Chapter 10

Mitochondrial DNA Variations in Tumors: Drivers or Passengers?

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Abstract

Mitochondrial DNA alterations, including point mutations, deletions, inversions and copy number variations, have been widely reported in many age-related degenerative diseases and tumors. However, numerous studies investigating their pathogenic role in cancer have provided inconsistent evidence. Furthermore, biological impacts of mitochondrial DNA variants vary tremendously, depending on the proportion of mutant DNA molecules carried by the neoplastic cells (the so-called heteroplasmy). The recent discovery of inter-genomic crosstalk between nucleus and mitochondria has reinforced the role of mitochondrial DNA variants in perturbing this essential signaling pathway and thus indirectly targeting nuclear genes involved in tumorigenic and invasive phenotype. Therefore, mitochondrial dysfunction is currently considered a crucial hallmark of carcinogenesis as well as a promising target for anticancer therapy. This chapter describes the role of different types of mitochondrial DNA alterations by mainly considering the paradigmatic model of colorectal carcinogenesis and, in particular, it revisits the issue of whether mitochondrial mutations are causative cancer drivers or simply genuine passenger events. The advent of high-throughput next-generation sequencing techniques, as well as the development of genetic and pharmaceutical interventions for the treatment of mitochondrial dysfunction in cancer, are also discussed.

Keywords: mitochondrial DNA variants, heteroplasmy, nuclear-mitochondrial crosstalk, oxidative stress, mtDNA copy number alterations, D-loop, cancer therapy, mitogenomics

1. Introduction

Mitochondria are highly dynamic organelles whose biogenesis and functions are tightly regulated by the nucleus through a constant bidirectional crosstalk. Indeed, only about 1%

of mitochondrial proteins are encoded by mitochondrial DNA (mtDNA), with all the others encoded by the nuclear genome, including proteins involved in mtDNA replication and transcription [1].

The human mtDNA is a small circular double-stranded DNA molecule of approximately 16.6 kb that encodes for 2 ribosomal RNAs (12S and 16S), 22 transfer RNAs required for protein synthesis and 13 essential protein subunits of the oxidative phosphorylation system (OXPHOS) (**Figure 1**) [2]. The electron transport chain, the primary metabolic pathway which generates energy in the form of ATP, is composed of five protein complexes (I–V) localized in the inner membrane of mitochondria, including complex II that is exclusively coded by the nuclear genome. This system includes seven subunits of respiratory enzyme complex I, one subunit of complex III, three subunits of complex IV and two subunits of complex V. As mentioned before, all other mitochondrial proteins, including those involved in mtDNA replication, transcription and translation, are encoded by nuclear genes and are targeted to the mitochondrion by specific transport systems. The discovery of over 2000 mitochondrial small non-coding RNAs (mitosRNAs), playing a pivotal role in the control of normal mitochondrial gene expression, revealed an underestimated level of mitochondrial functional complexity [3]. Furthermore, studies on antisense anti-termination tRNAs and delRNAs shed new light on novel mechanisms expanding the coding potential of mitogenome [4, 5].

Byproducts of the electron transport chain (ETC) constantly generate reactive oxygen species (ROS) that may severely damage the mitochondrial DNA. If not efficiently repaired, the accumulation of oxidative lesions in the mtDNA molecules lead to gradual mitochondrial dysfunction, which is reflected in changes in the number, morphology and functioning of mitochondria, as observed in cancer cells [6].

mtDNA is more susceptible to mutations than nuclear DNA, due to the lack of histones and chromatin protective structures, paucity of introns, less efficient mtDNA repair mechanisms and a higher exposure to deleterious ROS generated during ATP synthesis within the mitochondrial compartment [7].

Although low levels of intracellular ROS normally regulate cellular signaling and are essential for normal cell survival and proliferation, aberrant ROS production is frequently observed in neoplastic cells. In the mitochondrial free radical theory of aging accumulation of damaging mtDNA mutations, impairment of oxidative phosphorylation as well as an imbalance in the expression of antioxidant enzymes results in exponential overproduction of ROS. This elicited condition forms a "vicious cycle" that is the basis of a wide range of pathologies, termed as "free radical diseases" such as cancer, neurodegeneration, atherosclerosis, diabetes mellitus and chronic inflammation [8]. Importantly, besides the obvious induction of oxidative nucleotide damage to mtDNA, ROS promotes tumorigenesis through several other mechanisms, including stabilization of hypoxia-inducible factor (HIF)-α, increased calcium flux, inactivation of key phosphatases, such as Pten and PP2A, and activation of both the NRF2 and NF-κB transcription factors [9–11].

Since the Warburg theory of cancer postulated in 1956 [12], mitochondrial dysfunction has been regarded as a hallmark of cancer progression and as a promising target for anticancer therapies [13, 14]. For instance, enhancing complex I activity has been demonstrated to inhibit tumorigenicity and metastasis of breast cancer cells [15]. More recently, mitochondrial dysfunction

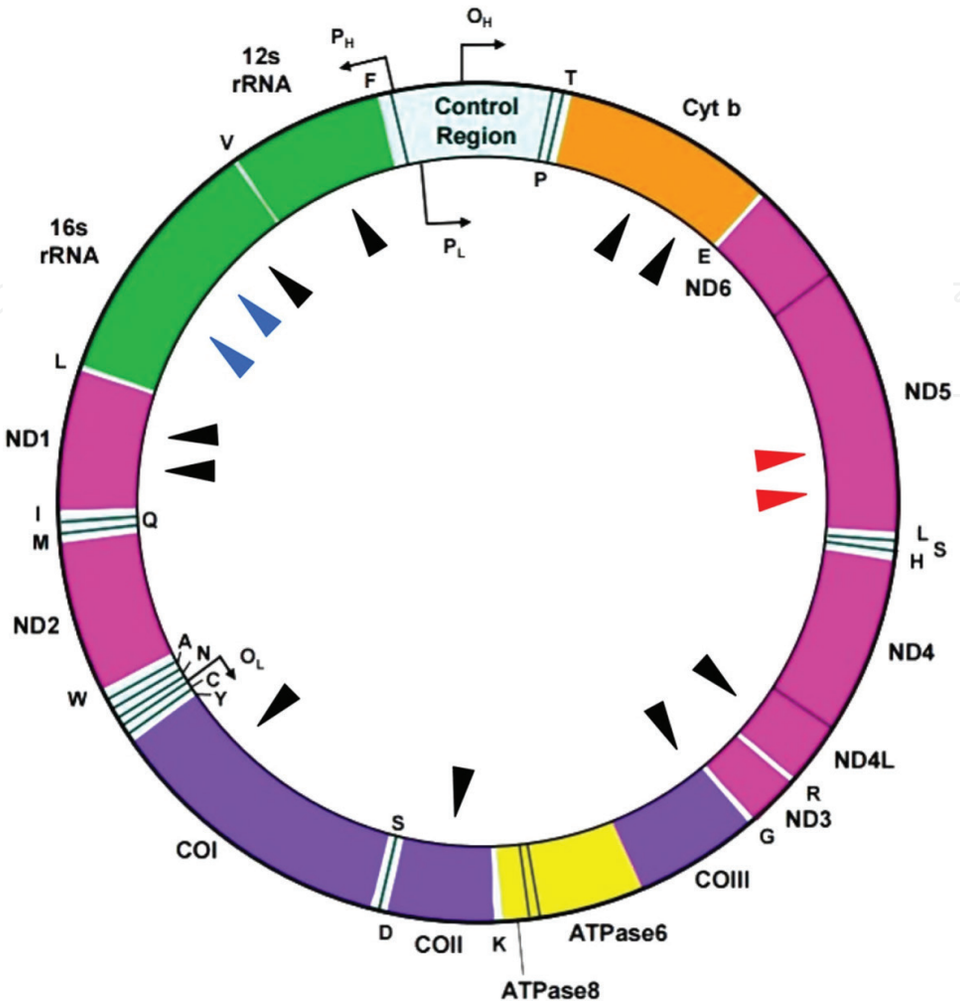

Figure 1. Map of the human mitochondrial DNA and distribution of somatic variants in colorectal cancer. mtDNA somatic mutations are mainly represented by homoplasmic alterations (black arrowheads), although rarer heteroplasmic substitutions (blue arrowheads) have been detected in the MT-RNR2 (16S) region or mixed homoplasmic/heteroplasmic variants (red arrowheads) in the MT-ND5 locus.

has also been associated with a crucial step for tumorigenesis, that is, epithelial-to-mesenchymal transition (EMT), enabling cancer dissemination and metastatic spread [16]. Importantly, mtDNA alterations may also disrupt the inter-genomic crosstalk between nucleus and mitochondrion and is associated with increased oxidative stress, ROS and cytosolic calcium accumulation, reduction of cell ATP levels and an imbalance in the NADH/NAD+ ratio. Moreover, ROS-induced oxidative stress may also affect the expression of nuclear genes involved in tumorigenic and invasive phenotypes, as it has been shown in colorectal cancer cells [17].

