



Cellular redox imbalance on the crossroad between mitochondrial dysfunction, senescence, and proliferation

Rumiana Bakalova^{a,*}, Ichio Aoki^a, Zhivko Zhelev^{a,b}, Tatsuya Higashi^a

^a Department of Molecular Imaging and Theranostics, National Institutes for Quantum Science and Technology (QST), Chiba, 263-8555, Japan

^b Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria & Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia, Bulgaria

ABSTRACT

Recent studies demonstrate that redox imbalance of NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ pairs due to impaired respiration may trigger two “hidden” metabolic pathways on the crossroad between mitochondrial dysfunction, senescence, and proliferation: “ β -oxidation shuttle” and “hydride transfer complex (HTC) cycle”. The “ β -oxidation shuttle” induces NAD^+/NADH redox imbalance in mitochondria, while HTC cycle maintains the redox balance of cytosolic NAD^+/NADH , increasing the redox disbalance of $\text{NADP}^+/\text{NADPH}$. Senescence appears to depend on high cytoplasmic NADH but low NADPH, while proliferation depends on high cytoplasmic NAD^+ and NADPH that are under mitochondrial control. Thus, activating or deactivating the HTC cycle can be crucial to cell fate – senescence or proliferation. These pathways are a source of enormous cataplerosis. They support the production of large amounts of NADPH and intermediates for lipid synthesis and membrane biogenesis, as well as for DNA synthesis.

1. Main text

The origin of pre-malignancy is still an enigma, which makes it impossible to define a clear and effective anticancer strategy. Over the last decade, studies indicate that mitochondrial redox state and energy homeostasis appear to determine the nuclear genome integrity and the opposite is less likely. For example, impairment of mitochondrial respiration underlies “aging and cancer phenomenon” [1]. Mitochondrial integrity and functionality decrease with age due to accumulation of mtDNA mutations and significant redox imbalance, which is accompanied by impaired oxidative phosphorylation (OXPHOS) and energy loss. This activates the substrate level phosphorylation (SLP) to compensate for this energy loss and keep the cell alive. It is generally accepted that OXPHOS and SLP are in cross-signaling to maintain adequate constant $\Delta G'_{\text{ATP}}$ (approximately -56 kJ/mol) for viability – when OXPHOS is compromised, SLP increases compensatory [2]. While OXPHOS depends on respiratory chain and mitochondrial proton motive force, SLP is associated with glycolysis and glutaminolysis. Therefore, prolonged dependence of cell on SLP for energy production may cause genomic instability, which eventually becomes irreversible [1]. Another piece of evidence is the viral origin of many cancers. mtDNA supposes to be more susceptible to viral attack than nuclear DNA, whose integrity is protected by histones and nucleotide excision repair mechanisms [3]. In addition, mtDNA damage as the result of inflammation and oxidative

stress is more extensive and lasts longer than nuclear DNA damage in human cells [3]. Viruses can be incorporated into the mitochondria of the host and disrupt mitochondrial respiration and energy metabolism. Thus, prolonged non-severe viral infections may alter the expression of tumor suppressor genes and oncogenes in the nuclear DNA and cause malignant transformation.

Interest in the mitoepigentic regulation of cancer is revived nowadays through the concept of “epigenetic priming” of cells, which is under mitochondrial control [4]. Damaged mitochondrial checkpoints and redox imbalance have been shown to cause epigenetic reprogramming in the nucleus via reversible or irreversible changes in the methylation and/or acetylation of nuclear genome [4].

At present, cancer cells and tumors are simply divided into two groups based on their ATP production: (i) dependent on glycolysis; and (ii) OXPHOS dependent. However, the generally accepted concepts for carcinogenesis did not address the case in which some cancer cells may have normal respiration (assessed by oxygen consumption) accompanied by greatly decreased oxygen-dependent ATP production via OXPHOS. For example, cells with high malignancy were found to have a higher oxygen consumption, but a lower mitochondrial ATP production compared to cells with lower malignancy [2,5]. This is consistent with the concept of mitochondrial uncoupling in cancer.

We would like to outline several important redox-dependent metabolic factors that could be a strong driver of malignant transformation,

* Corresponding author. Department of Molecular Imaging and Theranostics, National Institutes for Quantum Science and Technology (QST), 4-9-1 Anagawa, Inage-ku, Chiba, 263-8555, Japan.

E-mail address: bakalova.rumiana@qst.go.jp (R. Bakalova).

