Mitochondria and cancer relationship

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Cancer cells are characterized by an altered metabolism as compared with normal cells, on the point of view cancer as a metabolic disease, detected firstly by Otto Warburg in 1924, known as Warburg effect. Aerobic glycolysis is the central metabolic event involved in the carcinogenesis, and mitochondria are the main component of this process. The most crucial role of mitochondria in cancer is related to the recent evidences of the involvement in cell immortality and induction of metastasis from their origin site, spreading transformed cells for elsewhere in the body, originating other cancers. Summarizing, mitochondrial dysfunction has a main role in many diseases including cancer; however, the mechanisms involved are not fully known, in order to detect effective therapeutic targets. There are several interesting researches in progress, to throw light in understanding these mechanisms and the microscopy, together with other tools, gives doubtless a great contribution. Some recent references of methodologies for mitochondrial visualization in normal and cancer cells are presented, based on distinct technologies, complementing the review.

Keywords: Mitochondria; cancer; metastasis; Warburg effect; biosynthetic pathways; ion channels; therapeutic targets; cell death; apoptosis; microscopy

1. Introduction

Mitochondria were firstly described as sarcosomes, by Rudolf Albrecht von Koelliker in 1857, a Swiss anatomist. Richard Altman, a German pathologist suggested the condition of intracellular parasites in 1890; and Carl Benda, a German microbiologist designated these organelles as mitochondria in 1898 [1]. Beyond compartmentalizing the cellular bioenergetics processes of great complexity, mitochondria participate of several important activities like cell death by apoptosis, signal transduction pathways in the redox control of gene expression and biosynthetic pathways. Also, mitochondrial ion channels can influence organelle membrane potential, calcium homeostasis, ROS (reactive oxygen species) production, volume, and maybe morphology [2-5]. The mitochondrial involvement in cell immortality and induction of metastasis, spreading tumour cells for elsewhere in the body, given rise to other cancers is really challenger for researchers. Thus, mitochondrial dysfunction has a main role in many diseases including cancer; however, the mechanisms involved are not fully known, in order to detect effective therapeutic targets [6-7].

This review has aiming to present data of some advanced researches about mitochondria and its participation in cancer, to throw light in understanding these mechanisms. Some methodologies for mitochondrial visualization in normal and cancer cells, through confocal microscopy and transmission electron microscopy are presented, emphasizing the contributions of the microscopy methods in advances in the scientific research and education.

"All truths are easy to understand once they are discovered; the point is to discover them." Galileo Galilei (1564-1642)

2. Mitochondrial origin

Nowadays is well accepted the concept that eukaryotic cells evolved from bacterial ancestors by a series of symbiosis, and the origin of eukaryotic cells is seen as a special case of a general phenomenon, the evolution of microbial associations. Thus, the symbiosis drives to the innovation, and as opposed to prokaryotes, all eukaryotic cells are polygenic; because they contain several kinds of organelles that harbouring distinct genetic systems [9-12]. In an evolutionary concept, three classes of cellular organelles were once free-living bacteria: cilia, mitochondria, and chloroplasts [13-17]. The coevolution of partner species started around 3500 million years ago and is in course since then, until now [9].

Wallin wrote in 1927 about the mitochondrial legacy and cytoplasmic heredity, that "*The evidence for calling mitochondria bacteria, rests upon the following attributes: Their general behaviour in the cell is similar to that of known microorganisms which live symbiotically in the cells of higher organisms; for example the root-nodule bacteria of legumes. When grown independently in artificial culture media, they behave in all observed particulars like bacteria. They divide like bacteria. They are similar to bacteria in structure and shape. They exhibit no cultural characteristics foreign to bacteria.*" [9, 16-17].

Phylogenetically, mitochondria are closer relatives to proteobacteria (or purple bacteria), than they are to archaebacteria or other bacteria, either to the other cellular components in which they are integrated [9, 18-19]. Considering that all mitochondria are constituted by DNA, RNA, and ribosomes, the molecular mass of the DNA

around 10,000 to 100,000 kDa is not enough to codify all the proteins found in animal mitochondria. This fact raises the proposition that the completely developed mitochondria, as we currently know it, are products of the interaction of at least two genomes, mitochondrial and nuclear [9, 20]. Mitochondria have double-stranded circular DNA, composed by 37 genes that coordinate the production of enzymes and molecules of which the cell need to generate power, through Adenosine-Tri-Phosphate (ATP). Throughout the evolution of eukaryotic cells, some kinds of interaction between endosymbionts and nuclear DNA occurred, and currently the mitochondrial DNA (mtDNA) has 13 critical OXPHOS genes and the nuclear DNA (nDNA) has a complementary set at around 80 OXPHOS genes, required for ATP production, and some other genes related to the mitochondrial biogenesis and metabolism [4, 9].