2. mtDNA alterations: a focus on colorectal carcinogenesis

2.1. Somatic mtDNA variants

Cancer is caused by the accumulation of multiple genetic alterations, such as point mutations, copy number variations (CNVs), inversions and epigenetic modifications [18]. This multi-step

process has been depicted in detail for colorectal cancer, which represents an ideal paradigm of tumorigenesis. In 1990, Fearon and Vogelstein [19] postulated a multi-step model of colorectal carcinogenesis, the long established "adenoma-carcinoma sequence", in which the inactivation of the APC tumor-suppressor gene occurs first in normal colonic epithelial cells, followed by activating mutations in the KRAS gene and subsequent additional alterations in other tumor-suppressor genes, such as TP53 and TGF-β pathway genes.

Accumulating evidence emphasizes the functional role of mtDNA abnormalities in mitochondrial dysfunction and colorectal carcinogenesis. In a whole-genome comparative study of five different tumors, it has been demonstrated that the frequencies of deleterious non-synonymous somatic variants vary tremendously across tumor types, with the higher frequency (63%) in colorectal adenocarcinomas [20]. The vast majority of these mtDNA variants were represented by G > A and C > T transitions, the typical molecular fingerprint due to oxidative stress in mtDNA [21].

Thus far, mtDNA variants have been found to affect different regions with an essential role in mitochondrial protein synthesis machinery and oxidative phosphorylation (**Figure 1**) [22–24]. Importantly, it has been shown that mtDNA mutations may generate unprocessed transcripts by precluding RNA processing that impair mitochondrial biogenesis and energy maintenance [25, 26]. It is noteworthy to mention that mtDNA variants not only affect genes directly involved in the ETC, but also genes related to mitochondrial metabolism, such as tRNA genes, in which pathogenic mutations are 6.5 times more frequent than in other mitochondrial loci [27, 28].

MUTHY-associated polyposis (MAP) patients carry a significant increase of non-synonymous changes in conserved amino acid residues of the MT-CO_2 gene, particularly the hotspot m.7763G > A transition [29]. Nevertheless, there is no compelling evidence in the literature propending for a single common coding-region mtDNA variant or haplogroup that may strongly influence the risk of developing a colorectal adenocarcinoma. Alternatively, it is likely that mtDNA alterations influencing colorectal cancer risk may be in the form of heteroplasmic low frequency variants, possibly restricted to specific subsets of patients with colorectal cancer [30]. Curiously, it has been demonstrated that mutations disrupting the respiratory complex I in pituitary adenomas are somatic modifiers of tumorigenesis associated with less aggressive and genome-stable oncocytic lesions [31].

It is commonly believed that mtDNA variants arise due to positive selection of those "driver" variants conferring clonal growth advantage. Accordingly, we observed that likely non-pathogenic mtDNA variants ("passengers") reverted to the wild-type homoplasmic status during tumor progression in colorectal cancer patients [29]. On the contrary, the mtDNA variants that are positively selected during tumor progression might be considered the most tolerable alterations for neoplastic cells. However, a deleterious impact of mtDNA passenger variants on cancer progression may not be completely excluded, as it has been previously evidenced in nuclear DNA passenger alterations [32].

2.2. Mitochondrial DNA heteroplasmy

Mitochondrial DNA heteroplasmy has been involved in a large spectrum of human diseases. Beside classical mitochondrial diseases, such as mitochondrial myopathy, myoclonic epilepsy with ragged red fibers, and mitochondrial encephalomyopathy, lactic acidosis, and stroke-like

episodes (MELAS), mitochondrial heteroplasmy also plays a pivotal role in complex disorders, including type 2 diabetes mellitus, late-onset neurodegenerative diseases and cancer [30].

mtDNA variants are maternally-inherited or arise as *de novo* somatic mutations in a fraction (heteroplasmic) or all (homoplasmic) mitochondrial genomes within each cell containing hundreds of copies of mtDNA molecules. Over time, the proportion of the mutant mtDNA within the cell may vary and drift toward predominantly mutant or wild-type form to achieve homoplasmy. Accordingly, the biological impact of a mtDNA variant may fluctuate, depending on the proportion of mutant mtDNA molecules carried by the neoplastic cell. Moreover, the level of heteroplasmy increases significantly with age and may vary between tissues and ethnic groups [33, 34]. By using high-throughput sequencing technology, Guo et al. [35] showed that very low heteroplasmy variants, down to almost 0.1%, are generally inherited from the mother, thus implying their likely neutral effect, and that this inheritance begins to decrease at about 0.5%. Accordingly, it has been demonstrated that high heteroplasmic mtDNA mutation loads, generally above 80%, are required to trigger substantial dysfunctions in the oxidative phosphorylation process. For instance, the m.3571insC mutation in the MTND1 gene of respiratory complex I is commonly detected in oncocytic tumors, in which it causes a severe mitochondrial dysfunction when mutant load is above 83% [36]. Importantly, this mitochondrial threshold effect strictly regulates the balance between tumor growth and suppression [37]. Interestingly, low-level mitochondrial heteroplasmies are commonly found in healthy individuals, and the advent of next-generation sequencing (NGS) technologies revealed that 25–65% of the general population harbor at least one heteroplasmic variant across the entire mitochondrial genome [38, 39]. By studying human colorectal cancer cell lines, Polyak et al. [40] showed that the vast majority of mutations were ROS-related homoplasmic transitions, indicating that mtDNA molecules could rapidly become homogeneous under high clonal selection conditions. Nevertheless, several other *in vivo* studies demonstrated that mtDNA heteroplasmy is far more common in colorectal neoplasms [41–43]. As occasionally observed in the case of revertant mosaicism, a naturally occurring phenomenon involving spontaneous correction of a pathogenic mutation in a somatic cell, heteroplasmic somatic variants may also naturally revert to wild-type homoplasmy [44, 45].

2.3. mtDNA copy number alterations

Epidemiological studies have indicated significant association of leukocyte mtDNA copy number with risk of several malignancies, including glioma, colorectal and breast tumors, and its use has been proposed as a potential biomarker to select patients who benefit from adjuvant chemotherapy [46–50]. A reduced mtDNA content has also been correlated with lymph node metastasis and lower survival rates in patients with colorectal cancer [51].

In the past years, it has been demonstrated that mtDNA depletion leads to tumorigenesis by inducing changes in the redox status, membrane potential, ATP levels, gene expression, nucleotide pools, and increased chromosomal instability (e.g. translocations) [52, 53]. However, other findings reported a gain of mtDNA copy number, thus suggesting that mtDNA replication could be increased to compensate for detrimental metabolic effects caused by mtDNA variations and/or oxidative stress [54]. These conflicting data may be partly explained by the non-homogeneous timing of blood DNA analyses for mtDNA copy number determination. Interestingly, depletion

of mtDNA results in significant changes in methylation patterns of a number of nuclear-encoded genes, and these epigenetic modifications are reversed by the restoration of mtDNA content [55].

