

Mitochondria and Cancer

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Abstract

Mitochondria, as biosynthetic centers or powerhouses of the cell, are involved in many physiological and pathophysiological processes. They have emerged as therapeutic targets for cancer. Through the activity of TCA cycle, mitochondria contribute to synthesis of biomolecules, such as amino acids, lipids, and nucleotides. Other important mitochondrial functions are control of calcium homeostasis, the intrinsic pathway of apoptosis, energy metabolism, redox homeostasis, oncogenic signaling, innate immunity, and ROS production. Mitochondrial metabolic phenotypes have been linked to nuclear or mitochondrial genome (mtDNA) mutations. These mutations are linked to many pathological diseases and may be associated with decreased or increased risk of cancer. Mitochondrial biogenesis is upregulated in tumor cells, likely to match their increased metabolic demand. Also, mutations in many nuclear-encoded mitochondrial TCA cycle enzymes cause the synthesis of oncogenic metabolites and tumor progression. Here I discuss briefly the basics of mitochondria biology and provide an overview of the links between mitochondria and cancer.

Key words: mitochondria, TCA cycle, calcium homeostasis, intrinsic apoptosis, redox homeostasis, oncogenic signaling, innate immunity, ROS production, mtDNA

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List of Abbreviations

A

AA: Amino acid

Abl: Ableson leukemia oncogene cellular homolog

ACCase: Acetyl-CoA carboxylase

ACC1: Acetyl-CoA carboxylase 1

Ac-CoA: Acetyl-Coenzyme A

ACL: ATP citrate lyase

ACP: Acyl carrier protein

ADP: Adenosine diphosphate

AGA: Aspartate–glutamate antiporter

AGC family: Protein kinase A, G, and C family

AIF: Apoptosis-inducing factor

α KDH: α -ketoglutarate dehydrogenase

α KG: α -ketoglutarate

ALA: δ -aminolevulinic acid

ALAS: δ -aminolevulinic acid synthetase

ALL: Acute lymphoblastic leukemia

AMPK: AMP-activated kinase

ANT: Adenine nucleotide translocators

Apaf1: Apoptotic protease activating factor 1

ATM: Ataxia Telangiectasia Mutated

ATP: Adenosine triphosphate

B

BAD: Bcl-2-Associated Death Promoter

Bad: BH3-only domain

Bak: Bcl-2 homologous antagonist/killer

Bax: Bcl-2-associated X

Bcl-2: B-cell lymphoma 2

BclX_L: B-cell lymphoma-extra large

Bcr: breakpoint cluster region protein

BH3: Trihydridoboran

BH4: Tetrahydrobiopterin

Bid: BH3 domain-only death agonist protein

Bim: B-lymphocyte-induced maturation

BNIP3: BCL2/adenovirus E1B interacting protein 3

BR: Benzodiazepine receptor

C

CA9: Carbonic anhydrases 9

CA12: Carbonic anhydrases 12

CaM: calmodulin

CD: Cyclophilin D

Cit: citrate

CK: Creatine kinase

CLL: Chronic lymphocytic leukemia

c-Myc: Avian myelocytomatosis virus oncogene cellular homolog

CN: calcineurin

CO₂: Carbon Dioxide

CoA: coenzyme A

CoA-SH: Coenzyme A with a free sulphydryl group

CoQ: Coenzyme Q10

Cox: Cytochrome c oxidase

CPT: Carnitine palmitoyltransferase

Cpt1c: Carnitine palmitoyltransferase 1c

CS: Citrate synthase

CYP: Cytochrome P450

CYPD: Cyclosporine-A sensitive cyclophilin D

D

DAF-16: Dauer factor-16

DLBCL: Diffuse large B-cell lymphoma

DNA: Deoxyribonucleic acid

DRAM: Damage-regulated autophagy modulator

E

eIF4E: Eukaryotic translation initiation factor 4E

ENDOG: Mitochondrial endonuclease G

ER: Endoplasmic reticulum

ERK: extracellular regulated kinase

ETC: Electron transfer chain

F

FAD: Flavin adenine dinucleotide

***FADH*₂:** Flavin adenine dinucleotide (hydroquinone form)

FAS: Fatty acid synthase

FBP: Fructose-1,6-bisphosphate

FH: Fumarate hydratase

FMN: Flavin mono-nucleotide

FOXO: Forkhead box protein O

F6P: Fructose 6-phosphate

G

G1: Growth 1 phase

GDH: Glutamate dehydrogenase

Gln: Glutamine

GLS: Glutaminase

GLUD: Glutamate dehydrogenase

GLUT: Glucose transporter

G2/M: Growth 2 phase/Mitosis phase

GM-CSF: Granulocyte-macrophage colony-stimulating factor

GOT1: Glutamic-oxaloacetic transaminase 1

GOT2: Glutamic-oxaloacetic transaminase 2

G3P: Glyceraldehyde-3-phosphate

G6P: Glucose 6-phosphate

G6PDH: Glucose-6 Phosphate Dehydrogenase

GPX: Glutathione peroxidase

Grb2: Growth factor receptor-bound protein 2

GS: Glutamine synthetase

GSH: Glutathione

GSK: glycogen synthase kinase

GSSG: Oxidized glutathione dimer

GTP: Guanosine-5'-triphosphate

H

HIF-1: Hypoxia-inducible factor 1

HK: Hexokinase

HMOX1: Haemoxygenase 1

HMPDb: Human mitochondrial protein database

hnRNPs: Heterogeneous nuclear ribonucleoproteins

H-ras: Harvey rat sarcoma viral oncogene homolog

HSP-70: Heat-shock protein 70

HtrA2: high-temperature requirement 2

I

IAP2: Inhibitor of apoptosis 2

IDH1: Isocitrate dehydrogenase 1

IDH2: Isocitrate dehydrogenase 2

IDO: Indoleamine 2,3-dioxygenase

IL-2: Interleukin 2

IL-3: Interleukin 3

IMM: Inner mitochondrial membrane

IP3: Inositol trisphosphate

IP3R: Inositol trisphosphate receptor

J

JNK1: c-Jun N-terminal kinase

K

KAS: Ketoacyl-ACP synthase

KAS I: Ketoacyl-ACP synthase I

KAS III: Ketoacyl-ACP synthase III

K-ras: Kirsten rat sarcoma viral oncogene homolog

L

LAT1: L-type amino acid transporter 1

LDH: Lactate dehydrogenase

LDHA: Lactate dehydrogenase isoform A

LKB1: Liver kinase B1

M

MαA: Malate–αKG antiporter

MAC: Mitochondrial apoptosis-induced channel

MAM: Mitochondria-associated ER membrane

MAPK: Mitogen-activated protein kinase

MCT: Monocarboxylate transporter

MCU: Mitochondrial calcium uniporter

MDH: Malate dehydrogenase

miR-20: MicroRNA-20

MnSOD: Manganese superoxide dismutase

MOMP: Mitochondrial outer membrane permeabilization

MPC1: Mitochondrial pyruvate carrier 1

MPC2: Mitochondrial pyruvate carrier 2

MPT: Mitochondrial permeability transition

mRNA: Messenger ribonucleic acid

mtDNA: Mitochondrial DNA

MTHFD2: Methylenetetrahydrofolate dehydrogenase

mTOR: mechanistic target of rapamycin

mtPTP: Mitochondrial permeability transition pore

mtTFA: Mitochondrial transcription factor A

N

NAD⁺: Nicotinamide adenine dinucleotide (oxidized form)

NADH: Nicotinamide adenine dinucleotide (reduced form)

NADP⁺: Nicotinamide adenine dinucleotide phosphate

NADPH: Nicotinamide adenine dinucleotide phosphate (reduced form)

NFAT: Nuclear factor of activated T-cell

NNT: Nicotinamide nucleoside transhydrogenase

NRF1: Nuclear respiratory factor 1

NRF2: Nuclear factor erythroid 2–related factor 2

O

OAA: Oxaloacetate

OMM: Outer mitochondria membrane

OPA1: Optic atrophy type 1

Orai1: Calcium release-activated calcium ion channel protein 1

OSCP: Oligomycin sensitivity–conferring protein

OXPHOS: Oxidative phosphorylation

P

P27: Protein-27

P53: Protein-53 (Phosphoprotein-53)

P85: Protein-85

P130: Protein-130

PBR: Peripheral benzodiazepine receptor

PCD: Programmed cell-death

PDC: Pyruvate dehydrogenase complex

PDH: Pyruvate dehydrogenase

PDHK (PDK): Pyruvate dehydrogenase kinase

PDHP (PDP): Pyruvate dehydrogenase phosphatase

PEP: Phosphoenolpyruvate

PEPCK-M: Mitochondrial PEP carboxykinase

PFK: Phosphofructo Kinase

PFK1: Phosphofructo Kinase-1

6PG: 6-phosphogluconate

PGC1 α : Peroxisome proliferator-activated receptor gamma coactivator 1-
alpha

6PGDH: 6-Phosphogluconate Dehydrogenase

6PGL: 6-Phosphogluconase

PGM: Phosphoglycerate mutase

PH: Pleckstrin homology domain

PHD: Prolyl hydroxylase domain

PHGDH: Phosphoglycerate dehydrogenase

PI3K: Phosphatidylinositol 3-kinase

PINK1: PTEN-induced putative kinase 1

PIP3: Phosphatidylinositol 3, 4, 5-trisphosphate

PI (4, 5) P2: Phosphatidylinositol (4, 5)-phosphate

PI (3, 4, 5) P3: Phosphatidylinositol (3, 4, 5)-phosphate

PKB (Akt): Protein kinase B

PKC: Protein kinase C

PKM1: Pyruvate kinase Muscle isoform-1

PKM2: Pyruvate Kinase Muscle isoform-2

PP2A: Phospho-protein phosphatase 2

PPP: Pentose phosphate pathway

PT: Pyruvate transporter

PTEN: Phosphatase and tensin homolog

PTP: Permeability transition pore

R

Raf: Rapidly accelerated fibrosarcom

Ras: Redundant acronym syndrome

REDD1: regulated in development and DNA damage responses 1

Rheb: Ras homolog enriched in brain

(R)-2HG: *R* (-)-2-hydroxyglutarate

Rib-5-P: Ribose 5-phosphate

RNA: Ribonucleic acid

ROS: Reactive oxygen species

R5P: Ribose-5-phosphate

RPE: Ribulose-5-phosphate epimerase

RPI: Ribulose-5-phosphate isomerase

rRNA: Ribosomal ribonucleic acid

Ru5P: Ribulose-5-phosphate

S

S: Synthesis phase

SCO: Synthesis of cytochrome c oxidase

SCO2: Synthesis of cytochrome c oxidase 2

SCS: Succinyl-CoA synthetase

SDH: Succinate dehydrogenase

SDHA: SDH A subunit

SDHAF: SDHA factor

SDHB: SDH B subunit

SDHC: SDH C subunit

SDHD: SDH D subunit

SH2: Src homology-2 domain

Shc: SH2-containing collagen-related protein

SHIP: SH2-containing inositol phosphatase

SHMT2: Serine hydroxymethyl transferase

S6K: Small subunit ribosomal protein S6 kinase

SMAC: Second mitochondria-derived activator of caspase

SOS: Son of sevenless guanine nucleotide exchange factor

S7P: Sedoheptulose-7-phosphate

Src: Rous sarcoma oncogene cellular homolog

STIM1: Stromal interaction molecule 1

SUV3: Suppressor of Var1

T

TALDO: Transaldolase

TAT: Trans-activator of transcription

TAT-IDP: TAT-fused IP3R-derived peptide

TCA: Tricarboxylic acid

THF: Tetrahydrofolate

TIGAR: TP53-inducible glycolysis and apoptosis

TIM: Translocase of the inner membrane

TKT: Transketolase

TLK: Transketolase

TORC1 (mTORC1): Mechanistic target of rapamycin complex 1

TP53 (p53): Tumor protein-53

tRNA: Transfer ribonucleic acid

TRX2: Thioredoxin2

TSC: Tuberous sclerosis complex

TSC1: Tuberous sclerosis complex-1

TSC2: Tuberous sclerosis complex-2

TSPO: Translocator protein

U

UP: Ca²⁺ uniporter

UQ: Ubiquinone

UQH₂: Ubiquinone (reduced form)

UV: Ultraviolet

V

VDAC: Voltage dependent anion channel

VHL: Von Hippel-Lindau syndrome

W-Z

Xu5P: Xylulose-5-phosphate

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Chapter 1: Introduction

Origin and History of Mitochondria

The presence of mitochondria in eukaryotic cells was discovered after discovery of cell nuclei [1, 2]. Originally, they were called "bioblasts" by Altman. These essential organelles were isolated first and called mitochondrion (plural mitochondria) by Carl Benda in 1898. He concluded that mitochondrion is an organelle present in most eukaryotic cells and it contains proteins, lipids and other structural components [1]. The function of mitochondria in cellular respiration was studied by Kingsbury in 1912 [1]. Later on, Siekevitz described mitochondrion as the "powerhouse of the cell" [2]. In 1963, it was established that mitochondria had their own DNA or mtDNA [3]. The mtDNA was first sequenced in 1981 (16,569 base pairs) [4]. There are 37 genes that are encoded by mitochondrial genome (mtDNA). Out of 37, 24 genes have regulatory roles and 13 genes are protein-coding [5].

Mitochondria have been suggested to be a product of an endosymbiosis event between a hydrogen-dependent archaebacterium or eukaryotic cells (host cells) and a eubacterium (ancestor of modern mitochondria) approximately 2 billion years ago [6]. According to this theory, at first mitochondria were prokaryotic cells (free-living alpha-proteobacteria) that were capable of oxidative mechanisms (oxidative phosphorylation or cellular respiration). These cells were engulfed, as part

of an evolutionary process, by eukaryotic host cells and became endosymbionts (mitochondria) inside eukaryotic cells. This theory is supported by certain common features between mitochondria and prokaryotic cells. For example, prokaryotes and mitochondria both have their own DNA and basic proteins to form nucleoids that are responsible for DNA organization [7]. Also, mitochondria and prokaryotes both have a circular genomic DNA (1-7 Mbp) and both divide by binary fission [8].

Structure of mitochondria

Generally, mitochondria have five distinct parts; the outer mitochondria membrane (OMM), the intermembrane space (the space between the outer and the inner membranes), the inner mitochondrial membrane (IMM), cristae (invagination of the inner membrane) and the matrix (the internal space within the inner membrane). Mitochondrial inner and outer membranes consist of phospholipid bilayers and integral proteins, such as porins and ion channels [1, 9, 10]. There is contact or association between mitochondrial outer membrane and endoplasmic reticulum (ER) membrane, mitochondria-associated ER membrane (MAM). MAMs are important for calcium signaling and transportation of lipids and other substances between mitochondria and ER [11]. The intermembrane space is a small space between outer membrane and inner membrane [1] that contains several important proteins such as, cytochrome c and OPA1 (for mitochondrial fusion) [12] and other large proteins. These proteins

have a sequence for intermembrane space targeting. The intermembrane space is connected with cytosol through pumps, channels and large openings in the outer membrane such as, mitochondrial outer membrane permeabilization (MOMP) [13]. The inner membrane encloses the mitochondria inner space (matrix) [1], and contains several translocons such as, TIM complex for translocation of proteins into the matrix [9]. The inner membrane is responsible for transport of metabolites, ions and proteins into and out of the matrix [1].

The inner membrane also contains various respiratory complexes such as, complex I, II, III, IV and complex V (ATP synthase). These complexes perform redox reactions (oxidative phosphorylation) that cause the transport and generation of a gradient of protons in the intermembrane space and creation of electrochemical gradient for ATP synthesis [12].

Cristae are infoldings or invaginations of mitochondrial inner membrane and cristae abundance causes to high respiratory activity and increasing of ATP synthesis [1]. In cell types with high energy demands, such as muscle cells, the inner membrane surface is about five times larger than the outer membrane. To accommodate the extra surface area, the inner membrane is folded into cristae [10]. The protein-rich space inside the inner membrane of mitochondrial matrix contains a majority of mitochondrial proteins and enzymes, such as the citric acid cycle, pyruvate carboxylation and fatty acid oxidation enzymes [1].

One of the most important features of mitochondria is that they have their own genome or DNA. Mitochondrial genome is a circular DNA molecule of about 16 kilo-bases that resides inside the matrix. This mitochondrial genome contains 37 genes that encode some of the protein subunits of the respiratory complexes I, III, IV and complex V (ATP synthase), mitochondrial tRNA and rRNA for translation of mtDNA transcripts (mitochondrial mRNA). Most of the mitochondrial proteins are encoded by nuclear genes. These proteins are synthesized in the cytosol by cytoplasmic ribosomes and are translocated into the mitochondria [12].

There are high variations in mitochondrial shape, number and size and intracellular localizations based on tissue type and energetic demands. Also, cristae conformation is variable in different tissues. For example, the cardiac muscle cells and brown adipose tissues have more mitochondria and cristae than most cell types and their mitochondria are rich in cristae [1]. Mitochondria are dynamic organelles that move inside the cell during phases of cell activity, such as mitosis, based on the physiological and pathological state of the cell [14]. Mitochondrial structure and function could change in response to drugs or toxic substances or under stress conditions [1, 15]. There are some changes in mitochondrial shape and volume to form "mitochondrial swelling or contraction" [1].

Function of mitochondria

Mitochondria are dynamic and multifaceted organelles [14]. In addition to their critical role in the cellular respiration and energetics, they have other important functions in intracellular calcium homeostasis, heat production, programmed cell-death (PCD) and production of steroids and heme groups (Figure-1) [6, 16-20].

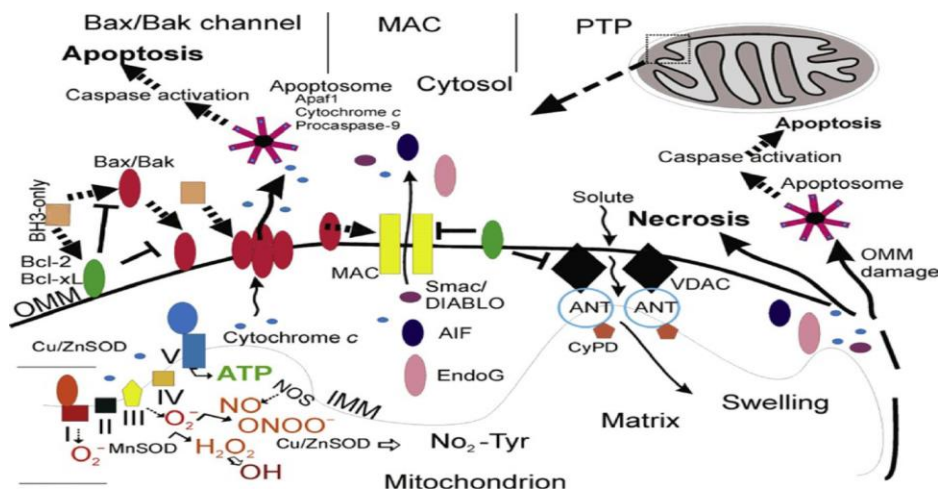


Figure1: Apoptosis regulation and ROS production by mitochondria in the cell, (Figure adopted from Martin, L.J. 2012 [20]).

