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Review

Mitochondria and cancer chemoresistance☆

Flora Guerra [b], Arnaldo A. Arbini [c], Loredana Moro [a,*]

[a] National Research Council, Institute of Biomembranes and Bioenergetics, Via Amendola 165/A, 70126 Bari, Italy
[b] Department of Biological and Environmental Sciences and Technologies (DiSTeBA), Università del Salento, Via Provinciale Lecce-Monteroni n. 165, 73100 Lecce, Italy
[c] Department of Pathology, NYU Langone Medical Center, 550 First Avenue, 10016 New York, NY, USA

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ABSTRACT

Mitochondria, known for more than a century as the energy powerhouse of a cell, represent key intracellular signaling hub that are emerging as important determinants of several aspects of cancer development and progression, including metabolic reprogramming, acquisition of metastatic capability, and response to chemotherapeutic drugs. The majority of cancer cells harbors somatic mutations in the mitochondrial genome (mtDNA) and/or alterations in the mtDNA content, leading to mitochondrial dysfunction. Decreased mtDNA content is also detected in tumor-initiating cells, a subpopulation of cancer cells that are believed to play an integral role in cancer recurrence following chemotherapy. Although mutations in mitochondrial genes are common in cancer cells, they do not shut down completely the mitochondrial energy metabolism and functionality. Instead, they promote rewiring of the bioenergetics and biosynthetic profile of a cancer cell through a mitochondria-to-nucleus signaling activated by "dysfunctional" mitochondria that results in changes in transcription and/or activity of cancer-related genes and signaling pathways. Different cancer cell types may undergo different bioenergetic changes, some to more glycolytic and some to more oxidative. These different metabolic signatures may coexist within the same tumor mass (intra-tumor heterogeneity). In this review we describe the current understanding of mitochondrial dysfunction in the context of cancer chemoresistance with special attention to the role of mtDNA alterations. We put emphasis on potential therapeutic strategies targeting different metabolic events specific to cancer cells, including glycolysis, glutaminolysis, oxidative phosphorylation, and the retrograde signaling, to prevent chemoresistance. We also highlight novel genome-editing strategies aimed at "correcting" mtDNA defects in cancer cells. We conclude on the importance of considering intratumor metabolic heterogeneity to develop effective metabolism-based cancer therapy that can overcome chemoresistance. This article is part of a Special Issue entitled Mitochondria in Cancer, edited by Giuseppe Gasparre, Rodrigue Rossignol and Pierre Sonveaux.

1. Introduction

Every cancer develops and evolves in a unique way conferring a distinctive sensitivity to given anti-cancer agents. Complicating this "inter-tumor" heterogeneity is the ascertained presence of a certain degree of cell diversity within the same tumor (intra-tumor heterogeneity), which encompasses tumor-initiating cells (TICs), as well as cells possessing slightly different degrees of differentiation. It has been proposed that TICs are the tumorigenic root of cancers and are responsible for relapses after therapy due to their ability to develop resistance to chemotherapy [1–3]. As such, TICs display low cell proliferation rate, high self-renewing ability, capacity to differentiate into highly proliferative cancer cells, as well as drug and/or radiotherapy resistance (for a review see [1]). The cell type heterogeneity within a tumor is accompanied by distinct, cell type-dependent changes in the metabolic profile, which may facilitate cancer cell ability to thrive under harsh microenvironmental conditions, such as during and after chemotherapeutic treatment [1,4,5].

Otto H. Warburg was the first to demonstrate that tumor cells have an altered energy metabolism: he observed that instead of using an oxidative metabolism, cancer cells convert glucose into lactate even in the presence of high oxygen tension. This "Warburg effect" is currently referred to as "metabolic reprogramming" and is a recognized hallmark of cancer [6]. In his pioneering research, Warburg hypothesized that mitochondrial dysfunction and subsequent cell inability to effectively oxidize glucose carbon to CO_2 was determinant in the metabolic shift onset, which occurs even in the presence of perfectly functional mitochondria [5]. The Warburg effect provides a biosynthetic advantage to tumor cells diverting energy substrates into key subsidiary biosynthetic reactions

Abbreviations: CI, Complex I; DCA, dichloroacetate; 2-DG, 2-deoxy-glucose; GDH, glutamate dehydrogenase; GLS, glutaminase; HK, hexokinase; LICs, Leukemia Initiating Cells; MDR, multidrug resistance; mtDNA, mitochondrial DNA; MSCs, mesenchymal stem cells; nDNA, nuclear DNA; OXPHOS, oxidative phosphorylation; PDK, pyruvate dehydrogenase kinase; PTX, paclitaxel; TALENs, transcription activator-like effector nucleases; TICs, tumor initiating cells; TNTs, tunneling nanotubes; ZFNs, Zinc Finger Nucleases.

