Neoplasia 2018 lecture 7

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ILOS

- 1. understand evading apoptosis as a hallmark of cancer.
- 2. listing changes in the apoptotic pathways that can lead to cancer.
- 3. apply this knowledge in clinical scenarios, like follicular lymphoma.
- 4. understand the metabolic changes occurring during tumorigenesis
- 5. understand the basic concepts behind PET scan examination
- 6. understand the effect of autophagy in cancer development
- 7. understand the concept of oncometabolites and their targeted therapy.

Intro

- Till now we know that tumor cells grow in an uncontrolled manner through being self-sufficient in growth signals, resisting growth inhibition and being able to replicate in a continuous manner.
- However.. These cells have acquired these characteristics via mutations that caused certain protein abnormalities.
- Normally mutated cells with abnormal proteins die by triggering death signals causing apoptosis.
- So: cancer cells need certain mechanisms to allow them to survive and evade(avoid) death.
- The first topic in this lecture is how tumor cells evade apoptosis.
- Then we will discuss another hallmark related to how cancer cells change their metabolism to help them survive. This involves several metabolic changes which we will discuss in detail.

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

Apoptosis

- Fragments of the apoptotic cells break off, giving the appearance that is responsible for the name (*apoptosis,* "falling off").

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

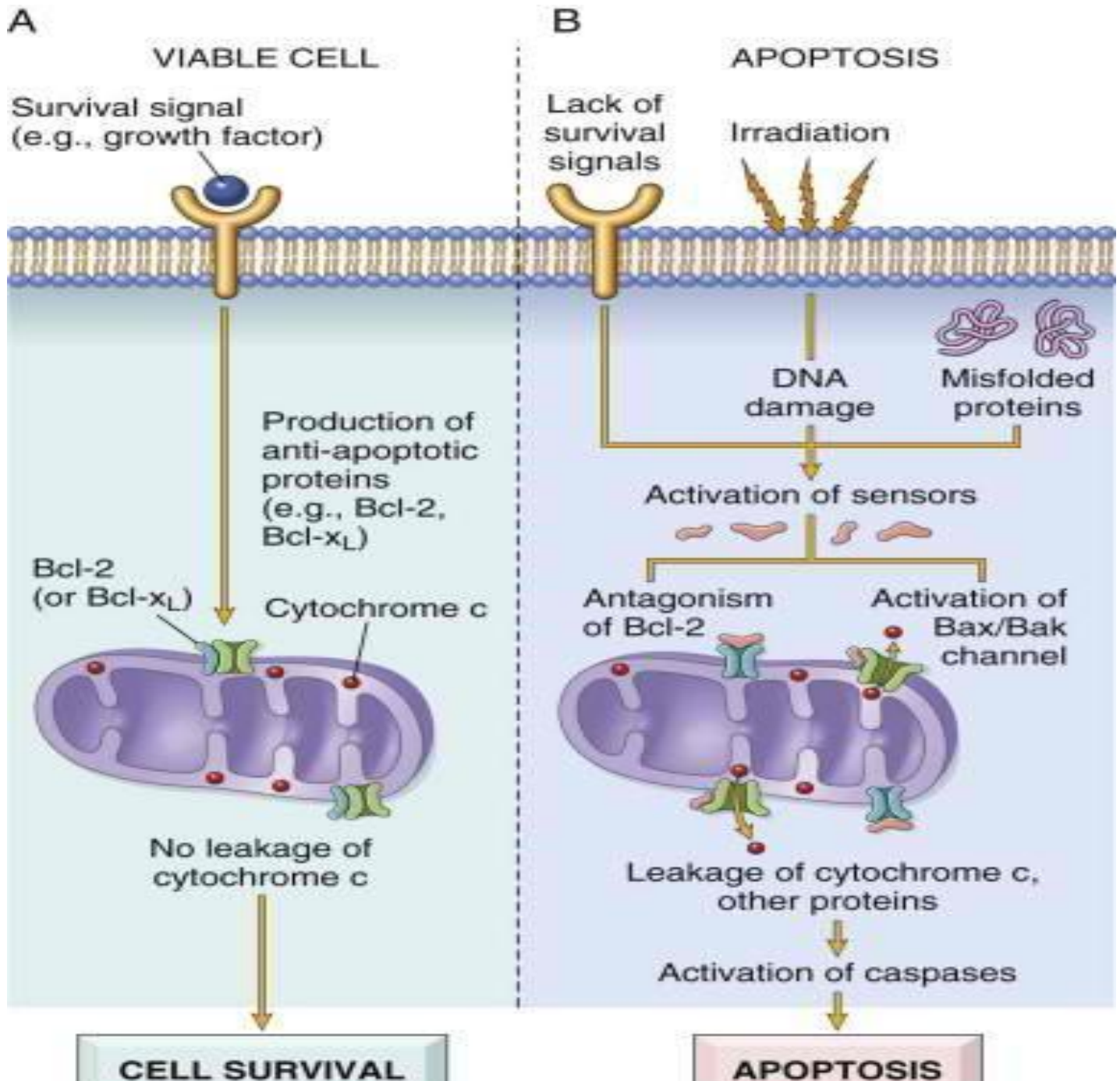
Mitochondrial permeability

- 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- Xl