The 16S ribosomal RNA from proteobacteria is also present in mitochondria. Through DNA primers like 16S ribosomal RNA gene, it is possible to assess samples of distinct surroundings concerning to its microbial inhabitants. A computational comparison of 13 samples from the Archaean, Bacteria until Eukarya have identified that 16S and 23S ribosomal RNA genes, and some transfer RNA can be considered the most conserved segments of DNA sequences across phylogeny, to be used to detect life on Earth and in other planets [9, 18-21].

In eukaryotic cells occur an exchanging of material in both senses, into and out from the nucleus to the cytoplasm, and vice versa; thereby, the DNA of mitochondria and chloroplasts are imported and integrated into the nuclear genome. The organellar sequences are inserted into nucleus during the repair process of double-stranded breaks. The inserted organellar DNA is modified by point mutations and deletions, leading sometimes to heritable diseases in humans. It is important to observe that this is not a rare event, with impact on nuclear genetic organization, affecting cellular metabolic processes. Under these circumstances, arises the question whether the mitochondrial DNA is beneficial to gene evolution [10-12].

There are many researchers around the world working and producing recent scientific advances, aimed the human evolution toward a future generation, free of existing hereditary diseases, hitherto known. The transmission of mitochondria occurs during the fertilization of the ovum to produce the zygote. Thus, inheritance of normal or dysfunctional mitochondria is attributed only by the maternal transmission for the progeny. Thinking about this, and in order to avoid the transmission of dysfunctional mitochondria, a strategy presented in the article published in 2014; where three-parent are necessary for *in vitro* fertilization, through gene replacement for the prevention of inherited mitochondrial diseases [22]. In addition, concerns about the efficacy of methodology, safety, ethic aspects and the regulatory issues are discussed [23].

3. Warburg effect

Otto Heinrich Warburg was born in Freiburg, Germany, in October 8, 1883; living until August 1, 1970; and was laureate with the Nobel Prize in Physiology or Medicine 1931 for his discovery of the nature and mode of action of the respiratory enzymes [24]. He was the first to observe that determined malignant cells were able to produce lactate from glucose in an atmosphere with normal levels of oxygen [25-27].

At this point, it is important to consider that fermentation is a type of anaerobic respiration, which occurs in the absence of oxygen. Respiration is the process of energy production in the cell by combustion of glucose. All combustion requires the presence of oxygen. In the case of anaerobic respiration, or fermentation more specifically, what happens is a conversion of one molecule to another, resulting in energy production in the absence of oxygen. For example, the conversion of pyruvate in lactate or lactic acid (Fig.1a), this type of respiration produces 2 ATP molecules, while aerobic respiration yields 36 ATP molecules for the cell.

Assaying the energy of respiration and fermentation, Warburg used ascites cancer cells from mouse to obtain an average respiration of 7 mm^3 of oxygen-consumed per mg/hour, and fermentation of 60 mm^3 of lactic acid-consumed per mg/hour. Converting in energy equivalents, means that cancer cells can obtain approximately the same amount of energy from fermentation as from respiration, whereas the normal body cells obtain much more energy from respiration than from fermentation. Exemplifying, the liver and kidney of an adult animal obtain hundred times, as much energy from respiration as from fermentation [27].

Warburg's researches describe the mechanisms of enzymes activity involved in the cellular respiration. These findings contributed to understand the metabolism of cancer cells in the absence of oxygen, until then unknown.

It was attributed the designation of Aerobic Glycolysis to this process, becoming known as Warburg Effect, which is the central metabolic event involved in the carcinogenesis, and mitochondria are the main component of this process (Fig.1b).
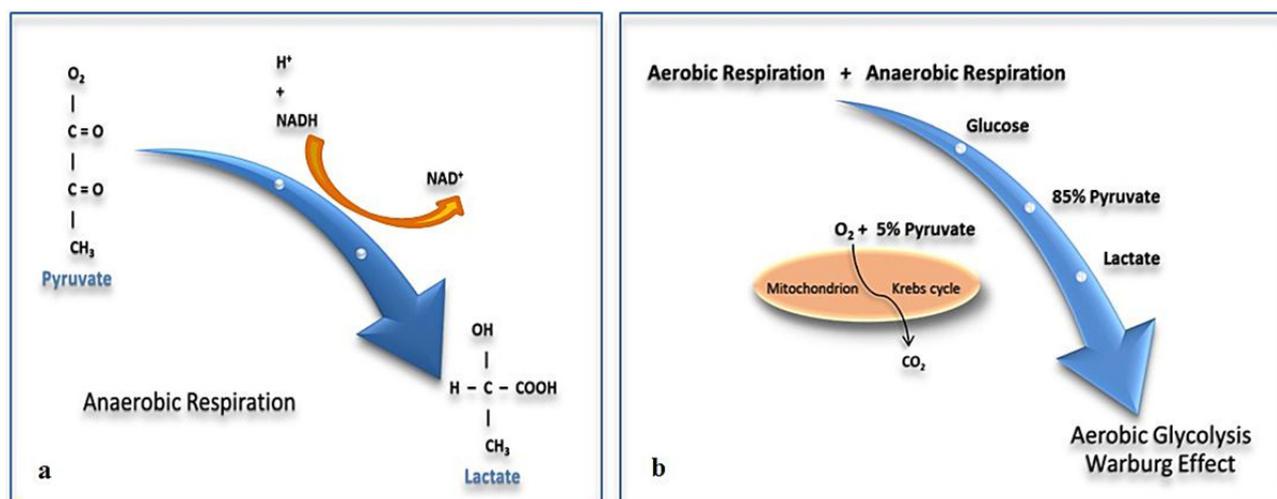
Fig. 1 Simplified schematic representation of Warburg Effect. **a.** Chemical reaction in one of the fermentation process, related to the conversion of pyruvate in lactic acid or lactate. This process is also known by anaerobic respiration and yields 2 ATP molecules per pyruvate molecule. **b.** Aerobic glycolysis yields 4 ATP molecules per glucose molecule. Aerobic respiration via mitochondrial enzymes of the Krebs cycle, also known as Tri Carboxylic Acid (TCA) cycle, or Citric Acid cycle, plus anaerobic respiration together yields 36 ATP molecules for cells.