The molecular mechanism altering mtDNA copy number is still under investigation. In a study of 65 colorectal cancers, it has been suggested that hypomethylation of specific sites on CpG islands of the D-loop promoter may be involved in the regulation of mtDNA copy numbers [56]. Moreover, it has been reported that polymorphisms within the nuclear-encoded polymerase gamma gene (POLG), which codifies for a key component of the mitochondrial genome maintenance machinery, may lead to a decrease in mtDNA content and mitochondrial dysfunction [57]. Curiously, a homozygous polymorphic insertion (AluYb8MUTYH) in the 15th intron of the MUTYH base excision repair gene has been associated with a significant reduction of the type 1 MUTYH protein that localizes to mitochondria as well as lowered mtDNA content in age-related diseases [58]. Since biallelic mutations of MUTYH are associated with the MAP syndrome, it might be speculated that homozygous or compound heterozygous MUTYH variants may correlate with the mtDNA content in colorectal cancer [30].

2.4. D-loop and mitochondrial instability

The non-coding D-loop region contains essential transcription and replication elements and is formed by two hypervariable regions, namely HV-I (nt. 16,024–16,383) and HV-II (nt. 57–333) [59]. The latter includes the D310 sequence, a polycytidine repeat (nt. 303–309), which is essential for mtDNA replication in virtue of the H-strand replication origin. Replication of the leading strand initiates at the origin of H-strand synthesis and proceeds unidirectionally, displacing the parental H-strand as single-stranded DNA [60]. The D-loop is a well-known hotspot for somatic mutations in many types of cancer, with a mutation rate 100- to 200-fold higher than nuclear DNA. This finding may be partly explained by considering the direct relationship between mutational frequency and single-strandedness during mitochondrial replication [61].

mtDNA variants in the D-loop region have been repeatedly associated with risk and survival rates in cancer patients and, thus, they have been proposed as valuable prognostic markers. However, it has been argued that most of these studies could be biased due to artifacts related to genotyping errors or inadequate experimental design [62]. Mitochondrial microsatellite instability (mtMSI), that is a change in length in the repetitive sequences of the D-loop segment between normal and tumor tissues, has been described as a frequent molecular event in different cancers, but its prognostic value is still debated [63]. The variation of the homopolymeric tract length mainly arises through replication slippage of mitochondrial DNA polymerase and, importantly, this process may affect mtDNA replication and transcription. Intriguingly, the oxidative damage to mitochondrial polymerase γ may also contribute to the alteration in the length of the polycytidine repeat by impacting on mtDNA replication [64].

Instability of the D-loop hypervariable region-II (HV-II) has been associated with variants specifically grouped inside the MT-CO_2 gene in MAP patients, thus suggesting that genome instability might contribute to drive non-random accumulation of MT-CO_2 variants in the early stages of MAP colorectal tumorigenesis [29]. Therefore, D-loop mutations probably do not directly drive carcinogenesis but are more likely an epiphenomenon, used as a universal clonal marker ("molecular clock") to estimate the relative mitotic history of tumors [65, 66].

3. Mitochondrial-nuclear crosstalk

Tight coordination between the nucleus and mitochondria is required for proper mitochondrial functioning and includes both anterograde (nucleus to mitochondria) and retrograde (mitochondria to nucleus) signals. This crosstalk is critical for the maintenance of cellular homeostasis, and accumulated mtDNA variants may perturb this subtle pathway [67]. It has been demonstrated that somatically acquired mitochondrial-nuclear genome fusion sequences are present in human cancer cells [68]. Although most of the genes encoding proteins of the OXPHOS machinery are transcribed in the nucleus (anterograde signaling), mitochondria may also exert retrograde regulatory control over the nucleus in terms of nuclear gene expression modulation [69]. This phenomenon suggests a strong association between nuclear and mitochondrial DNA alterations in driving tumor development and progression. Variants in nuclear-encoded mitochondrial genes, such as fumarate hydratase, iso-citrate dehydrogenase and succinate dehydrogenase) have been associated with a wide variety of human cancers, such as paragangliomas, uterine leiomyomas, renal carcinomas, breast cancers, gastrointestinal stromal cancers, leukemia, prostate cancer, glioblastomas and colorectal carcinomas [70–78]. Furthermore, it has been demonstrated that mtDNA changes and MAPK pathway alterations synergize to drive colorectal malignant transformation [79].

In a study on colorectal adenoma and adenocarcinoma samples, an increased number of mutations in nuclear genes encoding proteins involved in critical mitochondrial processes, such as fusion, fission and localization were found [80]. It has also been suggested that mtDNA depletion may disrupt crucial nuclear processes, leading to centrosome amplification and mitotic spindle multipolarity, both participating in cancer cell transformation [81, 82]. mtDNA variants have the potential to induce molecular signals through the mitochondrial-nuclear crosstalk mechanism, thereby promoting nuclear compensation in response to mitochondrial malfunction [67]. Interestingly, some typical nuclear transcription factors, such as the tumor-suppressor p53 and estrogen receptor (ER), are localized within mitochondria, where they exert various transcription-independent functions [83]. By using transmitochondrial cybrid systems (“cybrids”), Kaipparettu et al. [69] elegantly demonstrated that mitochondria derived from the non-transformed breast epithelial cell line MCF10A reverse the tumorigenic properties of osteosarcoma metastatic cells (e.g. cell proliferation and viability under hypoxic conditions, anchorage-independent cell growth, resistance to anticancer drugs) by suppressing several oncogenic pathways involving HER2, SRC, RAS and TP53; on the other hand, some of the tumor-suppressor genes including VHL, PTEN and RB1 were overexpressed in cytoplasmic hybrids (cybrids) with non-cancerous mitochondria.

Other studies suggested that mitochondrial dysfunction may induce epigenetic modifications within the nuclear genome, such as aberrant methylation patterns in CpG-rich regions [84, 85]. These epigenetic alterations, including DNA and chromatin modifications and signaling through small RNAs, may contribute to the maintenance of mitochondria-mediated oncogenic transformation. However, the mitochondrial signals that potentially might trigger these epigenetic changes in the nucleus remain still largely unknown [30].

ROS-induced mitochondrial deregulation has been reported to trigger a survival response by inducing the nuclear factor NF-κB pathway and stimulating the synthesis of anti-apoptotic molecules (such as Bcl-xL/Bcl-2), which in turn promote cell survival and proliferation [86].

Moreover, oxidative stress may also affect the expression of nuclear genes involved in tumorigenic and invasive phenotypes [87]. Altogether these findings suggest that targeting the retrograde signaling could be a successful therapeutic strategy for cancer.

4. Targeting mitochondria for cancer therapy

Numerous studies suggested that mtDNA alterations may contribute to chemotherapy resistance and affect radiotherapy outcome. For instance, Guerra et al. [88] showed that mutations in the NADH dehydrogenase subunit 4 (MT-ND4) lead to acquired chemoresistance during treatment with paclitaxel carboplatin.

In the last few years, spindle transfer, a promising emerging strategy aimed at generating clinical germline gene therapy against inherited mitochondrial disorders, has supported the idea of a possible gene therapy approach for the editing of somatic mtDNA alterations [89]. Ideally, repairing the mutated mtDNA sequence would also restore the normal mitochondrial function and likely induce tumor regression. Taylor et al. [90] proposed a strategy that aimed to specifically block the replication of the mutant mtDNA by peptide nucleic acid (PNA), thereby allowing the selective propagation of the wild-type DNA. Moreover, mitochondrial dysfunction might also be restored by stimulating the mitophagy process in order to eliminate the deleterious mtDNA variants [91]. Targeting DNA repair enzymes to mitochondria may be a suitable strategy to correct mtDNA mutations. For instance, cell transfection with an expression vector containing the gene coding the DNA repair enzyme human 8-oxoguanine DNA glycosylase/apurinic lyase (hOGG1) has been used to reduce free fatty acids (FFAs)-induced mtDNA damage [92]. Furthermore, overexpression of hOGG1 in mitochondria has been shown to attenuate breast cancer progression and metastasis in transgenic mice [93]. Although hOGG1 has been the most frequently employed enzyme to enhance mtDNA repair, alternative strategies targeting other proteins transferred to mitochondria, such as endonuclease III (EndoIII) and endonuclease VIII (EndoVIII), have been proposed in the last years [94–96]. Other therapeutic approaches for patients carrying mtDNA mutations are based on allotopic gene expression, as preliminary demonstrated in different mitochondrial disorders [97], and targeted restriction endonucleases. In this regard, SmaI and PstI have been used as a powerful tool for treatment of mitochondrial dysfunction, resulting in the elimination of the mutant mtDNA and restoration of normal mitochondrial functionality [98]. In the last decade, many other approaches and compounds targeting dysfunctional mitochondria have been experienced, such as signal peptides. Lipophilic cations, cell-penetrating peptides and nanoparticles. A promising approach is based on the reprogramming of energy metabolism in colorectal cancer cells, through specific mitochondria-targeting agents, such as the second-generation rosamine analogs that target complex II and ATP synthase activities of the mitochondrial oxidative phosphorylation pathway [99]. More recently, it has been argued that mitochondria of tumor-initiating cells (TICs), which play a prominent role in cancer initiation, metastasis and resistance to therapy, may be targeted by mitocan vitamin E succinate in a complex II-dependent manner [100]. Another original approach has been developed to trigger cell death signaling pathways in colorectal cancer cells [101], such as ROS-dependent apoptosis and autophagy [102]. The recent improvement of high-throughput drug-screening platforms allowed the identification of novel non-toxic mitochondrial inhibitors, as in the