Mitochondrion is a multifunctional organelle. One of the functions is oxygen and proton pump-driven ATP production by the electron transport chain. There are several respiratory chain proteins (complex I–IV) that establish an electrochemical gradient across the IMM through extruding protons out of the matrix into the intermembrane space with ATP production. Some ROS, such as superoxide is produced as a by-product of electron transport that is converted to hydrogen peroxide by MnSOD. Other function of mitochondria is to regulate cell death. Bcl-2 family members regulate apoptosis by modulating the release of cytochrome c from mitochondria into the cytosol. Cytochrome c, procaspase-9, and Apaf1 produce apoptosome that cause apoptosis with contribution of

mitochondrial apoptosis-induced channel (MAC), pro-apoptotic proteins, and Bax/Bak channel.

Mitochondria are also the main sites of reactive oxygen species (ROS), such as superoxide anion and hydrogen peroxide production [21], hydroxyl radical formation [22], and reactive nitrogen species formation [23]. They are also involved in regulation of metabolism, intracellular homeostasis, biosynthesis of lipids, nucleotides, carbohydrates, metabolism of metals (through Fe-S cluster and heme), neurotransmitter synthesis, and insulin secretion [24].

Mitochondrial metabolism

Glycolysis

The initial step of cellular respiration is glycolysis that occurs in the cytosol. Glycolysis converts glucose (6-C compound) into two pyruvate molecules (3-C compound) [25], two NADH and two ATP molecules [26].

In normal cells, Glycolysis is an anaerobic (independent of oxygen) process. When oxygen is present, pyruvate enters mitochondrial matrix for driving the TCA cycle and completes oxidation to carbon dioxide. When oxygen availability is limited (under hypoxic conditions), pyruvate molecules are converted to ethanol and carbon dioxide (alcohol fermentation) or are converted to lactate (lactate fermentation) [25].

During glycolysis, glucose is converted to glucose 6-phosphate (G6P) by hexokinase enzyme with hydrolysis of ATP to ADP. G6P is then converted to fructose 6-phosphate (F6P) by phosphoglucose isomerase; F6P is converted to fructose 1, 6-bisphosphate by phosphor fructose kinase with hydrolysis of ATP to ADP. Fructose 1, 6-bisphosphate is then hydrolyzed to two glyceraldehyde 3-phosphates by aldolase enzyme and then these two compounds are converted to two 1, 3-bisphosphoglycerate molecules with generation of two NADH by glyceraldehyde 3-phosphate dehydrogenase enzyme. The two 1, 3 bisphosphoglycerates are converted to two 3-phosphoglycerates with generation of two ATP by phosphoglycerate kinase. The 3-phosphoglycerate molecules are converted into two 2-

phosphoglycerates by phosphoglycerol mutase. The two 2-phosphoglycerates are converted to phosphoenolpyruvate (PEP) by the activity of the enolase enzyme. In the last step, the two PEP molecules are converted to two pyruvates with generation of two ATP by pyruvate kinase [25].

Oxidative decarboxylation of pyruvate

Pyruvate is a product of glycolysis [25], and is a central metabolic intermediate in most organisms [27]. The two pyruvate molecules, produced by glycolysis, are transferred from cytosol into mitochondrial matrix through pyruvate transporters. They are then converted to two acetaldehydes, generating two carbon dioxides by the action of pyruvate decarboxylase enzyme (pyruvate decarboxylation) [28-30]. Two acetyl-coenzyme A (acetyl-CoA) molecules are produced and two NADH molecules are generated through the catalytic action of pyruvate dehydrogenase [27, 31].

Krebs cycle (TCA or citric acid cycle)

Matrix is the mitochondrial protein-rich core that contains different proteins, enzymes and compounds for metabolic pathways including pyruvate dehydrogenase complex (PDC) and the TCA (citric acid) cycle enzymes. The TCA cycle was formulated by Krebs in 1937 [9].

The TCA cycle enzymes are encoded by nuclear DNA [32]. The Krebs or TCA cycle includes several chemical reactions in all aerobic organisms for ATP generation by oxidation of acetate (acetyl-CoA). These acetyl-CoA molecules derived from carbohydrate (glucose) or fats into two carbon dioxide molecules. This cycle is an important metabolic pathway for catabolism or hydrolysis of carbohydrates, fats and proteins and generation of energy (ATP). This cycle includes 8 different enzymes for complete oxidation of acetyl-CoA [33] that are in the mitochondrial matrix, except for succinate dehydrogenase which is embedded in the inner mitochondria membrane [32].

At first, coenzyme-A is dissociated from acetyl. The citric acid cycle starts with entrance of acetyl (2-C compound) and its combination with oxaloacetate (4-C compound) and generation of citrate (6-C compound) by citrate synthase enzyme [32-35]. The citrate molecule does chemical transformation and is changed to isocitrate by aconitase. Aconitase is a Fe-S cluster enzyme that catalyzes reversible isomerization of citrate to isocitrate that generate cis-aconitase intermediate [32, 36]. Then, isocitrate is converted to 2-oxoglutarate (5-C compound) by isocitrate dehydrogenase, losing of CO_2 accompanied by reduction of NAD^+ to NADH [32]. The 2-oxoglutarate is converted into succinyl-CoA (4-C compound) with losing of carboxyl groups as CO_2 molecule and reduction of NAD^+ to NADH molecule. Finally, succinate is synthesized with generation of energy and then succinate is changed to fumarate by succinate dehydrogenase with reduction of FAD to FADH_2 [32]. Fumarate is converted to malate by

fumarase or fumarate hydratase and malate is converted to oxaloacetate by malate dehydrogenase enzyme with reduction of NAD^+ to NADH . Oxaloacetate is regenerated and the cycle continues [32, 34, 35]. The first turn of the cycle produces one ATP (or GTP), three NADH molecules, one FADH_2 and two CO_2 molecules, so the two pyruvate molecules produced from one glucose molecule. These two pyruvate molecules are converted to two acetyl-CoA molecules that do Krebs cycle two times and generally, the stages of glycolysis, oxidative decarboxylation of pyruvate and Krebs cycle produce ten NADH molecules, two FADH_2 molecules, four ATP molecules and six CO_2 molecules [37].

Electron transfer chain

The mitochondrial inner membrane invaginates to form cristae [38]. This mitochondrial inner membrane contains various respiratory complexes such as, complex I, II, III, IV, known as the electron transfer chain, and complex V or the ATP synthase. In eukaryotic cells, the redox reactions (oxidative phosphorylation) occur in mitochondrial inner membrane (cristae). Also, the cristae includes electron transfer chain enzymes that cause the transport of protons across the inner membrane and creates a proton gradient (chemiosmotic potential) that drives ATP synthesis [12, 39]. In 1961, Mitchell proposed the chemiosmotic hypothesis of oxidative phosphorylation [40]. The respiratory chain contains three sites of

phosphorylation that these sites locate between NADH and cytochrome b, cytochrome b and cytochrome c and between cytochrome c and oxygen [1].

Electron transfer chain (ETC) or respiratory chain is located in the mitochondrial inner membrane and includes four complexes, including complex I, II, III, IV [12]. Also, Slater and Keilin found that electron transfer chain complexes comprised from several enzymes which made from ubiquinone, flavin, iron-sulfur and copper [41], and there is a link between respiratory electron flow, proton transfer and ATP synthesis [26, 42, 43].

In 1960, Mitchell described the relation between energy-generating electron-transfer with ATP synthesis by means of electrochemical proton gradient across the mitochondrial inner membrane (chemiosmotic hypothesis). This hypothesis includes four major features, 1. The electron transfer complexes located in inner membrane that are responsible for the translocation of protons across the membrane. 2. ATP synthase that generates ATP by using the proton gradient. 3. The gradient is formed across the mitochondrial inner membrane. 4. This membrane contains the proton-linked carriers, translocators and ion channels for osmotic stabilization and metabolite transportation. Also, there are several proton carriers (hydrogen atom carrier) such as, NADH and FADH_2 that do redox reactions in mitochondrial inner membrane. These compounds release their proton (hydrogen atom), and are oxidized and reduce other molecules in the membrane and many of these hydrogen atoms are transferred across the membrane [26].

One of the most important molecules in the mitochondrial inner membrane is ubiquinone (UQ) that receive hydrogen ion from NADH or FADH_2 and is reduced to UQ_2 molecules. These UQ_2 molecules can release their hydrogen ions and become oxidized to UQ. These hydrogen ions are transferred between complexes and across the membrane between matrix space and intermembrane space and also, these hydrogen ions are transferred or pumped into intermembrane space from the matrix via complexes I, III and IV [26, 32].

By 1960, the overall structure of four respiratory complexes had been predicted; complex I (NADH-ubiquinone reductase), complex II (succinate-ubiquinone reductase) [1], complex III (ubiquinol-cytochrome c reductase) [44], and complex IV (cytochrome c oxidase) [1, 45]. Complex V was identified later. This enzyme is responsible for ATP synthesis by utilizing the flow of protons back into the mitochondrial matrix [1, 40].

Function of OXPHOS complexes

Complex I

Complex I (NADH-ubiquinone oxidoreductase) is one of the electron transfer chain complexes that catalyzes the transfer of two electrons from NADH to ubiquinone and transfers protons from matrix to intermembrane space. This complex oxidizes NADH to NAD^+ and transfers electrons to ubiquinone and four hydrogen ions into intermembrane space [32, 46].

Complex I or NADH-ubiquinone oxidoreductase containing non-heme iron and FMN (flavin mono-nucleotide) and there are multiple iron-sulfur centers in its structure [1, 46].

Complex I acts as NADH dehydrogenase and removes hydrogen ions from NADH and oxidizes it. This dehydrogenation reaction is coupled to proton translocation across the mitochondrial inner membrane [26]. Complex I contains several subunits that are encoded by nuclear DNA or mtDNA [47].

Complex II

Complex II (succinate-ubiquinone reductase) is the second electron transfer chain complex that contains FAD (flavin di-nucleotides) and non-heme iron [1]. **Complex II or succinate-ubiquinone reductase** catalyzes the oxidation of FADH_2 to FAD and transfer of electrons and protons to ubiquinone (reduction of UQ to UQH_2). Its succinate is converted to

fumarate and reduced UQH_2 is oxidized again to UQ and transfer electrons and protons to complex III [46].

Complex III

Complex III (ubiquinol-cytochrome c reductase) [44] is the third electron transfer chain complex that contains cytochrome b and c_1 , ubiquinone and non-heme iron protein. Complex III is called cytochrome bc_1 or coenzyme Q or cytochrome c oxidoreductase that receives electrons and hydrogen ions from reduced ubiquinone (UQH_2) and transfers them to cytochrome c and reduces cytochrome c and pumps four protons into the intermembrane space [32].

This complex containing bovine enzyme that is composed of eleven subunits (three of these subunits are iron-sulfur protein, cytochrome c_1 and cytochrome b). These subunits are composed of redox-active prosthetic groups such as, Fe_2S_2 center and haem B and C and main core of this complex is the catalytic core as ubiquinol-cytochrome c oxidoreductase for proton transfer activities [48]. This complex transfers electrons between its subunits. First electron is transferred to the iron-sulfur protein and is passed via cytochrome c_1 to cytochrome c. Second electron is transferred to lower-potential haem B (b_L) and then reduces higher-potential haem b_H . This b_H molecules can reduce ubiquinone to the ubiquinol and causes proton translocation across the inner mitochondrial membrane [26].

Complex IV

Complex IV (cytochrome c oxidase) is the fourth electron transfer chain complex. This complex is composed of twelve subunits; three of them are encoded by mtDNA and the others nine subunits are encoded by nuclear DNA [32]. This complex (cytochrome c oxidase enzyme) spans in mitochondrial inner membrane and has O_2 -reactive site (cytochrome a_3) oriented toward the matrix side [1]. This complex transfers electrons from cytochrome c to molecular oxygen [26], and reduces molecular oxygen to water inside the matrix [45]. This reaction is slow and this complex IV is rate-limiting step of respiratory chain and is one of the main sites for ROS (reactive species oxygen) production [49].

Complex V

Complex V or the F1Fo ATP synthase is final enzyme of the OXPHOS pathway. ATP synthase utilizes the electrochemical potential gradient (proton motive force) that is formed across the mitochondrial inner membrane, as a function of ETC, for synthesis of ATP from ADP and inorganic phosphate inside the matrix [32].

Complex V is composed of two functional domains, F1, which is in the mitochondrial matrix and Fo, which is embedded in the mitochondrial inner membrane. In most eukaryotes, F1 is composed of three α subunits, three β subunits, one γ subunit, one δ and one ε subunit. Fo is composed of a c-

ring plus the a and b subunits. The d, F₆ and OSCP from the peripheral stalk which lies to one side of the complex (Figure-2) [50]. It's been shown that ATP synthase under pathological conditions can form a non-selective ion channel [50, 51]. Complex V affects several mitochondrial processes such as calcium buffering and cell death [52].

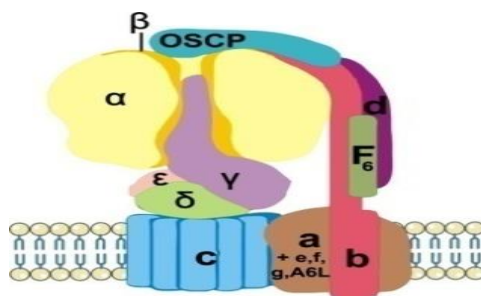


Figure2: The structure of mammalian mitochondrial ATP synthase (complex V), (Figure adopted from Jonckheere, A.I, and et al. 2012 [50]).

This complex V consists of two functional domains, F₁ and F₀. F₁ subunit comprises several different subunits (three α , three β , and one γ , δ and ϵ) and is placed in the mitochondrial matrix. F₀ subunit contains subunits c, a, b, d, F₆, OSCP and the accessory subunits e, f, g and A6L. Protons pass from the intermembrane space to the matrix through F₀, which transfers the energy created by the proton electrochemical gradient to F₁, where ADP is phosphorylated to ATP.

Oxidative phosphorylation

Transportation of protons by complexes I, III and IV from the matrix into the intermembrane space and then, pumping back of protons into the matrix by complex V (ATP synthase) are required for ATP production. Complex V transports protons into the matrix to synthesize ATP from ADP and P_i (chemiosmosis) [40, 53]. For first time, Peter Mitchell proposed the chemiosmotic hypothesis to explain the coupling between oxido-reduction by ETC and phosphorylation by ATP synthase. The chemiosmotic system consists of four basic parts: 1. The proton translocating, reversible ATPase system. 2. The proton translocating oxido-reduction chain. 3. The exchange diffusion systems, coupling proton translocation to **that of** anions and cations. 4. The ion-impermeable coupling membrane [40].

Evaluation of ATP synthesis yields from glucose oxidation

Aerobic glucose oxidation consists of glycolysis in the cytosol (yielding two NADH and two ATP molecules), oxidative decarboxylation of pyruvate (yielding two NADH molecules), Krebs or TCA cycle (yielding six NADH and two $FADH_2$ molecules and two GTP or ATP molecules) and electron transfer chain (ETC). For each NADH molecule, 3 ATP molecules are synthesized and for each $FADH_2$ molecule, 2 ATP are generated by oxidative phosphorylation. Generally, 38 ATP molecules are synthesized from a complete cycle of aerobic glucose oxidation [26]. This Mitochondrial ATP

production is the main energy source for intracellular metabolic pathways [54].

Regulation of intracellular calcium ion homeostasis

Mitochondria have basic functions in metabolic and physiological processes [55, 56] especially, in regulation of intracellular signaling and calcium homeostasis [57, 58]. Calcium ions activate many TCA cycle enzymes including the dehydrogenase enzymes inside the matrix [59]. Influx of calcium ions and their entry from outer **mitochondrial membrane (OMIM)**  **us**  through the high conductance voltage dependent anion channel (VDAC) [59, 60]. Calcium ions enter the matrix from the **mitochondrial membrane**  **(IMIM)** through the mitochondrial calcium uniporter (MCU) downward the direction of electric gradient through a high electronegative potential (- 180 mV) [55, 59].

Under physiological conditions, exit of calcium ion from the matrix is done mainly through $\text{Na}^+ / \text{Ca}^{2+}$ and $\text{H}^+ / \text{Ca}^{2+}$ exchanger (antiporter electroneutral). These exchangers are located in the IMM and enter sodium and hydrogen ions into the matrix and expel calcium ions from the matrix (Figure-3) [59], and (Figure-4) [55].

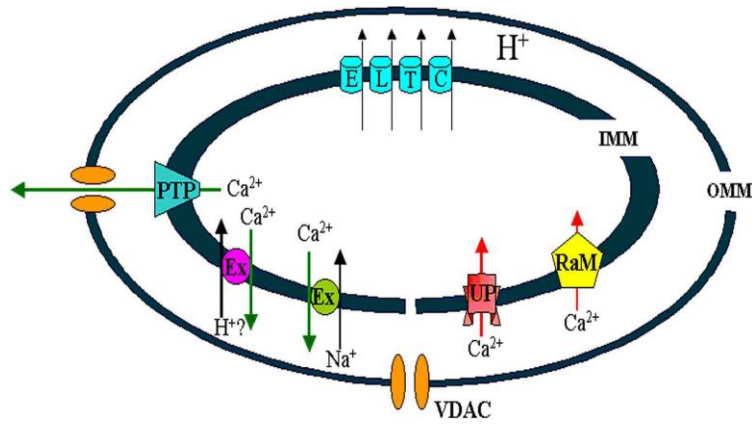


Figure3: Schematic representation of the Ca^{2+} transport pathways of mitochondria, (Figure adopted from Vandecasteele, G, and et al. 2001 [59]).

UP (Ca^{2+} uniporter), RaM (rapid uptake mode), Ex (exchangers), ELTC (electron transport chain), PTP (permeability transition pore), VDAC (voltage dependent anion channel), IMM (inner mitochondrial membrane), OMM (outer mitochondrial membrane).

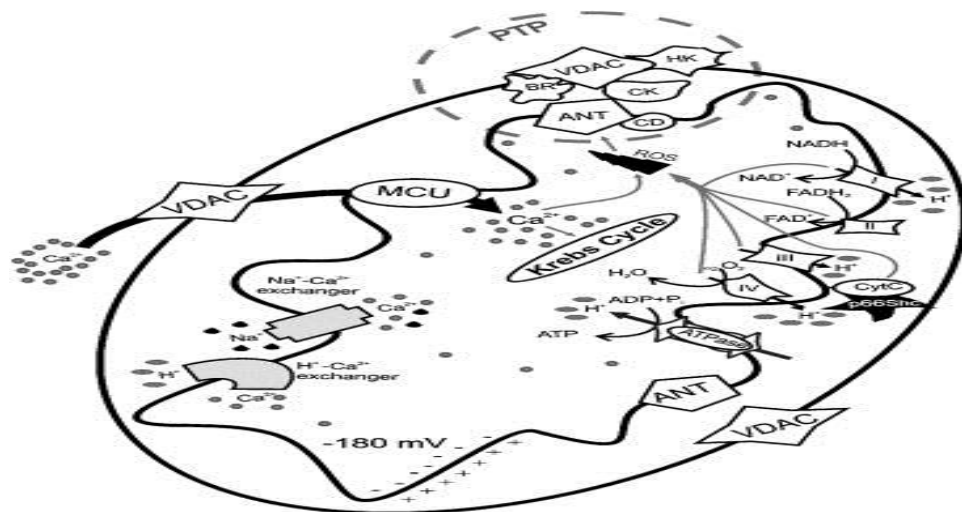


Figure4: Mitochondrial physiology and mitochondrial Ca^{2+} handling, (Figure adopted from Giorgi, C, and et al. 2012 [55]).