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

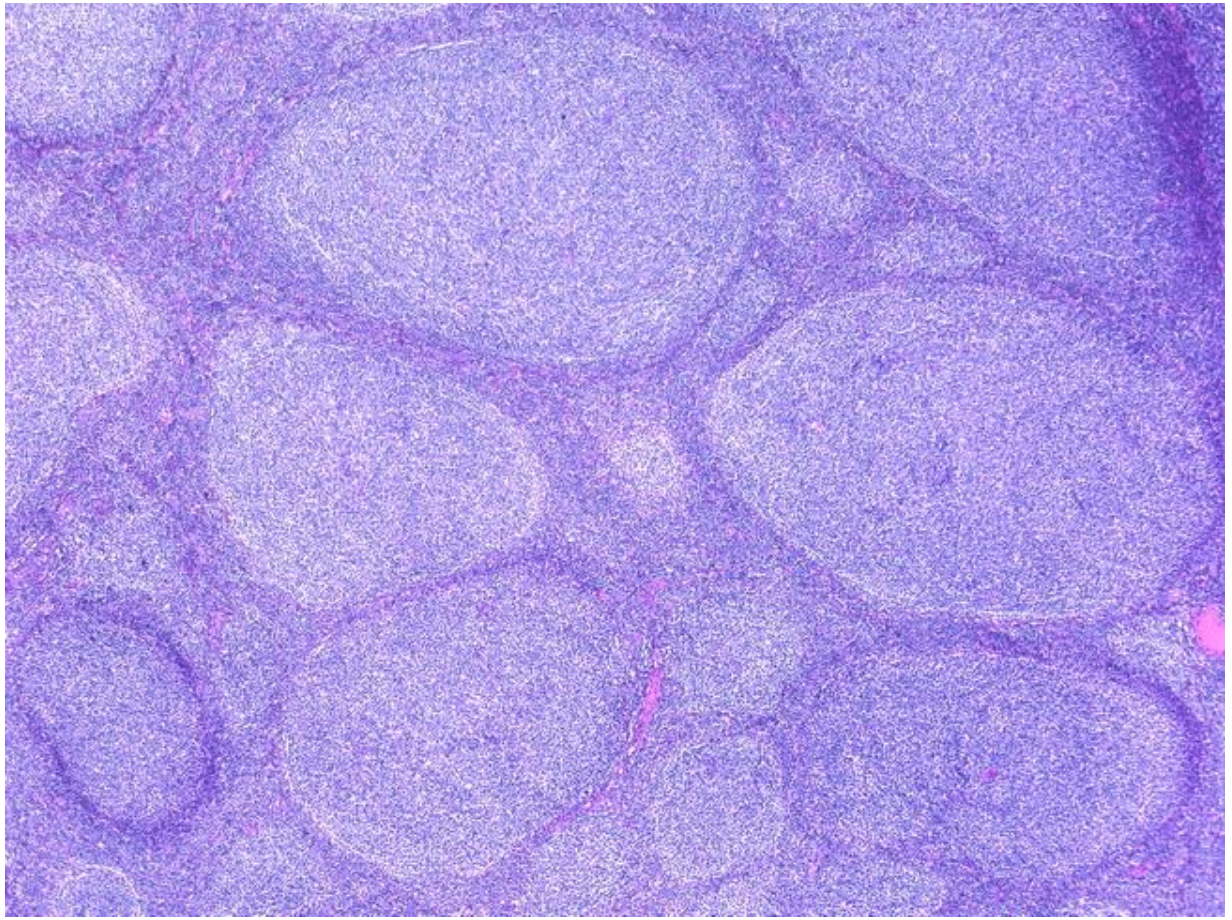
P53 and apoptosis

- 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

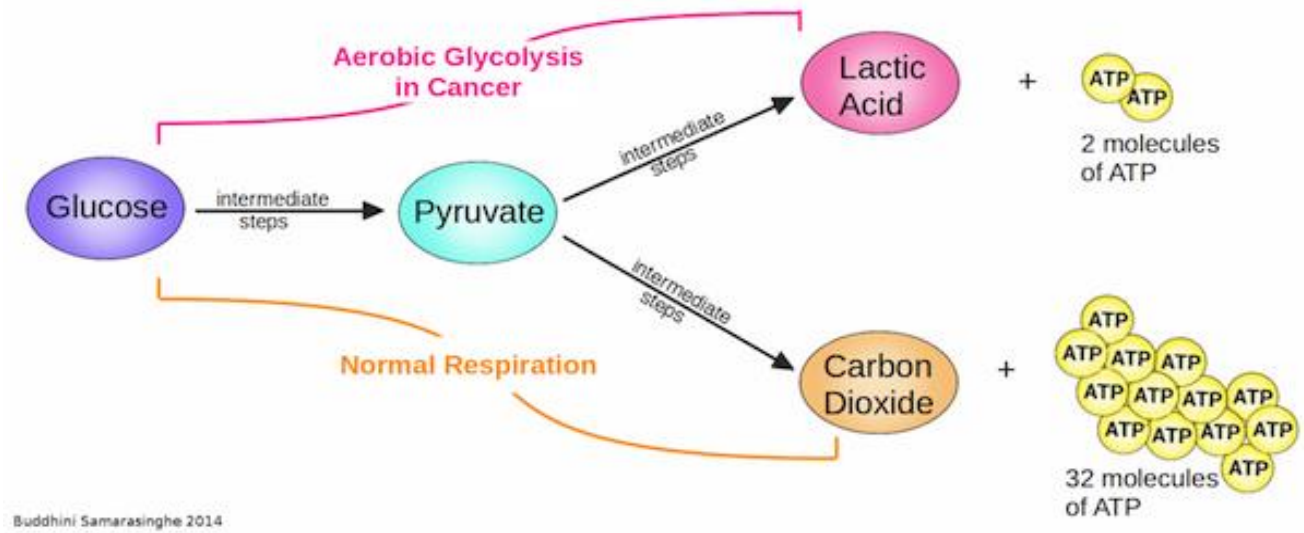
- Although less ATP is produced… the Warburg effect ensures that carbon atoms in glucose (which is converted to Pyruvate) are used for synthesis of organic compounds like lipids and proteins which are important in building new cells in the highly proliferative tumor.
- SO: Aerobic glycolysis provides 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.


Aerobic Glycolysis
in Cancer
Glucose
intermediate
steps
Pyruvate
intermediate
steps
Lactic
Acid
+
ATP
ATP
2 molecules
of ATP
intermediate
steps
Normal Respiration
Carbon
Dioxide
+
32 molecules
of ATP
Buddhini Samarasinghe 2014

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.