case of diphenyleneiodonium chloride (DPI), a strong inhibitor of mitochondrial complex I and II flavin-containing enzymes, which effectively depletes cancer stem-like cells (CSCs), one of the main drivers of poor clinical outcome in a wide variety of tumor types and especially in advanced disease states [103]. Interestingly, mitochondrial inhibition with VLX600 has also been proposed in combination with imatinib in the treatment of drug-resistant gastrointestinal stromal tumors (GISTs) [104].

Recently, morphological and ultrastructural changes in the mitochondrial cristae structure (cristae remodeling), for example, through the optic atrophy 1 (OPA1) pathway, represent an important step in apoptosis and autophagy, and a potential target for future pharmacological modulation in cancer [105].

Chromosomal translocations generating in-frame oncogenic gene fusions also represent successful examples of targeted cancer therapies, and recently it has been shown that the FGFR3-TACC3 (F3–T3) gene fusion—initially discovered in human glioblastoma and then reported in many other cancers—promotes oxidative phosphorylation, mitochondrial biogenesis and tumor growth [106–108].

5. Ultra-sensitive next-generation sequencing techniques and mitogenomics

Whole mitochondrial genome analysis by high-throughput next-generation sequencing (NGS) techniques enables the detection of low-level heteroplasmic mtDNA variants and completely revolutionized mitogenomics in the last few years [109]. This approach has been extensively applied to different mitochondrial disorders to carefully investigate the transmission dynamics of low-level maternal germline mtDNA variants across generations [110–112]. In a comparative analysis, it has been demonstrated that Sanger sequencing is valid for quantification of heteroplasmies with more than 10% of cells/mitochondria carrying the mutation, whereas NGS is capable of reliably detecting and quantifying heteroplasmic variants down to the 1% level [113]. Recently, a massive parallel sequencing (MPS) protocol reliably quantified low frequency, large mtDNA deletions in single cells with a lower detection limit of 0.5% [114]. mtDNA NGS has been also suggested as a useful quality check of pluripotent stem cells for drug discovery and regenerative medicine purposes [115].

Conventionally, DNA variants detected in a tumor sample but not in the germline counterpart (such as peripheral blood, buccal swab or saliva) are scored as somatic (likely pathogenic) mtDNA variants, otherwise they are considered as germinal variants (likely polymorphic/benign). High-throughput NGS approaches may unveil low-level germinal heteroplasmies having a tumoral tissue counterpart with higher heteroplasmy simply because of increased cell replication rate or random genetic drift phenomena and, therefore, without any deleterious oncogenic effect. The ultra-sensitive detection rate of NGS methods may be used to monitor even subtle shifts in the heteroplasmy levels of the tumor during time and potentially correlate them with tumor evolution [116]. Moreover, the possibility to easily analyze the circulating cell-free mtDNA isolated from plasma/serum ("liquid biopsy") or urine [117–119], may allow non-invasive serial sampling from the same patient.

6. Conclusions

In the last decades, evidence on the contribution of mtDNA variants to tumorigenesis has incredibly grown. Therefore, mitochondria are actually considered one of the most promising targets for novel anticancer therapies. Accordingly, mtDNA variants can be regarded as useful tumor biomarkers for clinical practice, whereas the tight communication between nuclear and mitochondrial genomes sheds new light on the molecular and functional mechanisms underlying the onset and progression of complex human diseases, such as cancer and neurodegenerative diseases.

Conflict of interest

The authors declare no conflicts of interest. This article does not contain any studies with human participants performed by the authors.

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References

[1] Bogenhagen DF. Mitochondrial DNA nucleoid structure. Biochimica et Biophysica Acta. 2012;**1819**(9-10):914-920. DOI: 10.1016/j.bbagrm.2011.11.005

[2] Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. Nature Reviews. Genetics. 2005;**6**(5):389-402. DOI: 10.1038/nrg1606

[3] Ro S, Ma HY, Park C, Ortogero N, Song R, Hennig GW, Zheng H, Lin YM, Moro L, Hsieh JT, Yan W. The mitochondrial genome encodes abundant small noncoding RNAs. Cell Research. 2013;**23**(6):759-774. DOI: 10.1038/cr.2013.37

[4] Seligmann H. Two genetic codes, one genome: Frameshifted primate mitochondrial genes code for additional proteins in presence of antisense antitermination tRNAs. Bio Systems. 2011;**105**(3):271-285. DOI: 10.1016/j.biosystems.2011.05.010

[5] Seligmann H. Codon expansion and systematic transcriptional deletions produce tetra-, pentacoded mitochondrial peptides. Journal of Theoretical Biology. 2015;**387**:154-165. DOI: 10.1016/j.jtbi.2015.09.030

[6] Gottfried E, Kreutz M, Mackensen A. Tumor metabolism as modulator of immune response and tumor progression. Seminars in Cancer Biology. 2012;**22**(4):335-341. DOI: 10.1016/j.semcancer.2012.02.009

[7] Druzhyna NM, Wilson GL, LeDoux SP. Mitochondrial DNA repair in aging and disease. Mechanisms of Ageing and Development. 2008;**129**(7-8):383-390. DOI: 10.1016/j.mad.2008.03.002

[8] Georgieva E, Ivanova D, Zhelev Z, Bakalova R, Gulubova M, Aoki I. Mitochondrial dysfunction and redox imbalance as a diagnostic marker of "free radical diseases". Anticancer Research. 2017;**37**(10):5373-5381. DOI: 10.21873/anticanres.11963

[9] Calvani M, Comito G, Giannoni E, Chiarugi P. Time-dependent stabilization of hypoxia inducible factor-1α by different intracellular sources of reactive oxygen species. PLoS One. 2012;**7**(10):e38388. DOI: 10.1371/journal.pone.0038388

[10] Gebremedhin D, Terashvili M, Wickramasekera N, Zhang DX, Rau N, Miura H, Harder DR. Redox signaling via oxidative inactivation of PTEN modulates pressure-dependent myogenic tone in rat middle cerebral arteries. PLoS One. 2013;**8**(7):e68498. DOI: 10.1371/journal.pone.0068498

[11] Nakahata S, Morishita K. PP2A inactivation by ROS accumulation. Blood. 2014;**124**(14):2163-2165. DOI: 10.1182/blood-2014-08-594093

[12] Warburg O. On the origin of cancer cells. Science. 1956;**123**:309-314

[13] Sharma LK, Fang H, Liu J, Vartak R, Deng J, Bai Y. Mitochondrial respiratory complex I dysfunction promotes tumorigenesis through ROS alteration and AKT activation. Human Molecular Genetics. 2011;**20**(23):4605-4616. DOI: 10.1093/hmg/ddr395

[14] Woo DK, Green PD, Santos JH, D'Souza AD, Walther Z, Martin WD, Christian BE, Chandel NS, Shadel GS. Mitochondrial genome instability and ROS enhance intestinal tumorigenesis in APC(min/+) mice. The American Journal of Pathology. 2012;**180**(1):24-31. DOI: 10.1016/j.ajpath.2011.10.003