Ca^{2+} ions enter in the mitochondrial matrix via a low affinity uniporter (MCU). There are two major routes ($\text{Na}^+/\text{Ca}^{2+}$ and $\text{H}^+/\text{Ca}^{2+}$ exchanger) for extrusion of Ca^{2+} . The gradient

across the inner membrane is established by the activity of the mitochondrial respiratory chain and it's important for the mitochondrial state. In the matrix, Ca^{2+} stimulates the activity Ca^{2+} sensitive dehydrogenases of the Krebs cycle. VDAC (voltage-dependent anion channel), ANT (adenosine nucleoside transporter), HK (hexokinase), CD (cyclophilin D), CK (creatine kinase), BR (benzodiazepine receptor), PTP (permeability transition pore).

Calcium ions  could play a role in metabolism by inducing dehydrogenase enzymes such as, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and isocitrate dehydrogenase inside the matrix. These enzymes induce Krebs (TCA) cycle and other pathways that cause an increase in cellular respiration and ATP generation. Also, calcium ions induce  contraction and secretion inside the cytosol [61, 62].

Steroid production

One of the important functions of mitochondrion is production of sex steroid hormones. These hormones have regulatory function on the mitochondria and this co-regulation relation between mitochondria and steroid hormones has important role in body physiology. Low production and defect in steroid hormones and mitochondrial dysfunction cause the tissue damage [63]. The main function of mitochondria is in first step of steroidogenesis  that mitochondria convert cholesterol to pregnenolone (precursor of steroid hormones). The cholesterol molecules enter from outer mitochondrial membrane (OMM) to inner mitochondrial membrane  (IMM) and then converted to pregnenolone [63] by cytochrome P450 (CYP)

which is located on IMM and oxidizes NADPH [64]. Then the pregnenolone molecules exported out from the mitochondria and converted to other steroid hormones by microsomal P450 enzymes [65]. One of the steroid hormones is estrogen which regulates mitochondrial function [63]. The estrogen attaches to the nuclear estrogen receptors and regulates expression of necessary genes for cell function. The estrogen regulates mitochondrial function through regulation of mitochondrial gene expression, activation of mitochondrial enzymes and proteins and activation of signaling cascade. Also, the estrogen protects mitochondria from oxidative damage and reduces ROS production (Figure-5) [63].

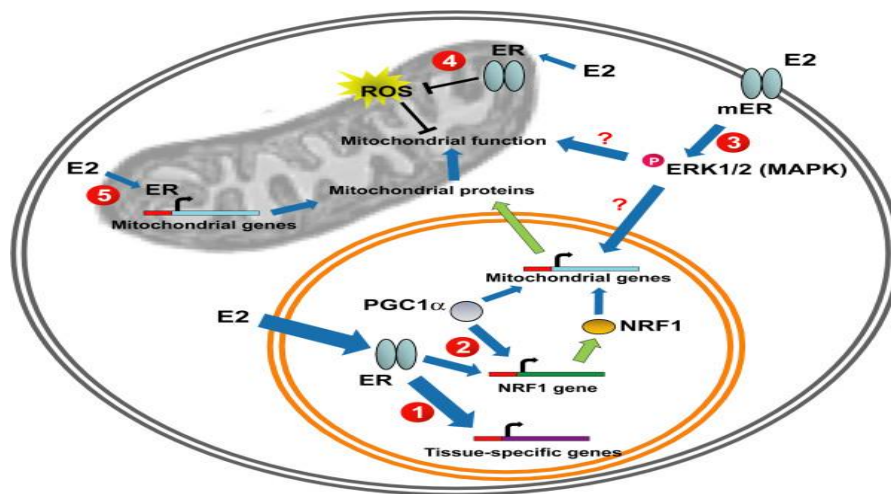


Figure5: Molecular targets of estrogen in regulating mitochondrial function, (Figure adopted from Velarde, M.C. 2014 [63]).

1. Estrogen hormone binds to nuclear estrogen receptors (ER) for regulation of tissue-specific expression in some functional genes.
2. Estrogen binds to nuclear ERs for regulation of expression in mitochondrial genes through gene expression of transcription factor NRF1 and increasing transcriptional activity of PGC1α.
3. Estrogen hormones bind to

membrane ERs and cause activation of signaling cascades that protect mitochondria from oxidative damage. 4. Estrogen also can bind to mitochondrial ERs and limits ROS generation in the mitochondria. 5. Estrogen hormone binds to mitochondrial ERs and regulates transcription of mitochondrial-encoded mitochondrial genes.

Programmed cell death (PCD) or apoptosis

One of the important morphological and biochemical energy-dependent processes is PCD or apoptosis. This process is one of the main components in cell turnover, growth, development and immunity processes. Any defect and dysfunction in PCD causes the different diseases such as, cancer, autoimmunity and neurodegenerative diseases [66]. The PCD process is controlled through many proteins and enzymes [66]. The PCD process occurs during the development and aging. This process is necessary for control and balance of cell numbers in tissue and destruction of damaged and diseased cells [66].

Irradiation and chemotherapy are the two main methods currently used as the standard of care for cancer [66]. Many morphological changes were detected during the apoptosis or PCD process such as, cell shrinkage and pyknosis by light and electron microscopes [67, 68]. Apoptosis or PCD process is complex and includes two main pathways, death receptor pathway (extrinsic) and mitochondrial pathway (intrinsic) and other T-cell mediated cytotoxicity and perforin-granzyme-dependent cell death (Fig. 8) [66].

In the perforin-granzyme pathway, the granzyme B molecules can use from mitochondrial pathway through activation of caspase-3 [66]. The mitochondrion has important function in apoptosis through intrinsic pathway (without necessary to death ligand inducer and death receptor). Some negative and positive signals activate intracellular signal cascade. These signals cause the changes in mitochondrial permeability transition (MPT) pore and enter two pro-apoptotic proteins to cytosol [69]. These two proteins are Smac/DIABLO (cytochrome c) and serine protease HtrA2/Omi [70]. These proteins activate caspase-dependent mitochondrial pathway and cytochrome c activates Apaf-1 molecule for apoptosome production [71]. These apoptosome molecules can activate caspase 9 and caspase 3 and cause the execution pathway [66]. The execution pathway causes the activation of endonuclease and protease enzymes and fragmentation of chromosomal DNA, nuclear proteins and cytoskeleton, cytoskeleton structure changing, cytomorphological alteration, chromatin condensation, nucleus fragmentation, apoptotic bodies' production and cell death (Figure-6) [66]. Also, in the apoptosis or PCD process, the extrinsic pathway can activate intrinsic pathway through activation of caspase 8 [66].

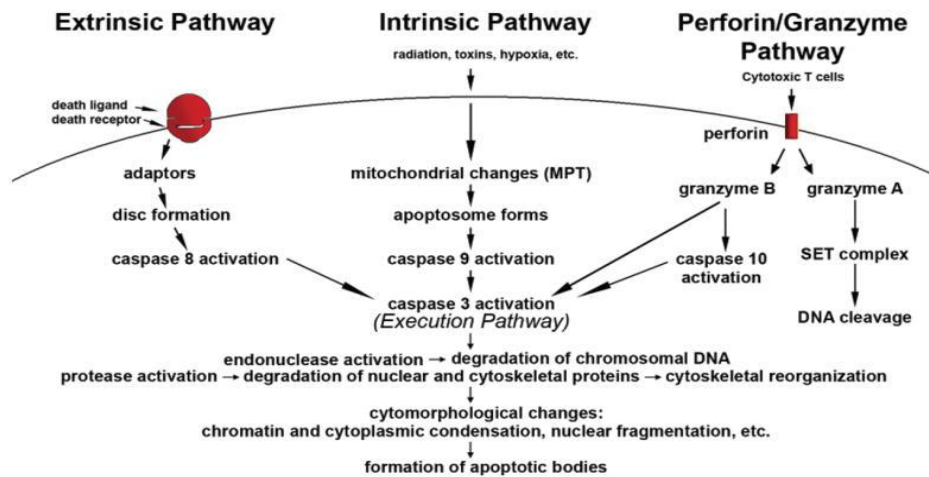


Figure6: Schematic representation of apoptotic events, (Figure adopted from Elmore, S. 2007 [66]).

Three pathways of apoptosis are extrinsic, intrinsic, and perforin/granzyme pathway. Each pathway requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway also activates its own initiator caspase that finally, cause activation of caspase-3. Also, the execution pathway results some cytomorphological features including cell shrinkage, chromatin condensation, formation of cytoplasmic blebs, apoptotic bodies, and phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophages.

Biosynthesis (biogenesis or anabolism)

Biogenesis or biosynthesis is a multi-step process. This pathway is catalyzed by some enzymes and substrates are converted into more complex products, such as conversion of amino acids to protein. In this process, simple compounds (monomers) are modified and converted into other compounds or to complex macromolecules (polymers). Biosynthesis consists of metabolic pathway and mitochondrion is one of the most important organelle that accomplishes these pathways. This biosynthetic or metabolic pathway needs to chemical energy (ATP), several catalytic enzymes and coenzymes or cofactors, such as metal ions and acetyl-CoA.

Lipid biosynthesis

Lipids are a group of naturally occurring molecules such as, waxes, glycerides and phospholipids and fats. Fatty acids are one of the main components of lipids. Lipid biosynthesis (lipogenesis) is creation of lipids or fatty acids from acetyl-CoA and malonyl-CoA precursors by fatty acid synthase enzyme [72, 73]. Lipids are hydrophobic small molecules. This character of lipids allows them to form structure such as vesicles and cell membrane in an aqueous environment [73]. The simplest structures of lipids are fatty acids. The fatty acids are hydrocarbons derivatives that contain a carboxyl group (hydrophilic head) and a hydrocarbon tail (hydrophobic tail). These lipids are one of the most important and essential constituents in both plant and animal cell and play important roles such as,

membrane synthesis and energy generation [74]. The mitochondrial pyruvate dehydrogenase synthesizes acetyl-CoA from pyruvate. The pyruvate molecules can be derived from the glycolytic pathway and side reaction of ribulose biphosphate carboxylase activity. Also, the acetyl-CoA molecules can be synthesized from the conversion of acetate and CoA (with consumption of ATP) to acetyl-CoA and generation of citrate in mitochondria by TCA cycle and **exit of citrate** and its conversion to acetyl-CoA. These acetyl-CoA molecules (2-Carbon compound) have central role in many metabolic pathways [74, 75].

After production of acetyl-CoA and in the first step of fatty acid biosynthesis, malonyl-CoA is synthesized from acetyl-CoA and CO_2 (with consumption of ATP) by acetyl-CoA carboxylase (ACCase). **This** ACCase enzyme is in eukaryotic cytosol. The other reactions in this pathway are done by multifunctional polypeptide complexes in **animals and fungi**. At first, malonyl group is transferred from CoA to a protein cofactor (acyl carrier protein or ACP) and malonyl-CoA is changed to malonyl-ACP. **This** malonyl-CoA molecule is condensed and CO_2 is removed from the molecule and **is converted to 3-ketoacyl-ACP** by 3-ketoacyl-ACP synthases (KAS). These condensing enzymes are required for generation of fatty acids with 18-Carbons [74]. Also, acetyl-CoA and malonyl-ACP are condensed to 3-ketobutyryl-ACP (4-Carbon compound) by losing of CO_2 . This reaction is catalyzed by 3-ketoacyl-ACP synthase III (KAS III) [76]. The KAS I enzyme is responsible for lengthening of carbon chain of fatty acid. 3-ketoacyl-ACP is then reduced to 3-hydroxybutyryl-ACP by 3-ketoacyl-ACP reductase. **This**

hydroxybutyryl-ACP molecule is dehydrated to butenoyl-ACP by 3-hydroxyacyl-ACP dehydrase. The butenoyl-ACP is reduced again to butyryl-ACP by enoyl-ACP reductase. This enzyme catalyzes reduction of trans-2 double bond to form a saturated fatty acid. The NADPH and NADH are electron donors in these reduction reactions. The butyryl-ACP is condensed to 3-ketoacyl-ACP and this cycle continues for generation of saturated and unsaturated fatty acids [74].

Amino acid biosynthesis

The amino acids are structural units or monomers for production of proteins (polymer). The basic elements of amino acids are hydrogen (H), carbon (C), oxygen (O) and nitrogen (N). All amino acids have an amine ($-NH_2$) group. The main problem for biological systems is that they cannot obtain nitrogen (N) in usable form. This problem is solved by some microorganisms such as, symbiotic bacteria that live in soil and plant organs (like root nodules). They can convert atmosphere nitrogen gas (N_2) to usable form such as, ammonia. Ammonia is a source of nitrogen (N) for production of all amino acids. The higher organisms use ammonia (fixed nitrogen) for synthesis of amino acids, nucleotides and other nitrogen-containing biomolecules.

Most amino acids are synthesized from transamination reactions. These reactions are chemical reactions between two molecules, one amino acid (with an $-NH_2$ group) and a keto acid (with a keto ($=O$) group). The

amine group and keto group are substituted in two molecules. These reactions are done by the aminotransferase enzyme. Most amino acids are synthesized from α -ketoacid. This molecule is transaminated from another amino acid (usually glutamate) and to synthesize amino acids [77]. Humans need 20 amino acids for their physiological functions. They cannot synthesize 8 amino acids that are called essential amino acids. The amino acids Arginine, Cysteine, Glycine, Glutamine, Histidine, Proline, Serine and Tyrosine are considered conditionally essential because these amino acids are not normally required in the diet, but the amino acids such as, Phenylalanine, Valine, Tryptophan and Lysine are essential and humans need to get them in their diet [78, 79].

An important transamination reaction for synthesis of most amino acids is reductive amination of α -ketoglutarate to glutamate. α -ketoglutarate is synthesized in TCA cycle from citrate and is converted to glutamate by glutamate dehydrogenase in mitochondria. Glutamate can be converted to glutamine, histidine and proline (three essential amino acids). Even arginine can be synthesized from this glutamate. Also, α -ketoglutarate can change to citrate in TCA cycle in mitochondria. Citrate can then change to oxaloacetate for synthesis of other non-essential amino acids [80]. Serine is synthesized from 3-phosphoglycerate (one of the glycolysis intermediate compound). This amino acid can be synthesized from glycine and formaldehyde in healthy adults and can be modified to produce glycine and cysteine [81]. Cysteine could also be synthesized from methionine, using the liver-specific trans-sulfuration pathway in healthy adults [81, 82].

Tyrosine is an aromatic amino acid that is synthesized by hydroxylation of phenylalanine [83, 84]. Also, alanine, leucine and valine are synthesized from pyruvate [80].

Nucleotide biosynthesis

Nucleotides are the structural units or monomers of nucleic acids, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). Nucleotides are organic molecules that are composed of a nitrogenous base (adenine, thymine, guanine and cytosine in DNA and adenine, uracil, guanine and cytosine in RNA), a five-carbon sugar (deoxyribose in DNA and ribose in RNA) and at least one phosphate group.

The nucleotides can be synthesized by de novo pathways or recycled via salvage pathways [85]. The nucleotides (purines and pyrimidines) are synthesized from intermediates of the degradative pathway during salvage pathway. This pathway recovers bases and nucleosides that are formed during degradation of DNA and RNA. These bases and nucleosides then can be converted to nucleotides [85]. Another nucleotide synthesis pathway is de novo; the components used in this pathway are derived from biosynthetic precursors of carbohydrates and amino acid metabolism [85]. One of the most important de novo pathways for synthesis of nucleotides is pentose phosphate pathway (PPP). The glucose is passed from the PPP pathway and is converted to ribose-5-phosphate for synthesis of nucleotides. Also, NADPH is synthesized during PPP pathway [80, 86].

Other de novo pathways for synthesis of nucleotides are initiated through the TCA cycle in the mitochondria and cytoplasm. The malate (that is produced during TCA cycle) leaves the mitochondria and is converted to **oxaloacetate** (OAA). OAA is converted to aspartate and is used for synthesis of nucleotides in the cytosol. Also, glutamate can be converted to aspartate which contributes to nucleoside and nucleotide synthesis (Figure-7) [80, 86]. Also, one of the effective pathways for nucleotides and antioxidant NADPH synthesis is increasing of pentose phosphate pathway (PPP) through alternative metabolism of glucose in cancer cells [2].

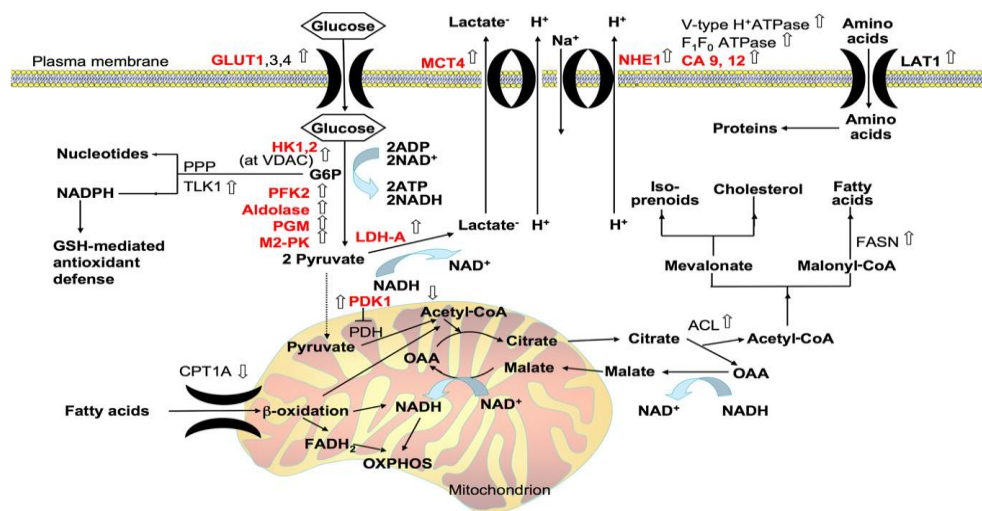


Figure7: Metabolic reprogramming in cancer cells, (Figure adopted from Kroemer, G, and et al. 2008 [86]).

