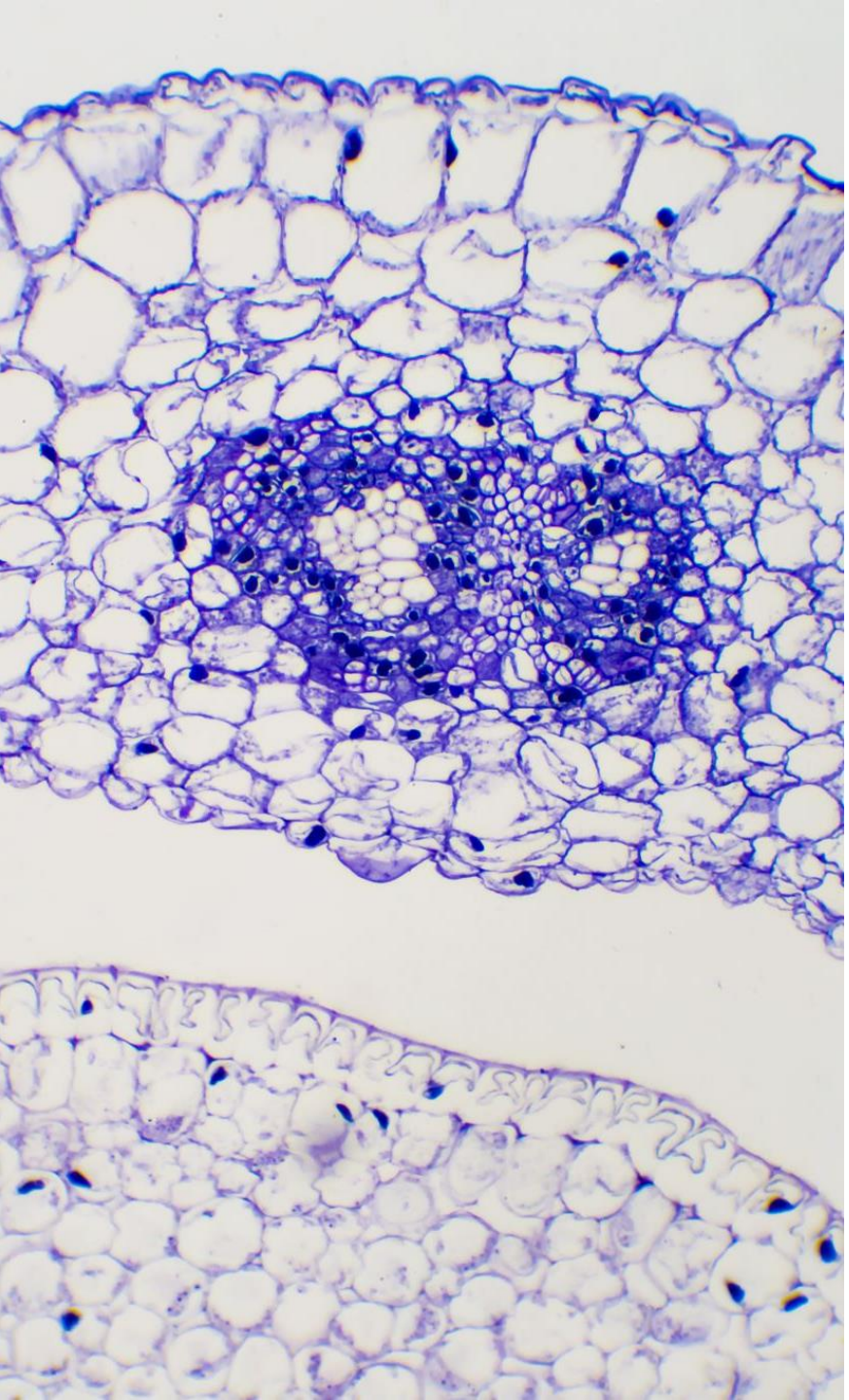
FIFTH HALLMARK: CHANGES IN CELL METABOLISM

- These changes include
- 1. reprogramming of energy metabolism to aerobic glycolysis
- 2. changes in autophagy
- 3. formation of oncometabolites



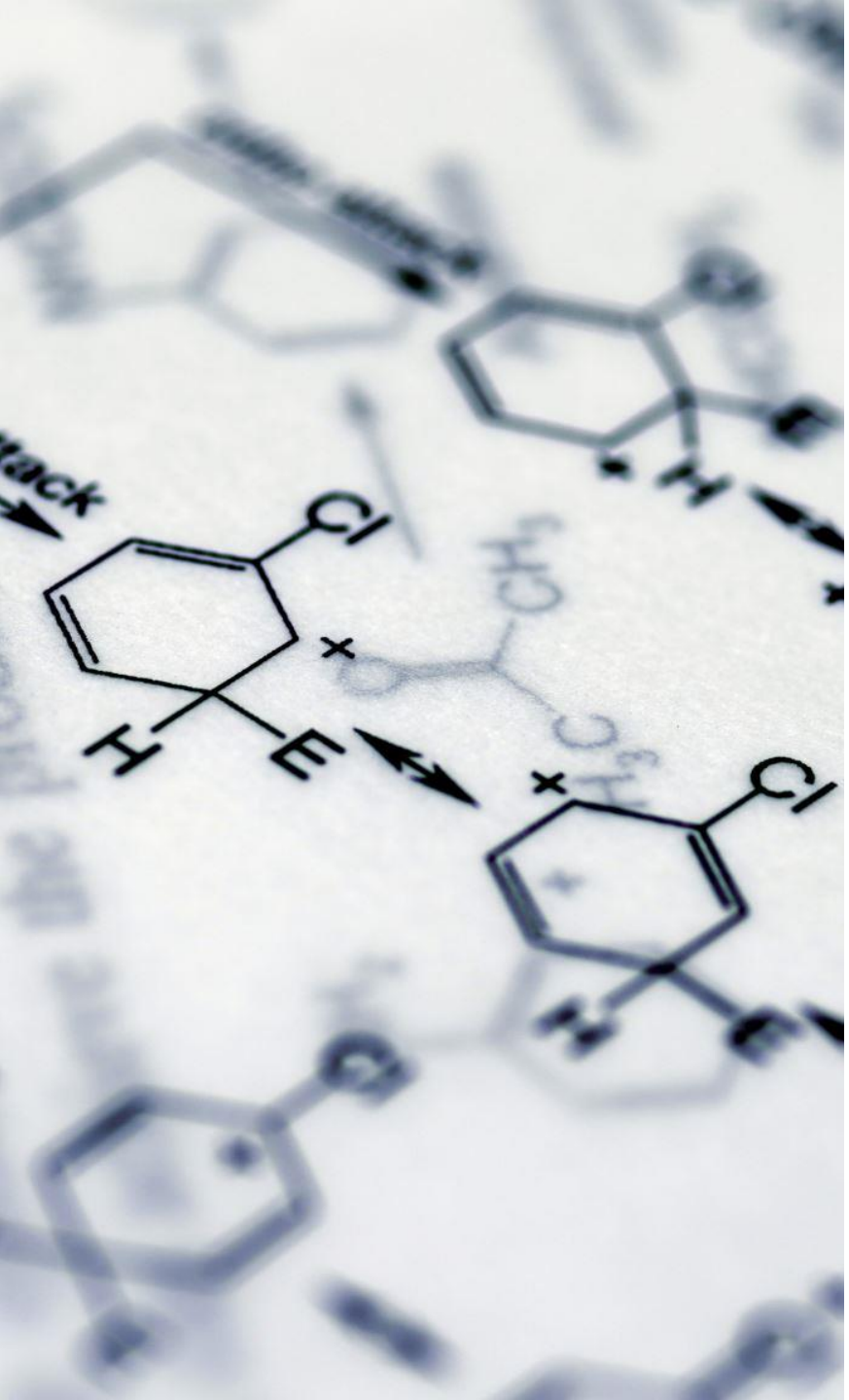
REPROGRAMMING OF ENERGY METABOLISM

- Normal cells obtain energy by:
- Oxidative phosphorylation if oxygen is available. In this process each glucose molecule used produces 36 ATP molecules.
- Anaerobic respiration if oxygen levels are low. In this process glucose is converted to lactic acid and for each glucose molecule used only 2 ATP molecules are produced.