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* Corresponding author.
E-mail address: l.moro@ibbe.cnr.it (L. Moro).

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required for differentiation, proliferation and growth [5,7–10]. As such, carbons derived from glucose are used for amino acid, fatty acid and nucleic acid synthesis, thus supporting cancer development. In turn, the increase in lactate production due to glucose fermentation would cause microenvironment acidification favoring local invasion and metastatic dissemination. Of note, under conditions of limited oxygen availability, *i.e.* in the hypoxic microenvironment of tumor tissues, glycolysis becomes the obligate pathway of energy production. Besides glucose, cancer cells use additional nutrients to support their biosynthetic needs. In this context, glutamine is a key nutrient that provides carbon for acetyl-CoA, citrate production and lipogenesis, nitrogen for purine, pyrimidine and DNA synthesis, and reducing power in the form of NADPH to support cell proliferation [5].

Mitochondria play a pivotal role in cancer metabolic reprogramming since most catabolic and anabolic pathways, including biosynthesis of amino acids, nucleic acids, lipids, and substrates such as NADH and NADPH, converge into these organelles [11,12]. In addition to regulating bioenergetics metabolism, mitochondria represent a central signaling and "integrator" hub controlling epigenetics, stemness, differentiation, initiation and execution of apoptosis [13,14]. These multiple functions of mitochondria allow them to sense cellular stress and contribute to cell adaptation to challenging microenvironment conditions, conferring a high degree of plasticity to tumor cells for growth and survival. Mitochondria possess their own genome, a 16,569 bp double-stranded, circular DNA molecule (mtDNA) which encodes for 13 polypeptides of the mitochondrial respiratory chain, 22 tRNAs and 2 rRNAs required for mitochondrial protein synthesis (Fig. 1). The mitochondrial genome also contains a non-coding displacement (D)-loop with regulatory regions for the transcription of the heavy and the light strands of the genome, the origin of replication of the heavy strand, binding sites for the mitochondrial transcriptional factor (mtTFA) and conserved sequence blocks (CSB I, II, III). Overall, the D-loop integrates nuclear-encoded events into the transcription and replication of the mtDNA. It is important to note that, being the mtDNA expressed as polycistronic transcripts, the entire mitochondrial-encoded genes may be potentially affected by a mutation in the D-loop region. In addition, mutations in the nuclear genome may also result in reduction/depletion of the mtDNA [15], particularly mutations in nuclear genes essential for mtDNA replication, such as the mitochondrial DNA polymerase γ (*POLG*), the DNA helicase *Twinkle*, and single-stranded DNA-binding protein (*mtSSB*) [15–17]. Recently, the methylation status of *POLG* has been reported to be an essential regulator of the mtDNA copy number in stem and cancer cells [17]. Mitochondria contain a variable number of mtDNA molecules, depending on the cell/tissue type, with an average of 5 molecules/mitochondrion. Within the same cell, some mitochondria may contain mtDNA mutations while others have wild-type mtDNA, a characteristic known as "heteroplasmy". The word "homoplasmy" instead indicates when a cell or a cell subpopulation contains a uniformly normal or mutant mtDNA pool. Although mitochondria contain their own genome, they are semi-autonomous because most of the proteins that reside in mitochondria are encoded by the nuclear genome. So far, a plethora of genetic and metabolic mitochondrial modifications have been described in cancer cells, affecting both the mitochondrial and the nuclear genome, and they have been implicated not only in metabolic reprogramming but also in the regulation of cancer cell response to chemotherapy (reviewed in [12,18–21]). There is, however, no unique scenario for the role of mitochondria in tumorigenesis and cancer progression as well as in the response of tumor cells to anti-cancer treatments. Instead, a range of mitochondrial functions may vary in cancer depending upon differences in genetics, microenvironment insults, and tumor/tissue type. Even within the same tumor, subclonal populations of cancer cells may display different mitochondrial functions that contribute to fostering tumor adaptation and therapeutic failure. In this review, we will focus on the potential role of defective mtDNA in the development of cancer chemoresistance and available therapeutic strategies aimed at targeting cancer cells with such genetic alterations. We will also discuss recent studies highlighting the importance of considering intra-tumor heterogeneity and microenvironment to develop effective metabolism-based anti-cancer therapeutic strategies that may overcome chemoresistance and cancer relapse.

