UNIVERZITA KARLOVA
PŘÍRODOVĚDECKÁ FAKULTA
KATEDRA BUNĚČNÉ BIOLOGIE

Studijní program Chemie
Studovaný obor Chemie se zaměřením na vzdělávání

Anna Růžičková

Oxidative phosphorylation addiction as a new approach to the therapy of neoplastic diseases

Závislost na oxidativní fosforylaci jako nový přístup k léčbě rakovinných onemocnění

Bakalářská práce

Vedoucí práce/Školitel: Prof. Ing. Jiří Neužil, CSc.

Praha 2017

Prohlášení:

Prohlašuji, že jsem tuto bakalářskou práci zpracovala samostatně pod vedením prof. Ing. Jiří Neužila, CSc. a že jsem uvedla všechny použité informační zdroje a literature. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, dne

Podpis: _____

Abstrakt

Nádorová onemocnění jsou dnes jednou z nejčastějších příčin úmrtí v industrializovaném světě, proto nalezení nových přístupů k jejich léčbě je velmi žádoucí. Nedávné výsledky ukazují na nezbytnou roli mitochondriální respirace pro růst nádorů. Je to zejména spojeno s objevem horizontálního přenosu mitochondrií mezi hostitelem a nádorovými buňkami s poškozenou mitochondriální DNA, který je nezbytný pro obnovu mitochondriální respirace, bez níž nádory nemohou růst. Zdá se, že stupeň respirace nutný pro růst nádorů se liší v jejich různých typech. Tuto hypotézu, kterou nazýváme ”závislost na oxidativní fosforylaci”, bude nutné ověřit, a na jejím základě bude možné navrhnout novou léčbu nádorových onemocnění za použití látek, které působí přímo na mitochondriální respirační komplexy.

Klíčová slova: mitochondrie, oxidativní fosforylace, horizontální transfer mitochondriální DNA, nádorová onemocnění, mitochondriálně cílené protirakovinné látky

Abstract

Neoplastic diseases belong at present time among the most frequent causes of premature death in industrialized countries. Discovery of novel approaches to their therapy is highly warranted. Recent results point to the requirement of mitochondrial respiration for tumor progression. This is linked primarily to recent discovery of horizontal transfer of mitochondrial transfer from the host to cancer cells with damaged mitochondrial DNA. This is a needed for the recovery of mitochondrial respiration, a prerequisite for tumor progression. It has appeared that the rate of respiration necessary for tumor progression differs in individual types of tumors. This hypothesis, which is refer to as 'oxidative phosphorylation addiction', however, needs to be verified. It could serve as the basis for proposing of novel therapeutic strategy for neoplastic diseases, using compounds that directly affect mitochondrial respiratory complexes.

Key words: mitochondria, oxidative phosphorylation, horizontal transfer of mitochondrial DNA, neoplastic pathologies, mitochondrially targeted anti-cancer agents

Content

List of used abbreviations

AIF Apoptosis-inducing factor

ADP Adenosine diphosphate

ANT Adenine nucleotide transporter

Apaf-1 Apoptotic protease activating factor 1

ATP Adenosine triphosphate

Bak Bcl-2 homologous antagonist/killer

Bax Bcl-2–associated X protein

Bcl-2 B-cell CLL/lymphoma 2

Bcl-xL B-cell lymphoma-extra large

BM Bone marrow

CAD Caspase dependent DNase

CCL21 Chemokine (C-C motif) ligand 21

CI Complex I

CII Complex II

CIII Complex III

CIV Complex IV

CV Complex V

CJ Crista junction

CoQ Ubiquinone

$CoQH_2$ Ubiquinol

CP Cytoplasm

CS Cigarette smoke

Cx43 Connexin 43

CypD Cyclophilin D

DCA Dichloroacetate

DR4 Death receptor

DR5 Death receptor

DTDP Deoxythymidine diphosphate

ECM Extracellular matrix

EndoG Endonuclease G

ETC Electron transport chain

EtBr Ethidum bromide

FMN Flavin mononucleotide

FAD Flavine-adenine dinucleotide

$FADH_2$ Reduced flavin-adenine dinucleotide

GJCs Gap junctional channels

HKII Hexokinase II

hMSCs Human mesenchymal stem cells

hsp2 Small heat shock protein

hsp1 Mitochondrial heat shock protein

HUVEC Human umbilical vein endothelial cells

HtrA2 Mitochondrially-located serine protease

IBM Inner boundary membrane

ICS Intercristal space

IMS Intermembrane space

IMM Inner mitochondrial membrane

iPSC-MSCs Human-induced pluripotent stem cell–derived MSCs

LDH Lactate dehydrogenase

LPS Lipopolysaccharide

lkB1 Liver kinase B1

MitoVES Mitochondrially targeted vitamin E succinate

MMP Matrix metalloproteases

MSCs Mesenchymal stem cells

mtDNA Mitochondrial DNA

NADH Reduced nicotine-amide-adenine dinucleotide

NADPH Reduced nicotine-amide-adenine dinucleotide phosphate

NGS Next generation sequencing

NK Natural killer cells

OMM Outer mitochondrial membrane

OXPHOS Oxidative phosphorylation

PDH Pyruvate dehydrogenase

PDK Pyruvate dehydrogenase kinase

PMF Proton motive force

PT Pyruvate transporter

PTPC Permeability transition pore complex

ROS Reactive oxygen species

SDH Succinate dehydrogenase

SDHA Succinate dehydrogenase subunit A

SDHB Succinate dehydrogenase subunit B

SDHC Succinate dehydrogenase subunit C

SDHD Succinate dehydrogenase subunit D

Smac/DIABLO Second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point

TCA Tricarboxylic acids cycle, Krebs cycle

TNF Tumor necrosis factor

TPP+ Triphenyl phosphonium

TRAIL TNF-related apoptosis-inducing ligand

VDAC Voltage-dependent anion channel

2-DG 2-deoxyglucose

3-BP 3-promopyruvat

α-TOS alpha tocopheryl succinate

1 Introduction

Nowadays, we are facing a renaissance of mitochondrial research from biochemical, metabolic and pathological point of view. This new wave of interest was started by Otto Warburg and his hypothesis about tumor bioenergetics and the very recent discovery that mitochondria can move between cells and restore mitochondrial function in damaged cells.

There is a large number of diseases linked to mitochondria, which clinically present an extremely heterogeneous group like heritable diseases caused by mutations that are found in mitochondrial and nuclear genes encoding mitochondrial proteins, to environmentally induced mutations in mtDNA causing many common acquired disorders. These include ischemic diseases of the heart and brain, neurodegenerative diseases, liver diseases, and certain cancers.

Cancer is characterized by deviations in energy metabolism involving not only genetic alterations in nDNA, mtDNA mutations and changes in mtDNA copy number. Mutations of mtDNA and nDNA that encode mitochondrial proteins, primarily the proteins of the electron-transport chain (ETC) compromise the process of oxidative phosphorylation (OXPHOS) and promote aerobic glycolysis that is specific for metastatic progression.

In this thesis, I will discuss the problematic of neoplastic diseases and the relevance of mitochondrial transfer in (patho)physiological processes. Starting from an overview of the functions of the mitochondrion and mechanisms of apoptosis, I will discuss the processes leading to neoplastic diseases, pathophysiological consequences of horizontal mitochondrial transfer and its potential for the treatment of neoplastic diseases, and finally in the last chapter, I will outline the potential of mitochondrial targeted drugs for cancer therapy.

2 Mitochondria

Mitochondria are organelles with the size of about 0.75 x 3 μm presented in almost most eukaryotic cells. They have two membranes, the inner mitochondrial membrane (IMM) and the outer mitochondrial membrane (OMM), with intermembrane space (IMS) between the two membranes and the space inside the mitochondrion referred to as the mitochondrial matrix (Rabl et al. 2009; Wiemerslage and Lee 2016).

The IMM creates structures called cristae inside the mitochondrion, which have been documented in recent years in the crista junction model using the electron-microscopic tomography. According to this model IMM can be divided into the cristae membrane (CM) that builds the cristea and the inner boundary membrane (IBM), that connects the cristae by the system of membrane tubules also known as cristae junctions (CJs). CJs have the diameter of ~30 nm and form a barrier for movement of

metabolites and proteins between inter-cristal (ICS) space and the IMS, therefore they are very important in regulation of the oxidative phosphorylation (OXPHOS) (Bogenhagen et al. 2008; Frey et al. 2002; Rabl et al. 2009). This barrier influences the diffusion of various metabolites, e.g. ADP, into ICS, and modifies the pH gradient through the inner membrane. The transport of synthetized ATP from the matrix to the IMS and the transport of ADP in the opposite direction is mediated by an ADP/ATP transporter of the IMM.

Mitochondria are not static entities. They often undergo fusion (connection of mitochondria) or fission (fragmentation of mitochondria). This continuous reorganization of the mitochondrial network is highly regulated and important to adjust to the energetic demands of the cell. Moreover, they can bind the cytoskeleton and travel long distances within the cell. This phenomenon occurs for example in neurons, where mitochondria are able to travel at the distance of one meter. On the contrary, mitochondria have more or less fixed position in the cells with a higher energetic demand, e.g. cells of the myocardium or the skeletal muscle, as well as in sperm cells, where mitochondria are situated only on the basis of the flagellum (Lodish et al. 2002).

Mitochondria participate in multiple important processes of the cell, which include cellular signalization, regulation of the cell cycle, apoptosis, production of reactive oxygen species (ROS), thermogenesis and homeostasis of Ca^{2+}. Probably the most important function of mitochondria is primarily the ability to transform the energy received from nutrients into ATP (Gogvadze and Zhivotovsky 2007; von Ballmoos et al. 2009).

2.1 Origin of the mitochondrion

Mitochondria are the remnants of archaebacterial that fused with primitive sells many million years ago. This hypothesis is confirmed by the presence of double lipid bilayer and mitochondrial DNA (López-García et al. 2017). During the evolution, mitochondria transferred almost all of their genes into nuclear DNA and, therefore, vast majority mitochondrial proteins are encoded by nuclear DNA. Mitochondrial DNA only code for 13 polypeptides.

2.2 Mitochondrial DNA

Mitochondrial DNA is a double stranded circular DNA. Contraty to nuclear DNA, mitochondrial DNA is present in a number of copies (hundreds to thousands) per cell, which are organized in mtDNA-protein complexes known as mitochondrial nucleoids. Mitochondrial genes are located in both strands of the mtDNA – one of them is mark as H (heavy) strand, the other one as L (light) strand (Chinnery et al. 2012). Transcription of mtDNA starts within the non-coding part of mtDNA known as D-loop (displacement loop) within promotors located on both heavy (HSP1 and HSP2) and light (LSP) strand (Bogenhagen et al. 2008; Bonawitz et al. 2006; Schon et al. 2012).