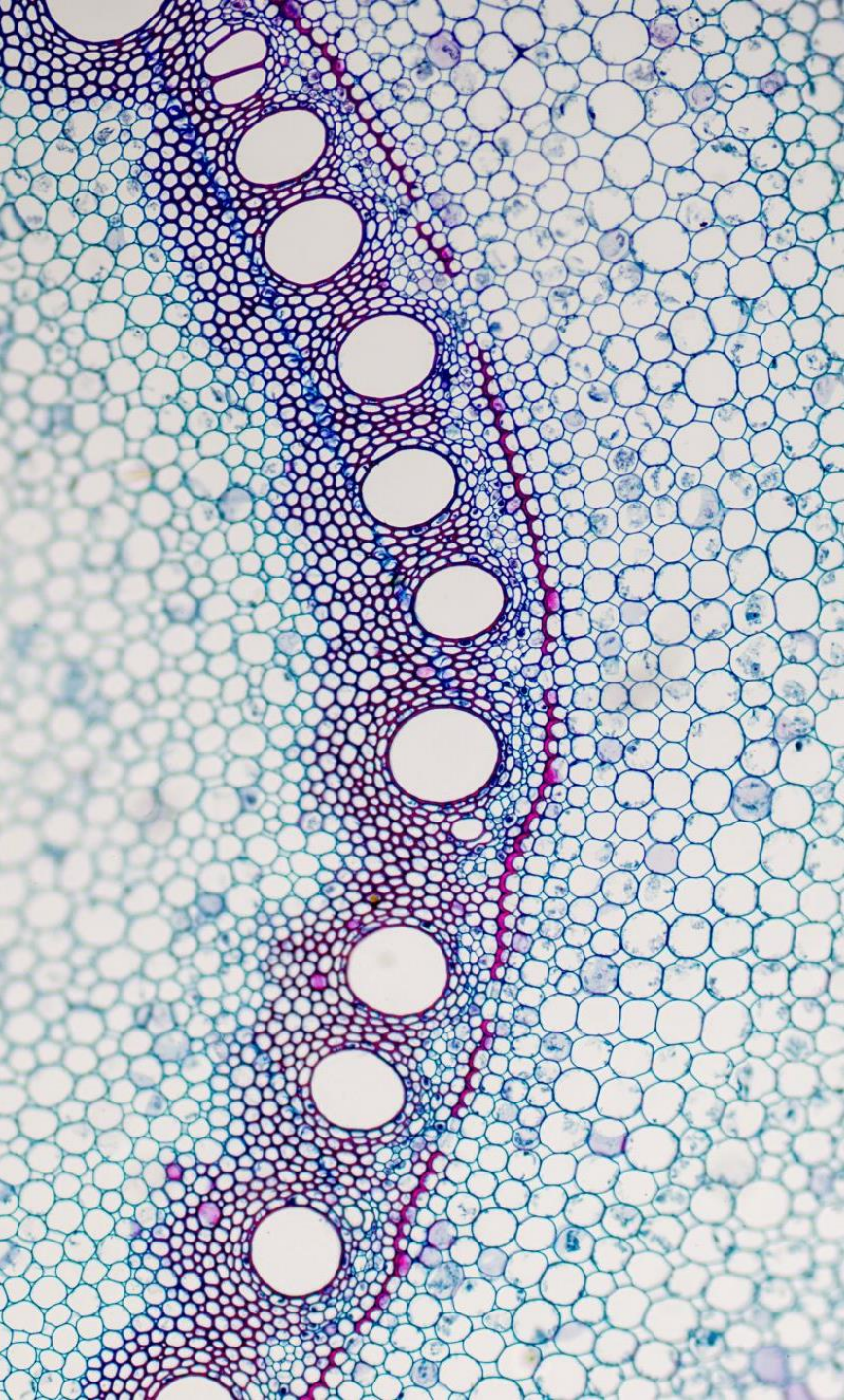
REPROGRAMMING OF ENERGY METABOLISM

- Cancer cells have a third way!
- They convert glucose to lactic acid **even in the presence of adequate oxygen**
- **This process is called : aerobic glycolysis or Warburg effect.**