In normal cells, aerobic glycolysis converts glucose (via pyruvate) into acetyl-CoA and oxidizes it to CO₂ and H₂O (which generates 38 ATP molecules per molecule of glucose) through the mitochondrion-localized TCA cycle and oxidative phosphorylation. In tumor cells, glycolysis is aborted. First, aerobic glycolysis in tumor cells converts glucose into pyruvate (which generates only two ATP molecules per molecule of glucose) and lactic acid

(waste product). Second, in tumor cells, acetyl-CoA can be introduced into a truncated TCA cycle, and can be exported into the cytosol as a building block for cell growth and proliferation. In this truncated TCA cycle, citrate is exported to the cytosol and is cleaved by ATP citrate lyase (ACL) to generate oxaloacetate and acetyl-CoA. Oxaloacetate is reduced to malate, that malate imported into mitochondria again and reconverted to oxaloacetate in the matrix (with generating NADH), and oxaloacetate reacts with acetyl-CoA to complete the substrate cycle. Small arrows pointing up or down indicate cancer-associated upregulation/activation or downregulation/inhibition of enzymes, respectively. CA9 and CA12 (carbonic anhydrases 9 and 12), CPT (carnitine palmitoyltransferase), GLUT (glucose transporter), GSH (glutathione), HIF (hypoxia-inducible factor), IDO (indoleamine 2,3-dioxygenase), HK (hexokinase), OXPHOS (oxidative phosphorylation), LAT1 (L-type amino acid transporter 1), LDHA (lactate dehydrogenase isoform A), MCT (monocarboxylate transporter), PDH (pyruvate dehydrogenase), PDK (pyruvate dehydrogenase kinase), PFK (phosphofructokinase), PI3K (phosphatidylinositol 3-kinase), PGM (phosphoglycerate mutase), PKM2 (pyruvate kinase isoform M2), PPP (pentose phosphate pathway), SCO2 (synthesis of cytochrome *c* oxidase 2), TLK (transketolase), VDAC (voltage-dependent anion channel).

Chapter 2: The mitochondrial metabolic changes in cancer cells

The growth and proliferation in unicellular and multicellular organisms

The unicellular organisms such as microbes grow and reproduce quickly when nutrients are enough in their environment. They use the nutrients and their elements such as Carbon and Nitrogen for generation of energy and synthesis of different biomolecules. The biomass production and proliferation in unicellular organisms is limited by nutrient availability. The multicellular organisms also have a control system for their growth and proliferation. They prevent uncontrolled proliferation of cells and don't take up nutrients from the environment unless they are stimulated by growth factors [87]. Genetic mutations and changes in signaling pathways can result in uncontrolled cellular growth and cancer [87].

The metabolism of cancer cells (Warburg effect)

In 1924, Otto Warburg postulated that cancer cells metabolize glucose differently from normal cells. Normal cells convert glucose to pyruvate (glycolysis) and then utilize mitochondrial oxidative phosphorylation for generation of energy (ATP) for cellular processes in presence of oxygen. Also, they do anaerobic glycolysis and ferment pyruvate to lactate in absence of oxygen. Most cancer cells, however, do aerobic glycolysis (Warburg effect) and convert most glucose to lactate in presence or

absence of oxygen. Aerobic glycolysis is an inefficient way for generation of ATP. The cancer cells facilitate the uptake and metabolism of nutrients into lipids, nucleotides and amino acids for generation of new cells [25].

Many unicellular organisms use fermentation of glucose and pyruvate to lactate (as an analogous to aerobic glycolysis in human cancer cells). The conversion of glucose to lactate (glycolysis) generates only 2 ATP molecules, but oxidative phosphorylation generates 36 ATP molecules per molecule of glucose. The aerobic glycolysis, therefore, is upregulated to provide sufficient energy for proliferation of cancer cells [25].

The features of cancer cells (mitochondrial pathway changes, physiology and bioenergetics, cancer cell metabolism and mutations)

Mitochondria are at the center of two prominent hallmarks of cancer, resistance to cell death and metabolic reprogramming [19]. Other hallmarks of cancer include: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, evasion of programmed cell death (apoptosis) [88]. The cancer cells generate most of their ATP through aerobic glycolysis (Warburg effect) [19]. There is a correlation between glycolytic ATP production and aggressiveness of cancer cells [89].

Otto Warburg first postulated the metabolic shift toward glycolysis in tumor cells. One of the main reasons for the metabolic shift is thought to be mitochondrial metabolism (defective oxidative phosphorylation) [90].

Cancer cells do high both aerobic and anaerobic glycolysis and have rapid growth and proliferation. This rapid growth and proliferation create hypoxia environment [19]. Cancer cells typically display high absorption and usage of glucose, but the mitochondria have efficient function for high ATP production [90].

The mitochondrial ability for oxidative phosphorylation (ATP production) is decreased under hypoxic conditions. Cancer cells upregulate glycolysis for compensation of ATP production through induction of hypoxia inducible factor (HIF-1) [91]. This factor induces glycolysis and controls angiogenesis, cell survival and invasion [19]. The HIF-1 factor starts apoptosis by inducing high concentrations of proapoptotic proteins (BNIP3). Also, this HIF-1 factor can induce anti-apoptotic proteins (IAP2) and downregulates pro-apoptotic protein Bax [92] in the hypoxia environment and creates a balance for induction and inhibition of apoptosis [19].

The ATP production, mitochondrial activity and its oxygen consumption is decreased in the tumor proliferating cells. One of the reasons for low ATP production is downregulation of catalytic subunit of ATP synthase (β -F1-ATPase). The reducing of cellular respiration and oxidative phosphorylation cause the glycolytic upregulation [93]. If the glycolysis is stopped in the cancer cells, these cells cannot upregulate oxidative phosphorylation and cause the defect in the mitochondria [94]. The inhibition and reduction of cellular respiration through the induced glycolysis is called Crabtree effect [95].

ATP production and low cellular respiration are observed in both tumor proliferating cells and fast-growing normal cells. Oxidative phosphorylation is believed to be insufficient **for** the proliferating (tumor) cells [19]. Mitochondria cannot produce enough ATP through the oxidative phosphorylation, electron transfer chain (ETC) and Krebs cycle so, the **mitochondria** increase the anaerobic glycolysis and ferment the glucose molecules for ATP production for the growing and proliferating cells requirement [19]. The summary of mitochondrial bioenergetics, cancer cell mutations and mitochondrial physiology are shown in the below figures, (Figure-8) and (Figure-9), respectively [96].

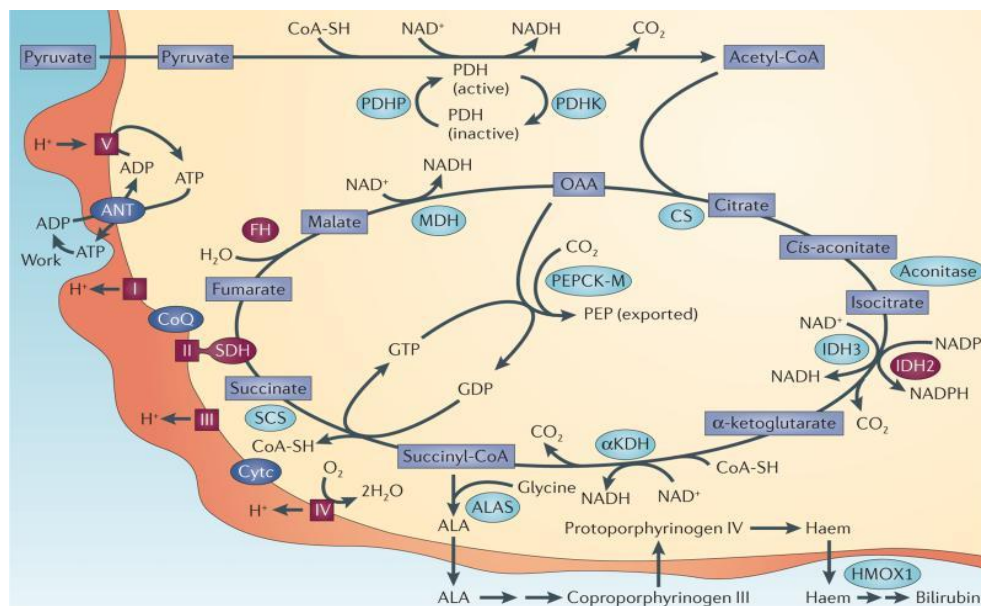


Figure8: Mitochondrial bioenergetics and cancer cell mutations, (Figure adopted from Wallace, D.C. 2012 [96]).

Pyruvate molecules from glycolysis are converted to acetyl-CoA by pyruvate dehydrogenase (PDH) with generating CO_2 and NADH. PDH kinase (PDHK) can inactivates PDH through phosphorylation, and PDH can be reactivated through dephosphorylation by PDH phosphatase (PDHP). Acetyl-CoA enters the tricarboxylic acid (TCA) cycle by the citrate

synthase (CS)-mediated reaction with oxaloacetate (OAA) to generate citrate. Citrate carbons can pass through the TCA cycle via the enzymes aconitase, isocitrate dehydrogenase (IDH2 or IDH3), α -ketoglutarate dehydrogenase (α KDH), succinyl-CoA synthetase (SCS), succinate dehydrogenase (SDH or complex II), fumarate hydratase (FH) and malate dehydrogenase (MDH). Mutations in IDH2, SDH, and FH (shown as red ovals) identified in cancer. Haemoxygenase 1 (HMOX1) degrades haem. δ -aminolevulinic acid (ALA) synthetase (ALAS) condenses succinyl-CoA and glycine to generate ALA. Conversion of succinyl-CoA to succinate generates GTP, which can drive the condensation of OAA and CO₂ to phosphoenolpyruvate (PEP) by mitochondrial PEP carboxykinase (PEPCK-M). NADH is generated by enzymes PDH, IDH3, α KDH and MDH, and can be oxidized by the electron transport chain (ETC). The ETC encompasses five multi-subunit complexes I–IV. NADH is oxidized by complex I (NADH dehydrogenase). The pumping of protons across the mitochondrial inner membrane generates the electrochemical gradient for condensing of ADP and inorganic phosphate (Pi) to ATP. The ADP and ATP are exchanged across the mitochondrial inner membrane by adenine nucleotide translocators (ANTs). Also, cancer cell mitochondrial DNA (mtDNA) mutations have been reported in genes for complexes I, III, IV and V (shown as red squares). Many of the mutations in mitochondrial gene are associated with oxidative phosphorylation (OXPHOS) and the redox regulation of reactive oxygen species (ROS) in cancer cells.

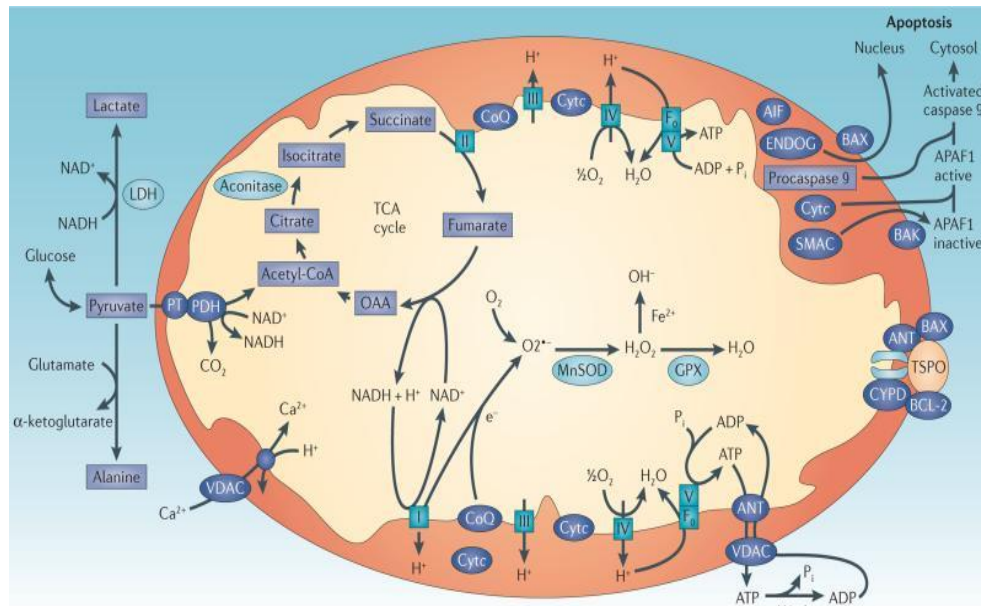


Figure9: Mitochondrial physiology, (Figure adopted from Wallace, D.C. 2012 [96]).

Mitochondria do most of biosynthetic pathways, produce much of the cellular energy through oxidative phosphorylation (OXPHOS), regulate Ca^{2+} concentrations and mitochondrial and cellular redox status, generate most of the reactive oxygen species (ROS), and can initiate apoptosis by the activation of the mitochondrial permeability transition pore (mtPTP). The mtPTP can be activated by a decreased membrane potential, high-energy phosphates (such as ADP), increased mitochondrial matrix Ca^{2+} , ROS concentrations, and a more-oxidized redox status. Acetyl-CoA enters the mitochondrion via pyruvate and fatty acids. Pyruvate is transported through the mitochondrial inner membrane by the pyruvate transporter (PT). Inhibition of mitochondrial function causes the pyruvate accumulation in the cytosol and it can be reduced to lactate. In the cytosol, fatty acids bind to CoA and are transferred to carnitine. Then they are transported through the outer and inner mitochondrial membranes. Many products and substrates of OXPHOS are transported through the outer membrane by the voltage-dependent anion channels (VDACs). Mitochondria donate excess electrons from complexes I and III directly to O_2 to generate ROS such as, superoxide anions (O_2^-). Mn superoxide dismutase (MnSOD or SOD2) dismutates matrix O_2^- to H_2O_2 , while intermembrane-space O_2^- is dismutated by Cu/Zn superoxide dismutase (Cu/ZnSOD or SOD1). H_2O_2 can be reduced to water by

glutathione peroxidase. Glutathione reductase reduces oxidized glutathione using NADPH as a reductant. The mtPTP is a protein complex. It includes the translocator protein (TSPO or PBR), an inner-membrane channel, adenine nucleotide translocators (ANTs) and the cyclosporine-A sensitive cyclophilin D (CYPD or PPID), with the BCL-2 pro- and anti-apoptotic family members. Activated mtPTP forms a channel between the inner and outer membranes that is associated with the aggregation of BAX and BAD in the mitochondrial outer membrane to form a megachannel. This megachannel releases pro-apoptotic proteins from the intermembrane space into the cytosol to initiate the degradation of the cellular proteins and DNA. AIF (apoptosis-inducing factor), CoA-SH (coenzyme A with a free sulphhydryl group), CoQ (coenzyme Q10), ENDOG (mitochondrial endonuclease G), GPX (glutathione peroxidase), LDH (lactate dehydrogenase), OAA (oxaloacetate), SMAC (second mitochondria-derived activator of caspase), TCA (tricarboxylic acid)

The PI3K-PTEN-AKT pathway

One of the pathways in the cancer cells is PI3K-PTEN-AKT. This signaling pathway causes the increasing of cell surface transporter expression (glucose and other nutrients transporters), increasing glycolytic and lipogenesis genes expression and stimulation of hexokinase and phosphofructokinase and other glycolytic enzymes [97, 98]. The cancer cells have enhanced glycolysis and produce high levels of pyruvate and convert most of these pyruvate molecules to lactate (lactic acid fermentation) by lactate dehydrogenase A (LDHA) [96]. The PI3K-PTEN-AKT pathway increases the fatty acid oxidation and phosphorylates hexokinase II which is attached to the porin (mitochondrial outer membrane voltage-dependent anion channel). Also, this pathway phosphorylates glucose to glucose-6-

phosphate for starting the glycolysis and pentose phosphate pathway (PPP) [96, 99].

In many organisms, AKT molecules phosphorylate many proteins, enzymes and other cofactors such as, DAF-16 in the *C. elegans* and FOXO in the mammals [96]. AKT activation stimulates many nuclear DNA-encoded mitochondrial genes for OXPHOS and antioxidant defense [100].

Pyruvate kinase muscle isozymes M1 and M2 (PKM1 and PKM2) in cancer

The PKM2 is an isoenzyme of the glycolytic enzyme pyruvate kinase. This enzyme dephosphorylates phosphoenolpyruvate (PEP) to pyruvate (with one ATP production) in the last step of glycolysis [96]. The mutation in PKM2 or inhibition of this enzyme through tyrosine kinase and oxidative stress (phosphorylation and inhibition of PKM2) causes the carbon atoms entrance to the anabolic pathways, i.e. the pentose phosphate pathway for ribose-5-phosphate production and nucleotide synthesis, NADPH production for the redox control, and for the glycerol-3-phosphate (lipid synthesis) [101]. The 3-phosphoglycerate (glycolytic intermediate) is a carbon source for amino acid and fatty acid synthesis. Also, the additional pyruvate molecules used for lipids and additional amino acids synthesis [97].

The PKM2 enzyme is active in the low oxidative stress, leading to pyruvate and lactate production and ATP synthesis, and is inactive in the high oxidative stress conditions, resulting in heightened PPP activity, NADPH

antioxidant production [96]. The isoforms PKM1 is expressed in terminally differentiated tissues (brain and muscle), which needs a large of ATP molecules. In cancer cells, PKM2 is over-expressed by oncoprotein c-Myc. This oncoprotein activates some hnRNPs (l, A1 and A2) that results in inhibition of PKM1 mRNA splicing [102]. Below figure illustrates metabolic pathway regulated by PKM2 in cancer cells (Figure-10) [102].

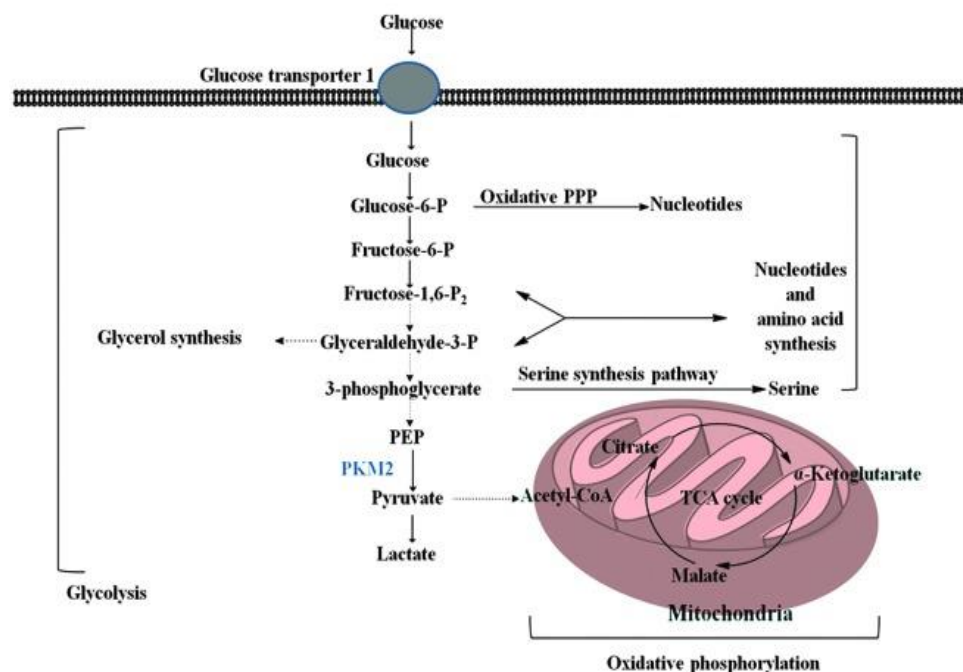


Figure10: Metabolic pathway regulated by PKM2 in cancer cells, (Figure adopted from Dong, G, and et al. 2016 [102]).