So, the ability of cancer cells to convert glucose in lactate in the presence of oxygen represents aerobic glycolysis or Warburg effect, distinguished from anaerobic glycolysis, where occurs like an adaptive response to low oxygen tension or hypoxia. In this case, adaptive responses promote hypoxia-inducible factor1 (HIF-1) and cell autonomous changes, which activate AKT, HIF-1, MYC or RAS oncogenes or inactivate the tumour suppressor P53 or VHL, all contributing to the Warburg effect [28].

Therefore, the metabolic degradation of glucose, one sugar composed by six carbons, is degraded in two molecules of pyruvate, composed by three carbons. This degradation occurs in a series of steps, catalysed by one of the specific enzymes. The intermediate product of each step serves as substrate to the next reaction. The metabolism of pyruvate subsequent to form carbon dioxide and water depends on a second series of reactions known as Krebs cycle. The Nobel Prize in Physiology or Medicine 1953 was divided equally between Hans Adolf Krebs for his discovery of the citric acid cycle, and Fritz Albert Lipmann for his discovery of co-enzyme A and its importance for intermediary metabolism [29].

Each pyruvate molecule is first converted in one acetate molecule with two carbons. Each molecule is combined with one molecule containing four carbons, named oxaloacetate, to produce another with six carbons. This new molecule is degraded in a series of steps, regenerating the oxaloacetate molecule, which combines with another acetate molecule, following to a new return to this cycle. In each two series of reactions occurs CO_2 liberation; these CO_2 together with another liberated in the pyruvate transformation into acetate correspond to the three carbons of the pyruvate molecule.

In five reactions of the Krebs cycle, the hydrogen atoms are transferred to coenzymes, NAD (nicotinamide-adenine-dinucleotide) or FAD (flavin-adenine-dinucleotide). In these reactions, coenzymes receive hydrogen atoms enzymatically free from substrates, being reduced to $NADH_2$ and $FADH_2$. These hydrogen atoms are transferred eventually to the oxygen.

The steps of glucose and pyruvate are anaerobic, i.e., do not require oxygen to occur. Almost all known organisms are able to degrade sugars by anaerobic via similar to described here. Nevertheless, there are cells that convert pyruvate molecules anaerobically in lactate molecules; this glucose to lactate via is called glycolysis. Thereafter, the aerobic metabolism of glucose, by Krebs cycle via uses the same steps of glucose to pyruvate, like in glycolysis.

The main source of cellular ATP is provided by correlated sugars and other carbohydrates. ATP is formed by the addition of one phosphate grouping to the ADP (adenosine diphosphate). The chemical binding formed in this reaction is designated as high-energy binding; and requires an energy input derived from metabolism. When this binding is broken, the energy became available. ATP is the main molecular form, by which the energy is stored and transferred to required places for the efficiency of cellular functions.

Reduced coenzymes are also carriers of metabolic energy. In the glucose metabolism, coenzymes are reduced in several reactions. These reduced coenzymes can be used in a special sequence of reactions that generate ATP, summarized as follows: 1) Electrons of hydrogen atoms carried by coenzymes enter at the sequence of reactions known as electrons transport. This involves an enzyme group denominated as respiratory chain; 2) Terminal reaction of the series results in the electron transfer plus the protons (H^+ ions) of hydrogen atoms until oxygen. This reaction produces water; 3) Many ATP is formed during electron transport. ATP formation process is called oxidative phosphorylation and establishes a binding between the oxygen consumption and the addition of phosphate to the ADP [30].

Thus, the aerobic via produces more ATP than anaerobic via; which reflects mainly in the coenzymes production in the Krebs cycle. The linkage of glucose metabolism to the ATP production by anaerobic cells involves three distinct sequences of reactions: pyruvate formation; degradation of pyruvate molecules in the Krebs cycle; the electron transport and the oxidative phosphorylation. The Nobel Prize in Chemistry 1978 was awarded to Peter D. Mitchell for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmosis theory [31]. For additional details of these mechanisms, see Lehninger [32].