[15] Santidrian AF, Matsuno-Yagi A, Ritland M, Seo BB, LeBoeuf SE, Gay LJ, Yagi T, Felding-Habermann B. Mitochondrial complex I activity and NAD+/NADH balance regulate breast cancer progression. The Journal of Clinical Investigation. 2013;**123**(3):1068-1081. DOI: 10.1172/JCI64264

[16] Guerra F, Guaragnella N, Arbini AA, Bucci C, Giannattasio S, Moro L. Mitochondrial dysfunction: A novel potential driver of epithelial-to-mesenchymal transition in cancer. Frontiers in Oncology. 2017;**7**:295. DOI: 10.3389/fonc.2017.00295

[17] Webb E, Broderick P, Chandler I, Lubbe S, Penegar S, Tomlinson IP, Houlston RS. Comprehensive analysis of common mitochondrial DNA variants and colorectal cancer risk. British Journal of Cancer. 2008;**99**(12):2088-2093. DOI: 10.1038/sj.bjc.6604805

[18] Wang C, Zhao S, Du Y, Guo Z. Single nucleotide polymorphisms in the D-loop region of mitochondrial DNA is associated with colorectal cancer outcome. Mitochondrial DNA. Part A, DNA Mapping, Sequencing, and Analysis. 2016;**27**(6):4361-4363. DOI: 10.3109/19401736.2015.1089502

[19] Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;**61**(5):759-767

[20] Larman TC, DePalma SR, Hadjipanayis AG, Cancer Genome Atlas Research Network, Protopopov A, Zhang J, Gabriel SB, Chin L, Seidman CE, Kucherlapati R, Seidman

JG. Spectrum of somatic mitochondrial mutations in five cancers. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(35):14087-14091. DOI: 10.1073/pnas.1211502109

[21] Kamiya H, Ueda T, Ohgi T, Matsukage A, Kasai H. Misincorporation of dAMP opposite 2-hydroxyadenine, an oxidative form of adenine. Nucleic Acids Research. 1995;**23**(5):761-766

[22] Abu-Amero KK, Alzahrani AS, Zou M, Shi Y. High frequency of somatic mitochondrial DNA mutations in human thyroid carcinomas and complex I respiratory defect in thyroid cancer cell lines. Oncogene. 2005;**24**(8):1455-1460. DOI: 10.1038/sj.onc.1208292

[23] Greaves LC, Preston SL, Tadrous PJ, Taylor RW, Barron MJ, Oukrif D, Leedham SJ, Deheragoda M, Sasieni P, Novelli MR, Jankowski JA, Turnbull DM, Wright NA, McDonald SA. Mitochondrial DNA mutations are established in human colonic stem cells, and mutated clones expand by crypt fission. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(3):714-719. DOI: 10.1073/pnas.0505903103

[24] Kassem AM, El-Guendy N, Tantawy M, Abdelhady H, El-Ghor A, Abdel Wahab AH. Mutational hotspots in the mitochondrial D-loop region of cancerous and precancerous colorectal lesions in Egyptian patients. DNA and Cell Biology. 2011;**30**(11):899-906. DOI: 10.1089/dna.2010.1186

[25] Rackham O, Busch JD, Matic S, Siira SJ, Kuznetsova I, Atanassov I, Ermer JA, Shearwood AM, Richman TR, Stewart JB, Mourier A, Milenkovic D, Larsson NG, Filipovska A. Hierarchical RNA processing is required for mitochondrial ribosome assembly. Cell Reports. 2016;**16**(7):1874-1890. DOI: 10.1016/j.celrep.2016.07.031

[26] Kuznetsova I, Siira SJ, Shearwood AJ, Ermer JA, Filipovska A, Rackham O. Simultaneous processing and degradation of mitochondrial RNAs revealed by circularized RNA sequencing. Nucleic Acids Research. 2017;**45**(9):5487-5500. DOI: 10.1093/nar/gkx104

[27] Mohammed F, Rezaee Khorasany AR, Mosaieby E, Houshmand M. Mitochondrial A12308G alteration in tRNA(Leu(CUN)) in colorectal cancer samples. Diagnostic Pathology. 2015;**10**:115. DOI: 10.1186/s13000-015-0337-6

[28] Seligmann H. Pathogenic mutations in antisense mitochondrial tRNAs. Journal of Theoretical Biology. 2011;**269**(1):287-296. DOI: 10.1016/j.jtbi.2010.11.007

[29] Errichiello E, Balsamo A, M1 C, Venesio T. Mitochondrial variants in MT-CO2 and D-loop instability are involved in MUTYH-associated polyposis. Journal of Molecular Medicine (Berlin). 2015;**93**(11):1271-1281. DOI: 10.1007/s00109-015-1312-0

[30] Errichiello E, Venesio T. Mitochondrial DNA variants in colorectal carcinogenesis: Drivers or passengers? Journal of Cancer Research and Clinical Oncology. 2017;**143**(10):1905-1914. DOI: 10.1007/s00432-017-2418-2

[31] Kurelac I, MacKay A, Lambros MB, Di Cesare E, Cenacchi G, Ceccarelli C, Morra I, Melcarne A, Morandi L, Calabrese FM, Attimonelli M, Tallini G, Reis-Filho JS, Gasparre G. Somatic complex I disruptive mitochondrial DNA mutations are modifiers of

tumorigenesis that correlate with low genomic instability in pituitary adenomas. Human Molecular Genetics. 2013;**22**(2):226-238. DOI: 10.1093/hmg/dds422

[32] McFarland CD, Korolev KS, Kryukov GV, Sunyaev SR, Mirny LA. Impact of deleterious passenger mutations on cancer progression. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(8):2910-2915. DOI: 10.1073/pnas.1213968110

[33] Sondheimer N, Glatz CE, Tirone JE, Deardorff MA, Krieger AM, Hakonarson H. Neutral mitochondrial heteroplasmy and the influence of aging. Human Molecular Genetics. 2011;**20**(8):1653-1659. DOI: 10.1093/hmg/ddr043

[34] Ramos A, Santos C, Mateiu L, Gonzalez Mdel M, Alvarez L, Azevedo L, Amorim A, Aluja MP. Frequency and pattern of heteroplasmy in the complete human mitochondrial genome. PLoS One. 2013;**8**(10):e74636. DOI: 10.1371/journal.pone.0074636

[35] Guo Y, Li CI, Sheng Q, Winther JF, Cai Q, Boice JD, Shyr Y. Very low-level heteroplasmy mtDNA variations are inherited in humans. Journal of Genetics and Genomics. 2013;**40**(12):607-615. DOI: 10.1016/j.jgg.2013.10.003

[36] Musicco C, Cormio A, Calvaruso MA, Iommarini L, Gasparre G, Porcelli AM, Timperio AM, Zolla L, Gadaleta MN. Analysis of the mitochondrial proteome of cybrid cells harbouring a truncative mitochondrial DNA mutation in respiratory complex I. Molecular BioSystems. 2014;**10**(6):1313-1319. DOI: 10.1039/c3mb70542k

[37] Gasparre G, Porcelli AM, Lenaz G, Romeo G. Relevance of mitochondrial genetics and metabolism in cancer development. Cold Spring Harbor Perspectives in Biology. 2013;**5**(2). DOI: 10.1101/cshperspect.a011411

[38] Sosa MX, Sivakumar IK, Maragh S, Veeramachaneni V, Hariharan R, Parulekar M, Fredrikson KM, Harkins TT, Lin J, Feldman AB, Tata P, Ehret GB, Chakravarti A. Next-generation sequencing of human mitochondrial reference genomes uncovers high heteroplasmy frequency. PLoS Computational Biology. 2012;**8**(10):e1002737. DOI: 10.1371/journal.pcbi.1002737

[39] Ye K, Lu J, Ma F, Keinan A, Gu Z. Extensive pathogenicity of mitochondrial heteroplasmy in healthy human individuals. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(29):10654-10659. DOI: 10.1073/pnas.1403521111

[40] Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nature Genetics. 1998;**20**(3):291-293. DOI: 10.1038/3108