PKM2 is an isoform of the glycolytic enzyme pyruvate kinase. The decreased enzymatic activity of PKM2 in the cytoplasm causes the accumulation of upstream glycolytic intermediates. This event converts these intermediates into anabolic pathways. PKM2 (pyruvate kinase muscle isozyme M2), P (phosphate), PEP (phosphoenolpyruvate), CoA (Coenzyme A), TCA (tricarboxylic acid), PPP (pentose phosphate pathway)

Acetyl-CoA regulation

The acetyl-CoA is necessary for lipid and ATP synthesis [87], for acetylation of proteins and enzymes and for signal transduction pathway [103]. Most of acetyl-CoA is synthesized inside the mitochondria through lipid oxidation and from the pyruvate by pyruvate dehydrogenase (PDH). The PDH kinase is an inhibitory enzyme for PDH. When the reducing agents in the ETC are limited, different enzymes such as PKC, retinol, P66 and cytochrome c inhibit **from** the PDH kinase and so, the production of acetyl-CoA is increased [96]. The fatty acyl-CoA is imported to the mitochondria through carnitine. The transfer from CoA to the carnitine is done by carnitine palmitoyltransferase. This carnitine palmitoyltransferase enzyme is upregulated in lung cancer cells for increasing of fatty acid oxidation (acetyl-CoA production in the mitochondria) [104].

The cytosolic acetyl-CoA is necessary for the fatty acid synthesis in the cancer cells. This acetyl-CoA is synthesized from the citrate (which is exported from the mitochondria to the cytosol) through ATP-dependent lyase [87]. The acetyl-CoA used for the fatty acid synthesis, cholesterol and etc in the cancer proliferating cells [97, 105].

The p53 function and mitochondria

P53 is a nuclear transcription factor that has a pro-apoptotic function. The mutant p53 has an oncogenic property [106]. Cells are exposed to many cellular stresses such as, DNA damage. These stresses induce cancer

development. In cells with DNA damage, p53 is induced and accumulated in nucleus. Some post-translational modifications such as acetylation and phosphorylation activate p53 molecules [106, 107]. These active p53 molecules induce cell cycle arrest and apoptosis [107]. The tumor suppressor protein p53 inhibits cell growth and causes cell death [97]. This protein also increases ATP production (by OXPHOS) and decreases the ROS production [96]. P53 stimulates TP53-inducible glycolysis and apoptosis regulator (TIGAR). TIGAR is responsible for downregulating glycolysis and changing the carbon pathway to pentose phosphate pathway (PPP) to increase NADPH production [108, 109]. Also, p53 downregulates AKT pathway and phosphoglycerate mutase and causes the inhibition from the glycolysis and increasing the antioxidant defense [96].

The hypoxia-inducible factor and mitochondria

The hypoxia-inducible factor (HIF-1) is a dimeric protein complex. This complex creates body's response to low oxygen concentration [110]. The hypoxia-inducible factor (HIF-1) stimulates glycolysis in hypoxic environments through upregulating the expression of genes for encoding glucose transporters, glycolytic proteins and enzymes, growth factors and by suppressing mitochondrial function [96]. HIF-1 stimulates the PDH kinase1 (PDHK1) and suppresses PDH enzyme (resulting in inhibition of pyruvate conversion to acetyl-CoA and lipid synthesis) and stimulates mitophagy [111, 112]. HIF-1 stimulates miR-20 transcription that causes the

downregulation of mitochondrial metabolism and function [113]. The HIF-1 factor affects on PKM2 transcription. This PKM2 enzyme is a co-transcriptional activator for the HIF-1. This mutual relation between HIF-1 and PKM2 is mediated through PHD3 that increases PKM2 attachment to the HIF1 α [114, 115]. Also, HIF-1 causes the survival and proliferation of cancerous cells through angiogenesis [110].

MYC regulators and glutaminolysis

The MYC oncoprotein has several functions in cell cycle, apoptosis, cellular transformation, cell proliferation and cancer [116, 117]. MYC oncogene causes many human cancers and increases the oxaloacetate (OAA) production. **Two** OAA and acetyl-CoA molecules are necessary for citrate synthesis. The increasing of citrate synthesis (for macromolecules synthesis) causes the **decreasing** of OAA level in the mitochondria and high MYC oncogene expression increases OAA synthesis. The MYC oncogene stimulates glutaminolysis and enters α -ketoglutarate to the TCA cycle for the OAA synthesis (anaplerosis) [105]. High MYC expression is observed in many cancers and tumors. This high expression causes oxygen consumption and mitochondrial biogenesis through stimulation of mtTFA transcription for expression of complex I subunits, uncoupling proteins and mitochondrial membrane proteins [118]. Also, high MYC expression causes glutaminase stimulation. This enzyme converts the glutamine to the glutamate and then the glutamate is converted to the α -ketoglutarate by glutamate

dehydrogenase. The α -ketoglutarate enters to TCA cycle and is converted to the OAA [98, 105, 119]. Generally, MYC oncogenes increase OAA concentration for macromolecules synthesis through citrate synthesis [105].

The metabolic requirements of proliferating cancer cells (oncogenes and Warburg effect inducers)

Two molecules of glucose and glutamine provide most of the free energy (ATP), nitrogen and carbon for cell growth and proliferation. The conversion of glucose to CO_2 during oxidative phosphorylation in mitochondria generates maximum amount of ATP. Also, some intermediate precursors such as acetyl-CoA and glycolytic intermediates and ribose sugar for synthesis of fatty acids, amino acids and nucleotides respectively are necessary and are synthesized during this oxidative phosphorylation (glucose metabolism). These intermediates, fatty acids, amino acids and nucleotides are necessary for proliferating cells [18]. Most of the glucose and glutamine is converted to lactate or alanine. These catabolic reactions generate NADH and the intermediate precursors required for synthesis of lipids and nucleotides in cancer proliferating cells. The cancer cells should adapt their metabolism for the high growth and proliferation. Also, these cells undergo metabolic adaptations for survival under stressful environments and develop resistance to cell death [97].

Many cancer cells do aerobic glycolysis for ATP production. The glucose is an important nutrient for ATP synthesis and is a carbon source for

macromolecule synthesis such as, proteins, lipids and DNA [120]. The aerobic glycolysis resolves biosynthetic and energetic requirements for anabolic growth [97]. The metabolic pathways and role of intermediates molecules such as, pyruvate, acetyl-CoA, OAA, and glutamine in cancer cell growth and proliferation is illustrated in below figure (Figure-11) [97].

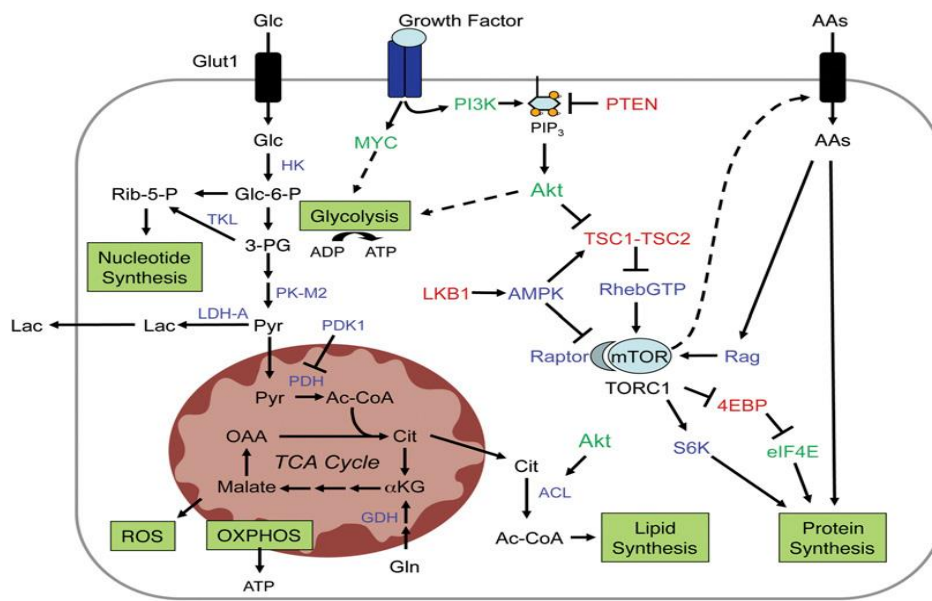


Figure11: Metabolic pathways of growth and proliferation, (Figure adopted from Jones, R.G, and et al. 2009 [97]).

Activation of the PI3K/Akt and c-Myc pathways change cellular metabolism for promoting of cancer cell growth and proliferation. These PI3K/Akt and c-Myc facilitate glucose uptake and glycolysis. Lactate dehydrogenase (LDH-A) converts excess pyruvate to lactate and regenerates NAD^+ for maintaining of glycolytic flux. Akt signaling increases expression of the glucose transporter Glut1 and enhances the activity of glycolytic enzymes. Glucose has important function in some processes such as, in glycolytic processing for production of ATP and pyruvate, processing the pentose phosphate shunt to generate ribose 5-phosphate (Rib-5-P) and NADPH for nucleotide biosynthesis, and ATP generation by the TCA cycle and electron transport chain (ETC). ATP citrate lyase (ACL) processes citrate (Cit) to acetyl-CoA (Ac-CoA) in the cytosol, which results in lipid production. Glutamine (Gln) is

deaminated to form glutamate. This glutamate can be processed in the mitochondria by glutamate dehydrogenase (GDH) to generate α -ketoglutarate (α KG). The TORC1 complex contains mTOR and its binding partner Raptor, and this complex regulates protein translation. The mTOR activity affects expression of amino acid (AA) transporters, and translation initiation. PI3K/Akt signals activate mTOR through inhibition of the TSC1–TSC2 complex. Also, bioenergetic signals inhibit the TORC1 complex through the LKB1–AMPK pathway. Oncogenes are shown in green, tumor suppressors in red, and effectors with undefined roles in tumorigenesis are shown in blue.

In addition to increased glucose absorption and use, the HIF-1 factors inhibit **from** the mitochondrial function in the cancer cells. **The HIF-1 factors provide a balance between glycolysis and oxidative phosphorylation.** This switch is controlled by pyruvate dehydrogenase (PDH) and lactate dehydrogenase (LDH). PDH function is to start the Krebs cycle and LDH converts pyruvate to lactate [19].

HIF-1 stimulates Pyruvate dehydrogenase kinase (PDK1). PDK1 inactivates PDH and inhibits the Krebs cycle and cellular respiration [121]. HIF-1 activates LDH enzyme for lactic acid fermentation [122]. Other important agents in balancing between glycolysis and oxidative phosphorylation are p53 and synthesis of cytochrome c oxidase 2 gene (SCO2) [19]. The mutations in p53 cause SCO2 downregulation suppress mitochondrial respiration and change the metabolic and energetic pathways of glycolysis. These mutations in p53 and cytochrome oxidase (COX) are found in many cancer cells [19]. The active (phosphorylated) p53 cause an increase in ATP production and mitochondrial respiration [19].

Some oncogenes are c-Myc, H-ras, Src and Akt. These oncogenes cause the stimulation of tumor cell growth and proliferation through excitation of anabolic signaling mechanisms and pathways [123].

The metabolic adaptations in cancer cells and effect of some tumor oncogenes and suppressors are shown in **the below figures** (Figure-12) [97]. Also, mTOR signaling pathway and mTOR function in cancer cells are shown in Figure-13 and 14, respectively [124].

Some other oncogenes are mTOR and Bcl2 that are inhibitors of autophagy and cell death [124]. These oncogenes increase the expression of HIF1 α and angiogenesis [125]. Akt2 is a substrate for mTOR and increases the expression of glycolytic enzyme PKM2 for Warburg effect [126].

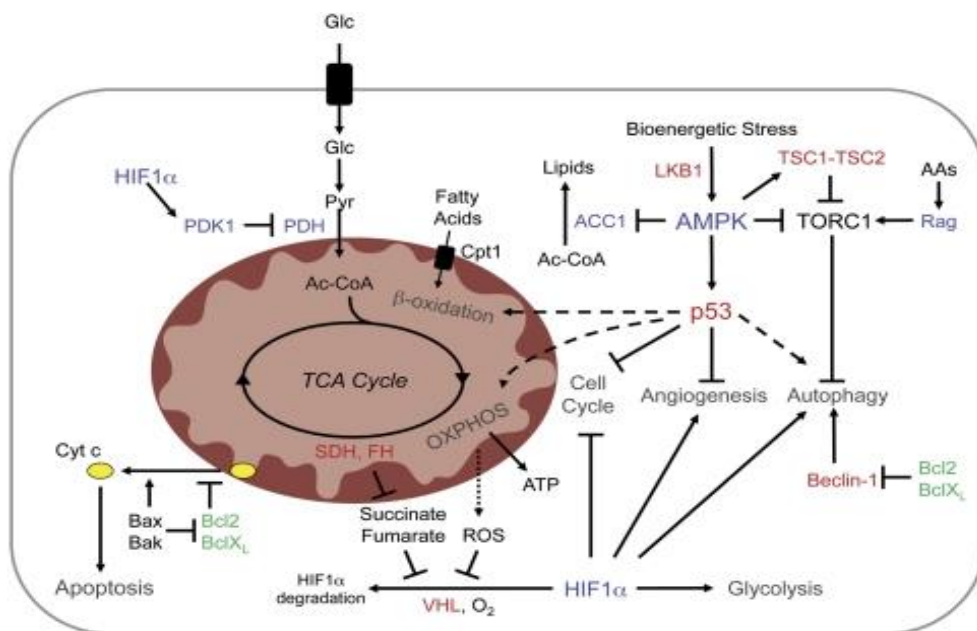


Figure12: Strategies of metabolic adaptation in cancer, (Figure adopted from Jones, R.G, and et al. 2009 [97]).

Intracellular sensors of energy, nutrients, and oxygen cause metabolic adaptation to stress during tumorigenesis. Some of these physiological strategies of metabolic adaptation

include inhibition of biosynthetic pathways (lipid, protein synthesis), cell cycle inhibition, increases in bioenergetic pathways (b-oxidation, glycolysis, and OXPHOS), and induction of autophagy. Bioenergetic stress activates AMPK through LKB-dependent manner. AMPK affects and mediates metabolic adaptation through several pathways, including down-regulation of fatty acid synthesis, inhibition of ACC1, activation of p53, and translation inhibition by blocking TORC1 activity, while tumor suppressors TSC1 and TSC2 inhibit TORC1 function. In deprivation of amino acids, Rag GTPases regulate TORC1 activity and autophagy. VHL tumor suppressor degrades HIF1 α . Low O₂, ROS, or accumulation of the TCA substrates succinate or fumarate promotes HIF1 α protein stabilization and transcriptional activity. Also, HIF1 α can activate PDK1 and blocks pyruvate entry into the mitochondrion by inhibition of PDH. Beclin-1 is required for autophagy induction and is suppressed by the anti-apoptotic proteins Bcl2/BclX_L. Cytochrome c also is released from mitochondria and causes induction of apoptosis. Oncogenes are displayed in green and tumor suppressors in red.

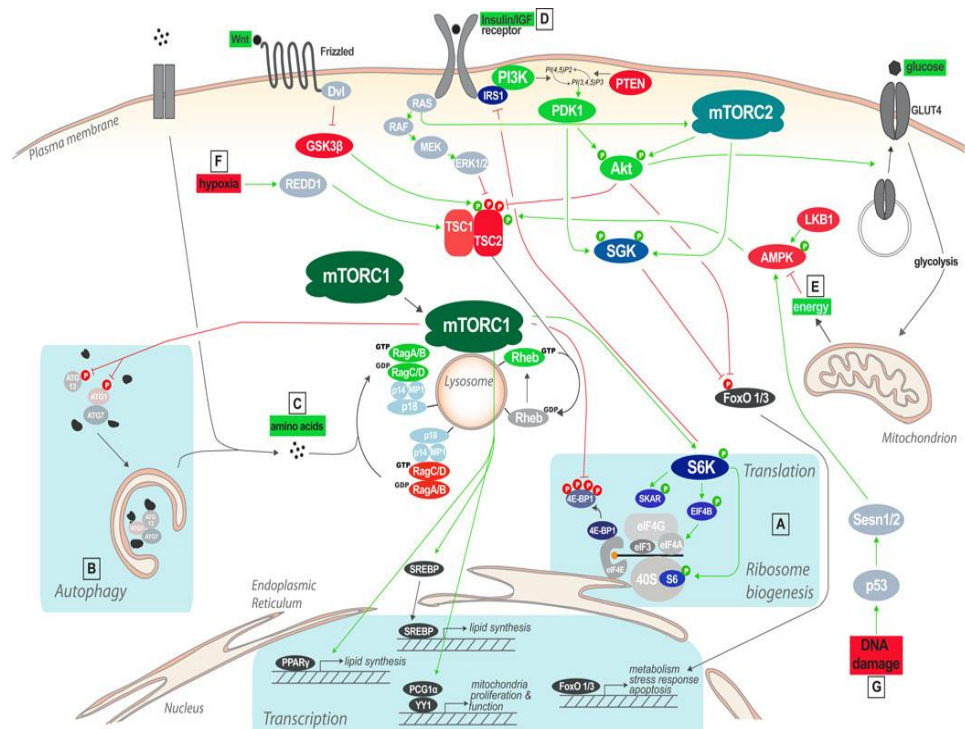
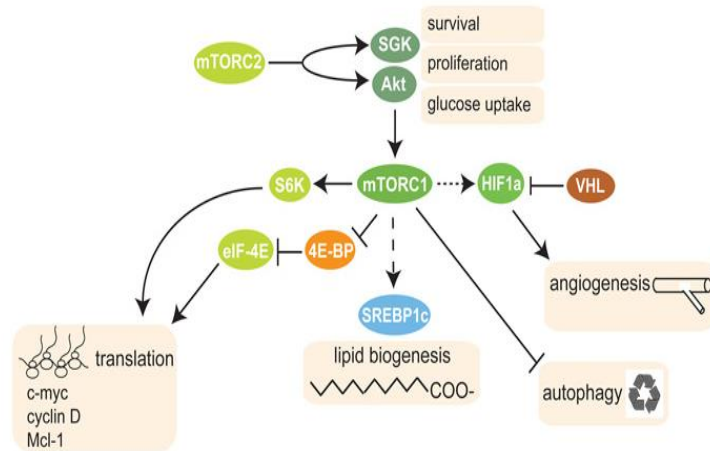


Figure13: The mTOR signaling pathway, (Figure adopted from Zoncu, R, and et al. 2011 [124]).

The mTORC1 can promote translation (A). It also inhibits autophagy (B), by integrating signals generated by amino acids (C), growth factor signals relayed by the insulin and insulin-like growth factors (D), energy signals acting through AMP-activated kinase (AMPK) (E) and various stressors including hypoxia (F) and DNA damage (G). The first level of signal integration is Akt and extracellular regulated kinase (ERK) 1/2 phosphorylate that inhibit Tuberous Sclerosis Complex (TSC), while AMPK and glycogen synthase kinase (GSK) 3-beta and hypoxic factor REDD1 activate it. The mTORC2 can phosphorylate and activate Akt and form a core component of the PI3K pathway. Second level of integration occurs at the lysosome and the Rag GTPases recruit mTORC1 to the lysosomal surface and lysosomal recruitment enables mTORC1 to interact with GTP-bound Rheb, the end point of growth factor, energy and stress inputs. Also, Growth factor receptors activate mTORC2 by Ras molecules.