Mitochondrial DNA of mammalian cells carries 37 genes, its size being 16,569 bp. These 37 genes are essential and include components of the apparatus for replication, transcription and translation of mtDNA. Two of these genes code for rRNA (12S and 16S); thirteen genes code for subunits of mitochondrial complexes. More specifically, the *MTND1–MTND6* and *MTND4L* code for subunits of complex I (CI), *MTCYB* codes for cytochrome b of complex III (CIII), three genes code for cytochrome c oxidase *MTCO1-MTCO3* of complex IV (CIV), and the genes *MTATP6* and *MTATP8* code for subunits of complex V (CV). There are 22 genes within mtDNA that code for tRNAa (Lodish H et al. 2000).

In mammals, mtDNA is predominantly maternally inherited and this uniparental germline inheritance reduces the benefit of recombination arising from sexual reproduction, also facilitating the appearance of maternally inherited disorders when a significant percentage of mtDNA mutations are transmitted to the offspring (Torralba et al. 2016).

2.3 Electron transport chain and oxidative phosphorylation (OXPHOS)

The electron-transport chain (ETC) consists of enzyme complexes I – IV and ATP-synthase (CV) that are incorporated in the IMM. Electrons from oxidized carbon-based cellular fuels gradually pass via mitochondrial complexes using the electron carrying molecules NAD+ and FADH+. An oxygen molecule as a terminal acceptor of electron receives the electrons and produces water. (Lodish H. et al. 2000).

While electrons are being transported, three of these complexes simultaneously pump H+ from mitochondrial matrix to the intramembrane space (IMS), whereby creating the proton motive force (PMF). PMF then generates an electrochemical gradient and drives the ATP synthase, indispensable cellular energy (Breuer et al. 2013; Lenaz and Genova 2010).

2.3.1 Complex I (NADH:ubiquinone oxidoreductase)

The largest complex of the ETC is the complex I, whose subunits are folded into a shape of letter L. The hydrophobic part of this complex, consisting of seven mitochondrially coded subunits ND1-ND6 and ND4L, is firmly anchored in the lipid bilayer of the IMM. The second hydrophilic part freely protrudes into the inner space of mitochondrion, the matrix. Other parts of this complex are eight Fe-S redox centers and a non-covalently bound flavin mononucleotide (FMN) molecule, that accept in the matrix an electron from reduced NADH + H^+. This electron is transported from FMN through a cascade of Fe-S transporters to ubiquinone. CI changes its conformation and transmits four protons to the IMS, thereby helping to generate proton gradient (Lenaz and Genova 2010; Santidrian et al. 2013; Sazanov 2015; Zickermann et al. 2015).

2.3.2 Complex II (succinate:ubiquinone oxidoreductase)

Complex II is by contrast the smallest complex of the ETC. It consists of four subunits which are encoded in the nuclear DNA. SDHC and SDHD make lipophilic anchor within the IMM and comprise two ubichinone binding sites. SDHA subunit binds covalently flavin adenine dinucleotide (FAD), and converts succinate into fumarate in the Krebs cycle located in the matrix, resulting in generation of two electrons. These two electrons are forwarded through the Fe-S redox centers toy reduce ubiqinone (CoQ) to ubiquinole ($CoQH_2$), which then moves on to complex III. There is no transport of protons into the IMS via CII (Dröse 2013; Lenaz and Genova 2010; Sun et al. 2005).

2.3.3 Complex III (ubiquinol:cytochrome c oxidoreductase)

This dimeric complex consists of eleven subunits; just one of them, MTCYB (cytochrome b), is encoded by mitochondrial DNA. CIII re-oxidized $CoQH_2$ back to CoQ, so two protons can be pumped into the IMS and two more electrons are acquired. These two electrons are transported to cytochrome c by the process known as the "Q cycle", which consists of two separate "half-cycles".

The first electron is transported to the first 2Fe-2S group of a Rieske's center, then to a hem group of cytochrome c1, and finally to the hem group of cytochrome c. Cytochrome c is soluble in the water, so it migrates in the IMS to complex IV. The molecule of cytochrome c is able to bind just one of the electrons, therefore the second electron cannot undergo the same pathway.

The second electron is transported to cytochrome b and passes through both of its hem groups and then reduces CoQ to the molecule known as semi-quinone radical ion $Q^{-}\bullet$. By this means, the second electron is also recycled. This semi-quinone radical is used in the second half-cycle, where $CoQH_2$ connects to CIII and re-oxidizes itself to CoQ. Two more electrons are acquired by this process, one undergoing the above mentioned pathway and the second one being recycled via $Q^{-}\bullet$. The radical uses two protons from the matrix to re-oxidize itself to CoQ to be used in the ETC again (Lenaz and Genova 2010; Solmaz and Hunte 2008).

2.3.4 Complex IV (cytochrome c oxidase)

The last complex of the ETC is cytochrome c oxidase. It consists of thirteen subunits, three of them (COXI, COXII, COXIII) being encoded mitochondrially. The nuclear encoded subunits are used to stabilize the structure of the complex.

Cytochrome c oxidase includes two hems (*a* a *a*3) groups and three atoms of Cu (2 Cu_A a Cu_B). CIII produced two reduced molecules of cytochromes c that are transported to CIV. They are re-oxidized and serve as transporters of two electrons to the Cu_A/Cu_A center, wherefrom they go through hem *a* to

hem *a*3, and finally one of the electrons is transported to Cu_B. This atom of copper is reduced from oxidation state +2 into +1. The second electron reduces hem *a*3.

If both hem a3 and CuB are in reduced form, they are able to bind the oxygen that creates a peroxide bridge between them. CIV uses electrons from another two molecules of cytochrome c and two protons from the matrix, which disconnects the peroxide bridge: the molecules of hem *a*3 an CuB are regenerated and two molecules of H_2O are formed. In total, four molecules of cytochrome c are re-oxidized, and one molecule of oxygen O_2 and four protons are used for generation of two molecules of water. CIV also creates the proton gradient in the IMS by transporting protons (Lenaz and Genova 2010; Solmaz and Hunte 2008).

2.3.5 Complex V (ATP synthase)

The terminal complex of the OXPHOS system is the F_OF_1-ATP synthase. This complex comprises sixteen subunits, two of which (α and β) are coded mitochondrially. ATP synthase has two functional domains – molecular motors F_O a F_1. F_1 is a hydrophilic motor, which is situated in the mitochondrial matrix. It consists of an αβ hexamer and γ, δ and ε subunits. The γ and ε subunits connect the hexamer to the F_O motor, and this central stalk rotates and catalyzes the addition of inorganic phosphate to ADP. The δ subunit connects the hexamer to the F_O domain through its subunit b, and keeps the hexamer in place, therefore this part of ATP synthase does not rotate. (Lodish et al. 2000).

The F_O domain is hydrophobic and is embedded in the lipid bilayer of the IMM. It serves as a channel for protons moving from the IMS back to the matrix of the mitochondrion. One of its parts, the c-ring complex of approximately 10 c subunits, promotes the spinning of the γε subunits of the F_1 domain.

The main function of ATP synthase is to synthesize ATP that is enabled by the proton gradient, which was created by complexes of the ETC pumping the protons from the matrix into the IMS. ATP synthesis is situated on the β subunits of the F_1 domain that change their conformation by the rotation of the γε subunits (Jonckheere et al. 2012; von Ballmoos et al. 2009).

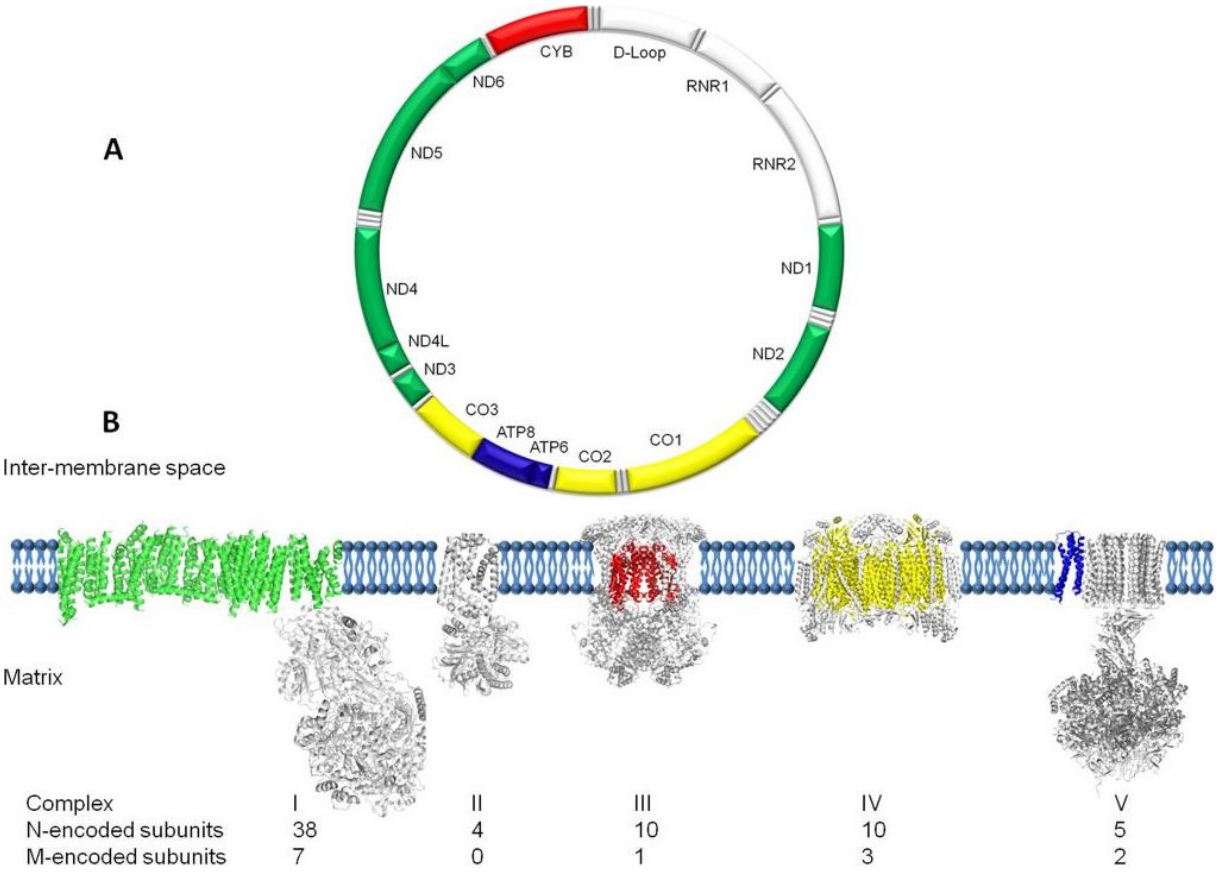

FIGURE 1 - Architecture of the mitochondrial genome and the respiratory chain.