Important pathways affecting reprogramming of cell metabolism: 1.oncogenic pathways:

1. Growth factor receptor signaling. In addition to transmitting growth signals to the nucleus, signals from growth factor receptors also influence metabolism by **upregulating glucose uptake and inhibiting the activity of pyruvate kinase,** which catalyzes the last step in the glycolytic pathway, the conversion of phosphoenolpyruvate to pyruvate.

Pyruvate kinase inhibition creates build up of upstream glycolytic intermediates, which are used for synthesis of DNA, RNA, and protein.

2. RAS signaling. Signals downstream of RAS upregulate the activity of glucose transporters and multiple glycolytic enzymes, thus increasing glycolysis; promote shunting of mitochondrial intermediates to pathways leading to lipid biosynthesis; and stimulate factors that are required for protein synthesis.

3. MYC. Among the MYC- regulated genes are those for several glycolytic enzymes and glutaminase, which is required for mitochondrial utilization of glutamine, a key source of carbon moieties needed for biosynthesis of cellular building blocks

Important pathways affecting reprogramming of cell metabolism: 2.Tumor suppressor genes pathway pathways

- p53, upregulates target genes that collectively inhibit glucose uptake, glycolysis, lipogenesis, and the generation of NADPH (a key cofactor needed for the biosynthesis of macromolecules).