14A



14B

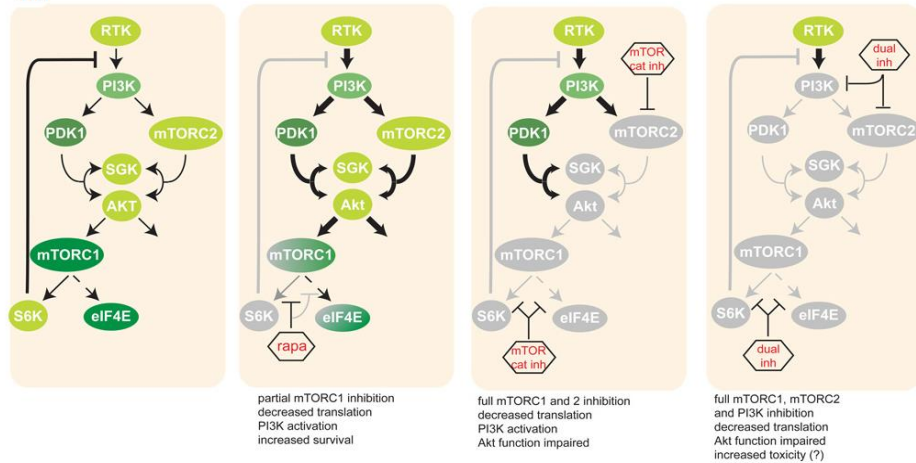


Figure14: The mTOR in cancer, (Figure adopted from Zoncu, R, and et al. 2011 [124]).

A) Playing of mTOR-regulated cellular processes in cancer, mTORC1 stimulates tumorigenesis through translation of oncogenes, inhibiting autophagy, upregulating angiogenesis, and enhancing the accumulation of lipids. The mTORC2 can activate Akt and other AGC family proteins which promote proliferation and survival. The mTORC2 plays a role in tumorigenesis by promoting Akt-mediated glucose uptake for cancer cells.

B) Therapeutic inhibition of mTOR activity by different compounds such as, rapamycin (rapa), mTOR catalytic inhibitors (mTOR cat inh), and dual PI3K-mTOR inhibitors (dual inh). Rapamycin only inhibits translation and activity of S6K but not eIF4E, so it only partially suppresses mTORC1 function. Also, inhibition of the S6K causes upregulation and activation of phosphatidylinositol 3 kinase (PI3K) and promotes cell survival. The mTOR catalytic inhibitors (mTOR cat inh) affect all known of mTORC1 as well as mTORC2. They inhibit translation more potently. Although PI3K over-activation still occurs, Akt phosphorylation by mTORC2 is impaired. Dual PI3K/mTOR inhibitors (dual inh) block all functions of PI3K, including PDK1- and mTORC2-mediated activation of Akt, so they have stronger effect, but might cause increased toxicity.

The Bcl-2 families of proteins are pro- and anti-apoptotic proteins that act on mitochondria and endoplasmic reticulum. The anti-apoptotic members of the Bcl-2 family cause cancer formation and progression through enhanced survival. Also, these proteins regulate calcium signaling and cause the cell proliferation and inhibition from apoptosis [127]. The main function of Bcl-2 family proteins is in cell death and proliferation and regulates these processes [128]. These Bcl-2 proteins upregulated in some cancer cells such as, chronic lymphocytic leukemia (CLL) and diffuse large B-cell lymphoma (DLBCL) (Figure-15) [127, 129].

retinoblastoma proteins for induction of arrest in the G1 phase. Bcl-2 phosphorylation (By JNK1 enzyme) leads to G2/M arrest [127]. These Bcl-2 proteins have inhibitory effect on calcineurin (CN), inositol 1, 4, 5-triphosphate receptors (IP3R) and nuclear factor of activated T-cells. Expression of growth factor genes (IL-2, IL-3 and GM-CSF) is suppressed or impaired and DNA synthesis in the S phase of cell cycle is affected and decreased in absence of these growth factors [127]. The interaction between Bcl-2 proteins and the cell cycle is shown in figure-16 [127].

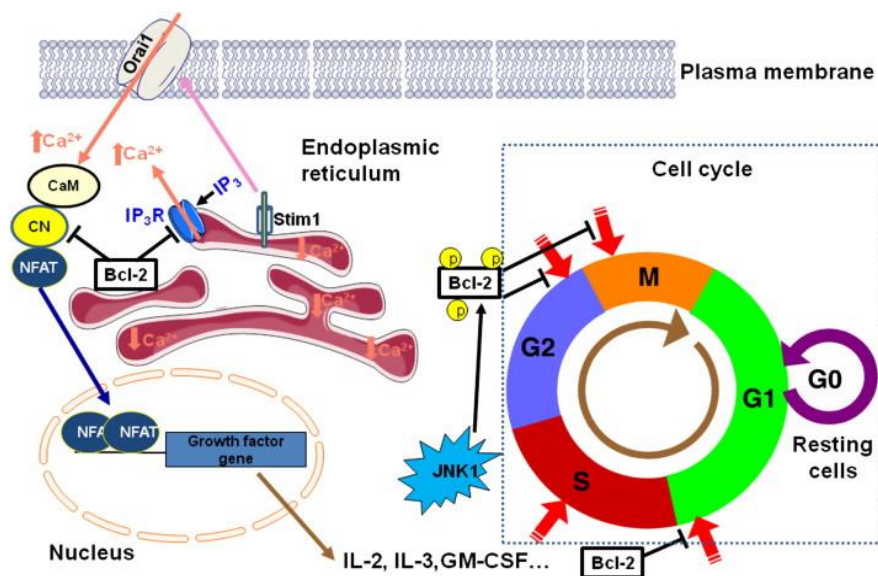


Figure16: Interaction between Bcl-2 and the cell cycle, (Figure adopted from Akl, H, and et al. 2014 [127]).

Bcl-2 can inhibit the proliferation of cells at different check points of the cell cycle (red arrows). JNK1 phosphorylates Bcl-2 that causes cell-cycle arrest at G₂/M. Also, Bcl-2 interacts with calcineurin (CN) and inhibits CN and IP₃Rs. Releasing of Ca²⁺ from the ER via IP₃R and STIM1-gated Orai1 channels causes the increasing of cytosolic Ca²⁺ levels and activation of Ca²⁺ /calmodulin (CaM)-dependent phosphatase CN, which will dephosphorylate NFAT proteins. The activated NFAT translocates to the nucleus and causes

the transcription of growth factor genes, like IL-2, IL-3 and GM-CSF. Bcl-2 has inhibitory effect on both CN and IP₃R, so NFAT nuclear translocation, and IL-2, IL-3 and GM-CSF expression are impaired. DNA synthesis in S phase of the cell cycle is affected, because of growth factors absence.

The Akt serine/threonine kinases comprise another group of important oncogene. These oncogenes stimulate aerobic glycolysis in the cancer cells. Also, the Akt proteins stimulate glucose metabolism and pentose phosphate cycle in cancer transformed cells and cause the continued cancer cell growth and survival [123, 130]. The Akt proteins regulate cell proliferation [131]. The Akt proteins are activated through amplification of PI3K (upstream activator of Akt) or deletion of PTEN (PI3K antagonist). These Akt molecules affect other oncogene such as Bcr-Abl and Ras [123].

The Akt molecules are one of the main components of PI3K/Akt signaling pathway. They phosphorylate many proteins and enzymes and stimulate cell proliferation and aerobic glycolysis [97, 132]. The activation of PI3K/Akt pathway and PI3K/Akt signaling pathway are shown in following Figure-17 and 18, respectively [132].

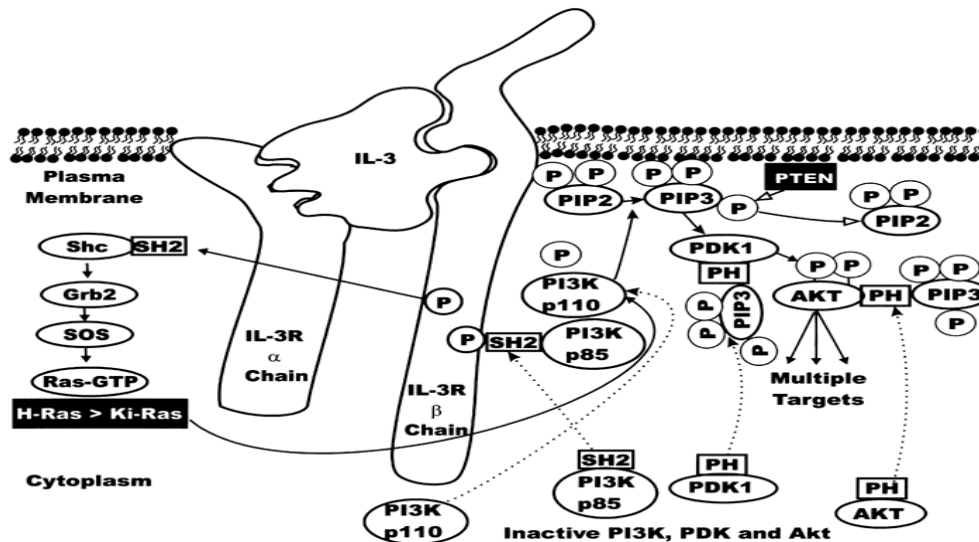


Figure17: Activation of the PI3K/Akt pathway, (Figure adopted from Chang, F, and et al. 2003 [132]).

Two mechanisms of PI3/Akt pathway activation are indicated. Activation of this pathway occurs near the cell membrane through interleukin-3 (IL-3) receptor and different compounds. Cytokine causes activation of receptor and a phosphorylated tyrosine residue on the IL-3 receptor activates catalytic subunit of PI3K, p110 and forms a complex. Also, other phosphorylated residue on the receptor activates Shc through SH2. Shc forms Grb-2 and SOS complex that activates Ras. Ras can induce membrane localization and activation of the p110 subunit of PI3K. conversion of phosphatidylinositol (4,5)-phosphate [PI(4,5)P₂] into phosphatidylinositol (3,4,5)-phosphate [PI(3,4,5)P₃] is done by Activated PI3K. This event causes the membrane localization of phosphotidylinositol-dependent kinase-1 (PDK1) via its pleckstrin homology (PH) domain. Also, PH domain recruits Akt to the lipid-rich plasma membrane and Akt is phosphorylated by PDK1 and another kinase. SH2 (Src homology-2 domain), PH (pleckstrin homology domain).

The tumor suppressors and their effects

Cancer is a metabolic disease. For growth and proliferation, cancer cells need the energy, necessary compounds and nutrient. There are many changes in the energy metabolic pathways and nutrient absorption in the cancer cells [97]. Some metabolic regulators act as tumor suppressor and inhibit the cancer cell growth and proliferation [97].

One of the tumor suppressors is the PTEN phosphatase. This enzyme dephosphorylates PIP3 and controls PI3K activity (The PI3K is a lipid kinase enzyme that regulates PIP3 in the plasma membrane and regulates Akt signaling pathway for biosynthesis) [133]. Mutation and alteration in PI3K/Akt signaling pathway inhibits the expression of different enzymes and proteins such as nutrients transporters, hexokinase and phosphofructokinase. Also, the altered PI3K/Akt signaling pathway suppresses glycolysis and lipogenesis and causes the inhibition of tumor growth [134].

The tumor suppressors are diverse; for example, the succinate dehydrogenase enzyme is a tumor suppressor enzyme [19]. This enzyme converts succinate to fumarate and participates in citric acid cycle and electron transfer chain [32]. The mutation in this enzyme causes the tumor stimulation [19].

One of the important enzymes in the lipid biosynthesis is ATP citrate lyase. This enzyme converts citrate to the cytosolic acetyl-CoA for lipid

synthesis and tumor stimulation, so any mutation and dysfunction in this enzyme suppresses tumor and cancer cell growth and proliferation [135].

One of the important tumor suppressors is p53 transcription factor. P53 mediates the cellular mechanisms and adaptations to the stress [136]. These p53 molecules phosphorylated by AMPK and causes the checkpoint initiation in the cell cycle and limits the tumor cells proliferation [137]. The AMPK/p53 pathway as tumor suppressor in metabolic adaptation is shown in following image (Figure-19) [97].

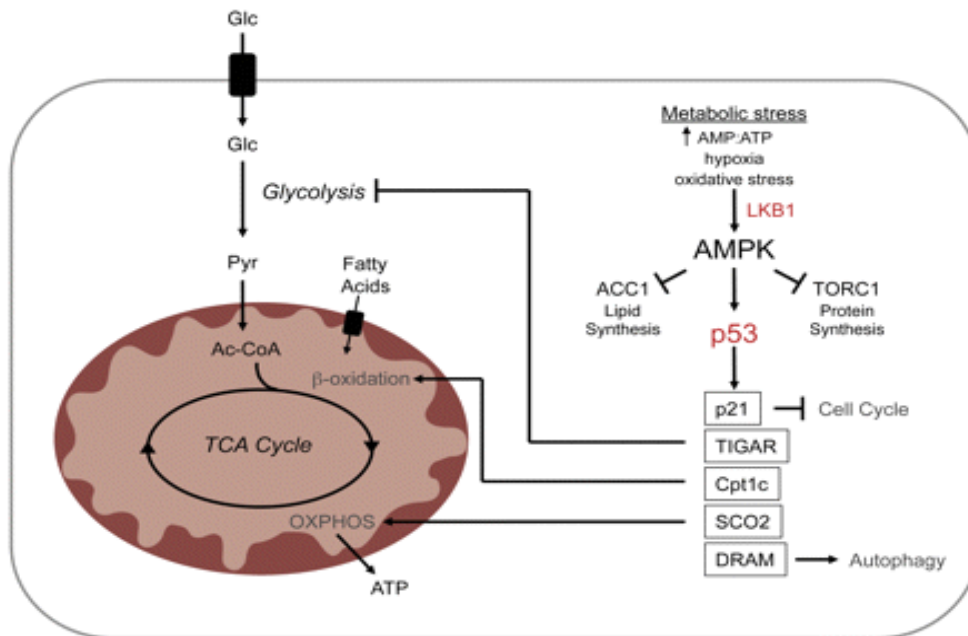


Figure19: The AMPK–p53 pathway in metabolic adaptation, (Figure adopted from Jones, R.G, and et al. 2009 [97]).

The p53 is a tumor suppressor and its activation is done by AMPK. Activation of p53 promotes physiological adaptation to metabolic stress (such as, hypoxia and oxidative stress) by multiple mechanisms. Activated AMPK inhibits lipid and protein synthesis. Also, AMPK-dependent p53 activation causes activation of p21 (cyclin-dependent kinase

inhibitor) that negatively regulates cell cycle progression. The p53 causes impairment of glycolytic flux by TIGAR. P53 also can activate β -oxidation and electron transport through expression of carnitine palmitoyltransferase 1c (Cpt1c) and SCO2, respectively, that finally results in a p53-dependent metabolic shift toward OXPHOS. The p53 regulates DRAM that induces autophagy.

The mechanistic target of rapamycin (mTOR) is a kinase enzyme. This enzyme regulates cell growth, proliferation and protein synthesis and causes the tumor growth initiation. Also, this enzyme inhibits the autophagy [124]. The mTOR-dependent mechanisms cause the growth of many sporadic cancers. Also, mTOR stimulates fatty acid synthase (FAS), which this enzyme involved in lipid synthesis for rapid growth and proliferation of cancer cells [124]. The Tsc1/Tsc2 and LKB1 are tumor suppressors. These molecules inhibit the mTOR activity (suppressing of the small GTPase Rheb). The AMPK enzyme phosphorylates Tsc1/Tsc2 complex and increases activity of this tumor suppressor [138].

Autophagy is a stress response mechanism that is considered a tumor suppressing mechanism. One of the important autophagy pathways is macroautophagy. During this mechanism, some aged and damaged intracellular components and organelles entered into the autophagic vesicles and are degraded by lysosome enzyme [97]. One of the stress conditions of cells is nutrients shortage (amino acids and glucose). These stress conditions stimulate autophagy mechanism. This pathway degrades

some macromolecules and products for energy production and continuing of cell viability [139].

Reactive oxygen species (ROS) impairment of mitochondrial function

2% of oxygen consumption is used for radical anion superoxide (ROS substrate) generation [19]. The O₂ molecules reduced to two water molecules by complex IV in the mitochondrial respiratory chain. Also, other complexes do redox reactions and transfer electrons to the molecular oxygen and cause superoxide generation [19].

The reactive oxygen species (ROS) generation increases under hypoxic environments of cancer cells through electron transfer complexes that are in reduced form [19]. The increasing of ROS production cause damage to the tumor cell components such as damage to the mitochondrial DNA [19].

ROS oxidizes lipids, proteins and nucleic acids and causes the cell dysfunction and death; for example, ROS can stimulate lipid peroxidation. This lipid peroxidation causes the damage to plasma membrane and mitochondrial DNA (oxidative DNA damage). Also, ROS mutate many respiratory enzymes and cause the mitochondrial dysfunction [19].

The hypoxic environments around the tumors cause ROS generation in the mitochondria. These hypoxia environments and ROS generation damage different macromolecules and enzymes such as, mtDNA and mitochondrial respiratory chain dysfunction and result in increased

glycolytic ATP production [19]. Some ROS molecules such as, superoxide, hydroxyl radicals and hydrogen peroxide are metabolic byproducts in cancer cells [2]. The NADPH molecules are necessary for reduction of ROS molecules such as H_2O_2 by glutathione peroxidase. Also, NADPH molecules cause regulation of protein activity through reduction of –SH functional group [96].

The increase in ROS molecules causes apoptosis. If cell death is stopped, the increase in ROS molecules causes neoplastic transformation [96]. Complex V (ATP synthase enzyme) generates ATP (through proton gradient). Any mutation and dysfunction in this enzyme causes the increasing of mitochondrial inner membrane polarity and inhibition from the electron transfer chain (ETC). Also, mutation in ATP synthase enzyme decreases the electrons transfers in the I and III complexes and causes the coenzyme Q shortage [96]. The extra electrons transferred to the O_2 molecules and generate superoxide. These superoxide molecules dismutated to the H_2O_2 through Mn superoxide dismutase (MnSOD) [96].

The mitochondrial defective enzymes in cancer cells

Many mutations in the nuclear DNA and mitochondrial enzymes cause tumor generation. The mutation in succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH) enzyme and other mitochondrial enzymes leads to tumor generation [96].

Succinate dehydrogenase (SDH) (complex II) is an integral respiratory enzyme in the mitochondrial inner membrane. This enzyme oxidizes succinate to fumarate. The SDH complex is composed of 4 subunits (SDHA, SDHB, SDHC and SDHD) [96]. Each subunit is composed of different cofactors and compounds and the assembly of SDH complex depends on SDHAF1 and SDHAF2 factors. Any mutation and dysfunction in these subunits, cofactors and factors causes tumor generation [140].