A. Schematic representation of the 16,569 bp human mitochondrial genome with two ribosomal RNAs (RNR1, RNR2), the protein-coding genes colored according to the complexes to which they contribute subunits (CI – green, CIII – red, CIV – yellow, CV – blue), 22 tRNAs and non-coding D-loop. **B.** This part shows the structure of mitochondriral complexes; the parts coded by the mitochondrial DNA are colored and the gray color represents the subunits encoded by the nuclear DNA. (Lloyd et al. 2013)

3 Apoptosis

One of the most important processes occurring during the lifespan of a cell is cell death. There are several types of cell death originating from different situations, but the major and most important one is apoptosis. Apoptosis is physiological, genetically determined and evolutionary conserved type of cell death occurring in all multi-cellular eukaryotes, where the unnecessary or damaged cells are eliminated (Ashkenazi and col., 1998). Apoptosis is defined as tightly regulated intracellular program, where the cells which are destined to die, activate enzymes that degrade critical cellular content including DNA and nuclear and cytoplasmic proteins.

It is a natural process with a robust physiological role in organism being prominent as an anti-tumor mechanism, development and in the immune system. In case the cells are exposed to high levels of stress (e.g. oxidative stress, chemical stress, UV), which could lead to tumorigenic proliferation of the cells, the apoptosis as a self-suicide mechanism takes place to protect the organism. The other role is for example during the embryonic stage, inevitable for the right development of the extremities of the fetus. Furthermore, apoptosis is also an integral part of development of the immune system, because it selects cytotoxic T-lymphocytes. There exists also hormonally directed apoptosis in the cells of the mammary gland during the post-lactation period (Taylor et al. 2008; Watson 2006).

The process of apoptosis is distinct from other types of cell death (necrosis) in that it is precisely controlled and in that it is not accompanied by the inflammatory reaction of the organism and damaging of the surrounding cells (Taylor, Cullen and Martin 2008).

3.1 Morphological features of apoptosis

During apoptosis cells undergo specific morphological changes. In the first step cell shrinkage occurs, the cytoskeleton breaks down and the inside of the cell becomes “more crowded”. There are also very important processes proceeding in the nucleus of the cell: fragmentation of the nucleus, chromatin condensation and the subsequent fragmentation of DNA, which forms the typical apoptotic ladder and, moreover, the breakdown of the nuclear membrane. The cytoplasmic membrane also undergo irreversible changes. It starts to form apoptotic bodies, the so called blebs, and at the end the cell falls apart to pieces. One of the key biochemical markers used for detection of apoptotic cells is externalization of phosphatidylserine (PS) that specifically binds annexin V. Mobilization of PS to the extracellular part of the plasma membrane serves as an “eat me” signal for macrophages, resulting in the clearance of dead cells and prevention of the inflammatory response of the immune system (Reed and Green 2002; Taylor et al. 2008).

3.2 Molecular mechanisms of apoptosis

The main characteristic of the apoptotic signaling pathway is the activation of cysteine-dependent aspartate-specific proteases known as caspases. Caspases and their relative meta- and para-caspases were identified in all multicellular organisms and even in prokaryotes, but in my work I have focused on the process of apoptosis in mammals (Boyce and col. 2004). The expression of caspases should be tightly regulated, which is the reason why these proteases are expressed as non-active pro-caspases known as zymogens and are activated during apoptotic signaling. Another key molecule in this type of cell death is caspase dependent DNase (CAD), which is activated by caspases and causes the catalytic dissociation of DNA. If the cell passes a point, when caspases are activated, it enters the commitment phase of programmed cell death, and from this moment it is not possible to stop the process of apoptosis, leading irreversibly to cell death (Taylor et al. 2008). There are two mechanisms of caspase-dependent apoptosis, which are activated by different types of pro-apoptotic signals: the extrinsic and the intrinsic pathway. Both pathways differ in type of initiation caspases, but activates the same effector caspases (Ashkenazi and col., 1998).

3.2.1 Mitochondrial (intrinsic) pathway

Mitochondrial or intrinsic pathway of apoptosis is activated by stress stimuli, such as UV, oxidative stress, chemical stress, etc. This process is strictly regulated by the large family of BCL-2 proteins. In healthy cells, apoptosis is inhibited by anti-apoptotic Bcl-2 proteins, mainly Bcl-2 and Bcl-xL, which inhibit the activation of pro-apoptotic proteins of the Bcl-2 family. In case the cell is 'somehow' damaged, it starts to produce and activate pro-apoptotic Bcl-2 proteins Bax and Bak, which form multimeric complexes and cause the outer mitochondrial membrane permeabilization (MOMP).

The permeabilization of outer mitochondrial membrane leads to the release of cytochrome c and other proteins from the intermembrane space (Smac/DIABLO, HtrA2/Omi, AIF). In the cytosol, the molecules of cytochrome c react with molecules of Apaf-1, and creates the special structure called the apoptosome. It serves as a platform for activation of initiation caspase-9, which activates the downstream effector caspase-3 (Garrido et al. 2006; Youle and Strasser 2008).

3.2.2 Extrinsic pathway

Unlikely the intrinsic pathway, the extrinsic pathway is activated by apoptogens coming from the outside of the cell. Extrinsic pathway is triggered by the binding of specific ligands to their cognate receptors, which are mostly cytoplasmic membrane-anchored proteins. These are: the Fas receptor interacting with the Fas ligand, the DR4 and DR5 receptors interacting with the TRAIL (tumor necrosis factor α-related apoptosis inducing ligand) ligand, or the TNF receptor 1 interacting with the TNFα Ligand. These receptors contain the important DED and DEATH domains, which serve as interaction domains with the other proteins involved in the apoptotic machinery. Binding of the ligands usually

results in oligomerization of the receptors, which recruits the adaptor proteins (FADD, TRADD, TRAF-2) and molecules of initiation pro-caspases (caspase-8/10). The latter are auto-cleaved and activated and then activate the downstream effector caspases (caspase-3/7), which afterwards cleave their specific substrate. This type of apoptotic pathway is often inter-connected with mitochondrial pathway (Elmore 2007; Ashkenazi a col. 1998).

3.2.3 Apoptosis-inducing factor

Caspase-independent cell death is primarily used by the cells of the nervous system and does not involve the activation of caspases, but involves the activation of the DNA-binding protein referred to as the apoptosis-inducing factor (AIF). In case the cell is damaged and the MOMP is formed, the AIF protein is transported from the IMS to the cytosol and then to the nucleus, where it binds to DNA, causing the DNA fragmentation and chromatin condensation, culminating in cell death.

The proteins Smac/DIABLO (second mitochondria-derived activator of caspases/direct inhibitor of apoptotic-binding protein with a low isoelectric point), EndoG (endonuclease G) and HtrA2 (high temperature requirement protein A2) act via a similar principle. The EndoG protein translocates to the nucleus similarly as does AIF, breaks down the DNA and causes apoptosis. Smac/DIABLO and HtrA2 are serine proteases that suppress the inhibitory activity of the anti-apoptotic proteins from the family of inhibitors of apoptosis proteins (IAP) (Adrain et al. 2001; Elmore 2007).

3.2.4 Activation via the PTPC channel

The permeability transition pore complex (PTPC) is a protein complex at the sites of contact between IMM and OMM. In healthy cells, the pore complex switches from open to closed state, so the small molecules can move between the cytosol and the matrix. In case there is a higher level of ROS or high concentration of Ca^{2+} ions in the cell, the PTPC becomes more permeable for small molecules, therefore they can uncontrollably enter the matrix. This causes swelling of the mitochondrion, the OMM comes apart and the inter-membrane apoptotic factors are released. There are three main proteins important for its formation: the voltage-dependent anion channel) (VADC) in the OMM, cyclophilin D (CypD) in the matrix and the adenine nucleotide transporter (ANT) in the IMM (Arif et al. 2016; Vianello et al. 2012; Zhivotovsky et al. 2009).

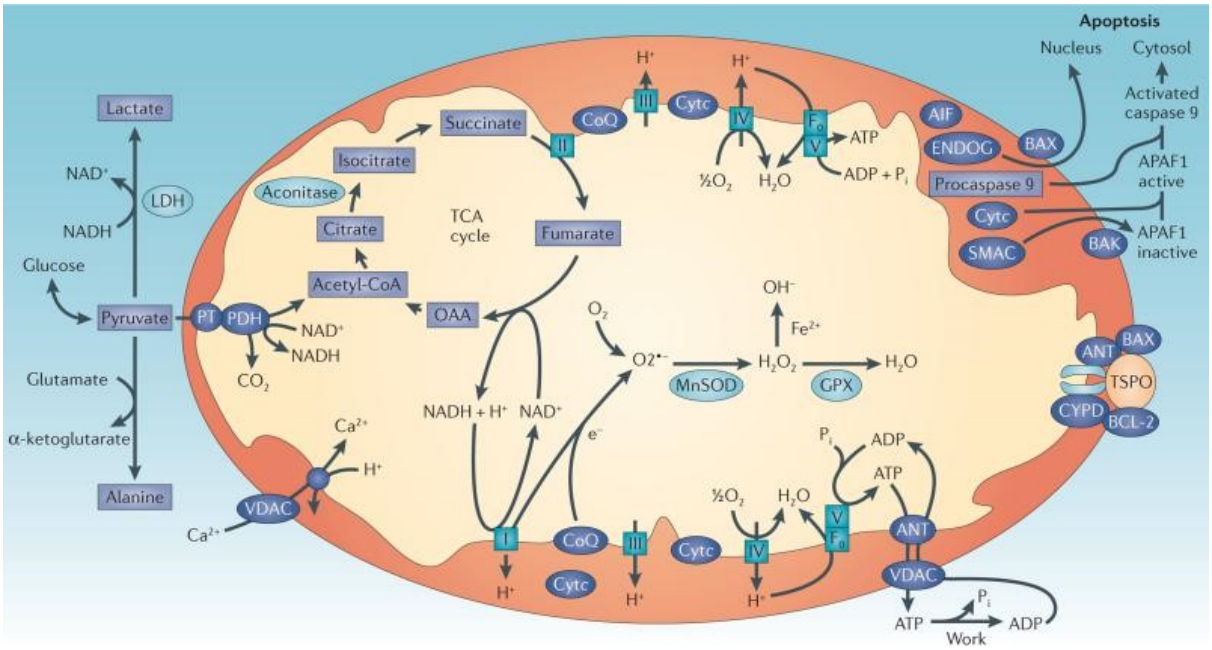

FIGURE 2 – Functions of the mitochondrion

Mitochondria are the crossroads of many biosynthetic pathways, and most of cellular ATP is produced here. Mitochondria also regulate redox processes, generate reactive oxygen species (ROS), regulate the level of Ca^{2+} ions and play the key role in the apoptosis induction. The energetic metabolism starts by converting glucose into pyruvate, which is transported to the mitochondrion by means of the pyruvate transporter (PT). Pyruvate dehydrogenase (PDH) then binds to the PT that catalyze the conversion of pyruvate to acetyl-coenzyme A (acetyl-CoA). Acetyl-CoA enters the Krebs cycle (tricarboxylic cycle, TCA cycle). Reducing cofactors (NADH and FADH2), that are generated in the TCA transmit the electrons to the ETC. The ETC results in production of ATP as a consequence of electron mobilization within the ETC. One of the possible ways to induce apoptosis is the permeabilization of OMM via the pore formed by Bax and Bak. This pore releases cytochrome c and Smac/DIABLO, which activate caspases and anti-apoptotic proteins. Fatty acids are transported to the mitochondrion with the help of coenzyme A and carnitine, and undergo the process of β-oxidation. ROS originate as by-products of OXPHOS, where excess electrons bind to molecules of O_2 and give rise to the superoxide anoin O_2^{-}• (Wallace 2012).