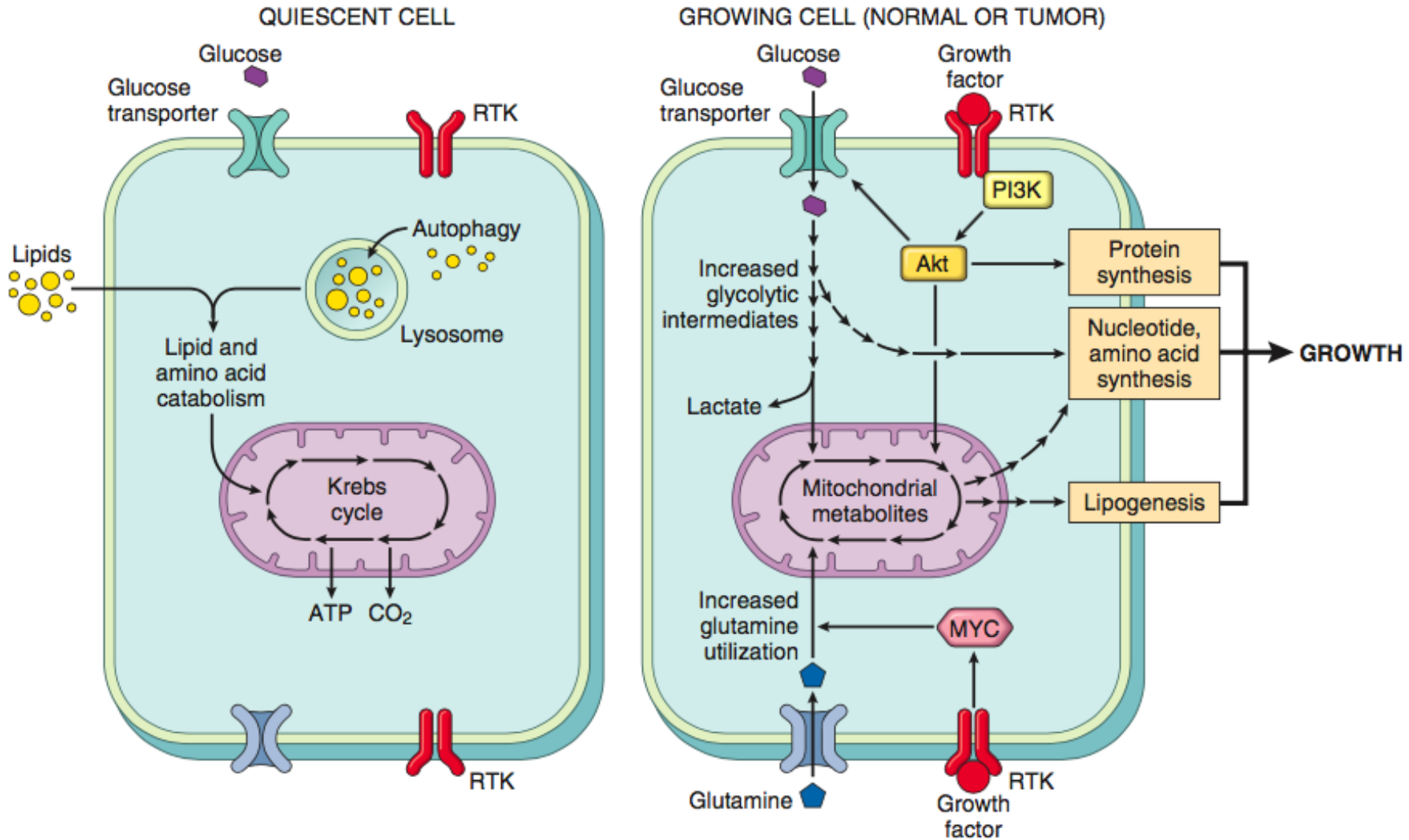


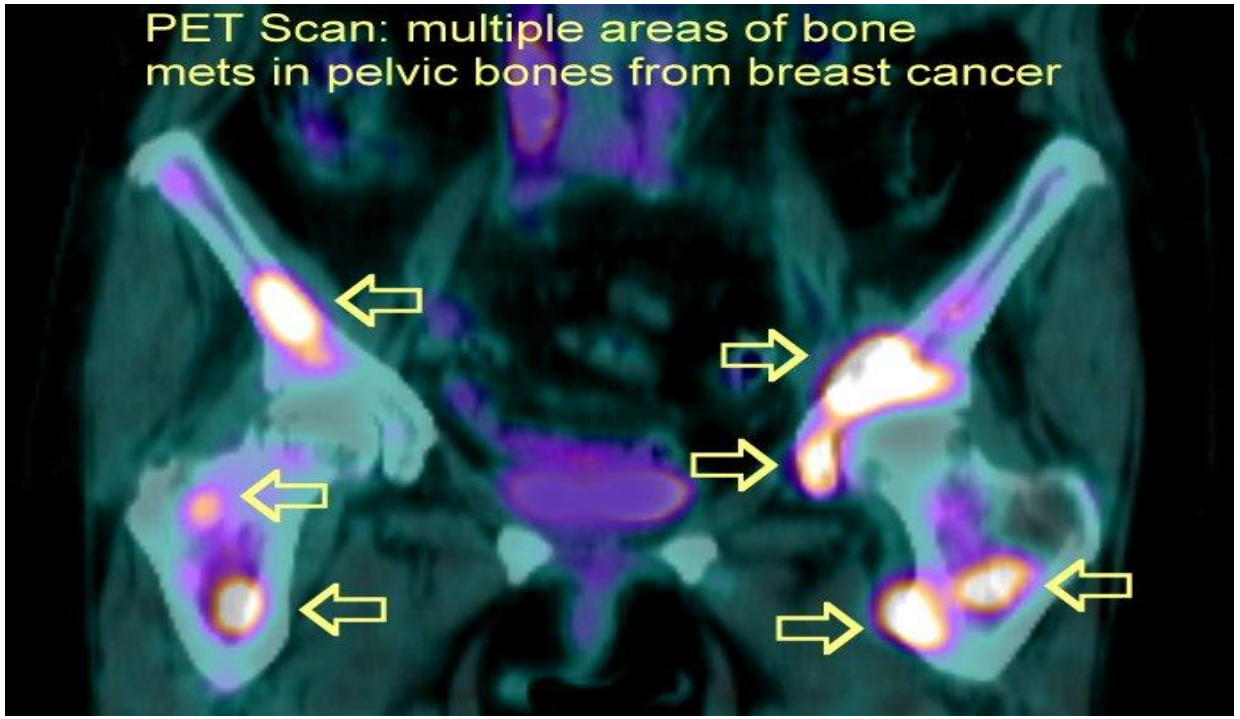
Fig. 6.23 Metabolism and cell growth. Quiescent cells rely mainly on the Krebs cycle for ATP production; if starved, autophagy (self-eating) is induced to provide a source of fuel. When stimulated by growth factors, normal cells markedly upregulate glucose and glutamine uptake, which provide carbon sources 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

PET scan


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

PET scan

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.