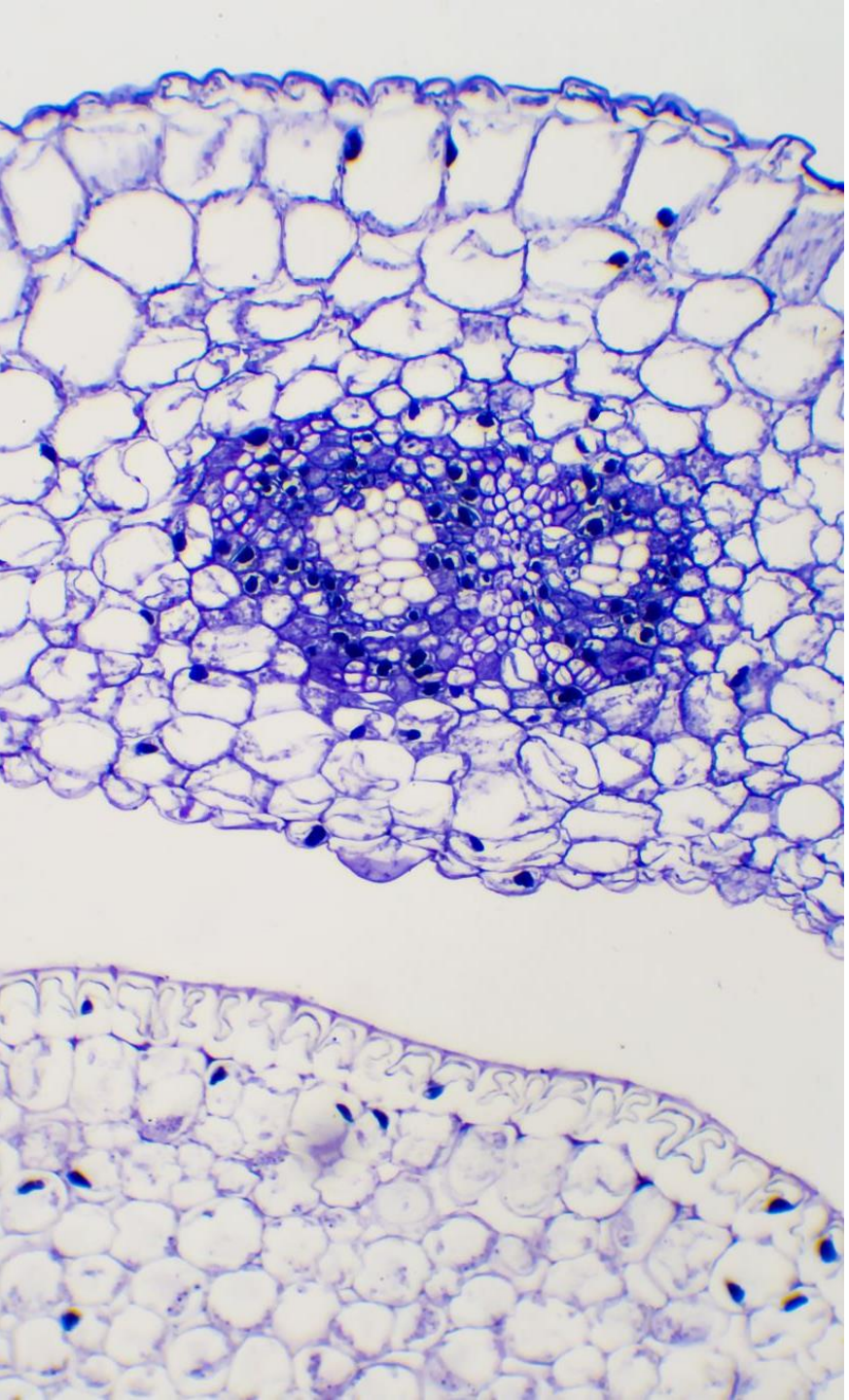
WARBURG EFFECT

- Although less ATP is produced… the Warburg effect ensures that carbon atoms in glucose (which is converted to Pyruvate) are used for synthesis of organic compounds like lipids and proteins which are important in building new cells in the highly proliferative tumor.
- SO: Aerobic glycolysis provides rapidly dividing tumor cells with metabolic intermediates that are needed for the synthesis of cellular components, whereas mitochondrial oxidative phosphorylation does not.



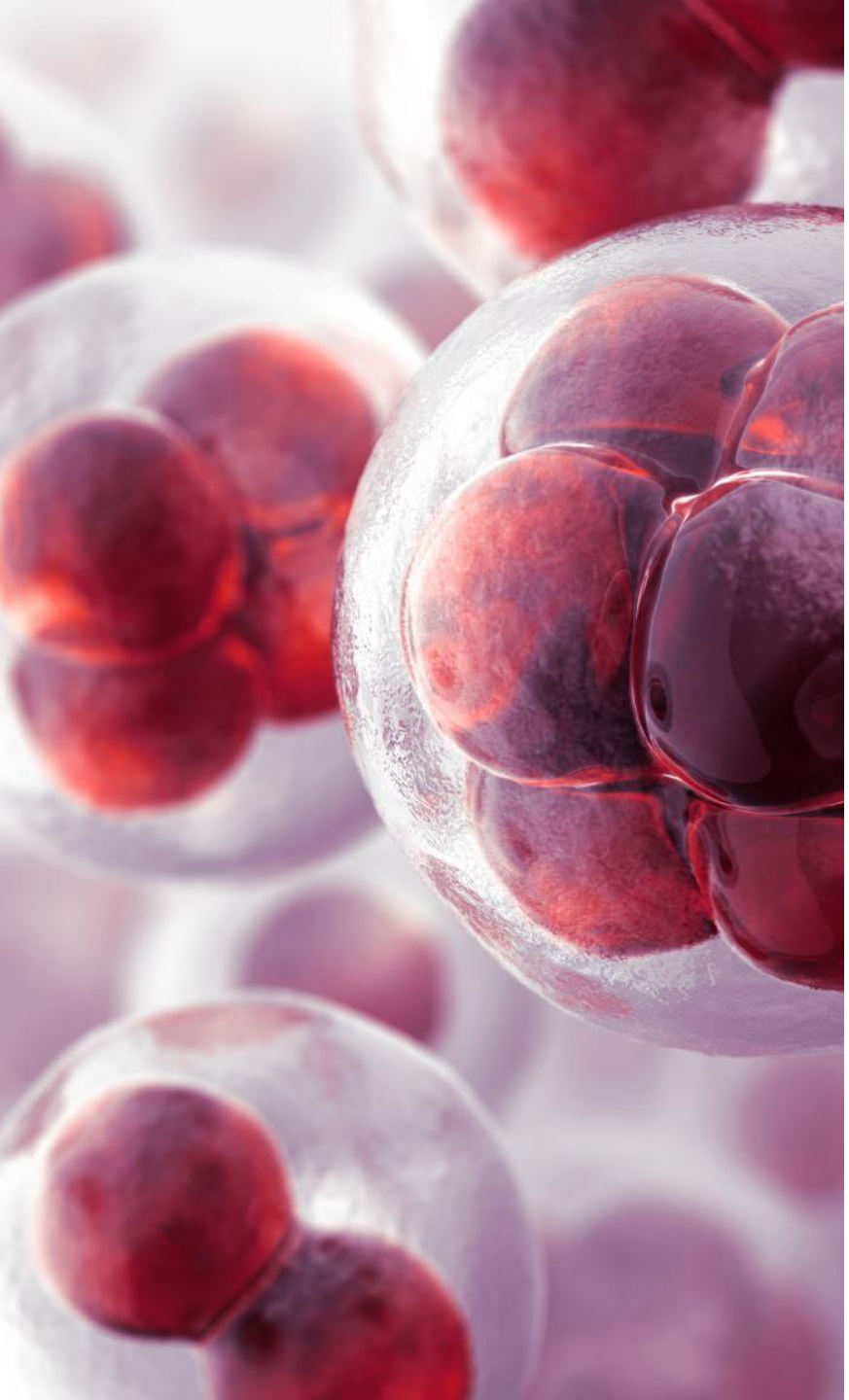
AEROBIC GLYCOLYSIS

- Cancer cells didn't invent aerobic glycolysis!
- *Actually, rapidly proliferating normal cells, like in embryonic tissues and lymphocytes during immune responses, rely on aerobic fermentation (glycolysis) .*
- So: "Warburg metabolism" is not cancer specific, but instead is a general property of growing cells.



NOTE:

- A growing cell must duplicate all of its cellular components—DNA, RNA, proteins, lipid, and organelles—before it can divide and produce two daughter cells.
- While oxidative phosphorylation yields abundant ATP, it fails to produce any carbon moieties that can be used to build the cellular components needed for growth (proteins, lipids, and nucleic acids). Even cells that are not actively growing must shunt some metabolic intermediates away from oxidative phosphorylation in order to synthesize macromolecules that are needed for cellular maintenance.



HOW DOES CANCER CELLS DO THIS SWITCH OF METABOLISM????

- **Metabolic reprogramming is produced by signalling cascades downstream of growth factor receptors, the very same pathways that are deregulated by mutations in oncogenes and tumors suppressor genes in cancers.**
- Thus, whereas in rapidly dividing normal cells aerobic glycolysis ceases when the tissue is no longer growing, in cancer cells this reprogramming persists due to the action of oncogenes and the loss of tumor suppressor gene function, including TP53 mutations.