Doctor of Chemistry (Berlin, 1906) and Medicine (Heidelberg, 1911), Warburg was able to detect the excess of lactate produced by tumour cells, even in the presence of oxygen, and he has supposed that this event was related to a mitochondrial dysfunction. Later it was confirmed that mitochondrial function is crucial for cancer cells viability [4].

Currently, from knowledge of the Warburg effect in cancer, associate with new technologies, like using hyperpolarized ^{13}C pyruvate and magnetic resonance spectroscopic imaging (MRSI), or Hyperpolarized [1- ^{13}C] pyruvate MRSI; and an analogue of the glucose, [Fluorine-18]-2-fluoro-2-deoxy-D-glucose (FDG), through positron emission tomography (PET) / computerized tomography (CT), or FDG-PET-CT system, it is possible to detect cancer cells, evaluate its metabolism and guide therapies [33-37].

4. Cell death

Mitochondria have a central role in programmed cell death, in the cell immortality and cancer, and providing power to the entire organisms work satisfactorily. These amazing organelles are composed of two membranes and a protein complex capable of converting approximately 10,000 to 50,000 times more energy per second than the sun, *mutatis mutandis* [38]. They were isolated firstly from fragmented cells, by the Belgium biochemist Albert Claude in 1944, through centrifugation and demonstrated that mitochondria catalyse the respiratory process [39-40]. After a long scientific career, he was laureate with the Nobel Prize in Physiology or Medicine 1974, together with George Palade and Christian De Duve, for their discoveries concerning the structural and functional organization of the cell [41].

Are Claude's reflexions: "*The power of respiration exists in a discrete state in the cytoplasm, a fact which led me to suggest that… mitochondria may be considered as the real power plants of the cell.*" [38, 42-43]. His researches opened new perspectives for mitochondrial bioenergetics studies in membrane-bound proteins, membrane potential, and to exploit the mitochondrial matrix and all its components.

As we can see, evolution of knowledge is multidisciplinary and walks side by side with the evolution of the technology. Exactly for it, each finding needs to be evaluated inside the context of time they were performed. Today is possible to say that mitochondria have crucial participation in the normal cell life, in cell death and in cell immortality, leading to the carcinogenesis. This is based on new knowledge from new methodologies that have emerged due to new reagents and more powerful equipment, including here the internet and all facilities related to the global bibliographic organization and accessibility.

Given the fact that cancer cells produce abnormally high rates of glycolysis and lactate in the presence of oxygen, Warburg suspected the existence of defects in mitochondrial functions, which would be responsible for the malignant transformation of cells. New studies assayed after the Warburg's era are demonstrating that the cancer as a metabolic disease occurs in some particular cases with the direct mitochondrial involvement, but not in general cancers. So, mitochondria have no direct effect in all of the carcinogenesis events, however, cancer cells can be benefited with the alterations in metabolism providing high levels of ATP production for the tumour's maintenance. Devoid of all the resources that we currently have, Warburg holds the merit to have established a correlation between cellular metabolism, mitochondrial bioenergetics, and tumour genesis almost seven decades ago [1, 4].

4.1 Apoptosis

Apoptosis or programmed cell death arose inside of the new concepts about mitochondria and its cancer relations. Thus, apoptosis can be seen as an intelligent mechanism of the body to get rid of unwanted or senescent cells, aiming to prevent damages for itself and on the opposite side, cancer cells develop distinct strategies to be kept in the body, in order to get benefits for themselves, in detriment of the body damages. In the dilemma that occurs within the body, many times the disease is the winner and begins the personal dilemma to find the cure.

In 1972, Kerr and collaborators proposed by first time the term apoptosis, to describe the mechanism of controlled cell deletion, well studied by light and electron microscopy [44-45]. They observed that the process could be complementary but with opposite role to mitosis in the regulation of animal cell populations, suggesting that this is a programmed phenomenon inherently active and may be initiated or inhibited by environmental stimuli of physiological and pathological nature. Morphologically apoptosis occurs in two stages: 1) nuclear and cytoplasmic condensation and cellular fragmentation, showing well preserved fragments seen by ultrastructure, linked at the nuclear and cytoplasmic membranes; 2) these fragments called apoptotic bodies are eliminated from the epithelium or surfaces coated and absorbed by phagocytes; which are attracted by the phosphatidylserine exposed on the membrane surface of apoptotic bodies, where they undergo a series of changes that resemble *in vitro* autolysis within phagosomes, and are rapidly degraded by lysosomal enzymes derived from cells that had ingested them [44].