4 Neoplastic diseases

The term neoplasia means new growth, and is commonly referred to as a tumor. Tumor cells divide at a much faster rate than non-malignant cells in a process that is not coordinated with the surrounding tissue.

4.1 Characteristics of neoplastic diseases

Tumors are abnormal pathological tissues in organism, which do not perform any physiological function. The tumors arise with changes in one cell or a small group of cells with abnormal pattern of growth. Tumors can be classified on the basis of their ability to invade the surrounding tissue.

The first type is benign tumor, which arises from well differentiated cells similar to the tissue of origin. This type of tumors does not invade to surrounding tissue and does not metastasize. The second type, malignant tumors, tend to be poorly differentiated. They are able to invade the surrounding tissue through the bloodstream or lymphatic system. Malignant tumors have also the ability to spread to other parts of the body so they can generate new, secondary tumors (metastases).

4.2 The hallmarks of cancer

The hallmarks of cancer are several mechanisms that must be breached by a cell on the path towards cancer. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, sustained angiogenesis, and activating invasion and metastasis (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).

4.2.1 Sustaining Proliferative Signaling

Under standard conditions, cell division is driven by the binding of growth factors to the growth factor receptors at the plasma membrane, which leads to activation of a cascade of signals. In cancer cells, DNA becomes damaged and the cells produce receptors, which do not need signaling by growth factors and they activate themselves. This then leads to uncontrolled growth of these cells and to tumor expansion (Hanahan and Weinberg 2000; Witsch et al. 2010).

4.2.2 Insensitivity to anti-growth signals

Cell cycle is a series of events that take place during cell division. During normal cell cycle the cell must be given by several coordinated signals and needs to cross certain checkpoints to produce two daughter cells. There are three checkpoints in the cell cycle, first is the G_1 checkpoint between the G_1 and the S phase, another is the G_2 checkpoint between the S and the G_2 phase, and the last one is the M checkpoint before the mitotic phase of cell division occurs. When the cell reaches a checkpoint, there

are mechanisms which should detect possible damage of DNA and hold the cell at the checkpoint to prevent further cell division: in order to achieve this, anti-growth signals are activated.

Cancer cells usually ignore these anti-growth signals. Merlin, the NF2 (neurofibromatosis type 2) tumor suppressor gene product and LKB1 (liver kinase B1) gene products limit the ability of cells to efficiently emit mitogenic signals or to organize epithelial structure, helping to maintain tumor integrity (Chiasson-MacKenzie et al. 2015). Most anti growth signals are channelled via the pRb (retinoblastoma protein) protein, a tumor suppressor protein that regulates the transition from the G_1 to the S phase. Disruption of this pathway is significant for most of the types of cancer.

In tumors there is no longer a balance between dying and dividing cells. Cells are usually not able to enter the G_0 phase of the cell cycle and therefore begin to divide without control (Hanahan and Weinberg 2000; Hanahan and Weinberg 2011).

4.2.3 Enabling replicative immortality

There are structures of DNA called telomers at the ends of chromosomes that do not code any proteins. These structures protect endings of DNA from damage. Most cells have certain restricted limit of division. This is known as the Hayflick limit, and is about 40 – 60 cell divisions. With every cell division, there is a small amount of DNA, which gets lost, and this patch of DNA is derived from telomers. The cells thus does not 'lose' DNA from the coding region. If this limit is disrupted/overcome, then also the coding sections in the DNA start to' disappear'. That leads to the production of defective proteins and to initiation of apoptotic cell death.

Cancer cells activate the enzyme called telomerase that ads the new bases at the end of the chromosome to make them longer. This allows the cell to keep dividing without destroying the coding part of the DNA for unlimited periods of time (Granger et al. 2002; Hanahan and Weinberg 2000).

4.2.4 Sustained angiogenesis

Every tissue in the body needs a steady supply of nutrients, which are accessible thanks to the circulation that is composed of blood vessels. The nutrients from bloodstream are able to diffuse into the tissue, but only at a shorter distance (about 1 millimeter), so that when the tissue reaches greater dimensions, it needs the blood vessels to spout into it in order to bring nutrients and oxygen. This is achieved by the process called angiogenesis that is characterized by growth of blood vessels. The ability to induce and maintain angiogenesis is activated at a stage when a particular tumor reaches a 'critical' mass. Switching to this phase allows rapid clonal expansion of cancer cells associated with the formation of macroscopic tumors, since without vascular supply tumors are unable to grow to larger dimensions (Hanahan and Weinberg 2000; Sennino and McDonald 2012).

4.2.5 Resisting cell death

Cells undergo apoptosis if they detect that they have damage their DNA, to ensure that the damaged cell will not survive and multiply carrying and propagating the ‘damage’. However, cancer cells ignore the signals, which trigger apoptosis thanks to mutations of many various genes that play an important role in the process of apoptosis. The well known role of apoptosis is e.g. the inactivating mutation in the tumor suppressor gene p53, or increased expression of inhibitors of apoptosis from the IAP family of proteins. Resistance to apoptosis is most likely a common characteristic of most (in not all) cancer types (Hanahan and Weinberg 2000).

4.2.6 Tissue invasion and metastasis

Tissues are formed by cells and extracellular matrix (ECM). ECM is a three-dimensional network composed of collagens, laminins, proteoglycans, fibronectin, elastin, and other glycoproteins. When cancer cells spreads into surrounding tissues, they use matrix metalloproteases (MMP) that act as 'molecular scissors' to cut through ECM that inhibit the movement of these migrating cells. Once cancer cells leave the original tissue, they move through the circulatory system until they find a suitable location to ‘settle‘ and re-enter the tissues where they start forming a new tumor. Whether a particular tumor has already metastasized or not is a crucial factor that determines the prognosis and predicts the response to treatment (Hanahan and Weinberg 2000).

4.2.7 Genome instability and mutations

There are many mechanisms to ensure the integrity of the genome in a cell. Many factors detect DNA damage that also start its repair machinery, other factors directly repair the damage or inactivate or intercept the mutagenic molecules before they could have damaged the DNA. If these housekeeping genes are mutated, other mutations occur and the progressing tumors are multiplied in the context of new genotypes. Progression of a tumor depends on the success of the mutation, and only the most successful genotypes (that will provide cancer cells with an advantage) will stabilize themselves (Hanahan and Weinberg 2011).

4.2.8 Tumor-promoting inflammation

It is common that some tumors are densely infiltrated by cells of both the innate and adaptive immunity. Although the inflammatory immune responses of the cell were thought to have primarily the defensive function, they can also be beneficial to the cell. Thanks to the inflammation, bioactive molecules may be supplied to the tumor microenvironment, including growth factors that sustain proliferative signaling, survival factors that limit cell death, pro-angiogenic factors and other hallmark-facilitating programs.

Cells of the immune system also release ROS that are mutagenic for nearby cancer cells, accelerating their genetic alterations toward states of increased malignancy (Hanahan and Weinberg 2011).

4.2.9 Reprogramming energy metabolism

This chapter is discussed in chapter 5.2 Warburg effect.

4.2.10 Evading immune destruction

The immune system has natural capacity to detect and destroy cancer cells that are able to avoid detection and destruction by several mechanisms based on expression of certain proteins on their surface that inactive immune cell, etc. Transplantation experiments have shown that cancer cells arising from immunosuppressed mice are not efficient at initiating secondary tumors in syngeneic immunocompetent mice, whereas cancer cells with the origin from immunocompetent mice are equally efficient at initiating transplanted tumors in both animal models. Highly immunogenic cancer cells are eliminated and only the cells with low immunogenicity survive; these cells can form tumors in both imunodeficient and immunoproficient mice.

Another way how to evade the immune system is by means of the release of immunosuppressive factors that counteract the activity of natural killer (NK) cells and cytotoxic T-lymphocytes. These factors are e.g. transforming growth factor β (TGF-β) or chemokine C-C motif ligand (CCL21) (Hanahan and Weinberg 2011).

5 Mitochondrial deregulations as significant markers in neoplasms

As already mentioned, mitochondria are important in many bioenergetic, anabolic and apoptotic processes in as major components of various signaling pathways in cells. Therefore, their dysfunction causes many different diseases, and mitochondrial deregulations are significant markers in some cancer cells (Imanishi et al. 2011; Wallace 2012). Different level of healthy and mutated mtDNA in a cell influences development and manifestation of the disease. After exceeding a certain threshold of mutated mtDNA, the physiological manifestations of multisystem diseases occur. These diseases affect mainly organs with high energy requirements such as the brain, muscle and heart (Rossignol et al. 2003; Wallace 2012).

5.1 Production of reactive oxygen species

Reactive oxygen species (ROS) are radicals, ions or molecules that have a single unpaired electron in their outermost shell of electrons. ROS can be categorized into two groups: free oxygen

radicals ($O2•^-$, •OH, NO•, R•, etc.) and non-radical ROS (H_2O_2, O_3, N_2O_2, NO_2^+, etc.). They are produced in normal differentiated cells in mitochondria and peroxisomes as well as in cancer cells, and play a role also in inflammatory processes (Fang et al. 2009).

In has been shown that oxidative stress-mediated signaling events have been reported to affect all characters of cancer cell behavior. The oxidative load causes damage to DNA and proteins, which leads to mutations and cancerous growth. Low doses of hydrogen peroxide and superoxide stimulate cell proliferation in a wide variety of cancer cell types (Dröse 2013; Lee et al. 2002; Martinon 2010).

5.2 Warburg effect

In contrast to non-cancer cells, which rely primarily on OXPHOS to generate the energy needed for sustained growth, metabolism, and reproduction, most cancer cells instead rely to a large extent on aerobic glycolysis, which is referred to as the Warburg effect (Vander Heiden et al. 2009). Under normal aerobic conditions, a healthy cell transforms glucose into pyruvate in the process called glycolysis. Pyruvate is afterwards further processed in the Krebs cycle and OXPHOS into carbon dioxide, water and energy produced as ATP. Under glycolytic conditions, pyruvate is converted into lactate in an energetically highly unbeneficial reaction. Unlike OXPHOS where 36 molecules of ATP are generated from just single molecule of glucose, only 2 molecules of ATP are formed under conditions of glycolysis.