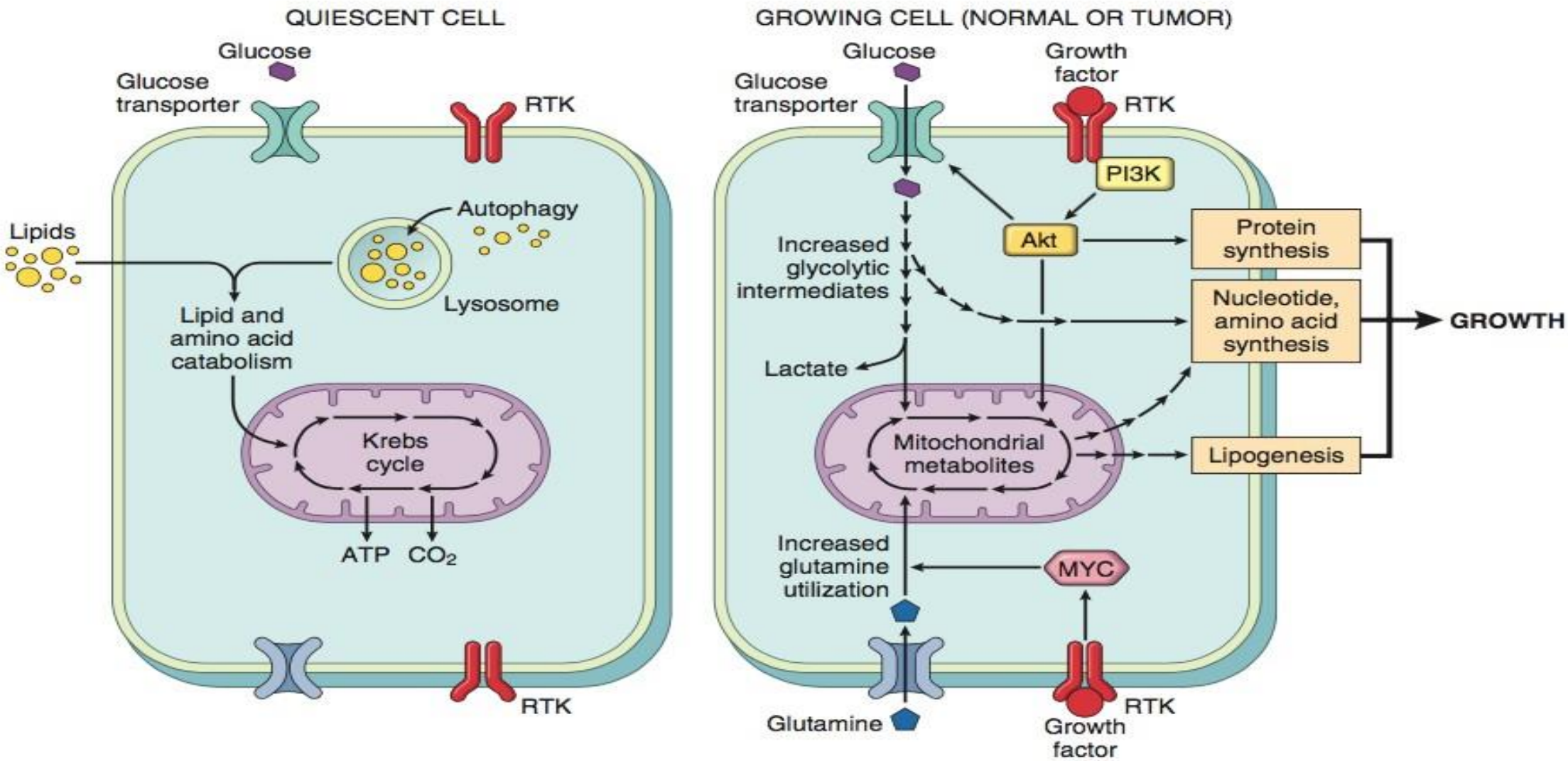
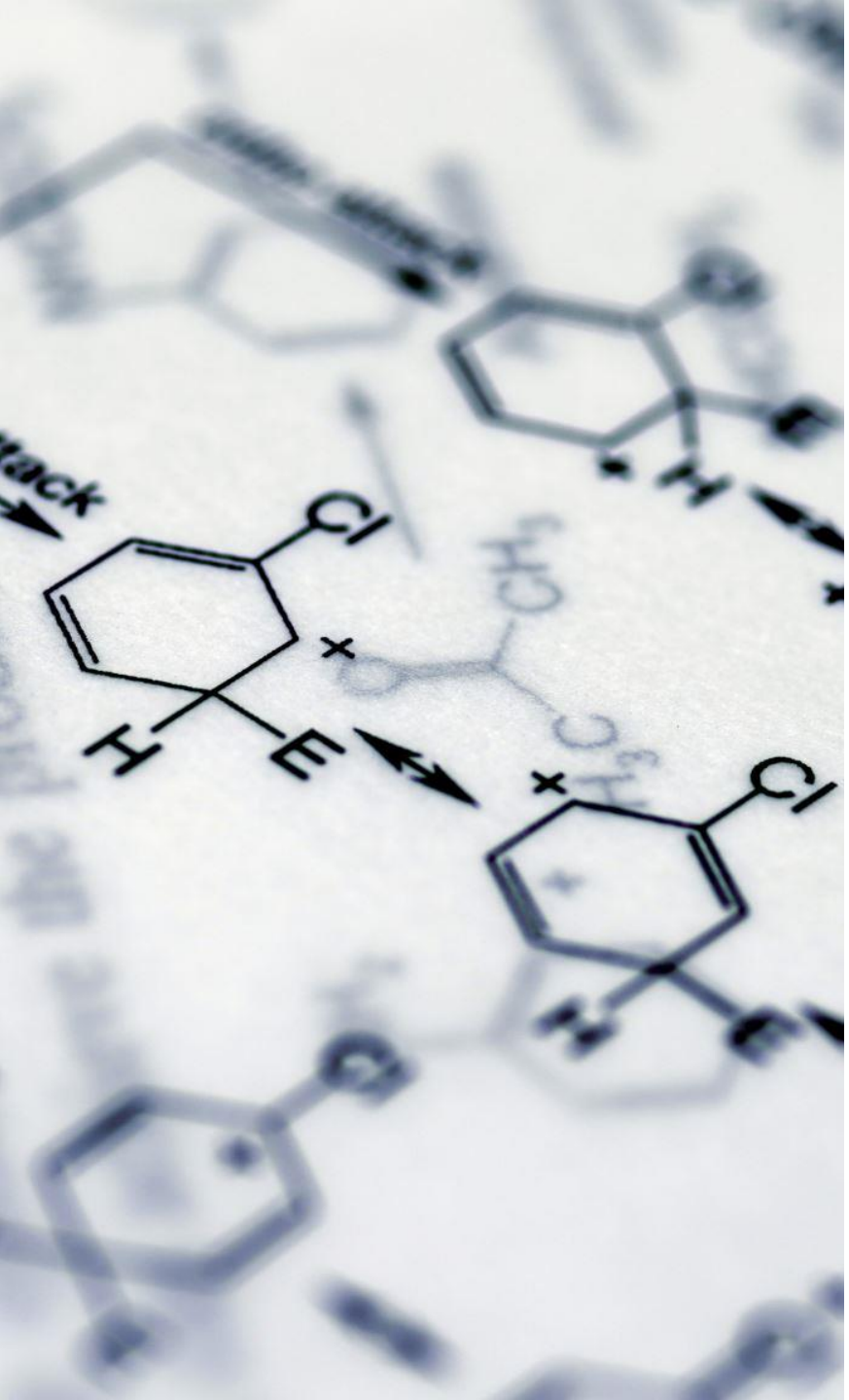


Fig. 6.23 Metabolism and cell growth. Quiescent cells rely mainly on the Krebs cycle for ATP production; if starved, autophagy (self-eating) is induced to provide a source of fuel. When stimulated by growth factors, normal cells markedly upregulate glucose and glutamine uptake, which provide carbon sources for synthesis of nucleotides, proteins, and lipids. In cancers, oncogenic mutations involving growth factor signaling pathways and other key factors such as MYC deregulate these metabolic pathways, an alteration known as the *Warburg effect*.