2. MtDNA defects and cancer chemoresistance

Most common chemotherapeutic drugs eliminate tumor cells by inducing apoptosis. Evasion from this form of programmed cell death is not only a requirement for cancer development [6], but also an acquired ability of cancer cells to escape the cytotoxic effect of chemotherapy. Mitochondria are the major regulators of apoptosis, thus studies on mitochondrial dysfunction in the context of cancer development, progression and response to therapy represent an appealing avenue in cancer research.

An insult to the mtDNA, including pathogenic point mutations, deletions and changes in copy number, has been shown to induce cancer progression to an advanced phenotype in several tumor types [15]. For example, mtDNA mutations and/or depletion in breast, prostate and colorectal cancers have been associated with cancer progression to a more malignant phenotype with adverse prognosis *in vivo* [22–27]. One of the first studies aimed at investigating the role of specific mtDNA mutations in tumorigenesis, cancer progression and resistance to apoptotic stimuli was performed by Ohta's lab [28]. They constructed trans-mitochondrial hybrids (cybrids) containing a common HeLa nucleus with or without a pathogenic homoplasmic point mutation in the mtDNA-encoded ATP synthase 6 protein (ATPase 6) derived from patients affected by mitochondrial encephalomyopathy, and assessed tumor growth and apoptosis in culture as well as in xenografts experiments in nude mice. Overall, the mutant cybrids showed increased tumorigenic potential and decreased apoptosis compared to wild-type cells both in culture and *in vivo*. Consistently, HeLa cybrids containing mutant mtDNA derived from the pancreatic cancer cell lines CFPAC-1 (mutations: T10970C [Trp to Arg] in *ND4*; T8696C [Met to Thr] and T9070G [Ser to Ala] in *ATPase 6*; A2905G in the *16S* rRNA gene) or CAPAN-2 (mutations: G6267A [Ala to Thr] in *COI*; G10176A [Gly to Ser] in *ND3*) were resistant to 5-fluorouracil and cisplatin [29]. Using such cybrids' technology, Ishikawa et al. [30] reported that point mutations in NADH dehydrogenase 6, which resulted in defective activity of mitochondrial respiratory Complex I (CI, NADH dehydrogenase) and increased reactive oxygen species (ROS) levels, conferred a highly metastatic potential to the recipient Lewis lung carcinoma murine cells, consistently with a role of certain mtDNA alterations in promoting cancer progression. In this study, CI defects were also observed in high metastatic fibrosarcoma B82M cells but not in high metastatic colon adenocarcinoma LuM1 murine cells, suggesting that metastatic tumors do not always display CI defects. In the same study, contribution of mtDNA to cancer cell metastasis was confirmed in human tumors, because transfer of mtDNA from the highly metastatic human breast cancer cell line MDA-MB-231 to the low metastatic HeLa cells induced CI defects, and increased ROS levels and the metastatic potential. It is interesting to note that mtDNA mutations were able to control the metastatic potential of some tumor cells but did not confer tumorigenic potential to nontransformed mouse NIH3T3 cells, supporting the hypothesis that mtDNA dysfunction may confer a "progression signal" to cancer cells instead of a "tumorigenic signal".

2.1. mtDNA depletion

MtDNA depletion or large mtDNA deletions have been detected *in vivo* in different cancer types, including hepatocellular, lung, gastric and colorectal cancers [31], prostate carcinoma [32,33] and breast cancer [34]. Lee et al. [31] have analyzed the D-loop sequence and mtDNA copy number in 54 hepatocellular carcinomas, 31 lung, 31 gastric and 25 colorectal cancers together with their corresponding non-tumoral