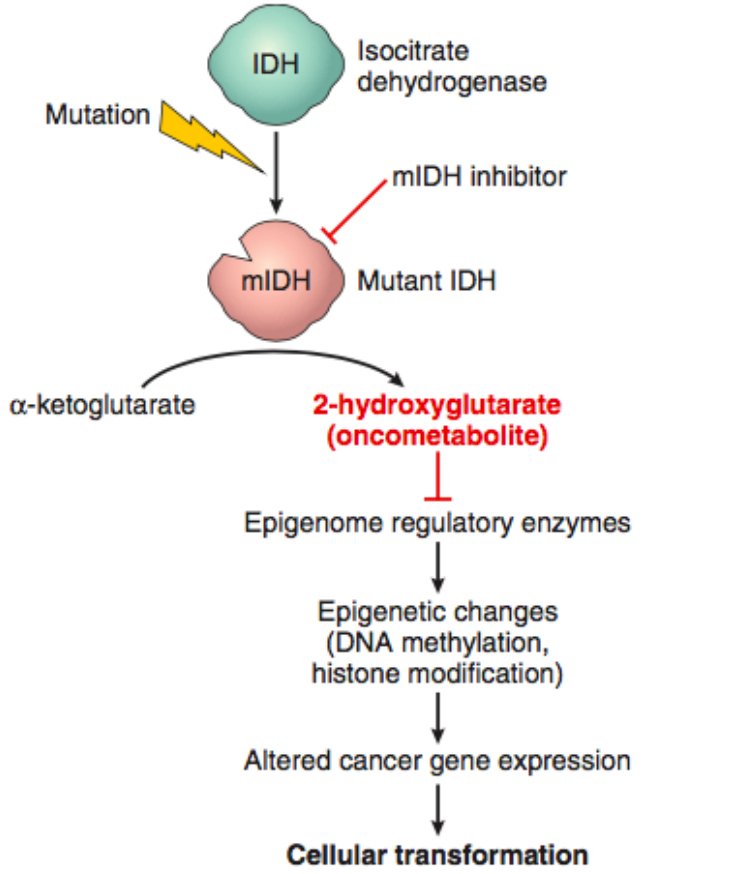


Fig. 6.24 Proposed action of the oncometabolite 2-hydroxyglutarate (2-HG) in cancer cells with mutated isocitrate dehydrogenase (mIDH).

- 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.

summary

- Evading apoptosis is an important trait of cancer cells. This occurs via blocking apoptotic or stimulating anti-apoptotic mechanisms.
- Tumor cells have altered metabolism that enhances their survival. These include: reprogramming of energy metabolism, autophagy changes and oncometabolite formation.
- Warburg phenomenon= aerobic metabolism= fermentation even with the presence of oxygen. This reduces ATP generated per gram glucose but provides carbon atoms needed for cell division and growth.
- This switch in metabolism is achieved via oncogenes overexpression and tumor suppressor genes inactivation. In both instances there is shift in glucose metabolism.
- Autophagy is evaded in tumors, but is upregulated during stress (chemotherapy or ischemia) to recycle cell components and enhance survival.
- IDH mutations ae a prototype for oncometabolites, they act by changing enzyme activity resulting in DNA methylation. New therapies are discovered to target these mutated enzymes.

Question

- A 54 year old male developed a brain tumor which was diagnosed as an astrocytoma. Immunohistochemical stains showed positive IDH staining implying a mutation in IDH. Through which of the following mechanisms this mutation resulted in cancer?
- A. increased micro RNAs
- B. oncogene amplification
- C. tumor suppressor gene downregulation
- D**. epigenetic changes… (it acts via DNA methylation)**
- E. Impairing DNA repair mechanisms


Thank
you!