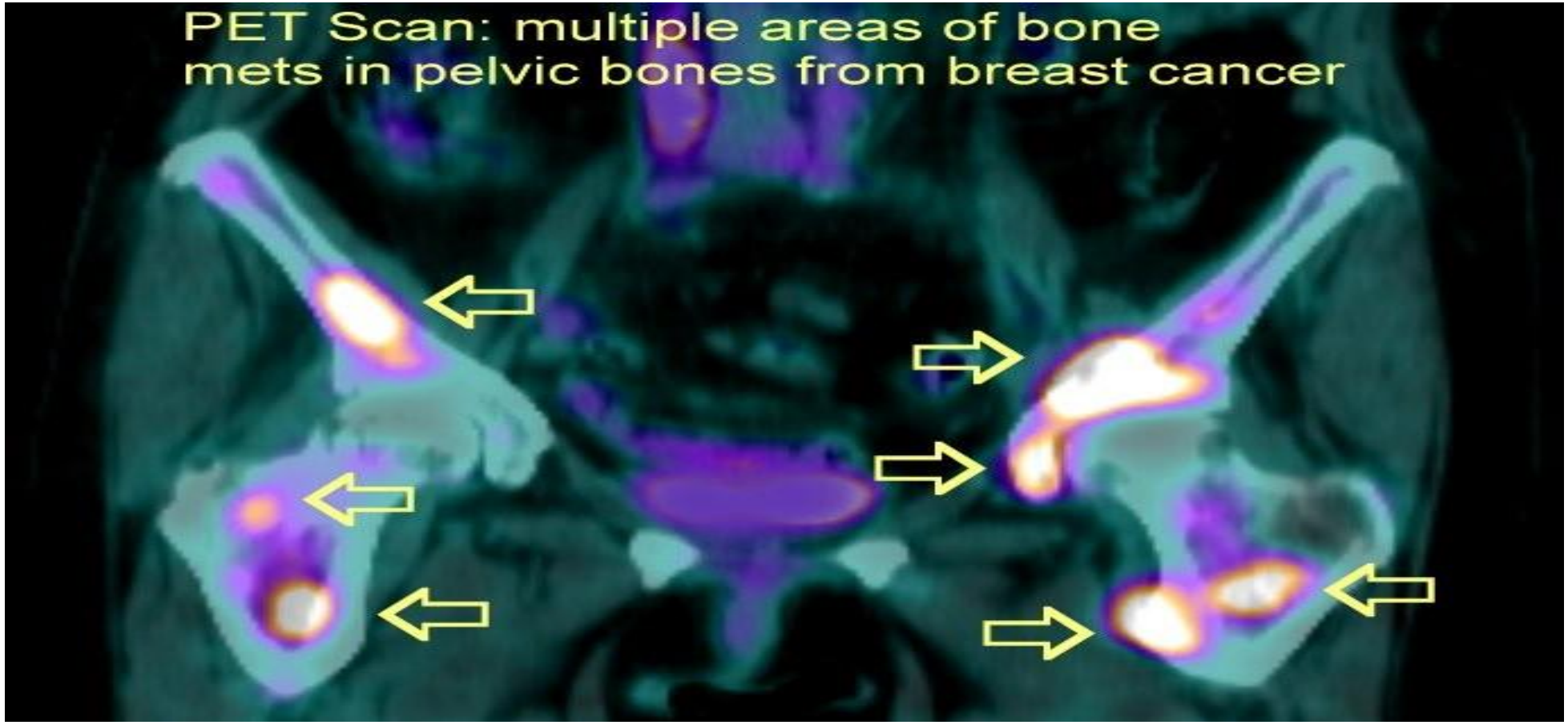
PET SCAN

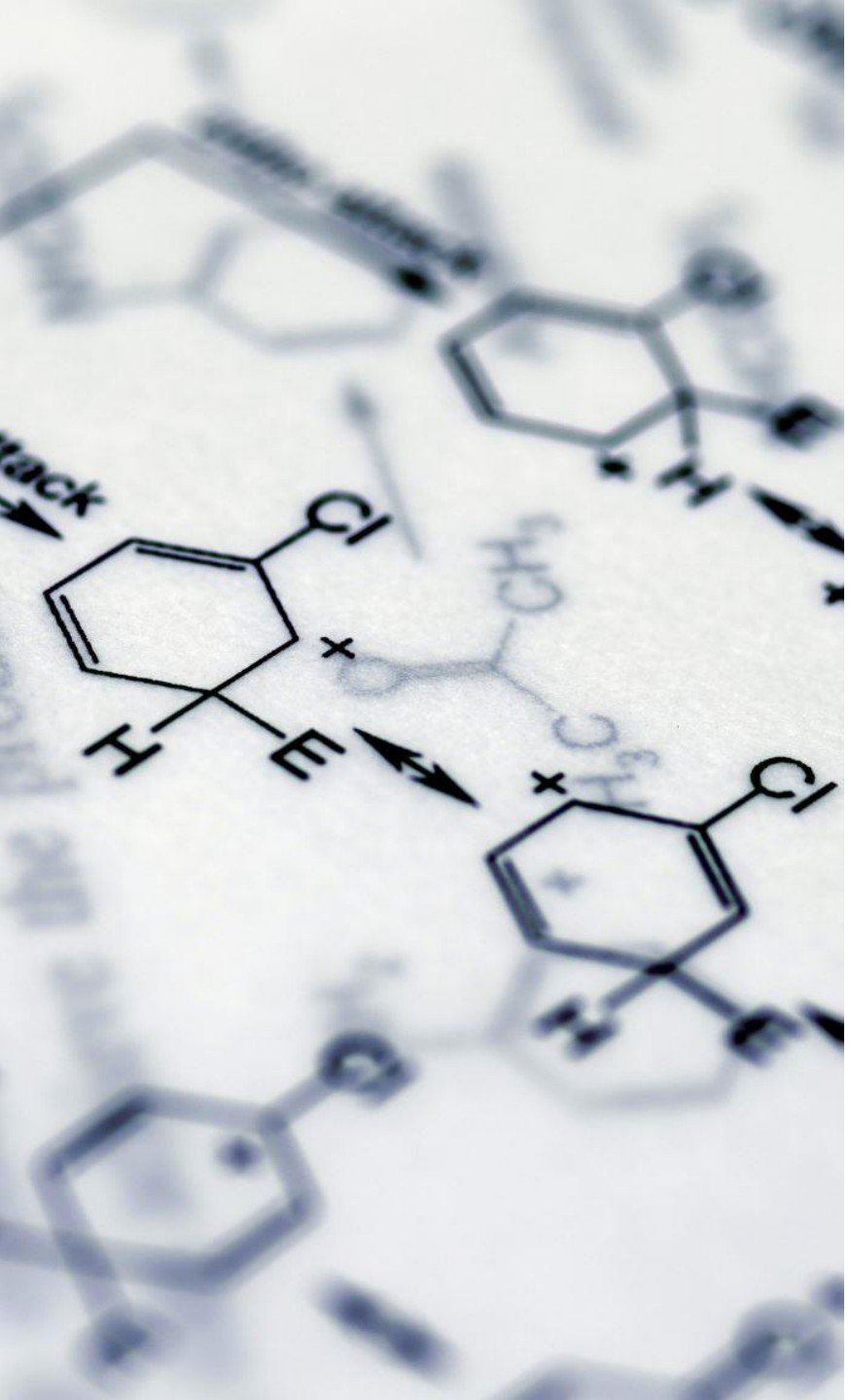
- Because of this reprogramming, tumor cells are "glucose hungry", they take loads of glucose, and this property is used in PET scans
- PET: positron emission tomography
- Patient is injected with a glucose derivative.. Tumor cells take this derivative more than normal cells and as such detected with the scan
- The more proliferative the tumor is… more uptake and more positivity with PET scan

PET SCAN

"I'm afraid the brain scan results confirm your worst fears, Mrs. Taylor."