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but are still out of the focus of researchers and seem to be neglected:

- (1) In addition to ATP, cancer cells need metabolites to grow and proliferate. Dysfunctional mitochondria are a source of enormous cataplerosis due to the disrupted Krebs cycle in certain segments. Cells with such mitochondria must utilize the large quantity of accumulated intermediates that are precursors for synthesis of lipids, proteins, and nucleic acids. This provokes mass accumulation (growth) and ultimately proliferation.
- (2) Accumulation of high amounts of NADPH in cancer cells due to accelerated metabolic pathways such as pentose-phosphate pathway and/or overexpressed regulatory factors such as nuclear factor erythroid 2-related factor 2 (NRF2). Cellular energy is stored not only in ATP but also in NADPH as energy for synthesis. It is generally accepted that NADPH maintains the steady-state level of reduced glutathione and other thiol-containing redox-active substances in cells (such as peroxiredoxins, thioredoxins, glutaredoxins, etc.), but it is also involved in many synthetic processes, including lipid synthesis. In addition, the level of these reducing equivalents and their pressure on the mitochondrial and cytosolic redox state may be a decisive factor in the fate of the cell – senescence or survival and immortality.
- (3) The fatty acid metabolism as a source of OXPHOS in cancer cells, as well as a source of intermediates for lipid synthesis (such as acetyl-CoA) and membrane biogenesis – an essential step of cell growth and proliferation. The connection between obesity and cancer can be noted here.

Recent articles demonstrate that cellular redox state is on the crossroad between mitochondrial dysfunction, senescence, and proliferation [6,7]. Redox imbalance of $NAD^+/NADH$ and $NADP^+/NADPH$ pairs may trigger two “hidden” pathways that drive metabolic reprogramming to overcome cell aging and induce carcinogenesis: (i) multi-enzymatic hydride transfer complex cycle – HTC cycle [6]; and (ii) “ β -oxidation-citrate-malate shuttle” or “ β -oxidation shuttle” for short [7]. These pathways support the production of large amounts of NADPH, and the “ β -oxidation shuttle” also supports the overproduction of acetyl-CoA – the main precursor of lipid synthesis.

The concept of the “ β -oxidation shuttle” is based on experimental evidence from the last decade for the pronounced mitochondrial fatty acid oxidation (mFAO) in many types of cancer cells and solid tumors, which was found to coexist with abnormally activated fatty acid synthesis (FAS) and the mevalonate pathway [7]. FAS is required for lipid synthesis and membrane biogenesis, while mevalonate pathway is closely related to cholesterol metabolism and universal lipid post-translational modification (prenylation) of oncoproteins and their intracellular membrane trafficking. It is assumed that partial combustion of fatty acids in mitochondria may be a major trigger for their redox imbalance and dysfunction. This well-founded hypothesis demonstrates that the impaired redox state of cancerous mitochondria can ensure the continuous operation of mitochondrial β -oxidation by disconnecting it from the Krebs cycle and connecting it to the citrate-malate shuttle. This could create a new metabolic pathway in cancer cells, called “ β -oxidation shuttle”, which forces them to proliferate (Fig. 1A). The “ β -oxidation shuttle” consists of a mitochondrial β -oxidation and a citrate-malate