The first assumption is that cancer cells have damaged mitochondria, so they have to use other biochemical pathways to produce sufficient amount of energy. However, recent studies have shown that mitochondria are, indeed, functional in most cancer cells, albeit in an aberrant manner. A possible reason for this bioenergetic alteration is the requirement to produce more nucleotides, lipids and amino acids to support the rapid growth of tumor cells.

The fact that cancer cells have increased level aerobic glycolysis independently on the availability of the oxygen was discovered Otto H. Warburg in 1924 (Krisher and Prather 2012; Vander Heiden, Cantley and Thompson 2009). Positron emission tomography (PET) has shown increased consumption of glucose, but this technique does not allow to evaluate the actual utilization of glycolysis and OXPHOS in cancer cell. At present, Warburg's “aerobic glycolysis” hypothesis has been challenged by a growing number of studies showing that mitochondria in tumor cells are not inactive *per se* but operate at low capacity or even supply most of the ATP in cancer cells (Jose et al. 2011; Rodríguez-Enríquez et al. 2010; Tan et al. 2016).

Cancer cells also need OXHOS, because mitochondrial metabolism allows for the generation of mitochondria-derived reactive oxygen species that are critical for anchorage-independent growth (Weinberg et al. 2010).

6 Intercellular transfer of mitochondrial DNA

Organelle exchange represents a special form of intercellular communication that allows unidirectional or bidirectional transfer, not only of signals, small molecules or ions, but also of defined intracellular structures such as mitochondria, lysosomes, endosomal vesicles and plasma membrane components. Intercellular transfer of mitochondria or their components is very useful for the cells because it rescues injured cells from mitochondrial dysfunctions, results in the initiation of stem cell differentiation, reprogramming of differentiated cells, or activation of inflammatory signaling pathways (Torralba et al. 2016).

6.1 Cellular mechanism of intercellular mitochondrial transfer

Transfer of mitochondria between cells can be mediated by various mechanisms. These include extracellular microvesicles, mitochondrial ejection, cytoplasmic fusion or GAP junctions. Another possible way is a transfer of mitochondria via the highly sensitive nanotubular structures formed *de novo* between cells, presenting complex networks described as tunneling nanotubes (TNTs) (Berridge et al. 2015; Rustom et al. 2004; Spees et al. 2006; Torralba et al. 2016).

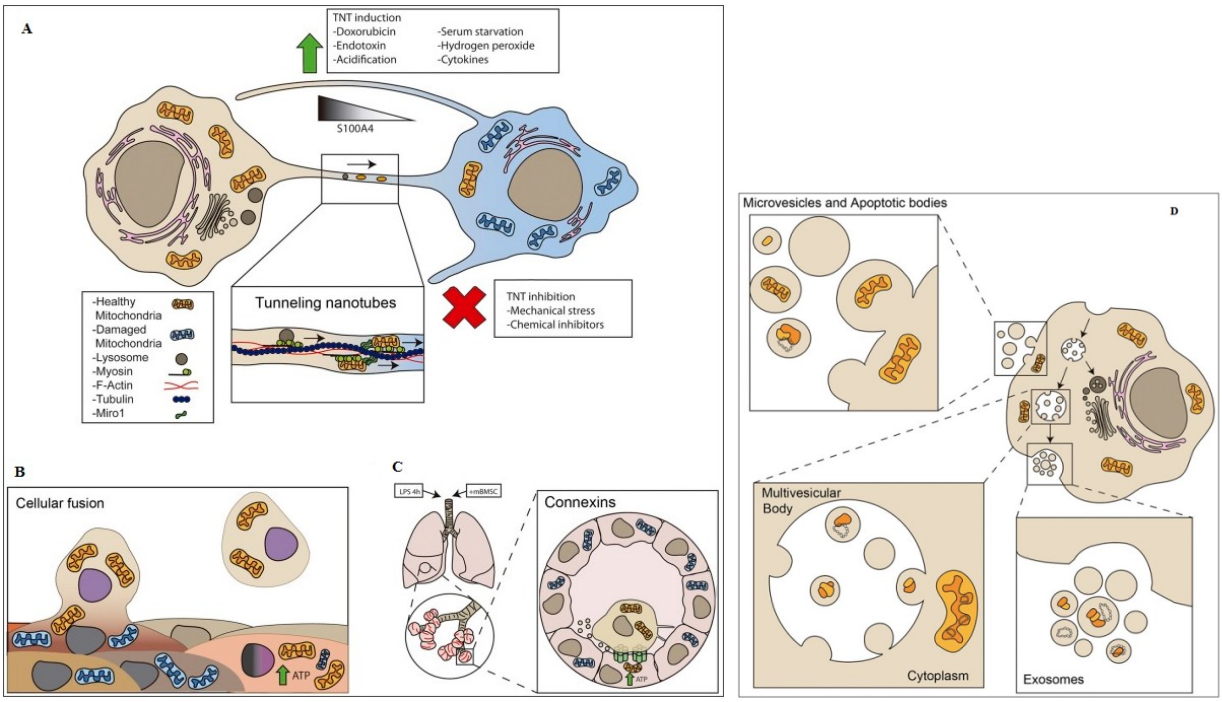

FIGURE 3 - Cellular mechanism of intercellular mitochondrial transfer

A. *Tunneling nanotubes (TNTs)* Ultrathin structures that mediate transfer between cells, containing microtubules and f-actin microfilaments, where the Miro1 protein regulates the efficiency of intercellular movement. **B.** *Cellular fusion* Another process, in which two independent cells fuse their membranes and share the cytosol and all their compounds except the nuclei. **C.** *GAP junctions* Intercellular transfer between BMSCs and alveolar epithelial cells caused by connexins (primarily Cx43). **D**. *Extracellular vesicles* EVs (extracellular vesicles) can contain mitochondrial fragments which include mitochondrial proteins and mtDNA. (Torralba et al. 2016)

6.1.1 Tunneling nanotubes (TNTs)

Tunneling nanotubes are ultrathin actin-based cell-to-cell cytoplasmic connections, which were first described in PC12, a cell line derived from rat pheochromocytoma (Rustom et al., 2004). These structures are usually 50 to 200 nm in diameter and often up to 100 μm in length. They contain actin microfilaments and microtubules, along which cellular organelles such as mitochondria can travel. (Rustom et al. 2004) Rho GTPases play an important role in organelle transfer through TNTs. Miro1 and microtubules have been involved in the transfer of mitochondria upon injury (Ahmad et al. 2014). Transfer of mitochondria through TNTs is usually unidirectional, from the cell that initiated the formation of TNT to the receptor cell (Rustom et al. 2004).

The study from Ahmad et al. showed that the movement of mitochondria from stem cells to recipient cells is regulated by Miro1, which is a protein connecting mitochondria to cytoskeletal motor proteins. Levels of Miro1 regulate the efficiency of intercellular movement of mitochondria: overexpression of Miro1 increased mitochondrial transfer via TNTs. The lack of Miro1 did not prevent formation of TNTs, but mitochondrial movement was significantly decreased (Ahmad et al. 2014).

In various cancer cells, gap-junctional intercellular communication is non-functional and becomes operative upon their differentiation. The transfer of cytoplasmic molecules and organelles across intercellular bridges may help synchronize cell differentiation. In DU 145 cells of human prostate cancer, it was observed that cell-to-cell communication via intercellular bridges can serve as a conduit for exchange of cellular material (Vidulescu et al. 2004).

Certain types of stress agents, such as cigarette smoke, doxorubicin, ethidium bromide, rotenone, or serum starvation, increase formation of TNTs and mitochondrial transfer (Ahmad et al. 2014; Cho et al. 2012; Li et al. 2014; Lou et al. 2012; Wang et al. 2011; Yasuda et al. 2010).

6.1.2 Extracellular vesicles

Extracellular vesicles (EVs) are secreted to the extracellular environment by almost every cell type as mediators of intercellular communication in various physiological and pathological processes. Ranging from 40 to 1,000 nm, they can be divided into exosomes (40–150 nm), microvesicles and apoptotic bodies. Mitochondrial components have been detected in EVs, although the mechanisms by which mitochondrial proteins and mtDNA are loaded in the diverse EVs are still unknown (Torralba, Baixauli and Sánchez-Madrid 2016). EVs participate in the mitochondrial transfer, thus it occurs through the transfer of either mitochondria-derived vesicles or intact mitochondria (Islam et al. 2012; Spees et al. 2006).

6.1.3 Cell fusion

In this process two independent cells fuse their membranes and share organelles and cytosolic compounds, while their nuclei remain intact (Aguilar et al. 2013). It was demonstrated that BMSCs fuse spontaneously with neural progenitors *in vitro*. Furthermore, bone marrow transplantation demonstrates that BMSCs fuse *in vivo* with hepatocytes in the liver, Purkinje neurons in the brain and cardiac muscle in the heart, resulting in the formation of multinucleated cell, which was the first *in vivo* evidence for cell fusion (Alvarez-Dolado et al. 2003).

Other studies improved correction of liver diseases in rat models after transplantation of bone-marrow-derived hepatocytes (Vassilopoulos et al. 2003; Wang et al. 2003). Further, it has been shown that stem cell therapy can cause a better recovery after myocardial infarction, most likely thanks to partial or total cell that takes place between cardiomyocytes and stem cells (Oh et al. 2003).

6.2 Mitochondrial transfer *in vitro*

Most studies used mesenchymal stem cells MSCs) as a donor for intracellular mitochondrial transfer. Mesenchymal stem (stromal) cells are self-renewing multipotent adult stem cells, progenitors of all connective tissue cells. Although usually isolated from the bone marrow (BM), MSCs have been identified also in other tissues and organs, including the skin and liver. In cell culture MSCs expand and differentiate into several tissue-forming cells such as bone, cartilage, fat, muscle, tendon, liver, kidney, heart, and even brain cells. There are unknown factors secreted from the cells with virtually absent mitochondrial function that might activate the MSC, prompting mitochondrial transfer by active cellular processes (Hsu et al. 2016; Jackson et al. 2016).

Several studies with cancer cells devoid of mtDNA and with damaged mtDNA showed transfer of mtDNA *in vitro* between BM-derived stem cells MSCs and tumor cells without mitochondria. It is possible to generate these ρ^0 cell lines that lack OXPHOS *in vitro* usually by long-term cultivation in low doses of ethidium bromide (EtBr). This substance is intercalates preferentially into mitochondrial DNA, which becomes became mutated and depleted, and the cells became incapable of aerobic respiration (Liu et al. 2014). Cancer cells without mitochondrial DNA (ρ^0 cells) that are unable to use their mitochondria for respiration, are able to grow in culture medium supplemented with uridine and pyruvate, although their growth is usually considerably slower than that of parental cells (Tan et al. 2015; Yu et al. 2007).

The first evidence of functional mitochondrial transfer between MSCs and mtDNA-depleted recipient cells was described in co-culture of hMSCs (adult nonhematopoietic stem/progenitor cells) from human bone marrow or skin fibroblasts with A549 ρ^0 (adenocarcinomic human alveolar basal epithelial cells that had defective or deleted mtDNA). The results showed that some of the A549 ρ^0 cells acquired functional mitochondria. The cells with mtDNA from hMSCs were able to proliferate in a restrictive medium similar to the parental cell line with functional mitochondria; genetic analysis of the

rescued clones from the co-cultures demonstrated that 97% of the clones contained mtDNA from the donor cells (Spees et al. 2006).