PET SCAN


PET Scan: multiple areas of bone
mets in pelvic bones from breast cancer

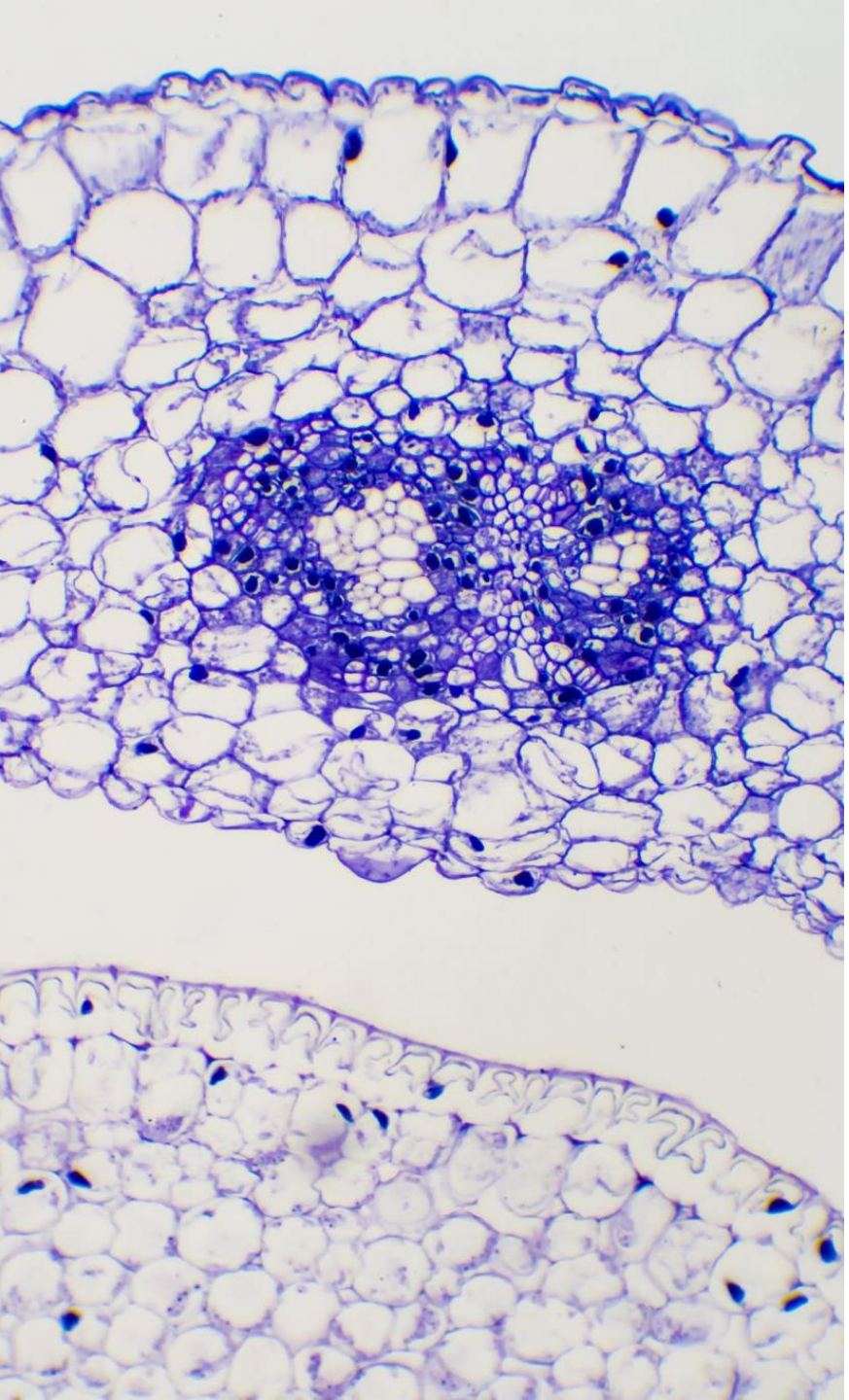


IMPORTANT NOTE

- Note that we agreed that ALL the phenotypes (cancer hallmarks) are needed to transform cells. But, it should be clear now that we don't need 8 mutations for the 8 hallmarks!
- Example: p53 mutations can cause insensitivity to growth signals, evasion of apoptosis and reprogramming of energy metabolism(three hallmarks from one mutation!)

AUTOPHAGY

- Autophagy is a catabolic process that balances synthesis, degradation and recycling of cellular products
- The recycling of the cell's organelles can produce energy needed for the stressed cells.
- This process can signal cell death if the cell cannot be rescued by the recycling process