The puzzle assembly of the apoptosis is running since then, until the present. Frequently a new molecule is found to be inserted as a new part to elucidate the process of high complexity. Nowadays, it is possible to say that some intrinsic stimuli can give the start for the mitochondrial release of the enzyme cytochrome c to the cytoplasm, or by extrinsic factors involving the activation of cell surface membrane receptors, like Fas and TNF (tumour necrosis factor) [46]. Fas are a surface receptor also known as APO-1, CD95, or TNFSF6, involved in apoptotic signal transmission. Fas interact with FasL, its natural ligand, starting the death signals cascade, which follows until the apoptotic cell death [47]. The next step is the formation of multiprotein complexes, the death-inducing signaling complex (DISC) and the apoptosome. These complexes have the compromise to enrol caspases effectors and to activate them efficiently, like caspase-8 for the extrinsic and caspase-9 for the intrinsic pathways, which converge into activation of the executors' caspase-3, caspase-6 and caspase-7 [48].

Described firstly by Robert Horvitz and collaborators in 80's decade, caspases are proteases constituted by cysteine; which are enzymes containing one cysteine residue able to cleavage other proteins after one aspartic acid residue, an uncommon specificity between proteases. Caspase is a denomination derived from this molecular characteristic: cysteine-aspartic-acid-proteases [49]. Studying nematodes *Caenorhabditis elegans* since then, they found recently that Xk-related protein 8 (Xkr8) and CED-8 (*C. elegans* protein, is the unique equivalent to Xk-family proteins) promote phosphatidylserine (PtdSer) exposure in response to apoptotic stimuli, supported by assays using mouse Xkr8-/- cells or human cancer cells in which Xkr8 expression was repressed by hypermethylation, they failed in to expose PtdSer ("eat me" signal) at the cell surface to start apoptosis and were inefficiently engulfed by phagocytes [50].

Apoptosis represents hitherto the cell death program most in use for therapeutic strategies search in cancer. The programmed cell death is generally activated in response to a specific stimulus, and the intrinsic apoptotic pathway starts with mitochondrial release of cytochrome c, which implicated furthermore in the Bcl-2 family involvement, through the modulation of anti-apoptotic *versus* pro-apoptotic members interactions [46]. These interactions will be responsible for cell destiny, if it will die or live. Bcl-2 (B cell lymphoma-2) family proteins and their effectors are known for act during apoptosis in the process of mitochondrial outer-membrane permeabilization (MOMP) induction. They operate through protein-protein interaction, and recent studies are demonstrating the existence of structural plasticity in the Bcl-2 proteins to achieve their functionality [51-52].

At this point, BH3-only proteins become like that important keys in the MOMP process. BH3-only proteins belong to the Bcl-2 family proteins, constituting essential inhibitors of apoptosis responsible by to disseminate extrinsic and intrinsic cell death signals [53]. Classified in canonical and non-canonical interactions, based on the ability of commitment or absence of the BH3 and C-terminus binding (BC) groove, this is a conserved, hydrophobic groove on the surface of the Bcl-2 core, positioned on one side of the globular domain by the BH1-BH3 regions. Canonical interactions at the BC groove involve binding of the BH3 ligands to form hetero-dimeric or homo-oligomeric complexes. Non-canonical interactions abroad the BC groove can allosterically activate or inhibit effectors, mediate effector oligomerization, modulate the affinity of BH3-only proteins for BC groove of anti-apoptotic binding partners, or control interactions with binding partners abroad the Bcl-2 family members [51, 53-54]. Accordingly, BH3-only proteins can act directly over activation of Bax and Bak, or by suppressing the anti-apoptotic proteins at the mitochondria and/or endoplasmic reticulum. In order to avoid formative cell death, BH3-only proteins are regulated by several mechanisms that include transcriptional and post-transcriptional changes, which control specific protein-protein interactions [53].

4.2 Autophagy

Another relevant pathway of cell death is called autophagy, which have also starts by a signal controlled by BH3-only proteins. It seems that after activation, BH3-only proteins interact with mitochondrial and/or endoplasmic reticulum membranes, controls linkage to other Bcl-2 family members, directing for a defined function [46, 53].

Autophagy (ATG) or autophagocytosis is a catabolic lysosomal via well conserved through evolution, responsible by recycling of intracellular components and organelles, with several roles in the control of tumour genesis. Thus, the process of autophagy has a tumour suppressive effect or anti-tumour action, being able to blockage the beginning of oncogenesis by delimitation of the cytoplasmic damage, genomic instability and inflammation, beyond to the loss of certain autophagy's genes that can lead to cancer. On the other hand, autophagy is able to aid cells to overcome stressful metabolic environments, providing cancer cells survival. Indeed, several kind of cancer has need of autophagy to stay alive and evolve. Therefore, autophagy plays a significant role in cancer, because it is also linked to the major cancer networks, which include those directed by p53, RAS, mammalian target rapamycin (Tor/mTOR), and glutamine metabolism. ATG can be induced by internal or external stimuli, may be inhibited by nutrient-rich conditions and hence stimulated by innutrition. Also, ATG has mTOR as an important key regulator of induction, aside of the innutrition or mTOR inhibitors e.g. rapamycin [55-56].