The mutation and dysfunction in SDH causes an increase in succinate concentration, PHD enzyme suppression and stabilization of HIF1 α (hypoxia stimulation) and its transfer to the nucleus. Mutations in SDH enzyme causes the shift of energy metabolism from oxidative pathway to glycolysis [103]. Also, the mutated SDH enzyme in the cancer cell causes increase in ROS generation and oxidative stress [140].

The fumarate hydratase (FH) enzyme converts fumarate to malate. Mutation and dysfunction in this enzyme causes increased concentration of fumarate and succinate and a decrease of malate and citrate concentration [96]. Also, increased fumarate concentration causes the HIF1 α stabilization (hypoxia stimulation). The mutation in the FH enzyme is found in some renal and cutaneous and uterine leiomyomas [96].

The mutation in FH enzyme increases succinyl CoA concentration. Also, this mutation stimulates heme oxygenase 1 (HMOX1). This HMOX1 enzyme combines succinyl CoA and glycine and synthesizes δ - aminolevulinic acid (ALA) through ALA synthetase (ALAS) enzyme. This

pathway causes porphyrin synthesis. This porphyrin converted to the Haem group and degraded by HMOX1 and is converted to the bilirubin [141].

The extra succinyl-CoA is converted to the succinate through succinyl-CoA synthetase with GTP generation. These GTP molecules are used for conversion of oxaloacetate (OAA) to phosphoenolpyruvate (PEP). PEP enters the cytosol for pyruvate and ATP generation [141, 142], so extra succinyl-CoA causes an increase in PEP and pyruvate synthesis and reduces OAA for the citrate synthesis and lipid biogenesis [96].

The mutation in the cytosolic isocitrate dehydrogenase 1 (IDH1) and mitochondrial IDH2 enzymes is found in many gliomas, astrocytomas and chondromas [96]. These mutations cause the increasing of the oncometabolite (R) (-)-2-hydroxyglutarate (2HG). This compound activates the prolyl hydroxylase domain enzymes (PHD1 and PHD2) and decreases the HIF1 α . This compound causes cancer cell proliferation and clone formation [143]. Also, the (R)-2HG molecules cause the alteration of methylation, genome transcription patterns and chromatin modification changes [144]. IDH1 and IDH2 do oxidative decarboxylation of isocitrate to α -ketoglutarate. Also, they can reduce NADP⁺ instead of NAD⁺ and generate NADPH [96]. The altered IDH activity generates a wasteful cycle, so mutation in the IDH1 and IDH2 causes NADPH and α -ketoglutarate waste and expends the NADPH for conversion of α -ketoglutarate to (R)-2HG [96]. The reduction of NADPH inhibits the glutathione peroxidase enzyme. Also, NADPH reduction causes the increasing of H₂O₂, redox status

across the mitochondrial inner membrane into the cytosol by the citrate carrier. Citrate can be converted to cis-aconitate and isocitrate by cytosolic aconitase and then to α KG by cytosolic NADP⁺-linked IDH1 with producing cytosolic NADPH. Also, excess amount of α KG and glutamate can then be imported into the mitochondrion. In the cytosol, the NADPH can be used to reduce glutathione. Oncogenic mutations in IDH1 or IDH2 (shown as the red ovals) can change this metabolic pathway and result in a neomorphic function. The mutant mitochondrial IDH2 and cytosolic IDH1 convert α KG to *R*(-)-2-hydroxyglutarate ((*R*)-2HG) with generating NADP⁺. This event wastes NADPH that causes increasing ROS production and altering the regulation of nuclear transcription factors. AGA (aspartate–glutamate antiporter), GSH (reduced glutathione monomer), GSSG (oxidized glutathione dimer), M α A (malate– α KG antiporter), OAA (oxaloacetate), PEP (phosphoenolpyruvate), PKM (pyruvate kinase isoform M).

The reverse Warburg effect

Different tumor cells have diverse bioenergetics regulations. Many tumor cell types undergo Warburg effect and some others regulate their metabolism differently [96].

There are interactions between tumor cells and neighboring cells. For example, breast cancer cells generate H₂O₂ that affects stromal fibroblast cells and converts the oxidative mechanism to the glycolytic mechanism. These H₂O₂ molecules cause mitophagy and decreased mitochondria in the stromal cells. Also, an increase in glycolysis causes the synthesis of lactate and ketone in the stromal cells. The cancer cells use from these compounds (lactate and ketone) for their energy production, proliferation and

biogenesis. This intercellular cooperation between epithelial-stromal cells is called reverse Warburg effect [145, 146].

Targeting amino acids metabolism of cancer cells

One of the important amino acids for the proliferation of cancer cells is glutamine. Some cancer cells cannot survive without glutamine. This non-essential amino acid synthesized from glucose. Glutamine is nitrogen donor to some metabolic enzymes that used for purine and pyrimidine synthesis and anabolic growth [147]. Also, glutamine is an mTORC1 translational regulator [148], and is an important substrate for TCA cycle [147].

Some enzymes such as, L-asparaginase expel glutamine and asparagine from the cells. These enzymes have anti-tumor activity and are used for cancer therapy such as, acute lymphoblastic leukemia (ALL) therapy [147].

Other important amino acids are glycine and serine in the breast cancer cells. The phosphoglycerate dehydrogenase (PHGDH) enzyme converts 3-phosphoglycerate to the phosphohydroxy pyruvate for serine and glycine synthesis. This enzyme is upregulated in many tumor cells [149].

Pentose phosphate pathway (PPP)

The pentose phosphate pathway or hexose monophosphate shunt starts in the first step of glycolysis [150]. In this step, glucose is converted to

glucose-6-phosphate by hexokinase. In the second step, glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate to 6-phosphogluconolactone with reduction of NADP^+ to NADPH. Then 6-phosphogluconolactone is converted to 6-phosphogluconate by 6-phosphogluconase and in the next step, 6-phosphogluconate is converted to ribulose-5-phosphate with again reduction of NADP^+ to NADPH. These NADPH molecules are used for lipid synthesis and ROS detoxification by glutathione (GSH) [150]. In the next steps, ribulose-5-phosphate isomerase (RPI) enzyme isomerizes ribulose-5-phosphate to ribose-5-phosphate or this ribulose-5-phosphate is epimerized to xylulose-5-phosphate by ribulose-5-phosphate epimerase (RPE). The ribose-5-phosphate molecules converted to phosphoribosyl pyrophosphate for ribonucleotides synthesis [150]. In the non-oxidative pentose phosphate pathway, transketolase and transaldolase enzymes transfer carbon atoms between different compounds. In this pathway, the fructose-6-phosphate and glyceraldehyde-6-phosphate are generated that used for glycolysis or for the pentose phosphate pathway with conversion of fructose-6-phosphate to glucose-6-phosphate (Figure-21) [150].

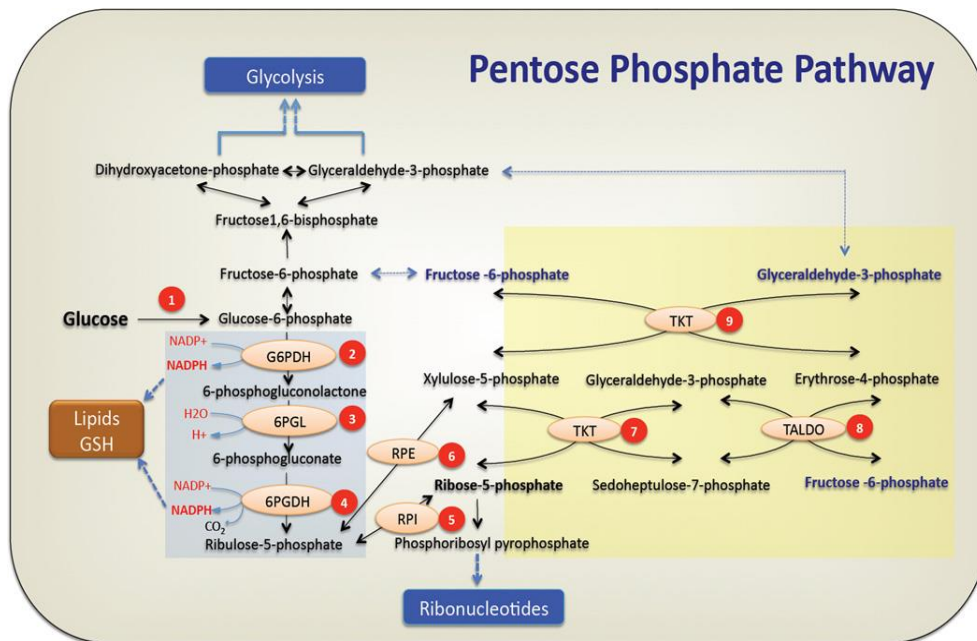


Figure21: The pentose phosphate pathway, (Figure adopted from Patra, K.C, and et al. 2014 [150]).

1. The conversion of glucose to glucose-6-phosphate (G6P) by hexokinases is oxidative part of PPP. 2. G6P is converted (oxidized) to 6-phosphogluconolactone by Glucose-6 Phosphate Dehydrogenase with reducing NADP^+ to NADPH. 3. The 6-phosphogluconolactone is hydrolyzed to 6-phosphogluconate (6PG) by 6-Phosphogluconase (6PGL). 4. This step is oxidative decarboxylation of 6-phosphogluconate to ribulose-5-phosphate by 6PGDH with generating NADPH. These NADPH produced in oxidative PPP can be utilized for lipid biosynthesis or ROS detoxification (GSH). 5, 6. Ribulose-5-phosphate (Ru5P) is isomerized by Ribulose-5-Phosphate Isomerase (RPI) or epimerized by Ribulose-5-Phosphate Epimerase (RPE) to generate ribose-5-phosphate (R5P) or xylulose-5-phosphate (Xu5P), respectively. Ribose-5-phosphate can be converted to phosphoribosyl-pyrophosphate that is backbone for ribonucleotide synthesis. 7. In the non-oxidative PPP, transketolase (TKT) transfers two carbon units from xylulose-5-phosphate to ribose-5-phosphate to generate sedoheptulose-7-phosphate (S7P) and glyceraldehyde-3-phosphate (G3P). 8. Also, Transaldolase (TALDO) transfers three carbon units from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to generate erythrose-4-phosphate and fructose-6-phosphate. 9. Also, TKT catalyzes other reaction and it transfers two carbon units from xylulose-5-phosphate to

erythrose-4-phosphate for generating of other fructose-6-phosphate and glyceraldehyde-3-phosphate. Fructose-6-phosphate (F6P) can be used for glycolysis and also, can be converted back to G6P to replenish the oxidative PPP. Glyceraldehyde-3-phosphate (G3P) also can be used in glycolysis.

The pentose phosphate pathway and cancer

The pentose phosphate pathway (PPP) generates NADPH and pentose phosphate molecules for fatty acids and nucleic acid synthesis, respectively. Also, this pathway contributes to cellular survival under stress conditions. The cancer cells express high level of glucose-6-phosphate dehydrogenase enzyme for increasing of PPP and NADPH generation. Also, cancer cells express 6-phosphogluconate dehydrogenase (6PGDH) for increasing NADPH and ribulose-5-phosphate synthesis, especially in the lung cancer cells. P53 inhibits the 6PGDH activity and causes the cancer cells senescence [150].

The ribulose-5-phosphate isomerase and ribulose-5-phosphate epimerase cause the conversion of ribulose-5-phosphate to ribose-5-phosphate and xylulose-5-phosphate, respectively. **Theribose**-5-phosphate and xylulose-5-phosphate are used for ribonucleotides synthesis. Also, these two compounds cause an increase in glycolysis in the proliferating cancer cells. The transketolase and transaldolase enzymes are expressed highly in cancer cells. These two enzymes are important in non-oxidative PPP for fructose-6-phosphate and glyceraldehyde-3-phosphate synthesis. This non-oxidative pathway causes the initiation of oxidative PPP and

glycolysis (more generation of NADPH and ribonucleotides). 80% of ribonucleotides originated from the non-oxidative PPP (Figure-22) [150].

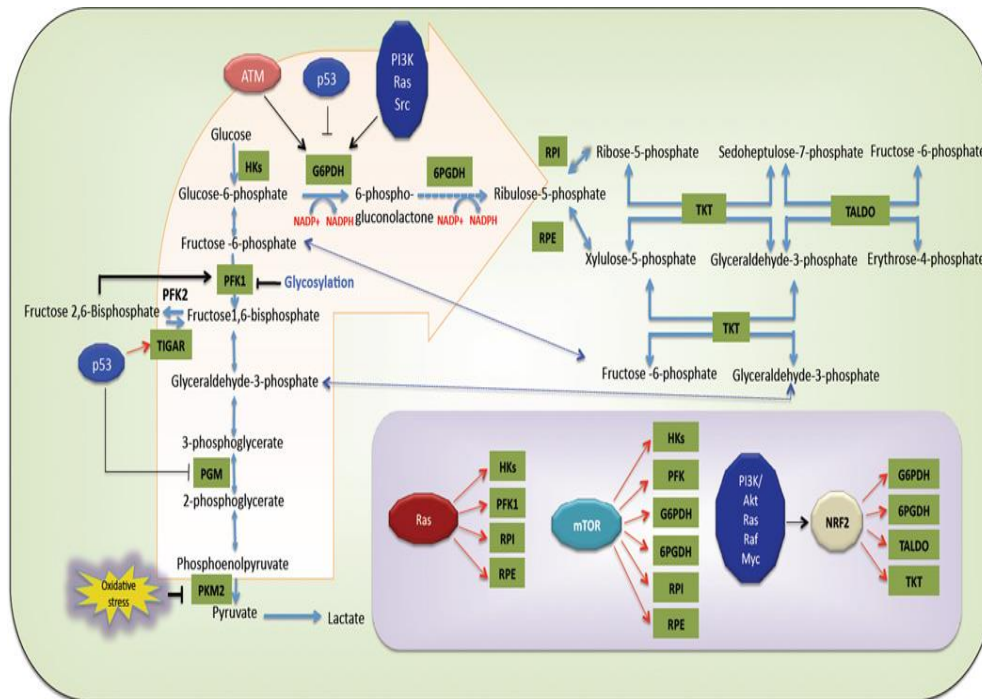


Figure22: Oncogenic regulation of the pentose phosphate pathway, (Figure adopted from Patra, K.C, and et al. 2014 [150]).

Activation of TIGAR and p53 causes increasing the flux of glucose to the PPP. Also, p53 inhibits G6PDH activity, while ATM, Ras, PI3K/Akt and Src positively influence G6PDH activity through several mechanisms. Glycosylation and oxidation inhibit the glycolytic enzymes PFK1 and PKM2, respectively, and cause increasing the glycolytic flux towards oxidative PPP. Also, several oncoproteins modulate the PPP by transcriptional up-regulation of several enzymes that affect the PPP (transcriptional regulations are showed by red arrows.). HKs (Hexokinases), PFK1 (Phosphofructo Kinase-1), TIGAR (TP53-inducible glycolysis and apoptosis regulator), PGM (Phosphoglycerate Mutase), PKM2 (Pyruvate Kinase Muscle isoform-2), ATM (Ataxia Telangiectasia Mutated), G6PDH (Glucose-6 Phosphate Dehydrogenase), 6PGDH (6-Phosphogluconate Dehydrogenase), RPI (Ribulose-

5-Phosphate Isomerase), RPE (Ribulose-5-Phosphate Epimerase), TKT (Transketolase), TALDO (Transaldolase), mTOR (Mammalian target of rapamycin), NRF2 (Nuclear factor erythroid 2-related factor 2).

The effect of mtDNA mutations in cancer cells

The somatic and germline mtDNA mutations found in many cancer types such as, renal adenocarcinoma, head and neck tumors, thyroid and breast and ovarian tumors and etc. The importance of mtDNA in cancer cells because, if change the cancer cells mtDNA with normal cells mtDNA, the cancer cells phenotype is changed. If we delete the mtDNA from the cancer cells, the tumor and colony generation and growth decreased in these cancer cells [96].

MtDNA is placed in the nucleoid region of mitochondria and is attached to the mitochondrial transcription factors A (mtTFA). These factors are mutated in some cancer types and are associated with mtDNA depletion. MtTFAs enter the nucleus in the prostate cancer cells and their high expression causes cancer cells proliferation [96]. Also, there are other proteins and enzymes that associate with mtDNA such as, nuclear RNA helicase and SUV3 helicase that affect on mtDNA transcription and replication [151].

The knockdown mutations in these SUV3 helicase enzymes cause a decrease in mtDNA numbers and downregulation of OXPHOS enzymes and ATP production [152]. Functional mitochondria and mtDNA are necessary

for tumor growth and tumorigenesis. Also, mtDNA mutations and defect in OXPHOS and other enzymes and proteins are other features of cancer cells [96].

The mitochondria and cancer (past, present, and future)

Mitochondria are dynamic multifunctional organelles [14]. They have several important roles in the cell, especially in metabolism, energy (ATP) production, cellular homeostasis, and oxidative phosphorylation [153]. Any defect and dysfunction in mitochondrion causes disorder in metabolism and many diseases such as cancer and neurodegeneration [54]. Mitochondria play a multifunctional role in tumor progression, making them an appealing therapeutic target for cancer therapy. The mutation in the nuclear-encoded TCA cycle enzymes causes the some cancer. These mutations cause the oncogenic metabolites production. Also, the mitochondrial metabolism and biogenesis are upregulated in cancer cells [154].

The mitochondrion has its own DNA that encodes tRNA, rRNA and some important proteins for respiration. This organelle is wild-type or mutant-type in the cells. The mitochondrion is very important for cell function and human health and is responsible for biosynthesis and energy production. Mitochondria disease can affect nervous system, heart and muscle tissues. These diseases can be originated from the defect in mtDNA or nuclear-encoded mitochondrial enzymes [155]. The high ROS production

and decreasing of oxidative phosphorylation and increasing of glycolysis are stimulators of tumor growth through mitochondria [156].

Warburg found that increasing of aerobic glycolysis and glucose absorption are important advantages of cancer cells for growth and proliferation in the unsuitable and deadly environment for other normal cells [157]. Also, the high level of glycolysis causes the increasing of glycolytic intermediates and metabolites for macromolecules synthesis (glycogen, phospholipid, malate, carbohydrates, and fatty acids), and anabolic reactions that are necessary for cancer cells growth and proliferation [2].

Absorption of glucose and its fermentation to the lactate through aerobic glycolysis is feature of most cancer cells. These cancer cells have defect in mitochondrial respiration [154]. There are some mutations in many other cancer cells such as, activation of K-ras, c-Myc and PI3 kinase and inactivation of P53 and phosphatase. These mutations cause the mitochondrial respiration defect and increase the aerobic glycolysis [25]. Some cancer cells do oxidative phosphorylation highly, but some retain mitochondrial function (respiration) [154].

The cancer cells restrict oxidative phosphorylation for ATP generation. They can survive in the hypoxia environment and proliferate. These cancer cells generates high lactic acid and bicarbonic acid, so cancer cells environment is acidic [2].

One of the energy sources for cancer cells is glutamine. The glutamine is converted to glutamate in the cytoplasm by glutaminase enzyme. The glutamate transferred to the mitochondria and converted to α - ketoglutarate during TCA cycle for macromolecules synthesis [158].