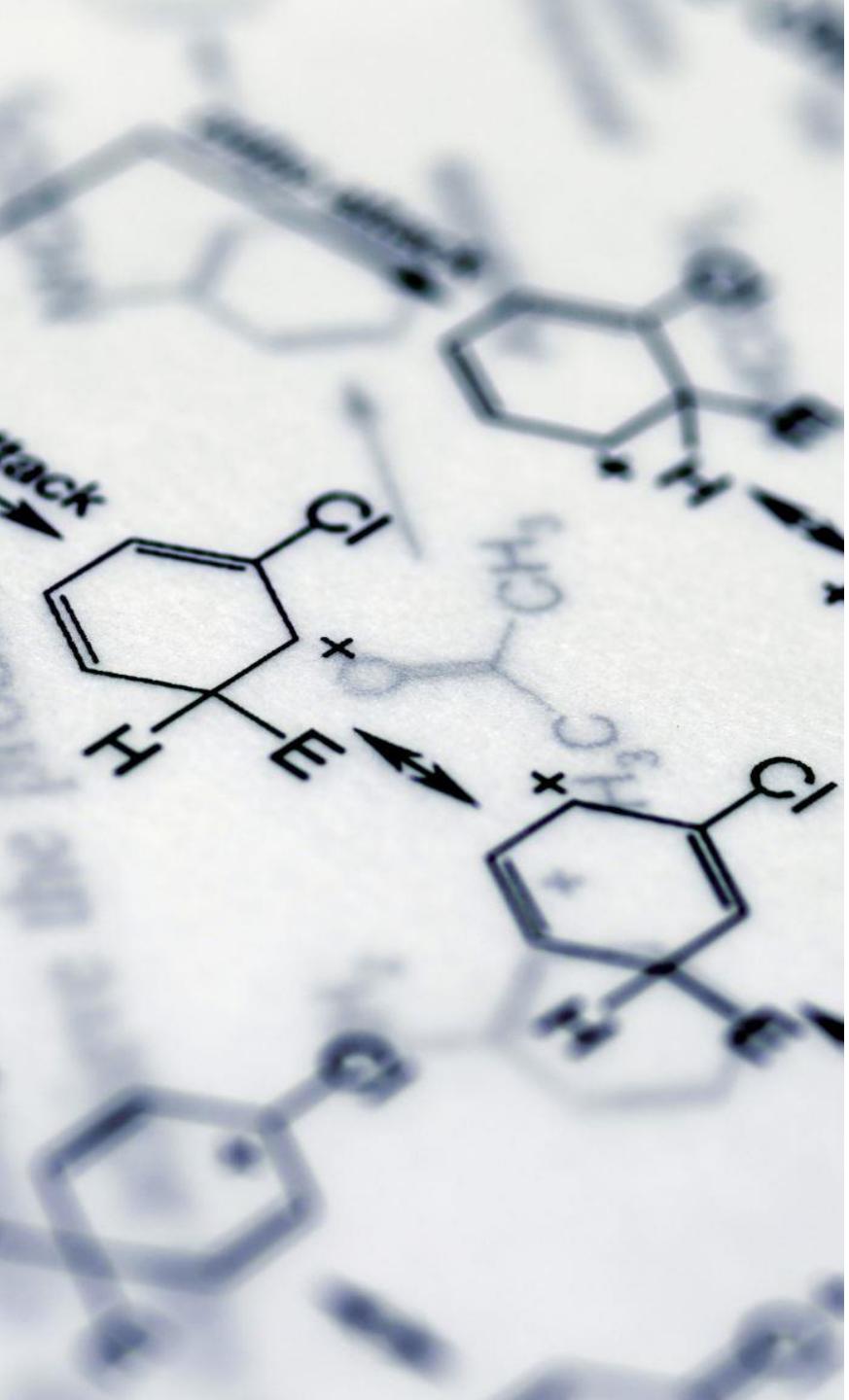
AUTOPHAGY

- ***Autophagy* is a state of severe nutrient deficiency in which cells not only arrest their growth, but also cannibalize their own organelles, proteins, and membranes as carbon sources for energy production**).
- If this adaptation fails, the cells die.
- Tumor cells grow under marginal environmental conditions without triggering autophagy, suggesting that the pathways that induce autophagy are deranged.
- In keeping with this, several genes that promote autophagy are tumor suppressors.

NOTE

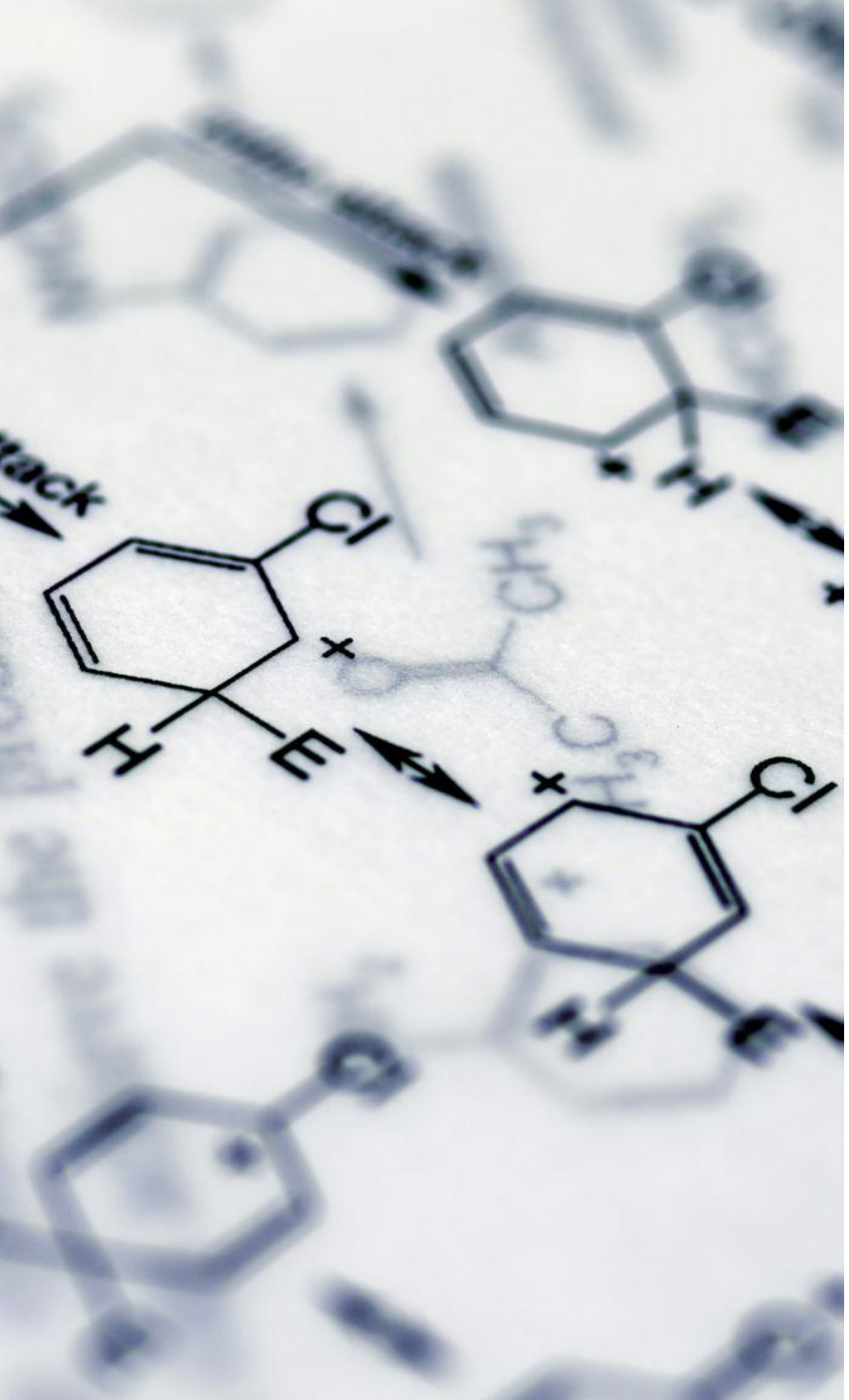
Although autophagy is an anti-tumor process….. Later on if there is a tumor mass formed, autophagy can help the tumor to survive if it's used to recycle organelles to be used as an energy source .

Autophagy can help tumor cells to survive during unfriendly climates: for example during chemotherapy treatment.



ONCOMETABOLISM

- This is a new concept, which was discovered through finding certain mutations in enzymes that participate in the Krebs cycle. Of these, mutations in isocitrate dehydrogenase (IDH) is the most studied.



HOW A MUTATION IN IDH CAUSES CANCER?

- IDH acquires a mutation involving the active site of the enzyme, so it loses its ability to function as an isocitrate dehydrogenase and instead acquires a new enzymatic activity that catalyzes the production of 2-hydroxglutarate (2-HG).
- 2-HG in turn acts as an inhibitor of several other enzymes that are members of the TET family, including TET2.
- TET2 regulate DNA methylation, which is an epigenetic modification that controls normal gene expression.
- Abnormal DNA methylation in turn leads to mis-expression of currently unknown cancer genes, which drive cellular transformation and oncogenesis.

ONCOMETABOLITE: A METABOLIC PRODUCT CAUSING ONCOGENESIS.