The central structure of ATG process is constituted for at least 30 ATG-related proteins, and for the autophagosome's generation is necessary the composition of 2 complexes, as follow, ATG6 (Beclin1) interaction with the Class III P13K proteins complexes and ATG14, which form the first complex, and ATG12, ATG16, ATG5, ATG7, forming the second complex. This step is crucial for the involvement of ATG8 (LC3) in the induction of autophagy, and owing to this,

cytosolic LC3-I (ATG8) is cleaved and lipidated to structure LC3-II. Therefore, LC3 is a manufacturer of the autophagosome membrane. In the next step, the fusion between autophagosome and lysosome and the subsequent breakdown of autophagic vesicle is less well defined, although there is an essential role for the LAMP2 protein in this process. So, similar to that occurs in apoptosis, autophagy is also a complex puzzle, which is being deciphered. Since 2008, Landes Bioscience has publishing guidelines for accessing autophagy, recommended for additional information [57-60].

Summarizing, the visualization of intersecting points between autophagy and apoptosis occurred after the elucidation of Beclin-1 and its associated molecules, which led to the Bcl-2 recognition as a core regulator in both processes, through the interaction with Beclin-1 and Bax/Bak respectively. On some cell stimuli, Bcl-2 is shifted from Beclin-2 and Bax, leading to the start of autophagy and apoptosis. Bcl-2 inhibits autophagy whenever situated in the mitochondria and endoplasmic reticulum. In sequence, the complexes' breaking occurs by BH3-only proteins and by post-translational changes. Mitochondria participate in both cell death processes; however, the bonding mechanisms between them have not been completely elucidated [61].

Table 1   Effectors mediate MOMP [51]

Pro-apoptotic family members [Class I]
Bcl-2 antagonist killer 1 (BAK)
Bcl-2-associated X protein (BAX)
Bcl-2-related ovarian killer (BOK)
Anti-apoptotic family members [Class II]
Bcl-2
Bcl-xL
Bcl-w
Myeloid cell leukaemia 1 (Mcl-1)
Bcl-2-related gene A1 (A1)
Subfamily BH3-only proteins [Class III] [sharing only the third BCL-2 homology (BH) domain]
Class III regulates Classes I and II by activating the pro-apoptotic effectors, for example:
Bcl-2 interacting domain death agonist (BID)
Bcl-2 interacting mediator of cell death (BIM)
p53-upregulated modulator of apoptosis (PUMA)
or, by blocking the activity of the anti-apoptotic proteins without directly engaging the pro-apoptotic effectors, for example:
Bcl-2 antagonist of cell death (BAD)
Bcl-2 interacting killer (BIK)
Bcl-2 modifying factor (BMF)
Derepressors or sensitizers: Hara-kiri (HRK) and Noxa, because they disrupt existing anti-apoptotic complexes or occupy binding sites in anti-apoptotic proteins, respectively, without directly causing MOMP.

5. Cell immortality and cancer

The maintenance of the homeostatic balance amongst cell survival and death is considered crucial for the establishment of the oncogenic process [46]. Through of the genetics point of view, cancers are result of a mutations succession and epimutations (epigenetics changes) in the genome of normal cells, decisive for their destiny. It seems that epigenetic mechanisms can accomplishes the activation of oncogenes, inactivation of tumour suppressor genes and cause genomic instability.

A landmark recorded in cancer biology studies in the 1940 decade with the researches performed by Earle's team, was about the production of malignancy *in vitro* [62-63].

Alterations in the DNA base sequence typical in genetic mutations do not occur in epimutations, which seem to be reversionary. Conversely, epimutations and genetic mutations share specific patterns of gene expression, which are hereditable through cell divisions [64]. According to the Knudson hypothesis the inactivation of tumor suppressor genes requires two steps with the first step occurring in both somatic cells (sporadic cancer) or germ line (hereditary cancer) and the second is always somatic [65].

Table 2   Proteins participating in both autophagy and apoptosis [61]