Another study on human osteosarcoma 143B cells and 143B ρ^0 mtDNA-depleted cells has shown that mitochondrial transfer is triggered by an almost complete absence of mitochondrial function, such as mtDNA depletion or treatment with mitochondrial non-genetic toxin R6G (rhodamine 6G), since transfer is not detected in cells harboring pathogenic mutations that partially affect mitochondrial function (Cho et al. 2012).

In the *in vitro* models of ischemia-reperfusion (the cells were cultured under glucose-oxygen deprivation – OGD and then reoxygenated – RO) MSCs restored cell damage by transferring their mitochondria to HUVECs (human umbilical vein endothelial cells). HUVECs were also co-cultured with MSCs with dysfunctional mitochondria; in this case, mitochondrial function was not restored. This proves that that MSCs have a protective effect. MSCs can thus transfer intact mitochondria using the TNT-like structures to rescue aerobic respiration and prevent the forthcoming cell death (Liu et al. 2014).

Mitochondrial transfer seems to be a distinct way of cell therapy effect for heart disorders. MSCs can donate functional mitochondria to cardiomyocytes and restore their energetic state under the conditions when their own mitochondria are damaged or dysfunctional. If so, this may be an essential repair mechanism of mitochondria in heart cells damaged both under infarction and ischaemia as well as due to genetic defects underlying cardiomyopathies (Plotnikov et al. 2008).

Another evidence of mitochondrial transfer *in vitro* and *in vivo* was observed between human-induced pluripotent stem cell-derived MSCs (iPSC-MSCs) and cells in which damage was induced using cigarette smoke (CS). The study used a CS-exposed rat model and human bronchial epithelial cell line (BEAS-2B), and showed that iPSC-MSCs functionally attenuated CS-induced lung damage and also identified the superiority of iPSC-MSCs over adult bone marrow MSCs in attenuating CS-induced lung damage, which may be attributed to a higher capacity for mitochondrial transfer (Li et al. 2014).

6.3 Mitochondrial transfer *in vivo*

Findings of another study show the first evidence of mitochondrial transfer from BMSCs to lung epithelial cells, and that this phenomenon is critical for the ensuing protective effects against alveolar lung injuries. BMSCs grafted into the trachea of lipopolysaccharide (LPS) - treated mice forming connexin 43 (Cx43)-containing gap junctional channels (GJCs) with the alveolar epithelium, releasing mitochondria-containing microvesicles that the epithelium engulfed. Cx43 regulates inter-cellular mitochondrial transfer by promoting the attachment of BMSCs to LPS-injured alveolar epithelial cells, leading to the formation of TNTs and microvesicles that mediate mitochondrial transfer. These results implicated mitochondrial transfer as a driver of *in vivo* benefits of mesenchymal stem cell therapy in models of acute lung injury and other inflammatory diseases, enhancing cellular bioenergetics and improving organ function (Islam et al. 2012; Torralba et al. 2016).

The host origin of mtDNA was documented in cancer cells isolated from primary tumors derived from 4T1ρ^0 and B16ρ^0 cells based on next generation sequencing (NGS) analysis. The only plausible explanation for this phenomenon is the transfer of mtDNA from host cells to tumor cells with compromised mtDNA (Dong et al. 2017; Tan et al. 2015).

7 Mitocans – mitochondrially targeted anticancer agents

The word "mitocan" came from the terms "mitochondrion" and "cancer". It described a group of small molecules that have anti-cancer activity, which is exerted by interfering with the mitochondrial function (Biasutto et al. 2010; Neuzil et al. 2013).

TABLE 1 - Classification and site of action of individual groups of mitocans

Class	Type	Examples
I	Hexokinase inhibitors	3BP, 2DG, cyclophilin D
II	Compounds targeting Bcl-2 family proteins	gossypol, epigallocatechingallate
III	Thiol redox inhibitors	PEITC, BMH, DTDP, PAO
IV	VDAC/ANT targeting drugs	lonidamine, arsenites, CD437
V	Electron redox chain targeting drugs	α-TOS, MitoVES, tamoxifen,
VI	Lipophilic cations targeting inner membrane	Rhodamine-123, F16
VII	Drugs targeting the tricarboxylic acid cycle	DCA
VIII	Drugs targeting mtDNA	Vitamin K3, MitoVES

7.1 Classification of mitocans

The first group includes inhibitors of hexokinase (ATP: D-hexose 6-phospho-transferase), which are enzymes that catalyze the conversion of glucose into glucose-6-phosphate (G6P), which is a substrate for metabolic pathways ultimately coupled with ATP generation. These enzymes are overexpressed in cancer cells, similarly as found for VDAC, a trans-membrane protein in the OMM (Neuzil et al. 2013).

Two substances, 3-bromopyruvate (3-BP) and 2-deoxyglucose (2-DG), destabilize the complex of hexokinase and VDAC, so that Bax can interact with VDAC to trigger apoptosis (Chen et al. 2009; Neuzil et al. 2007; Simons et al. 2007). Anther way to release hexokinase II from the complex with VDAC may be mediated by inactivation of cyclophilin D via small interfering RNA or through the cyclophilin inhibitor, so that Bax can initiated apotosis (Machida et al. 2006).

The second group of mitocans are compounds targeting the Bcl-2 family of proteins. They include gossypol and epigallocatechingallate. They act as mimetics of the Bcl-2 homology-3 (BH3) domains, integral parts of Bcl-2 family of proteins, binding to the BH3 domains of anti-apoptotic proteins Bcl-2 and Bax_L. Therefore, these anti-apoptotic proteins cannot bind to the Bax and Bak proteins, that have pro-apoptotic activity, so that apoptosis is no longer inhibited (Degli Esposti and Dive 2003; Esposti et al. 2003; Neuzil et al. 2013; Youle and Strasser 2008).

Thiol redox inhibitors PEITC, BMH, DTDP, PAO are the third group of mitocans. They modify the redox states of thiols, which play key role in the membrane permeability in mitochondria. They influence the transmembrane channels VDAC and ANT, which contain two or more cysteine bridges, whose change in conformation can influence their function.

Another group of mitocans are VDAC/ANT targeting drugs lonidamide, arsenic and CD437. They also modify the ANT channel, but unlike the third group, they bind directly to the ANT and cause its conformational change and activation of the PTPC channel (Neuzil et al. 2013).

ETC-targeting drugs form the fifth group of mitocans. These substances influence the components of the ETC. Due to the large electron flow, ETC si also the main producer of ROS, which may selective induce apoptosis. This group includes the vitamin E analog α-tocopheryl succinate (α-TOS) that modulates the function of CII. α-TOS has high affinity to the binding site of ubiquinone, which results in increased combination of electrons and oxygen to form superoxide radicals. The higher level of ROS results in induction of apoptosis. Although α-TOS acts on mitochondria, it does not discriminate between the different membranous compartments within the cell. This was the reason for tagging α-TOS with the positively charged triphenylphosphonium group (TPP^+). This new compound is referred to as mitochondrially targeted vitamin E succinate (MitoVES). Experiments with mouse models with HER2-overexpressing breast cancer showed high anti-tumor effects of MitoVES, which was more effective than α-TOS. Another substance in this group is tamoxifen, which induces apoptosis in breast cancer cells via CI resulting in H_2O_2 production (Neuzil et al. 2013).

The sixth group of mitocans are molecules with delocalized cations such as TPP+ including rhodamine-123 orF16, which accumulate in the mitochondrial matrix more than in the cytoplasm thanks to the negative potential the IMM. This process is much more distinct in the cancer cell with aberrant bio-energetic management (Modica-Napolitano and Aprille 2001; Sommerwerk et al. 2017).

Mitocans of the seventh group act within the Krebs cycle. Dichloro-acetate (DCA) inhibits pyruvate dehydrogenase kinase (PDK), thereby modulating the glycolytic and oxidative metabolism in cancer cells. That is why NADH, the donor of electrons for CI, accumulates in the cell, leading to higher production of ROS. ROS then cause oxidative damage of CI, resulting in induction of apoptosis via the PTPS channel (Neuzil et al. 2013).

The last group of mitocans includes substance that influences mtDNA or the DNA polymerases, which are important enzymes for the maintaining the integrity of the genome. Menandione (VK3), a

synthetic analog of vitamin K, inhibits DNA polymerase γ and causes production of ROS, which leads to cell death. Another substance is MitoVES that inhibits transcription and replication of mtDNA and also proliferation of cancer cells (Aoganghua et al. 2011; Sasaki et al. 2008; Truksa et al. 2015).

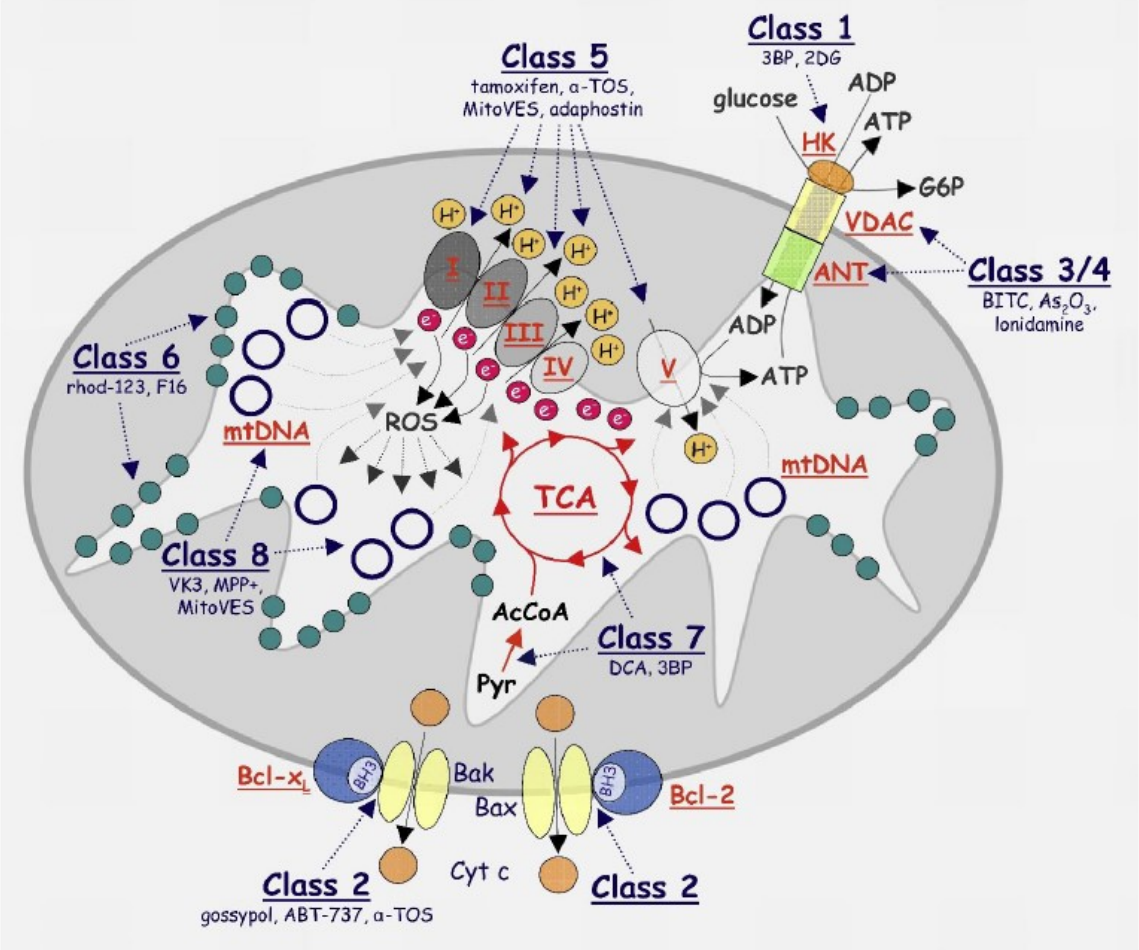

FIGURE 3 - Schematic illustration of the groups of mitocans.