[41] Chatterjee A, Mambo E, Sidransky D. Mitochondrial DNA mutations in human cancer. Oncogene. 2006;**25**:4663-4674. DOI: 10.1038/sj.onc.1209604

[42] He Y, Wu J, Dressman DC, Iacobuzio-Donahue C, Markowitz SD, Velculescu VE, Diaz LA Jr, Kinzler KW, Vogelstein B, Papadopoulos N. Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. Nature. 2010;**464**(7288):610-614. DOI: 10.1038/nature08802

[43] Mehrabi S, Akwe JA, Adams G Jr, Grizzle W, Yao X, Aikhionbare FO. Analysis of mtDNA sequence variants in colorectal adenomatous polyps. Diagnostic Pathology 2010;**5**:66. DOI:10.1186/1746-1596-5-66

[44] JE1 L-C, McGrath JA, Uitto J. Revertant mosaicism in skin: Natural gene therapy. Trends in Molecular Medicine. 2011;**17**(3):140-148. DOI: 10.1016/j.molmed.2010.11.003

[45] Errichiello E, Berrino E, Panero M, Sapino A, Venesio T. Frequent Homoplasmic Wild-Type Reversion of Cytochrome b Variants in MUTYH-Associated Polyposis. Conference "Mitochondrial Medicine: Developing New Treatments for Mitochondrial Disease". Hinxton, Cambridge, UK: Wellcome Genome Canpus Conference Center; 4-6 May 2016. P28

[46] Thyagarajan B, Wang R, Barcelo H, Koh WP, Yuan JM. Mitochondrial copy number is associated with colorectal cancer risk. Cancer Epidemiology, Biomarkers & Prevention. 2012;**21**(9):1574-1581. DOI: 10.1158/1055-9965.EPI-12-0138-T

[47] Huang B, Gao YT, Shu XO, Wen W, Yang G, Li G, Courtney R, Ji BT, Li HL, Purdue MP, Zheng W, Cai Q. Association of leukocyte mitochondrial DNA copy number with colorectal cancer risk: Results from the Shanghai Women's health study. Cancer Epidemiology, Biomarkers & Prevention. 2014;**23**(11):2357-2365. DOI: 10.1158/1055-9965.EPI-14-0297

[48] Hofmann JN, Hosgood HD 3rd, Liu CS, Chow WH, Shuch B, Cheng WL, Lin TT, Moore LE, Lan Q, Rothman N, Purdue MP. A nested case-control study of leukocyte mitochondrial DNA copy number and renal cell carcinoma in the prostate, lung, colorectal and ovarian cancer screening trial. Carcinogenesis. 2014;**35**(5):1028-1031. DOI:10.1093/carcin/bgt495

[49] Chen Y, Zhang J, Huang X, Zhang J, Zhou X, Hu J, Li G, He S, Xing J. High leukocyte mitochondrial DNA content contributes to poor prognosis in glioma patients through its immunosuppressive effect. British Journal of Cancer. 2015;**113**(1):99-106. DOI: 10.1038/bjc.2015.184

[50] Jiang H, Zhao H, Xu H, Hu L, Wang W, Wei Y, Wang Y, Peng X, Zhou F. Peripheral blood mitochondrial DNA content, A10398G polymorphism, and risk of breast cancer in a Han Chinese population. Cancer Science. 2014;**105**(6):639-645. DOI: 10.1111/cas.12412

[51] Cui H, Huang P, Wang Z, Zhang Y, Zhang Z, Xu W, Wang X, Han Y, Guo X. Association of decreased mitochondrial DNA content with the progression of colorectal cancer. BMC Cancer. 2013;**13**:110. DOI:10.1186/1471-2407-13-110

[52] Delsite R, Kachhap S, Anbazhagan R, Gabrielson E, Singh KK. Nuclear genes involved in mitochondria-to-nucleus communication in breast cancer cells. Molecular Cancer. 2002;**1**:6

[53] Desler C, Munch-Petersen B, Stevnsner T, Matsui S, Kulawiec M, Singh KK, Rasmussen LJ. Mitochondria as determinant of nucleotide pools and chromosomal stability. Mutation Research. 2007;**625**(1-2):112-124. DOI: 10.1016/j.mrfmmm.2007.06.002

[54] Feng S, Xiong L, Ji Z, Cheng W, Yang H. Correlation between increased copy number of mitochondrial DNA and clinicopathological stage in colorectal cancer. Oncology Letters. 2011;**2**(5):899-903. DOI: 10.3892/ol.2011.322

[55] Smiraglia DJ, Kulawiec M, Bistulfi GL, Gupta SG, Singh KK. A novel role for mitochondria in regulating epigenetic modification in the nucleus. Cancer Biology & Therapy. 2008;**7**(8):1182-1190

[56] Gao J, Wen S, Zhou H, Feng S. De-methylation of displacement loop of mitochondrial DNA is associated with increased mitochondrial copy number and nicotinamide adenine dinucleotide subunit 2 expression in colorectal cancer. Molecular Medicine Reports. 2015;**12**(5):7033-7038. DOI: 10.3892/mmr.2015.4256

[57] Linkowska K, Jawień A, Marszałek A, Malyarchuk BA, Tońska K, Bartnik E, Skonieczna K, Grzybowski T. Mitochondrial DNA polymerase γ mutations and their implications in mtDNA alterations in colorectal Cancer. Annals of Human Genetics. 2015;**79**:320-328. DOI: 10.1111/ahg.12111

[58] Guo W, Zheng B, Cai Z, Xu L, Guo D, Cao L, Wang Y. The polymorphic AluYb8 insertion in the MUTYH gene is associated with reduced type 1 protein expression and reduced mitochondrial DNA content. PLoS One. 2013;**8**(8):e70718. DOI: 10.1371/journal.pone.0070718

[59] Tipirisetti NR, Govatati S, Pullari P, Malempati S, Thupurani MK, Perugu S, Guruvaiah P, Rao KL, Digumarti RR, Nallanchakravarthula V, Bhanoori M, Satti V. Mitochondrial control region alterations and breast cancer risk: A study in south Indian population. PLoS One. 2014;**9**(1):e85363. DOI: 10.1371/journal.pone.0085363

[60] Brown TA, Cecconi C, Tkachuk AN, Bustamante C, Clayton DA. Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. Genes & Development. 2005;**19**(20):2466-2476. DOI: 10.1101/gad.1352105

[61] Seligmann H. Coding constraints modulate chemically spontaneous mutational replication gradients in mitochondrial genomes. Current Genomics. 2012;**13**(1):37-54. DOI: 10.2174/138920212799034802

[62] Salas A, García-Magariños M, Logan I, Bandelt HJ. The saga of the many studies wrongly associating mitochondrial DNA with breast cancer. BMC Cancer. 2014;**14**:659. DOI: 10.1186/1471-2407-14-659

[63] Venderbosch S, van Vliet S, Craenmehr MH, Simmer F, de Haan AF, Punt CJ, Koopman M, Nagtegaal ID. Mitochondrial microsatellite instability in patients with metastatic colorectal cancer. Virchows Archiv. 2015;**466**(5):495-502. DOI:10.1007/s00428-015-1733-8

[64] Graziewicz MA, Day BJ, Copeland WC. The mitochondrial DNA polymerase as a target of oxidative damage. Nucleic Acids Research. 2002;**30**(13):2817-2824

[65] Máximo V, Soares P, Lima J, Cameselle-Teijeiro J, Sobrinho-Simões M. Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology a study with emphasis on Hürthle cell tumors. The American Journal of Pathology. 2002;**160**(5):1857-1865. DOI: 10.1016/S0002-9440(10)61132-7

[66] Schwartz S Jr, Alazzouzi H, Perucho M. Mutational dynamics in human tumors confirm the neutral intrinsic instability of the mitochondrial D-loop poly-cytidine repeat. Genes, Chromosomes & Cancer. 2006;**45**(8):770-780. DOI:10.1002/gcc.20340

[67] Horan MP, Cooper DN. The emergence of the mitochondrial genome as a partial regulator of nuclear function is providing new insights into the genetic mechanisms underlying age-related complex disease. Human Genetics. 2014;**133**(4):435-458. DOI: 10.1007/s00439-013-1402-4