Protein	Autophagy	Apoptosis
Autophagic proteins		
mTOR	Inactive form involves in initiation	mTOR regulates apoptosis
Beclin-1	Autophagosome nucleation	Cleaved C-fragment induces mitochondrial apoptosis
UVRAG Up regulates	Vps34–Beclin1 interaction	Anti-apoptotic, inhibits Bax translocation from cytosol to mitochondria
AMBRA	Up regulates Vps34–Beclin1 interaction	Regulate mitochondrial apoptosis; cleaved by caspases and calpains
Atg3	Conjugates with Atg12	Regulates mitochondrial cell death
Atg5	Conjugates with Atg12, autophagosome elongation	Interacts with FADD to inhibit apoptosis, cleaved N-fragment induces mitochondrial apoptosis
Atg12	Autophagosome elongation	Stimulates mitochondrial apoptosis by inactivating Bcl-2 and Mcl-1
Atg4D	LC3 processing	Cleaved Atg4D localize to mitochondria and induces apoptosis
p62	Binds with LC3, promotes degradation of polyubiquitinated	Caspase-8 protein aggregates processing and activation
Apoptotic proteins		
Bcl-2, Bcl-xL	Interacts with Beclin-1 and inhibit autophagy	Anti-apoptotic
Bad, Bak, BNIP3, Nix	Pro-autophagic, disrupting Beclin-1/Bcl-2 interaction	Pro-apoptotic
Bax, PUMA	Pro-autophagic, non-canonical type	Pro-apoptotic
p53	Inhibits by cytoplasmic p53 Induces by nuclear p53 through DRAM	Pro-apoptotic Pro-apoptotic
Noxa	Induces autophagy by disrupting Mcl-1/Beclin-1 interaction	Pro-apoptotic
Bim	Sequesters Beclin-1, inhibits autophagy	Pro-apoptotic
XIAP	Inhibits by Mdm2-p53 signalling	Inhibits caspases 3, 7
cFLIP	Prevent interaction between Atg3 and LC3	Inhibits caspase 8

Epimutations enclosing methylation and demethylation events on the CpG islands of the promoter regions of genes, affecting their expression, histone modifications, and microRNA (miRNA)-based regulation, which may lead a normal cell to become a cancer cell. Histone tails undergo covalent changes and the local structure of chromatin, as well as gene expression are regulated by complexes that remodel nucleosomes with the requirement of ATP, being the remodeling of nucleosome an epigenetic regulation [64-66].

Genomic instability is typical for nearly every cancer, but the molecular events involved are not well established. Chromosomal instability (CIN) occurs in several cancers but there are other forms like microsatellite instability (MSI or MIN), hereditary non-polyposis colon cancer (Lynch syndrome), germ line mutations in breast cancer, and many others. When characterized by the occurrence of CIN, the genomic instability may likewise assigned to mutations in DNA repair genes. The human cancers development mainly determined by genomic instability occurrence in CIN, but there are other ways for genomic instability, which include MSI. In this case, expansion or contraction in the oligonucleotide repetition occurs in the microsatellite sequence. Other genomic instability can be determined by the increase of base pairs mutations [66].

In hereditary cancers, the genomic instability seems to be the first event, followed by the establishment of the others, culminating with cancer. In the sporadic (non-hereditary) cancers, growth-regulating genes seems to be deregulated, constituting the first event, followed by DNA damage and DNA replication stress leading to the genomic instability and selective pressure for tumour suppressor p53 (TP53) inactivation. This fact conducted to the evasion from cell death, providing additional mutations and the establishment of the sporadic or non-hereditary cancer [66-67].

Accordingly, cancer is a multistep and mutagenic process in which normal cells acquire new functionalities as unbounded proliferative capacity, control of growth signals, and resistance to anti-proliferative and apoptotic actions. Despite some oncogenes and tumour suppressors oftentimes detected in cancer cells mutations, like p53, Rb, p16INK4a, PI3K, PTEN and Ras, other low-frequency alterations are involved in the oncogenesis. DNA damage and DNA replication stress, proteotoxic stress, mitotic stress, metabolic stress and oxidative stress constitute the complementary phenotype of cancer establishment and evolution [67].

Modern molecular biology is closely related to SV40-induced transformation and tumour genesis. The simplified viral system has provides several opportunities to understand the complexity of the eukaryotic DNA replication, gene expression and mRNA translation. For extension, SV40 provides the contributions to the better knowledge of the molecular basis of cancer. The discovery that some viruses hold the ability of normal cell transformation and its immortalization, besides to induce tumour formation in some laboratory animals, opened a new gate for basic and applied researches [68].

The first evidence that cells infected by Rous sarcoma virus transformed *in vitro* and immortalized for an oncogenic retrovirus, was decisive to the beginning of a series of studies in cancer biology and virology, through cultured isolated cancer cells. It was due to does not growth prevention or not cease movements when cells in contact with other normal cells or with a surface [69-70]. Some premises have driven the studies, as follow [68]:

1. SV40 induces tumours in laboratory animals and transforms cells in culture;
2. The *A* gene of SV40 encodes large tumour antigen;
3. SV40-transformed cells contain integrated viral DNA;
4. Large T antigen is essential for transformation;
5. Large T antigen: genetic analysis of a multifunctional protein;
6. The relationship between viral replication and cellular transformation;
7. SV40 induces transformation by binding key cellular proteins and altering their activities;
8. Combining mouse and viral genetics: how does SV40 action on cellular targets contribute to tumour genesis?