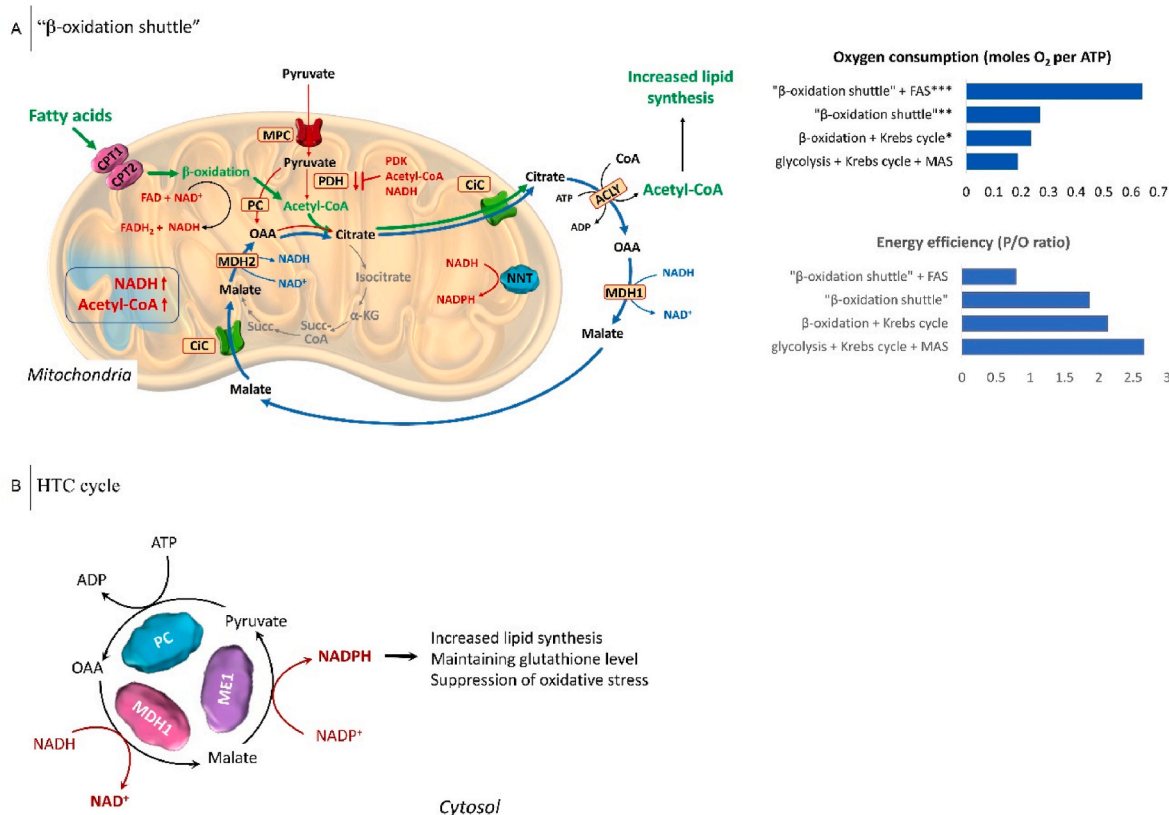


Fig. 1. Schematic representation of the “ β -oxidation shuttle” in mitochondria (A) and the “hydride transfer complex (HTC) cycle” in cytosol (B). In (A): The green arrows indicate the metabolic flux from fatty acids. The red arrows indicate the metabolic flux from glucose. The red blunt ends indicate the inhibition of a particular enzyme. The blue arrows indicate the malate-citrate shuttle. Phosphate/oxygen (P/O) ratio and oxygen consumption in the combustion of glucose and palmitate in the “ β -oxidation shuttle” and other metabolic pathways are given on the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Abbreviations: ACLY: ATP citrate lyase; CIC: citrate-isocitrate carrier; CPT1 and CPT2: carnitine palmitoyl transferases 1 and 2; FAS – fatty acid synthesis; α -KG: α -ketoglutarate; MAS: malate-aspartate shuttle; ME1: malic enzyme 1; MPC: mitochondrial pyruvate carrier; NNT: NAD(P) transhydrogenase; OAA: oxaloacetate; PDK: pyruvate dehydrogenase kinase; PDH: pyruvate dehydrogenase; PC: pyruvate carboxylase; Succ: succinate.

shuttle. In turn, the citrate-malate shuttle consists of a transmembrane transporter (citrate-isocitrate carrier, CIC) and several enzymes: ATP-citrate lyase (ACLY) and two malate dehydrogenases (MDH1 and MDH2). The four proteins have been found to be overexpressed in cancer cells [7].

Calculation of the phosphate/oxygen ratio indicates that the “ β -oxidation shuttle” is inefficient as an energy source and must consume significantly more oxygen per mole of ATP produced when combined with acetyl-CoA consuming pathways, such as the FAS and mevalonate pathway (Fig. 1A). The possible inefficient synthesis of ATP in the “ β -oxidation shuttle” provides conditions for some solid tumors (such as gliomas) to be dependent simultaneously on OXPHOS and glycolysis [2,8,9]. The “ β -oxidation shuttle” is unconventional mFAO that leads to overproduction of acetyl-CoA and increased lipid synthesis. In addition, this pathway is tightly connected to activation of oxidative and reductive glutaminolysis and aspartate synthesis – key factors for accelerated DNA replication and proliferation [7]. Briefly, the “ β -oxidation shuttle” is a source for biomass accumulation, accelerated oxygen consumption, and proliferation. This unconventional mFAO may represent the metabolic “secret” of cancer underlying hypoxia and genomic instability.

It should be noted that the altered NAD^+/NADH ratio in the mitochondrial matrix as a result of mitochondrial dysfunction is the trigger of the “ β -oxidation shuttle”. In turn, the “ β -oxidation shuttle” may further decrease this ratio in the mitochondrial matrix, aggravating redox imbalance.