[68] Ju YS, Tubio JM, Mifsud W, Fu B, Davies HR, Ramakrishna M, et al. Frequent somatic transfer of mitochondrial DNA into the nuclear genome of human cancer cells. Genome Research. 2015;**25**(6):814-824. DOI: 10.1101/gr.190470.115

[69] Kaipparettu BA, Ma Y, Park JH, Lee TL, Zhang Y, Yotnda P, Creighton CJ, Chan WY, Wong LJ. Crosstalk from non-cancerous mitochondria can inhibit tumor properties of metastatic cells by suppressing oncogenic pathways. PLoS One. 2013;**8**(5):e61747. DOI: 10.1371/journal.pone.0061747

[70] Dwight T, Mann K, Benn DE, Robinson BG, McKelvie P, Gill AJ, Winship I, Clifton-Bligh RJ. Familial SDHA mutation associated with pituitary adenoma and pheochromocytoma/paraganglioma. The Journal of Clinical Endocrinology and Metabolism. 2013;**98**(6):E1103-E1108. DOI: 10.1210/jc.2013-1400

[71] Lehtonen R, Kiuru M, Vanharanta S, Sjöberg J, Aaltonen LM, Aittomäki K, et al. Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors. The American Journal of Pathology. 2004;**164**(1):17-22. DOI: 10.1016/S0002-9440(10)63091-X

[72] Ricketts C, Woodward ER, Killick P, Morris MR, Astuti D, Latif F, Maher ER. Germline SDHB mutations and familial renal cell carcinoma. Journal of the National Cancer Institute. 2008;**100**(17):1260-1262. DOI: 10.1093/jnci/djn254

[73] Janeway KA, Kim SY, Lodish M, Nosé V, Rustin P, Gaal J, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. Proceedings of the National Academy of Sciences of the United States of America. 2011;**108**(1):314-318. DOI: 10.1073/pnas.1009199108

[74] Simões RV, Serganova IS, Kruchevsky N, Leftin A, Shestov AA, Thaler HT, Sukenick G, Locasale JW, Blasberg RG, Koutcher JA, Ackerstaff E. Metabolic plasticity of metastatic breast cancer cells: Adaptation to changes in the microenvironment. Neoplasia. 2015;**17**(8):671-684. DOI: 10.1016/j.neo.2015.08.005

[75] Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Krönke J, Bullinger L, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. Journal of Clinical Oncology. 2010;**28**(22):3636-3643. DOI: 10.1200/JCO.2010.28.3762

[76] Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;**321**(5897):1807-1812. DOI: 10.1126/science.1164382

[77] Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell. 2010;**18**(1):11-22. DOI: 10.1016/j.ccr.2010.05.026

[78] Li WL, Xiao MS, Zhang DF, Yu D, Yang RX, Li XY, Yao YG. Mutation and expression analysis of the IDH1, IDH2, DNMT3A, and MYD88 genes in colorectal cancer. Gene. 2014;**546**(2):263-270. DOI: 10.1016/j.gene.2014.05.070

[79] Venesio T, Balsamo A, Errichiello E, Ranzani GN, Risio M. Oxidative DNA damage drives carcinogenesis in MUTYH-associated-polyposis by specific mutations of mitochondrial and MAPK genes. Modern Pathology. 2013;**26**(10):1371-1381. DOI: 10.1038/modpathol.2013.66

[80] de Araujo LF, Fonseca AS, Muys BR, Plaça JR, Bueno RB, Lorenzi JC, Santos AR, Molfetta GA, Zanette DL, Souza JE, Valente V, Silva WA Jr. Mitochondrial genome instability in colorectal adenoma and adenocarcinoma. Tumour Biology 2015;**36**(11):8869-8879. DOI:10.1007/s13277-015-3640-7

[81] Donthamsetty S, Brahmbhatt M, Pannu V, Rida PC, Ramarathinam S, Ogden A, Cheng A, Singh KK, Aneja R. Mitochondrial genome regulates mitotic fidelity by maintaining centrosomal homeostasis. Cell Cycle. 2014;**13**(13):2056-2063. DOI: 10.4161/cc.29061

[82] Saunders W. Centrosomal amplification and spindle multipolarity in cancer cells. Seminars in Cancer Biology. 2005;**15**(1):25-32. DOI: 10.1016/j.semcancer.2004.09.003

[83] Wickramasekera NT, Das GM. Tumor suppressor p53 and estrogen receptors in nuclear-mitochondrial communication. Mitochondrion. 2014;**16**:26-37. DOI: 10.1016/j.mito.2013.10.002

[84] Bellizzi D, D'Aquila P, Giordano M, Montesanto A, Passarino G. Global DNA methylation levels are modulated by mitochondrial DNA variants. Epigenomics. 2012;**4**(1):17-27. DOI: 10.2217/epi.11.109

[85] Minocherhomji S, Tollefsbol TO, Singh KK. Mitochondrial regulation of epigenetics and its role in human diseases. Epigenetics. 2012;**7**(4):326-334. DOI: 10.4161/epi.19547

[86] Formentini L, Sánchez-Aragó M, Sánchez-Cenizo L, Cuezva JM. The mitochondrial ATPase inhibitory factor 1 triggers a ROS-mediated retrograde prosurvival and proliferative response. Molecular Cell. 2012;**45**(6):731-742. DOI: 10.1016/j.molcel.2012.01.008

[87] Kang KA, Zhang R, Kim GY, Bae SC, Hyun JW. Epigenetic changes induced by oxidative stress in colorectal cancer cells: Methylation of tumor suppressor RUNX3. Tumour Biology. 2012;**33**(2):403-412. DOI: 10.1007/s13277-012-0322-6

[88] Guerra F, Perrone AM, Kurelac I, Santini D, Ceccarelli C, Cricca M, Zamagni C, De Iaco P, Gasparre G. Mitochondrial DNA mutation in serous ovarian cancer: Implications

for mitochondria-coded genes in chemoresistance. Journal of Clinical Oncology. 2012;**30**(36):e373-e378. DOI: 10.1200/JCO.2012.43.5933

[89] Tachibana M, Amato P, Sparman M, Woodward J, Sanchis DM, Ma H, et al. Towards germline gene therapy of inherited mitochondrial diseases. Nature. 2013;**493**(7434):627-631. DOI: 10.1038/nature11647

[90] Taylor RW, Chinnery PF, Turnbull DM, Lightowlers RN. Selective inhibition of mutant human mitochondrial DNA replication in vitro by peptide nucleic acids. Nature Genetics. 1997;**15**(2):212-215. DOI: 10.1038/ng0297-212

[91] van Gisbergen MW, Voets AM, Starmans MH, de Coo IF, Yadak R, Hoffmann RF, Boutros PC, Smeets HJ, Dubois L, Lambin P. How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. Mutation Research, Reviews in Mutation Research 2015;**764**:16-30. DOI:10.1016/j.mrrev.2015.01.001

[92] Hashizume M, Mouner M, Chouteau JM, Gorodnya OM, Ruchko MV, Potter BJ, Wilson GL, Gillespie MN, Parker JC. Mitochondrial-targeted DNA repair enzyme 8-oxoguanine DNA glycosylase 1 protects against ventilator-induced lung injury in intact mice. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2013;**304**(4):L287-L297. DOI: 10.1152/ajplung.00071.2012

[93] Yuzefovych LV, Kahn AG, Schuler MA, Eide L, Arora R, Wilson GL, Tan M, Rachek LI. Mitochondrial DNA repair through OGG1 activity attenuates breast Cancer progression and metastasis. Cancer Research. 2016;**76**(1):30-34. DOI: 10.1158/0008-5472.CAN-15-0692

[94] Rachek LI, Grishko VI, Alexeyev MF, Pastukh VV, LeDoux SP, Wilson GL. Endonuclease III and endonuclease VIII conditionally targeted into mitochondria enhance mitochondrial DNA repair and cell survival following oxidative stress. Nucleic Acids Research. 2004;**32**(10):3240-3247. DOI: 10.1093/nar/gkh648