One of the important elements in cancer and tumor progression is mitochondrial dysfunction because of mtDNA numerous and mtDNA deletions [2]. There are two mtDNA mutation types in the cancer cells, homoplasmic and heteroplasmic. The homoplasmic mutations are identical in all mtDNA, but the heteroplasmic mutations occurred in some mtDNA and not in others [159]. The different cancer cells have different types and locations of mtDNA mutations. The accurate analyzing and sequencing of mtDNA cause detecting of mutations and sequences alterations for improving of treatment [2].

Mitochondria have many functions in catabolism, anabolism, and signaling. They are biosynthetic and bioenergetic organelles that do TCA cycle, fatty acid oxidation, ETC and respiration and generate ATP with synthesis of amino acids, lipids, heme clusters and nucleotides and NADPH. The mitochondrial functions in biosynthesis and signaling are shown in the following image (Figure-23) [154].

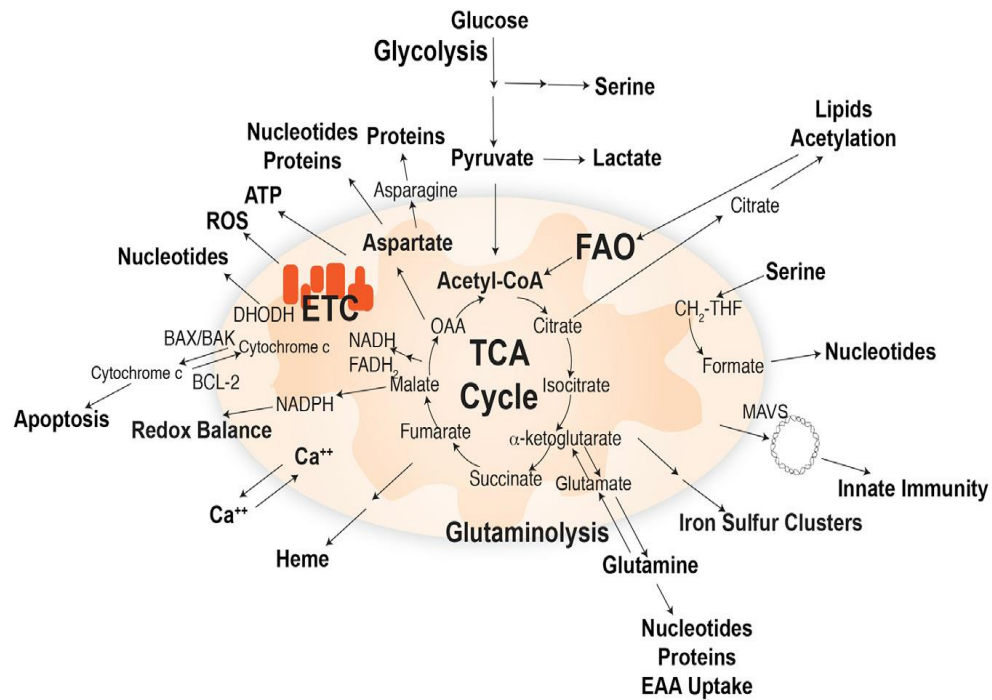


Figure23: Mitochondria as a biosynthetic, bioenergetic and signaling organelle, (Figure adopted from Zong, W.X, and et al. 2016 [154]).

The TCA cycle is occurred in mitochondria. This cycle uses different substrates from glycolysis, fatty acid oxidation, and amino acid catabolism to generate building blocks and high-energy electrons (NADH and FADH₂) to power the ETC. Major biosynthetic products produced by mitochondria and signaling pathways regulated by mitochondrial function are indicated in this figure.

One of the byproducts of ETC in the mitochondria is ROS. These ROS compounds can activate some signaling pathway such as, MAP kinase (MAPK) and the excess of ROS can cause the cell death. Also, mitochondria regulate Ca²⁺ releasing for control of signaling and cell function [154].

There are some mitochondrial outer membrane proteins such as, BCL 2 family of proteins. These proteins control programmed cell death by

apoptosis. Also, two proapoptotic proteins (BAX and BAK) cause the releasing of cytochrome c from the mitochondrial intermembrane space. These cytochrome c molecules cause the apoptosome and caspase protease activation for cell death [154].

The mitochondria are biosynthetic centers in the cell. Many amino acids, lipids and nucleotides are synthesized in the mitochondria. The mitochondria condense 2C and 4C compounds to form of citrate (6C compound). This citrate can start the TCA cycle for synthesis of other intermediates. Also, this citrate can be exported to the cytosol and converted to oxaloacetate and acetyl-CoA for lipid biosynthesis and protein modifications [154].

One of the mitochondrial functions is metabolism of 1C compounds for purine, methionine and thymine synthesis. The tetrahydrofolate (THF) is essential to carry 1C units for synthesis of thymine and purine bases, so the folate vitamin is essential for cell growth. Some antifolate compounds and aminopterin (folate analog) are effective as chemotherapy agents for cancer treatment [160]. The major metabolism of 1C compounds occurred in cytosol and also, some mitochondrial pathways are important in this metabolism. There are some 1C donors such as serine, glycine and betaine [161]. These compounds are main sources of 1C units. There are some mitochondrial 1C enzymes such as, SHMT2 and MTHFD2 that are important in 1C metabolism [162]. SHMT2 enzyme loads a 1C unit to THF with using of serine [163]. This enzyme expressed highly in some tumor cells and causes

the cancer cell survival [164]. The MTHFD2 function is in formyl-THF production. **This** mitochondrial formyl-THF can be used for three goals; facilitating of mitochondrial translation initiation (by producing of formyl-methionine tRNA), generation of mitochondrial NADPH (through formyl-THF dehydrogenase enzyme) and releasing the free formate [154]. **These** formate molecules are important in 1C metabolism and used in nucleotide synthesis and methylation in **the** cytosol [154]. SHMT2, MTHFD2 and formyl-THF dehydrogenase are three important enzymes that are expressed highly in the cancer cells, so targeting and inhibition **from** these enzymes are important in cancer therapy [154].

Glutamine is the most important and abundant amino acid in the blood and it converted to the glutamate by glutaminase (GLS) in mitochondria. The glutamate can enter to TCA cycle for ATP production through conversion to α -ketoglutarate by glutamate dehydrogenase (GLUD) [154]. The α -ketoglutarate causes the ATP and oxaloacetate (4C compound) generation in the TCA cycle. Also, α -ketoglutarate can be converted to isocitrate and citrate through reductive carboxylation. This pathway is used for fatty acid synthesis [165]. The glutamine is important carbon and nitrogen **sources** for synthesis of different macromolecules such **as**, fatty acid, purine, pyrimidine, asparagine, NAD and oxaloacetate and etc [154]. The glutamine is very important in cancer cells for anabolic pathways. The proto-oncoprotein C-Myc induces the expression of glutamine transporters and GLS enzyme for glutamine metabolism [154]. The mutation in GLS enzyme suppresses tumor growth, so targeting of GLS is important for

cancer therapy [166, 167]. The glutamine synthetase (GS) condenses ammonia and glutamate into glutamine and is responsible for glutamine synthesis in the muscle and liver. This glutamine is transported to the tumor tissue for anabolic pathways. In many cancers, the GS enzyme is over-expressed and is very active for cell survival and growth [154]. Also, proto-oncoprotein C-Myc induces the expression GS enzyme through for cancer cells [154].

Other important amino acid is aspartate that is built from oxaloacetate (TCA cycle intermediate). Aspartate is very important in both protein and nucleotide synthesis. Most cells can uptake minimal amount of aspartate, so this amino acid should be built in the cells. The aspartate is built through transamination of oxaloacetate by glutamate. There are two aspartate transaminase isozymes, cytosolic (GOT1) and mitochondrial (GOT2). In an aspartate-malate shuttle, aspartate is synthesized by GOT2 in the mitochondria and then converted to the oxaloacetate by GOT1 in the cytosol. This oxaloacetate is reduced to the malate by NADH. This malate is fuel for malic enzyme for NADPH synthesis [154]. Other role of GOT1 is in cell growth and proliferation (aspartate synthesis) when ETC is impaired [168].

The mitochondrial genome in cancer

Some cancer types are created because of changes and mutations in nuclear DNA or mitochondrial genome. The types of somatic mutations are

diverse such as, rearrangement, insertions or deletions of small or large DNA segments, breaking of DNA and rejoining. The somatic mutations are responsible for many cancer types. In addition to somatic changes, the cancer cells can be created because of epigenetic changes (from exogenous sources such as, UV radiation or viruses) that alter chromatin and genome structure and gene expression [169]. The increasing of mutation frequency in cell genome such as, replication errors and carcinogen exposure and cancer driver mutations cause the increasing of cancer and its growth [154].

DNA in normal cells is damaged by internal and external mutagens during division, but most of these mutation and damages are repaired through repair mechanisms. Some of these mutations and damages are fixed, converting normal cells to cancer cells. The external sources of mutagens (carcinogens) such as, tobacco smoke, chemicals (like aflatoxins) and UV light increases the mutation rates in the normal cells [169].

Quality control of mitochondria in cancer

The communication between nucleus and mitochondria is necessary for proper functions of cells [170]. The nuclear genes control mitochondrial biogenesis and autophagy. There are some nuclear genes that can control mitochondrial biogenesis and metabolism. These genes cause the mitochondria synthesis in the cell. There are some nuclear autophagy or mitophagy genes that cause the mitochondrial destruction. These two pathways of biogenesis and mitophagy cause the control of mitochondrial

mass, function and quality. Some effects of mitophagy are depolarization of outer **mitochondrial membrane (OMM)**, phosphorylation and ubiquitination of mitochondrial proteins [154].

Depolarization of OMM causes the activation of PINK1 kinase that phosphorylates ubiquitin and activates E3 ligase PARKIN [171]. This enzyme ubiquitinylates mitochondrial proteins and cause the autophagy. The mechanism of autophagy (mitophagy for mitochondria) degrades **these** mitochondria in lysosome [154]. The mechanism of autophagy is very important in quality control of mitochondria in cancer cells. This mechanism provides substrates and micromolecules for biogenesis and metabolism. **Also, autophagy is important for tumor progression to malignant growth** [154].

Mitochondrial proteomic

The most mitochondrial proteins encoded through nuclear genome. These proteins enter to mitochondria for their function. The mitochondrial proteomic represents complex relations between nucleus and mitochondria [2]. The HMPDb (human mitochondrial protein database) detected useful information about mtDNA sequencing, polymorphism, three-dimensional structure of proteins and disease-related proteins. **Also, this way is effective for treatment of human mitochondrial diseases** [2]. **The dysfunction and mutation in many proteins, glycolytic and PPP enzymes such as, cytochrome oxidase and malate dehydrogenase stimulate tumor growth** [2].

There are many heat-shock proteins (HSP-70) in some cancers such as, gastric adenocarcinoma and hepatocarcinoma and this HSP-70 protein is a mediator for cellular proliferation and senescence and inhibits the p53 and stimulates the Ras-Raf pathway and tumor growth [2].

Nowadays and in future, the mitochondrial genome (mtDNA) and its mutations and proteins and enzymes will be studied for treatment of cancers and other diseases [2]. The molecular studies in mitochondria cause the many progressions in treatment of prostate cancer [172].

Chapter 3: Conclusion

Mitochondria as biosynthetic centers of the cell are responsible for many physiological and pathophysiological processes. They contribute to synthesis of biomolecules, such as lipids, amino acids, and nucleotides through the TCA cycle, and they are principal site of ATP production. Mitochondria play a critical role in successful conquest of cancer [24]. In recent decades, many groups had worked on importance of mitochondrial metabolism in cancer. In addition to generating 95% of cell fuel through carbohydrate breakdown (TCA cycle), mitochondria perform several activities such as ROS production and apoptosis. Most of these pathways are altered during malignant transformation [2].

Therapeutically, there are a few ways to interfere with the mitochondria-dependent anabolic processes. At the first, TCA cycle can be suppressed through inactivation of one of the TCA enzymes to inhibit making substrates. Glucose and glutamine are two main molecules that need to be catabolized to supply most of the carbon, nitrogen, and free energy and to support cell growth and proliferation. Glutamine can be converted to glutamate by glutaminase to enter the TCA cycle with conversion of glutamate to α -ketoglutarate (glutaminolysis), so suppression of glutaminase enzyme might interfere with this process. Another important substrate in TCA cycle is malate, which can be converted to oxaloacetate to start the cycle. One of the ways to interfere with the cycle is to inhibit malate production through suppression of succinate dehydrogenase. Also, TCA cycle can be prevented through suppression of

citrate synthase and citrate production. Stopping the production of citrate also interferes with lipid synthesis because citrate is an important precursor for lipid synthesis [25]. One of the important enzymes in glycolysis pathway is phosphofructokinase that phosphorylates fructose-6-phosphate to fructose-1, 6-bisphosphate (FBP) and then FBP molecules converted to glyceraldehyde-3-phosphate. Glyceraldehyde-3-phosphate can participate in nucleotide synthesis and TCA cycle through production of ribose-5-phosphate and pyruvate, respectively. LKB1/AMPK signaling pathway can suppress proliferative metabolism during periods of low energy availability. Following figure-24 illustrates metabolic pathways active in proliferating cells are directly controlled by signaling pathways involving known oncogenes and tumor suppressor genes [25].

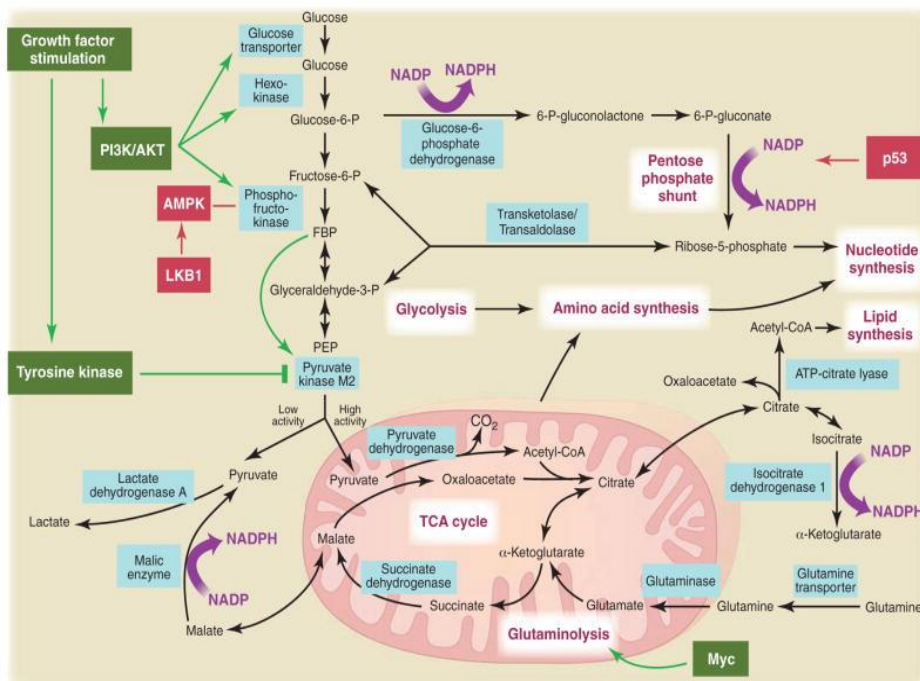


Figure24: Metabolic pathways active in proliferating cells are directly controlled by signaling pathways involving known oncogenes and tumor suppressor genes, (Figure adopted from Vander Heiden, M.G, and et al. 2009 [25]).

This image shows interconnection of glycolysis, oxidative phosphorylation, the pentose phosphate pathway, and glutamine metabolism together in proliferating cells. This metabolic pathway allows for both NADPH production and acetyl-CoA flux to the cytosol for lipid synthesis. Main steps in these metabolic pathways can be influenced by signaling pathways. Activation of growth factor receptors causes driving of both tyrosine kinase signaling and PI3K pathways. Activation of AKT/PI3K stimulates glucose uptake and flux through the early part of glycolysis. Tyrosine kinase signaling suppresses the late steps of glycolysis, making glycolytic intermediates available for macromolecular synthesis with NADPH production. Myc activates glutamine metabolism with supporting NADPH production. LKB1/AMPK signaling pathway and p53 decrease metabolic flux through glycolysis in response to cell stress, and this decreased glycolytic flux may be an adaptive response to shut off proliferative metabolism during periods of low energy (ATP) availability or oxidative stress. Tumor suppressors (LKB1/AMPK and p53) are shown in red, and oncogenes (Myc, PI3K/AKT, and tyrosine kinase) are in green. Key metabolic pathways are labeled in purple with white boxes, and the critical controlling enzymes in these pathways are shown in blue. Some of these enzymes are targeted as novel therapeutic targets in cancer.

Secondly, transportation and entry of some substrates can be stopped. Inhibition of glutamine transportation into mitochondria (suppression of glutamine transporters) suppresses glutaminolysis (conversion of glutamine to glutamate and α -ketoglutarate) and TCA cycle for ATP and intermediates production [25].

Suppression of mitochondrial Pyruvate transport also can be used in cancer therapy. Pyruvate is a major substrate for oxidative metabolism (TCA cycle), and an important point for anabolic pathways (glucose, lactate, fatty

acid, cholesterol, and amino acid synthesis). Pyruvate for metabolism should pass through the OMM and IMM and enter mitochondrial matrix. There are two proteins, mitochondrial pyruvate carriers (MPC1 and MPC2) that form a hetero-oligomeric complex in the IMM to facilitate pyruvate transport [173]. The exact identity of these carriers causes identification of novel modulator compounds that modulate MPC activity and also, identification of novel therapeutic ways for treating diabetes, cardiovascular disease, cancer, and neurodegenerative diseases. Pyruvate should pass through the OMM by large and non-specific VDAC channels or porin [174]. Human cells deficient in VDAC1 show impaired pyruvate oxidation and ATP production [175]. Some anthracycline chemotherapeutic drugs such as, doxorubicin inhibit pyruvate transport through IMM and suppress the MPC complex in heart mitochondria [173]. Also, the Antibiotic gatifloxacin inhibits mitochondrial pyruvate uptake by renal and hepatic mitochondria, and silibinin inhibit the MPC complex in liver mitochondria [173].

Warburg hypothesis caused a new understanding of tumor metabolism features. The exact metabolic transformation of cancer cells is not clear now, but it's obvious that many tumor cells perform oxidative phosphorylation when forced. Also, glucose metabolism is increased in most cancer cells. This increased glucose metabolism is required for high production of ATP and metabolites. In brief, we concluded that mitochondria play an important role in tumorigenesis, and their

dysfunctions cause tumorigenesis and cancer metabolic transformation [176].

New studies on the function of mitochondria in cancer are revealing novel approaches to targeted therapy. Many studies have shown that glycolysis and lactate production is high in oxygenated cancer cells, and defects in mitochondrial function and metabolism are main reasons for malignancy in cancer cells [176]. Essentially, metabolism is involved directly or indirectly in everything that cells do [25]. Despite many studies, there are still many questions about the complex connection between metabolism and proliferation. Understanding this aspect of cell biology is likely to have a major impact on many aspects of cancer therapy [25].

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