Recently, another metabolic pathway has been described (HTC cycle), concerning the cytoplasmic NAD^+/NADH ratio, which determines the cell choice – senescence or proliferation [6]. This cycle consists of three enzymes: MDH1, malic enzyme 1 (ME1), and cytosolic pyruvate carboxylase (PC) that are found to be overexpressed in cancer [6,7]. HTC cycle reprograms NAD metabolism and overcomes cell senescence, consuming ATP (Fig. 1B). It was discovered in cytosolic phase-separated bodies of prostate cancer cells and fibroblasts growing in hypoxic conditions. Inactivation of the HTC cycle causes senescence, while its exogenous expression bypasses senescence and triggers transformation of primary embryonic fibroblasts allowing colony formation [6].

This cycle compensates for the reductive pressure of NADH in the cytoplasm, which is the result of mitochondrial dysfunction. Dysfunctional mitochondria are known to be characterized by high levels of NADH. This is accompanied by an increase in cytosolic NADH and a decrease in the NAD^+/NADH ratio in the cytoplasm, which is a prerequisite for senescence. The malate-aspartate shuttle (MAS) is known to link the redox state of mitochondrial NADH/NAD^+ with the cytosolic NADH/NAD^+ (“Borst cycle”) [10]. The efflux of aspartate from mitochondria by the MAS is dependent on the proton-motive force generated by the respiratory chain: for every aspartate effluxed, mitochondria take up one glutamate and one proton. Thus, the MAS transports reducing equivalents from the cytosol to the mitochondria, against the concentration gradient of NADH. This makes the MAS unidirectional toward oxidation of cytosolic NADH and explains why the NADH/NAD^+ ratio is much higher in the mitochondria than in the cytosol. We consider this shuttle as the main mechanism for clearing the cytosol from high concentrations of NADH when the mitochondria are functioning normally and there is enough oxygen in the cell [7]. However, in cells with dysfunctional mitochondria and hypoxia, the transport of reducing equivalents from MAS is disrupted, leading to accumulation of NADH in the cytosol [7]. In addition, inborn errors have been found in MAS in cancer cells [10].

Igelmann et al. have shown that the HTC cycle transfers reducing equivalents from cytosolic NADH to NADP^+ and increases the NAD^+/NADH ratio, which is accompanied by the production of large amounts of NADPH (Fig. 1B) [6]. This is a prerequisite for increased lipid synthesis, maintenance of glutathione metabolism, suppression of oxidative stress, and ultimately suppression of cell senescence. Their study shows

that the transitions between NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ redox pairs is a crucial factor in cell fate. This transition is also catalyzed by nicotinamide nucleotide transhydrogenase (NNT) which is overexpressed in cancer progression and its deficiency dysregulates mitochondrial retrograde signaling and impedes proliferation [11].

Dysfunctional mitochondria could be repaired by processes like mitochondrial biogenesis, mitophagy, fission and fusion. However, if the dysfunction is generalized to all mitochondria and they cannot be repaired, the cell faces two choices – senescence or transformation and uncontrolled proliferation. Tumor suppressor p53 appears to inhibit pathways for adjustment of cellular redox state and prevents proliferation [6]. Genetic instability, which may result from impaired redox state of NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ pairs and possibly increased mitochondrial ROS production, may affect the function of these tumor suppressors. The ability of some tumor suppressors, such as p53, to inhibit the HTC cycle and lipid synthesis, marks the key metabolic segments that can be attacked to fight cancer (Fig. 2).

The two metabolic pathways described above are not energetically beneficial, but are sources of enormous cataplerosis and energy for synthesis in form of NADPH, which forces cells to grow and proliferate. The “ β -oxidation shuttle” also illustrates that high oxygen consumption could be accompanied by very low oxygen-dependent ATP production, which means that increased OXPHOS may coexist with increased glycolysis and glutaminolysis. The HTC cycle illustrates how cancer cells can bypass senescence by modulating the redox state of NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ pairs in the cytosol, saving dysfunctional mitochondria and cell in general. In both metabolic pathways, redox imbalance of dysfunctional mitochondria appears to be the crucial factor in triggering mitochondrial-mediated “epigenetic priming” and malignant transformation.