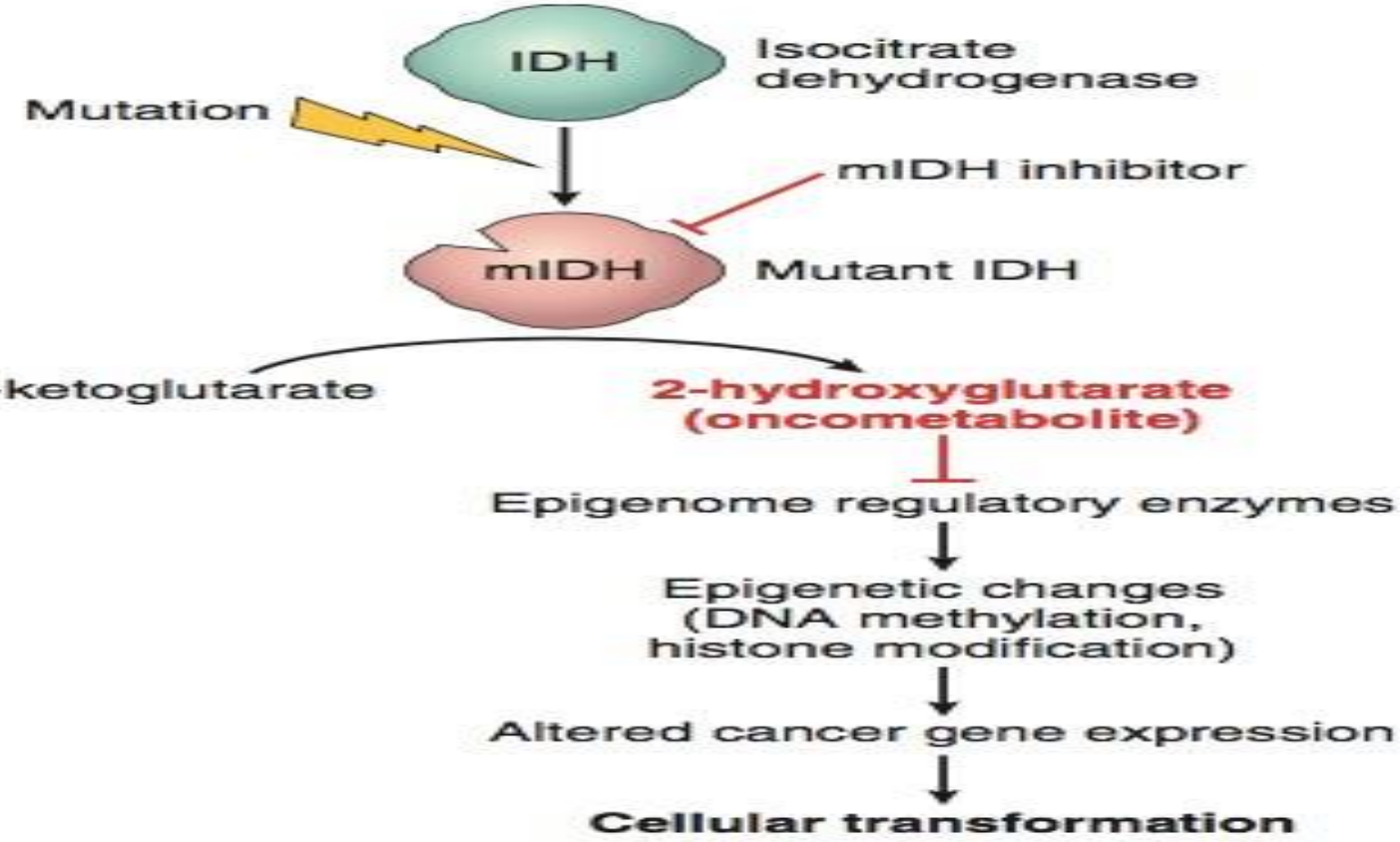
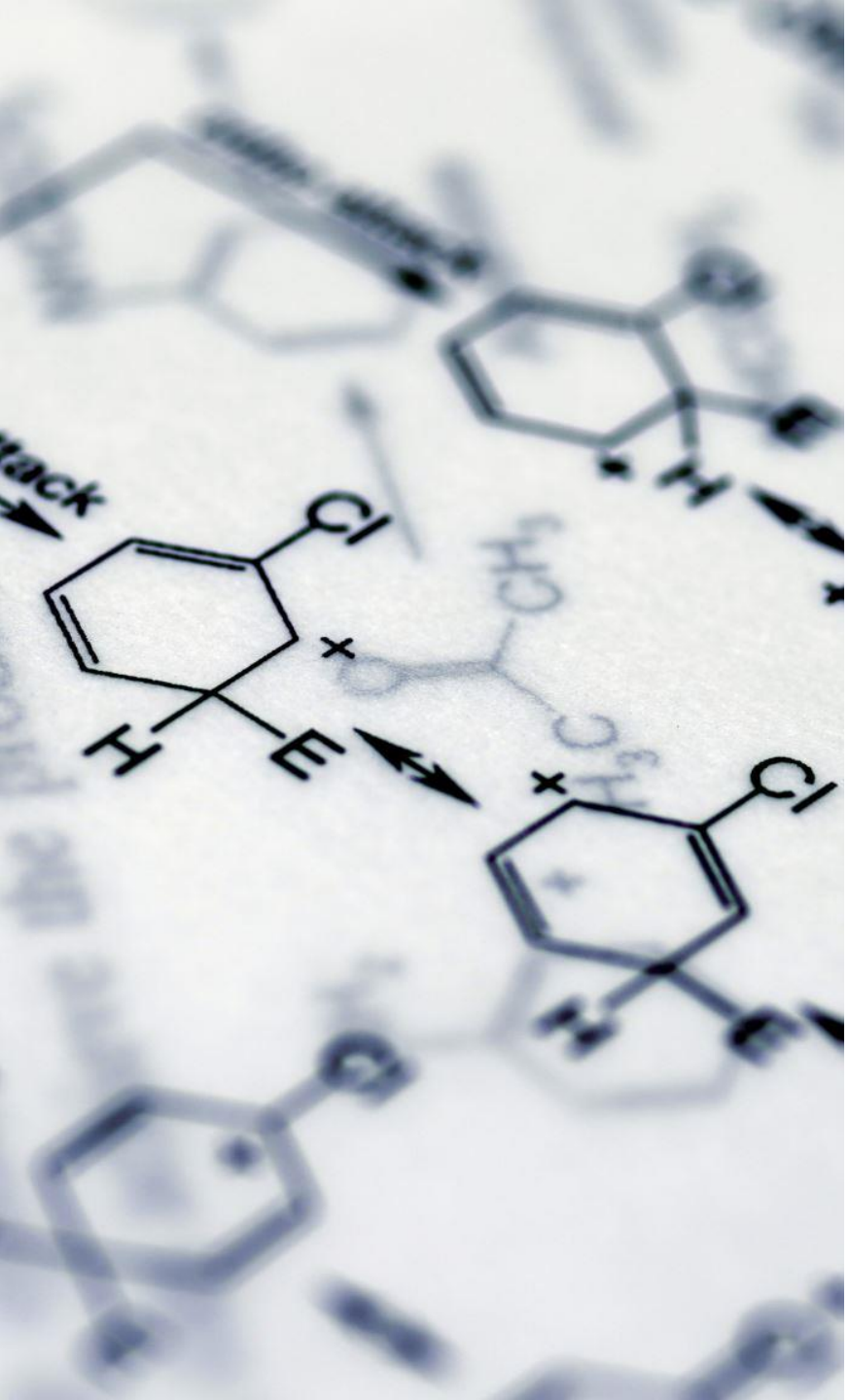


Fig. 6.24 Proposed action of the oncometabolite 2-hydroxyglutarate (2-HG) in cancer cells with mutated isocitrate dehydrogenase (mIDH).



- IDH mutations are found in gliomas, acute myeloid leukaemia, and sarcomas.
- the mutated IDH proteins have an altered structure ,so it has been possible to develop drugs that inhibit mutated IDH and not the normal IDH enzyme.
- These drugs are now being tested in cancer patients and have produced encouraging therapeutic responses.

REFERENCE: ROBIN BASIC PATHOLOGY, 10TH EDITION

THANK YOU

After 61 slides