Class 1 - Inhibitors of hexokinases, Class 2 - Compounds targeting Bcl-2 family proteins, Class 3 - Thiol redox inhibitors, Class 4 - VDAC/ANT targeting drugs, Class 5 - Electron-transporting chain targeting drugs, Class 6 - Lipophilic cations targeting the inner membrane, Class 7 - Drugs targeting the tricarboxylic acid cycle, Class 8 - Drugs targeting mtDNA. (Neuzil et al. 2013)

8 Conclusions

Mitochondria are able to pass across cell boundaries and thus be horizontally transferred between cells. This assumption could indicate that genetic defects in mtDNA in various diseases can be rescued by transfer of normally functioning mitochondria or mtDNA from the donor cell to the recipient cell with damaged mitochondrial function. Although the complete physiological relevance of mitochondrial transfer is not known, researchers have shown that donation of mitochondria rescues mitochondrial respiration defects in recipient cells and regulate signaling, proliferation or chemotherapy resistance *in vitro* and *in vivo*.

Due to mitochondrial transfer in the direction of the damaged cells, it is essential to find a way to facilitate the transfer to cells with mitochondrial dysfunctions and exploit its potential in the therapy of various diseases, including cancer. The strategies in cancer therapy could be in the future focused at the ability to interfere with the specificities of cancer cell's bioenergetics. It is necessary to determine the bioenergetics profile of the relevant cancer cell, then discover the specific biochemical pathway that is primarily used for the energy production. In the next step the therapy should efficiently target such a pathway. Tumors can potentially be treated by mitochondrial-targeted drugs that seem to be selective for tumor cells for many different biochemical processes associated with mitochondria, or in the case of glycolytic types of tumors by mitochondrial transfer and subsequent re-activation of mitochondrial oxidative metabolism by means of ‘donated’ mitochondria.

Future research in this field is likely to open new horizons for the treatment of cancer, and I plan to focus on some of its aspects in my future research.

9 References

ADRAIN, C., E. M. CREAGH AND S. J. MARTIN Apoptosis-associated release of Smac/DIABLO from mitochondria requires active caspases and is blocked by Bcl-2. EMBO J, Dec 2001, 20(23), 6627-6636.

AGUILAR, P. S., M. K. BAYLIES, A. FLEISSNER, L. HELMING, et al. Genetic basis of cell-cell fusion mechanisms. Trends Genet, Jul 2013, 29(7), 427-437.

AHMAD, T., S. MUKHERJEE, B. PATTNAIK, M. KUMAR, et al. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. EMBO J, May 2014, 33(9), 994-1010.

ALVAREZ-DOLADO, M., R. PARDAL, J. M. GARCIA-VERDUGO, J. R. FIKE, et al. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature, Oct 2003, 425(6961), 968-973.

ALBERTS B, JOHNSON A, LEWIS J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

AOGANGHUA, A., S. NISHIUMI, K. KOBAYASHI, M. NISHIDA, et al. Inhibitory effects of vitamin K_3 derivatives on DNA polymerase and inflammatory activity. Int J Mol Med, Dec 2011, 28(6), 937-945.

ARIF, T., Y. KRELIN AND V. SHOSHAN-BARMATZ Reducing VDAC1 expression induces a non-apoptotic role for pro-apoptotic proteins in cancer cell differentiation. Biochim Biophys Acta, Aug 2016, 1857(8), 1228-1242.

ASHKENAZI, A., DIXIT, V. M. Death receptors: Signaling and modulation. Science, Aug 1998, 281 (5381),1305-1308.

BERRIDGE, M. V., L. DONG AND J. NEUZIL Mitochondrial DNA in Tumor Initiation, Progression, and Metastasis: Role of Horizontal mtDNA Transfer. Cancer Res, Aug 2015, 75(16), 3203-3208.

BIASUTTO, L., L. F. DONG, M. ZORATTI AND J. NEUZIL Mitochondrially targeted anti-cancer agents. Mitochondrion, Nov 2010, 10(6), 670-681.

BOGENHAGEN, D. F., D. ROUSSEAU AND S. BURKE The layered structure of human mitochondrial DNA nucleoids. J Biol Chem, Feb 2008, 283(6), 3665-3675.

BONAWITZ, N. D., D. A. CLAYTON AND G. S. SHADEL Initiation and beyond: multiple functions of the human mitochondrial transcription machinery. Mol Cell, Dec 2006, 24(6), 813-825.

BOYCE, M., DEGTEREV, A., YUAN, J. Caspases: an ancient cellular sword of Damocles. Cell Death Differ, Jan 2004, 11 (1), 29-37.

BREUER, M. E., W. J. KOOPMAN, S. KOENE, M. NOOTEBOOM, et al. The role of mitochondrial OXPHOS dysfunction in the developement of neurologic diseases. Neurobiol Dis, Mar 2013, 51, 27-34.

CHEN, Z., H. ZHANG, W. LU AND P. HUANG Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. Biochim Biophys Acta, May 2009, 1787(5), 553-560.

CHIASSON-MACKENZIE, C., Z. S. MORRIS, Q. BACA, B. MORRIS, et al. NF2/Merlin mediates contact-dependent inhibition of EGFR mobility and internalization via cortical actomyosin. J Cell Biol, Oct 2015, 211(2), 391-405.

CHINNERY, P. F., H. R. ELLIOTT, G. HUDSON, D. C. SAMUELS, et al. Epigenetics, epidemiology and mitochondrial DNA diseases. Int J Epidemiol, Feb 2012, 41(1), 177-187.

CHO, Y. M., J. H. KIM, M. KIM, S. J. PARK, et al. Mesenchymal stem cells transfer mitochondria to the cells with virtually no mitochondrial function but not with pathogenic mtDNA mutations. PLoS One, 2012, 7(3), e32778.

DEGLI ESPOSTI, M. AND C. DIVE Mitochondrial membrane permeabilisation by Bax/Bak. Biochem Biophys Res Commun, May 2003, 304(3), 455-461.

DONG, L. F., J. KOVAROVA, M. BAJZIKOVA, A. BEZAWORK-GELETA, et al. Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. Elife, Feb 2017, 6.

DRÖSE, S. Differential effects of complex II on mitochondrial ROS production and their relation to cardioprotective pre- and postconditioning. Biochim Biophys Acta, May 2013, 1827(5), 578-587.

ELMORE, S. Apoptosis: a review of programmed cell death. Toxicol Pathol, Jun 2007, 35(4), 495-516.

ESPOSTI, M. D., I. M. CRISTEA, S. J. GASKELL, Y. NAKAO, et al. Proapoptotic Bid binds to monolysocardiolipin, a new molecular connection between mitochondrial membranes and cell death. Cell Death Differ, Dec 2003, 10(12), 1300-1309.

FANG, J., T. SEKI AND H. MAEDA Therapeutic strategies by modulating oxygen stress in cancer and inflammation. Adv Drug Deliv Rev, Apr 2009, 61(4), 290-302.

FREY, T. G., C. W. RENKEN AND G. A. PERKINS Insight into mitochondrial structure and function from electron tomography. Biochim Biophys Acta, Sep 2002, 1555(1-3), 196-203.

GARRIDO, C., L. GALLUZZI, M. BRUNET, P. E. PUIG, et al. Mechanisms of cytochrome c release from mitochondria. Cell Death Differ, Sep 2006, 13(9), 1423-1433.

GOGVADZE, V. AND B. ZHIVOTOVSKY Alteration of mitochondrial function and cell sensitization to death. J Bioenerg Biomembr, Feb 2007, 39(1), 23-30.

GRANGER, M. P., W. E. WRIGHT AND J. W. SHAY Telomerase in cancer and aging. Crit Rev Oncol Hematol, Jan 2002, 41(1), 29-40.

HANAHAN, D. AND R. A. WEINBERG The hallmarks of cancer. Cell, Jan 2000, 100(1), 57-70.

HANAHAN, D. AND R. A. WEINBERG Hallmarks of cancer: the next generation. Cell, Mar 2011, 144(5), 646-674.

HSU, Y. C., Y. T. WU, T. H. YU AND Y. H. WEI Mitochondria in mesenchymal stem cell biology and cell therapy: From cellular differentiation to mitochondrial transfer. Semin Cell Dev Biol, Apr 2016, 52, 119-131.

IMANISHI, H., K. HATTORI, R. WADA, K. ISHIKAWA, et al. Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. PLoS One, 2011, 6(8), e23401.

ISLAM, M. N., S. R. DAS, M. T. EMIN, M. WEI, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nat Med, Apr 2012, 18(5), 759-765.

JACKSON, M. V., T. J. MORRISON, D. F. DOHERTY, D. F. MCAULEY, et al. Mitochondrial Transfer via Tunneling Nanotubes is an Important Mechanism by Which Mesenchymal Stem Cells Enhance Macrophage Phagocytosis in the In Vitro and In Vivo Models of ARDS. Stem Cells, Aug 2016, 34(8), 2210-2223.

JONCKHEERE, A. I., J. A. SMEITINK AND R. J. RODENBURG Mitochondrial ATP synthase: architecture, function and pathology. J Inherit Metab Dis, Mar 2012, 35(2), 211-225.

JOSE, C., N. BELLANCE AND R. ROSSIGNOL Choosing between glycolysis and oxidative phosphorylation: a tumor's dilemma? Biochim Biophys Acta, Jun 2011, 1807(6), 552-561.

KRISHER, R. L. AND R. S. PRATHER A role for the Warburg effect in preimplantation embryo developement: metabolic modification to support rapid cell proliferation. Mol Reprod Dev, May 2012, 79(5), 311-320.