[95] Gebb SA, Decoux A, Waggoner A, Wilson GL, Gillespie MN. Mitochondrial DNA damage mediates hyperoxic dysmorphogenesis in rat fetal lung explants. Neonatology. 2013;**103**(2):91-97. DOI: 10.1159/000342632

[96] Yang XM, Cui L, White J, Kuck J, Ruchko MV, Wilson GL, Alexeyev M, Gillespie MN, Downey JM, Cohen MV. Mitochondrially targeted endonuclease III has a powerful anti-infarct effect in an in vivo rat model of myocardial ischemia/reperfusion. Basic Research in Cardiology. 2015;**110**(2):3. DOI: 10.1007/s00395-014-0459-0

[97] Manfredi G, Fu J, Ojaimi J, Sadlock JE, Kwong JQ, Guy J, Schon EA. Rescue of a deficiency in ATP synthesis by transfer of MTATP6, a mitochondrial DNA-encoded gene, to the nucleus. Nature Genetics. 2002;**30**(4):394-399. DOI: 10.1038/ng851

[98] Tanaka M, Borgeld HJ, Zhang J, Muramatsu S, Gong JS, Yoneda M, et al. Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. Journal of Biomedical Science. 2002;**9**(6 Pt 1):534-541. DOI: 64726

[99] Lim SH, Wu L, Kiew LV, Chung LY, Burgess K, Lee HB. Rosamines targeting the cancer oxidative phosphorylation pathway. PLoS One. 2014;**9**(3):e82934. DOI: 10.1371/journal.pone.0082934

[100] Yan B, Stantic M, Zobalova R, Bezawork-Geleta A, Stapelberg M, Stursa J, Prokopova K, Dong L, Neuzil J. Mitochondrially targeted vitamin E succinate efficiently kills breast tumour-initiating cells in a complex II-dependent manner. BMC Cancer. 2015;**15**:401. DOI: 10.1186/s12885-015-1394-7

[101] Koehler BC, Jäger D, Schulze-Bergkamen H. Targeting cell death signaling in colorectal cancer: Current strategies and future perspectives. World Journal of Gastroenterology. 2014;**20**(8):1923-1934. DOI: 10.3748/wjg.v20.i8.1923

[102] Wilson TR, McEwan M, McLaughlin K, Le Clorennec C, Allen WL, Fennell DA, Johnston PG, Longley DB. Combined inhibition of FLIP and XIAP induces Bax-independent apoptosis in type II cancer cells. Oncogene. 2009;**28**(1):63-72. DOI: 10.1038/onc.2008.366

[103] Ozsvari B, Bonuccelli G, Sanchez-Alvarez R, Foster R, Sotgia F, Lisanti MP. Targeting flavin-containing enzymes eliminates cancer stem cells (CSCs), by inhibiting mitochondrial respiration: Vitamin B2 (riboflavin) in cancer therapy. Aging (Albany NY). 2017;**9**(12):2610-2628. DOI: 10.18632/aging.101351

[104] Vitiello GA, Medina BD, Zeng S, Bowler TG, Zhang JQ, Loo JK, Param NJ, Liu M, Moral JA, Zhao JN, Rossi F, Antonescu CR, Balachandran VP, Cross JR, DeMatteo RP. Mitochondrial inhibition augments the efficacy of imatinib by resetting the metabolic phenotype of gastrointestinal stromal tumor. Clinical Cancer Research. 2018;**24**(4):972-984. DOI:10.1158/1078-0432.CCR-17-2697

[105] Burke PJ. Mitochondria, bioenergetics and apoptosis in cancer. Trends in Cancer. 2017;**3**(12):857-870. DOI: 10.1016/j.trecan.2017.10.006

[106] Costa R, Carneiro BA, Taxter T, Tavora FA, Kalyan A, Pai SA, Chae YK, Giles FJ. FGFR3-TACC3 fusion in solid tumors: Mini review. Oncotarget. 2016;**7**(34):55924-55938. DOI: 10.18632/oncotarget.10482

[107] Lasorella A, Sanson M, Iavarone A. FGFR-TACC gene fusions in human glioma. Neuro-Oncology. 2017;**19**(4):475-483. DOI: 10.1093/neuonc/now240

[108] Frattini V, Pagnotta SM, Tala FJJ, Russo MV, Lee SB, et al. A metabolic function of FGFR3-TACC3 gene fusions in cancer. Nature. 2018;**553**(7687):222-227. DOI: 10.1038/nature25171

[109] Briscoe AG, Hopkins KP, Waeschenbach A. High-throughput sequencing of complete mitochondrial genomes. Methods in Molecular Biology. 2016;**1452**:45-64. DOI: 10.1007/978-1-4939-3774-5_3

[110] Li M, Schönberg A, Schaefer M, Schroeder R, Nasidze I, Stoneking M. Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. American Journal of Human Genetics. 2010;**87**(2):237-249. DOI: 10.1016/j.ajhg.2010.07.014

[111] Goto H, Dickins B, Afgan E, Paul IM, Taylor J, Makova KD, Nekrutenko A. Dynamics of mitochondrial heteroplasmy in three families investigated via a repeatable re-sequencing study. Genome Biology. 2011;**12**(6):R59. DOI: 10.1186/gb-2011-12-6-r59

[112] Dames S, Chou LS, Xiao Y, Wayman T, Stocks J, Singleton M, Eilbeck K, Mao R. The development of next-generation sequencing assays for the mitochondrial genome and 108 nuclear genes associated with mitochondrial disorders. The Journal of Molecular Diagnostics. 2013;**15**(4):526-534. DOI: 10.1016/j.jmoldx.2013.03.005

[113] Kloss-Brandstätter A, Weissensteiner H, Erhart G, Schäfer G, Forer L, Schönherr S, et al. Validation of next-generation sequencing of entire mitochondrial genomes and the diversity of mitochondrial DNA mutations in oral squamous cell carcinoma. PLoS One. 2015;**10**(8):e0135643. DOI: 10.1371/journal.pone.0135643

[114] Zambelli F, Vancampenhout K, Daneels D, Brown D, Mertens J, Van Dooren S, Caljon B, Gianaroli L, Sermon K, Voet T, Seneca S, Spits C. Accurate and comprehensive analysis of single nucleotide variants and large deletions of the human mitochondrial genome in DNA and single cells. European Journal of Human Genetics. 2017;**25**(11):1229-1236. DOI: 10.1038/ejhg.2017.129

[115] Perales-Clemente E, Cook AN, Evans JM, Roellinger S, Secreto F, Emmanuele V, Oglesbee D, Mootha VK, Hirano M, Schon EA, A1 T, Nelson TJ. Natural underlying mtDNA heteroplasmy as a potential source of intra-person hiPSC variability. The EMBO Journal. 2016;**35**(18):1979-1990. DOI: 10.15252/embj.201694892

[116] Pagani IS, Kok CH, Saunders VA, Van der Hoek MB, Heatley SL, Schwarer AP, Hahn CN, Hughes TP, White DL, Ross DM. A method for next-generation sequencing of paired diagnostic and remission samples to detect mitochondrial DNA mutations associated with Leukemia. The Journal of Molecular Diagnostics. 2017;**19**(5):711-721. DOI: 10.1016/j.jmoldx.2017.05.009

[117] Yu M. Circulating cell-free mitochondrial DNA as a novel cancer biomarker: Opportunities and challenges. Mitochondrial DNA. 2012;**23**(5):329-332. DOI: 10.3109/19401736.2012.696625

[118] Lu H, Busch J, Jung M, Rabenhorst S, Ralla B, Kilic E, Mergemeier S, Budach N, Fendler A, Jung K. Diagnostic and prognostic potential of circulating cell-free genomic and mitochondrial DNA fragments in clear cell renal cell carcinoma patients. Clinica Chimica Acta. 2016;**452**:109-119. DOI: 10.1016/j.cca.2015.11.009

[119] He WS, Bishop KS. The potential use of cell-free-circulating-tumor DNA as a biomarker for prostate cancer. Expert Review of Molecular Diagnostics. 2016;**16**(8):839-852. DOI: 10.1080/14737159.2016.1197121