An important concept arises from these studies in 1980, related to the ability of SV40 genome to be cloned and propagated in bacteria as a recombinant plasmid, and rescued from that plasmid to regenerate infectious SV40 virions [71]. This was the first demonstration that oncogenes, in this case the SV40 T antigens, can induce transformation when expressed from a plasmid that had been propagated in bacteria. With the advent of cloning, T antigen mutants that were defective for virus growth could be amplified in bacteria prior to study in mammalian cells. The processing functions of T antigen regarding genetic studies provided detached views. Complexes formed by T antigen with p53 were identified both in SV40-transformed and infected cells. It was observed that T antigen mutants defective for p53 binding results also in transformation defective. T antigen and p53 interaction precludes p53-dependent gene expression, and probably can prevent p53-dependent transcription and growth-arrest by mechanisms not well known. In addition, this interaction hampers the replicative functions of T antigen. The association of Rb and p53 with T antigen is necessary for transformation, but not enough [68].

Therefore, the knowledge provided by SV40 researches represents a scientific milestone for the beginning of genetic engineering and recombinant DNA technology, broadly exploited nowadays for the production of heterologous proteins in bacteria, yeast, eukaryotic cells, to the development of new drugs and vaccines.

Inside of the context of cell immortality and cancer, we have the Sirtuins (SIRT1-SIRT7), a highly conserved family of mammalian proteins NAD+-dependent deacetylases, which act as central regulators of metabolic function and cell survival. SIRT3, SIRT4, and SIRT5 are found in the mitochondria. Despite it's a very challenging issue the regulation of lifetime, the sirtuins appear to be involved in several diseases, including age-related diseases. SIRT3 seems to act as a central regulator of mitochondrial adaptation in health and disease [7].

When SIRT3 is scanty, mitochondrial proteins step into hyper acetylated state, driving to mitochondrial dysfunction. Hyper acetylation of SIRT3 substrates promote enhancement in oxidative stress, the aperture of mPTP (mitochondrial permeability transition pore), and reduction of ATP production, directing cells to genomic instability and cell death. SIRT3 is also required to assemble adaptive mitochondrial responses to stress. The inability to protect cells against stress can lead to development of cancer, metabolic disorders and neurodegenerative diseases. Therefore, SIRT3 is involved in various aspects of the regulation of mitochondrial function, metabolism, ATP generation, and in the modulation of the oxidative stress response [72-74]. Advances in the knowledge of SIRT3 function are crucial for identify SIRT3 targets and the implications of its loss for the cell-protective mechanisms, with the view of improving mitochondrial function for the adequate therapy development as a consequence. This represents a great opportunity for new applications in researches.

6. Metastasis

The most crucial role of mitochondria in cancer is related to the recent evidences of the involvement in cell immortality and induction of metastasis from their origin site, spreading transformed cells for elsewhere in the body, originating other cancers. Therefore, mitochondria provide ATP for cancer cells growth, to work in favour of their immortality and

metastasizing [6]. During hypoxia/reoxygenation (Hyp/RO) and after intense generation of reactive oxygen species (ROS) and caspases activation, cell death usually occurs. Alterations of mitochondrial function observed when Bax inhibitor-1 (BI-1), an anti-apoptotic protein overexpressing in tumour cells, enhances cancer metastasis through changings in glucose metabolism and activation of sodium-hydrogen exchanging [75-76]. The metabolic stress also regulates cytoskeletal dynamics and metastasis in cancer cells [77] and the metastasis suppressor KISS1 seems to reverse the Warburg Effect through improving mitochondrial biogenesis [78]. At this point, is appropriate to discuss if cancer is a metabolic disease. Recent findings with p53 are giving support to the close relation between metabolic changes and tumour development and progression with metastasis. In addition to disrupting the intrinsic mitochondrial apoptosis in metastatic cancer cells, other indirect mechanisms may be due to a disruption of p53 signalling pathway. The mutations of the p53 gene found in more than half of human tumours were undergoing metastasis. Beyond to transcription of downstream effects associated with the p53 gene, the p53 mutation also affects the activation of Bak in the outer mitochondrial membrane, leading to disrupt the apoptotic machinery. Recent studies reported that mutant p53 protein can create amyloid aggregates and fibrils with prion-like action in cancer tumour and metastasis. These observations suggest that over-expression of anti-apoptotic proteins is associated with cancer metastasis and chemo-resistance [76-85]. Based on the scientific literature the association of mitochondria with cellular metabolism, ROS production and apoptotic pathways seems to be evident regarding cancer metastasis. Anyway, additional studies are necessary in order to add more evidences on this issue, which is quite promising for the detection of new therapeutic targets for the cure of diverse cancers, which are so aggressive to the human beings.

7. Microscopy

Mitochondria and its relation to the cancer constitute a complex theme that arose recently in evidence, and all tools in microscopy and other methodologies for assessment of intra-organellar components of the cell are fundamental for the better understanding of their relationships in health and disease. Nowadays it is well known that mitochondria play a key role in both situations. There are many references describing methods in cell biology, aiming to access mitochondria by different microscopy technology, like through light, laser confocal, atomic force and electron microscopy. Also, the selected references include methods in flow cytometry, biochemistry, molecular biology and structural biology to add more evidence and reliability in researches [4, 57-60, 86-100].

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