LEE, I., E. BENDER AND B. KADENBACH Control of mitochondrial membrane potential and ROS formation by reversible phosphorylation of cytochrome c oxidase. Mol Cell Biochem, 2002 May-Jun 2002, 234-235(1-2), 63-70.

LENAZ, G. AND M. L. GENOVA Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. Antioxid Redox Signal, Apr 2010, 12(8), 961-1008.

LI, X., Y. ZHANG, S. C. YEUNG, Y. LIANG, et al. Mitochondrial transfer of induced pluripotent stem cell-derived mesenchymal stem cells to airway epithelial cells attenuates cigarette smoke-induced damage. Am J Respir Cell Mol Biol, Sep 2014, 51(3), 455-465.

LIU, K., K. JI, L. GUO, W. WU, et al. Mesenchymal stem cells rescue injured endothelial cells in an in vitro ischemia-reperfusion model via tunneling nanotube like structure-mediated mitochondrial transfer. Microvasc Res, Mar 2014, 92, 10-18.

LLOYD RE, MCGEEHAN JE. Structural Analysis of Mitochondrial Mutations Reveals a Role for Bigenomic Protein Interactions in Human Disease. Bai Y, ed. PLoS One. 2013, 8(7) e69003.

LODISH H, BERK A, ZIPURSKY SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000.

LOU, E., S. FUJISAWA, A. MOROZOV, A. BARLAS, et al. Tunneling nanotubes provide a unique conduit for intercellular transfer of cellular contents in human malignant pleural mesothelioma. PLoS One, 2012, 7(3), e33093.

LÓPEZ-GARCÍA, P., L. EME AND D. MOREIRA Symbiosis in eukaryotic evolution. J Theor Biol, Feb 2017.

MACHIDA, K., Y. OHTA AND H. OSADA Suppression of apoptosis by cyclophilin D via stabilization of hexokinase II mitochondrial binding in cancer cells. J Biol Chem, May 2006, 281(20), 14314-14320.

MARTINON, F. Signaling by ROS drives inflammasome activation. Eur J Immunol, Mar 2010, 40(3), 616-619.

MODICA-NAPOLITANO, J. S. AND J. R. APRILLE Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. Adv Drug Deliv Rev, Jul 2001, 49(1-2), 63-70.

NEUZIL, J., L. F. DONG, L. RAMANATHAPURAM, T. HAHN, et al. Vitamin E analogues as a novel group of mitocans: anti-cancer agents that act by targeting mitochondria. Mol Aspects Med, 2007 Oct-Dec 2007, 28(5-6), 607-645.

NEUZIL, J., L. F. DONG, J. ROHLENA, J. TRUKSA, et al. Classification of mitocans, anti-cancer drugs acting on mitochondria. Mitochondrion, May 2013, 13(3), 199-208.

OH, H., S. B. BRADFUTE, T. D. GALLARDO, T. NAKAMURA, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc Natl Acad Sci U S A, Oct 2003, 100(21), 12313-12318.

PLOTNIKOV, E. Y., T. G. KHRYAPENKOVA, A. K. VASILEVA, M. V. MAREY, et al. Cell-to-cell cross-talk between mesenchymal stem cells and cardiomyocytes in co-culture. J Cell Mol Med, 2008 Sep-Oct 2008, 12(5A), 1622-1631.

RABL, R., V. SOUBANNIER, R. SCHOLZ, F. VOGEL, et al. Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g. J Cell Biol, Jun 2009, 185(6), 1047-1063.

REED, J. C. AND D. R. GREEN Remodeling for demolition: changes in mitochondrial ultrastructure during apoptosis. Mol Cell, Jan 2002, 9(1), 1-3.

RODRÍGUEZ-ENRÍQUEZ, S., L. CARREÑO-FUENTES, J. C. GALLARDO-PÉREZ, E. SAAVEDRA, et al. Oxidative phosphorylation is impaired by prolonged hypoxia in breast and possibly in cervix carcinoma. Int J Biochem Cell Biol, Oct 2010, 42(10), 1744-1751.

ROSSIGNOL, R., B. FAUSTIN, C. ROCHER, M. MALGAT, et al. Mitochondrial threshold effects. Biochem J, Mar 2003, 370(Pt 3), 751-762.

RUSTOM, A., R. SAFFRICH, I. MARKOVIC, P. WALTHER, et al. Nanotubular highways for intercellular organelle transport. Science, Feb 2004, 303(5660), 1007-1010.

SANTIDRIAN, A. F., A. MATSUNO-YAGI, M. RITLAND, B. B. SEO, et al. Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. J Clin Invest, Mar 2013, 123(3), 1068-1081.

SASAKI, R., Y. SUZUKI, Y. YONEZAWA, Y. OTA, et al. DNA polymerase gamma inhibition by vitamin K3 induces mitochondria-mediated cytotoxicity in human cancer cells. Cancer Sci, May 2008, 99(5), 1040-1048.

SAZANOV, L. A. A giant molecular proton pump: structure and mechanism of respiratory complex I. Nat Rev Mol Cell Biol, Jun 2015, 16(6), 375-388.

SCHON, E. A., S. DIMAURO AND M. HIRANO Human mitochondrial DNA: roles of inherited and somatic mutations. Nat Rev Genet, Dec 2012, 13(12), 878-890.

SENNINO, B. AND D. M. MCDONALD Controlling escape from angiogenesis inhibitors. Nat Rev Cancer, 10 2012, 12(10), 699-709.

SIMONS, A. L., I. M. AHMAD, D. M. MATTSON, K. J. DORNFELD, et al. 2-Deoxy-D-glucose combined with cisplatin enhances cytotoxicity via metabolic oxidative stress in human head and neck cancer cells. Cancer Res, Apr 2007, 67(7), 3364-3370.

SOLMAZ, S. R. AND C. HUNTE Structure of complex III with bound cytochrome c in reduced state and definition of a minimal core interface for electron transfer. J Biol Chem, Jun 2008, 283(25), 17542-17549.

SOMMERWERK, S., L. HELLER, C. KERZIG, A. E. KRAMELL, et al. Rhodamine B conjugates of triterpenoic acids are cytotoxic mitocans even at nanomolar concentrations. Eur J Med Chem, Feb 2017, 127, 1-9.

SPEES, J. L., S. D. OLSON, M. J. WHITNEY AND D. J. PROCKOP Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci U S A, Jan 2006, 103(5), 1283-1288.

SUN, F., X. HUO, Y. ZHAI, A. WANG, et al. Crystal structure of mitochondrial respiratory membrane protein complex II. Cell, Jul 2005, 121(7), 1043-1057.

TAN, A. S., J. W. BATY, L. F. DONG, A. BEZAWORK-GELETA, et al. Mitochondrial genome acquisition restores respiratory function and tumorigenic potential of cancer cells without mitochondrial DNA. Cell Metab, Jan 2015, 21(1), 81-94.

TAYLOR, R. C., S. P. CULLEN AND S. J. MARTIN Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol, Mar 2008, 9(3), 231-241.

TORRALBA, D., F. BAIXAULI AND F. SÁNCHEZ-MADRID Mitochondria Know No Boundaries: Mechanisms and Functions of Intercellular Mitochondrial Transfer. Front Cell Dev Biol, 2016, 4, 107.

TRUKSA, J., L. F. DONG, J. ROHLENA, J. STURSA, et al. Mitochondrially targeted vitamin E succinate modulates expression of mitochondrial DNA transcripts and mitochondrial biogenesis. Antioxid Redox Signal, Apr 2015, 22(11), 883-900.

VANDER HEIDEN, M. G., L. C. CANTLEY AND C. B. THOMPSON Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science, May 2009, 324(5930), 1029-1033.

VASSILOPOULOS, G., P. R. WANG AND D. W. RUSSELL Transplanted bone marrow regenerates liver by cell fusion. Nature, Apr 2003, 422(6934), 901-904.

VIANELLO, A., V. CASOLO, E. PETRUSSA, C. PERESSON, et al. The mitochondrial permeability transition pore (PTP) - an example of multiple molecular exaptation? Biochim Biophys Acta, Nov 2012, 1817(11), 2072-2086.

VIDULESCU, C., S. CLEJAN AND K. C. O'CONNOR Vesicle traffic through intercellular bridges in DU 145 human prostate cancer cells. J Cell Mol Med, 2004 Jul-Sep 2004, 8(3), 388-396.

VON BALLMOOS, C., A. WIEDENMANN AND P. DIMROTH Essentials for ATP synthesis by F1F0 ATP synthases. Annu Rev Biochem, 2009, 78, 649-672.

WALLACE, D. C. Mitochondria and cancer. Nat Rev Cancer, Oct 2012, 12(10), 685-698.

WANG, X. AND H. H. GERDES Transfer of mitochondria via tunneling nanotubes rescues apoptotic PC12 cells. Cell Death Differ, Jul 2015, 22(7), 1181-1191.

WANG, X., H. WILLENBRING, Y. AKKARI, Y. TORIMARU, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature, Apr 2003, 422(6934), 897-901.

WANG, Y., J. CUI, X. SUN AND Y. ZHANG Tunneling-nanotube developement in astrocytes depends on p53 activation. Cell Death Differ, Apr 2011, 18(4), 732-742.

WATSON, C. J. Involution: apoptosis and tissue remodelling that convert the mammary gland from milk factory to a quiescent organ. Breast Cancer Res, 2006, 8(2), 203.

WEINBERG, F., R. HAMANAKA, W. W. WHEATON, S. WEINBERG, et al. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. Proc Natl Acad Sci U S A, May 2010, 107(19), 8788-8793.

WIEMERSLAGE, L. AND D. LEE Quantification of mitochondrial morphology in neurites of dopaminergic neurons using multiple parameters. J Neurosci Methods, Mar 2016, 262, 56-65.

WITSCH, E., M. SELA AND Y. YARDEN Roles for growth factors in cancer progression. Physiology (Bethesda), Apr 2010, 25(2), 85-101.

YASUDA, K., H. C. PARK, B. RATLIFF, F. ADDABBO, et al. Adriamycin nephropathy: a failure of endothelial progenitor cell-induced repair. Am J Pathol, Apr 2010, 176(4), 1685-1695.

YOULE, R. J. AND A. STRASSER The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol, Jan 2008, 9(1), 47-59.

YU, M., Y. SHI, X. WEI, Y. YANG, et al. Depletion of mitochondrial DNA by ethidium bromide treatment inhibits the proliferation and tumorigenesis of T47D human breast cancer cells. Toxicol Lett, Apr 2007, 170(1), 83-93.

ZHIVOTOVSKY, B., L. GALLUZZI, O. KEPP AND G. KROEMER Adenine nucleotide translocase: a component of the phylogenetically conserved cell death machinery. Cell Death Differ, Nov 2009, 16(11), 1419-1425.

ZICKERMANN, V., C. WIRTH, H. NASIRI, K. SIEGMUND, et al. Structural biology. Mechanistic insight from the crystal structure of mitochondrial complex I. Science, Jan 2015, 347(6217), 44-49.