Mitochondrial dysfunction can occur because of genetic, metabolic, and/or regulatory pathways and factors, but the result is the same – the accumulation of large amounts of NADH and overproduction of ROS (mostly superoxide) in the impaired mitochondria. Many redox-related theories of aging and malignant transformation have been described. ROS is considered a major factor in these two processes, but it is not clear why ROS increases in cell senescence as well as in carcinogenesis. An interesting hypothesis has been described by Pervaiz & Clement [12]. The authors consider superoxide as “oncogenic ROS” and hydrogen peroxide as “onco-suppressive ROS”. They suggested that cellular redox state, where the ratio tilts predominantly in favor of superoxide, inhibits apoptosis, and promotes survival and proliferation. If the ratio tilts in favor of hydrogen peroxide, this creates an intracellular environment suitable for senescence, induction of apoptosis and cell death. However, this hypothesis does not explain the following paradox: Why some of the most aggressive and rapidly proliferating tumors contain high levels of superoxide dismutase [13,14], which should be accompanied by decrease of superoxide and increase of hydrogen peroxide. In this context, it is interesting to note the role of NADPH-dependent pathways involved in hydroperoxide metabolism and activating the “adaptive antioxidant response” in cancer cells, such as glutathione peroxidase and transcriptional factor NRF2, which counteract oxidative stress. Much has been written about the role of glutathione, so we would like to briefly discuss the role of NRF2 in the context of our hypothesis. NRF2 is considered a factor that overcomes mitochondrial damage, increases the oxidative metabolism of mitochondria, and regulates the cellular redox homeostasis and cytoprotective responses, allowing adaptation and survival under conditions of stress [15]. NRF2 activates all metabolic pathways and enzymes leading to NADPH synthesis, including mFAO [15]. This is directly related to the activation of the “ β -oxidation shuttle” in dysfunctional mitochondria. Most likely, NRF2 also activates the HTC cycle, but this needs experimental evidence.

The interplay between NRF2 and peroxiredoxins could also be a decisive factor in cell choice – senescence or genomic instability [16]. The role of peroxiredoxins in linking aging to genome instability and

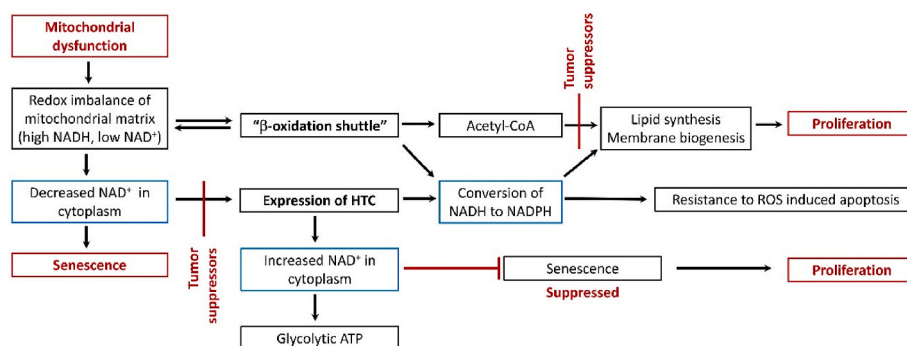


Fig. 2. “Beta-oxidation shuttle” and “hydride transfer complex (HTC) cycle” on the crossroad between mitochondrial dysfunction, senescence, and proliferation: Role of the redox state of NAD^+/NADH and $\text{NADP}^+/\text{NADPH}$ pairs for cell fate.

cancer is well described [17]. It should be noted that NADPH maintains the reduced state of peroxiredoxins, glutathione, and many other thiol-containing redox active compounds of the adaptive antioxidant response. However, NADPH is also involved in the production of ROS (mainly “pro-oncogenic” superoxide) by the NADPH-dependent oxidase complex (NOX) [18]. The balance between NADPH-mediated redox-sensitive pathways seems crucial for cell fate. The collapse of antioxidant defense systems and severe oxidative stress will lead to senescence and cell death. However, when high levels of NADPH are accompanied by tolerable inflammation and sufficiently good adaptive antioxidant response, this allows cells to maintain ROS above a critical level, causing genomic instability but below the threshold for inducing apoptosis, which may trigger malignant transformation.

Our hypothesis focuses on survival and malignant transformation, rather than senescence, as the “β-oxidation shuttle” and the HTC cycle are mainly related to proliferation. However, it explains the causal relationship between proliferation and senescence in cells with mitochondrial dysfunction. We assume that the causal relationship and the reason for cell differentiation/dedifferentiation lie in the redox state of NADH/NAD^+ and $\text{NADPH}/\text{NADP}^+$ pairs. The discovery of the key factor and/or key regulatory mechanism that switches the redox state of these two redox pairs from senescence mode to survival mode and vice versa, which determines cell choice – senescence or proliferation, could help clarify the root cause of aging and carcinogenesis. This is the subject of future analyses, and hypotheses.

Declaration of competing interest

No potential conflicts of interest are disclosed.

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