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Hypothesis

Hypothetical Hydrogenase Activity of Human Mitochondrial Complex I and Its Role in Preventing Cancer Transformation

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Abstract

Cancer research has made a magnificent progress in past decades with an advancement of molecular biology. However, the mechanisms of cancer transformation are still not fully revealed. Thus, we must think about if there are some unknown factors playing a causative role in the cancer formation. Mitochondrial complex I oxidizes NADH to NAD^+ and reduces ubiquinone to ubiquinol, regenerated NAD^+ keeping pyruvate dehydrogenase and Krebs cycle function. Hydrogenases are widespread in nature, they occur in bacteria, archaea, and some eukarya. It is unknown whether hydrogenase activity exists in human mitochondria. The complex I shares a last common ancestor with hydrogenases, and is closely related with hydrogenase in sequence and modular structure. The hydrogenase activity has been observed recently in complex I of higher plants. Based on these observations, I propose a hypothesis that mitochondrial complex I in human may also retain the hydrogenase activity. The hypothetical hydrogenase activity could release excessive reducing equivalents of NADH from electron transport chain when a cell is in hypoxia, decreased oxidative phosphorylation or a low ATP demand. Loss of the hydrogenase activity may result in aerobic glycolysis, activation of pentose phosphate pathway, elevated lipid synthesis, and activations of oncoproteins via acetylation, all of these alterations lead to cell proliferations and cancer transformation. Reducing mitochondrial NADH/NAD^+ ratio or recovering the hydrogenase activity would reverse the cell transformation.

Introduction

Complex I (NADH:ubiquinone oxidoreductase, E.C.1.6.5.3) is the first enzyme in the respiratory chain of mitochondria and bacteria, which catalyzes the oxidation of NADH and the reduction of quinone. Close relations between complex I and group 4 membrane-bound [NiFe] hydrogenase suggest that complex I arose from the association of a soluble nicotinamide adenine dinucleotide (NAD^+)-reducing hydrogenase with a Mrp-like antiporter.[1] In addition, Bovine mitochondrial complex I has sequence similarity with a bacterial NAD^+-reducing hydrogenase.[2] Moreover, hydrogenase activity has been detected in higher plants mitochondria and is closely related to complex I.[3] Based on these observations and ubiquity of mitochondria in eukaryotes, it is likely that human mitochondrial complex I also possesses hydrogenase activity. On the other hand, it is generally accepted that aerobic glycolysis is a hallmark of cancer cells. Warburg initially suggested that the aerobic glycolysis was caused by mitochondrial defect.[4] However, later studies show that cancer cells still exhibit certain extent of oxidative phosphorylation (OXPHOS) to meet the cellular energy demand. Other studies indicate that the aerobic glycolysis may result from upregulation of oncogenes, such as c-*Myc* and Hypoxia-Inducible Factor *(HIF*), or loss of *p53* tumor suppressor.[5] Alternatively, the hydrogenase activity hypothesis may provide an explanation of why cancer cells show upregulation of aerobic glycolysis.

The hypothesis

Based on the similarity of complex I with hydrogenase and the observation of hydrogenase activity in complex I of higher plants, I hypothesize that human mitochondrial complex I may also possess the hydrogenase activity. Loss of it is likely resulting in cancer

Keywords: Cancer; Complex I; Hydrogenase activity; NADH; Glycolysis and pentose phosphate pathway; Oncogenes.

Abbreviations: NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; PDC, pyruvate dehydrogenase complex; PDKs, pyruvate dehydrogenase kinases; ETC, electron transport chain; TCA, tricarboxylic acid; PPP, pentose phosphate pathway; ACLY, ATP citrate lyase.

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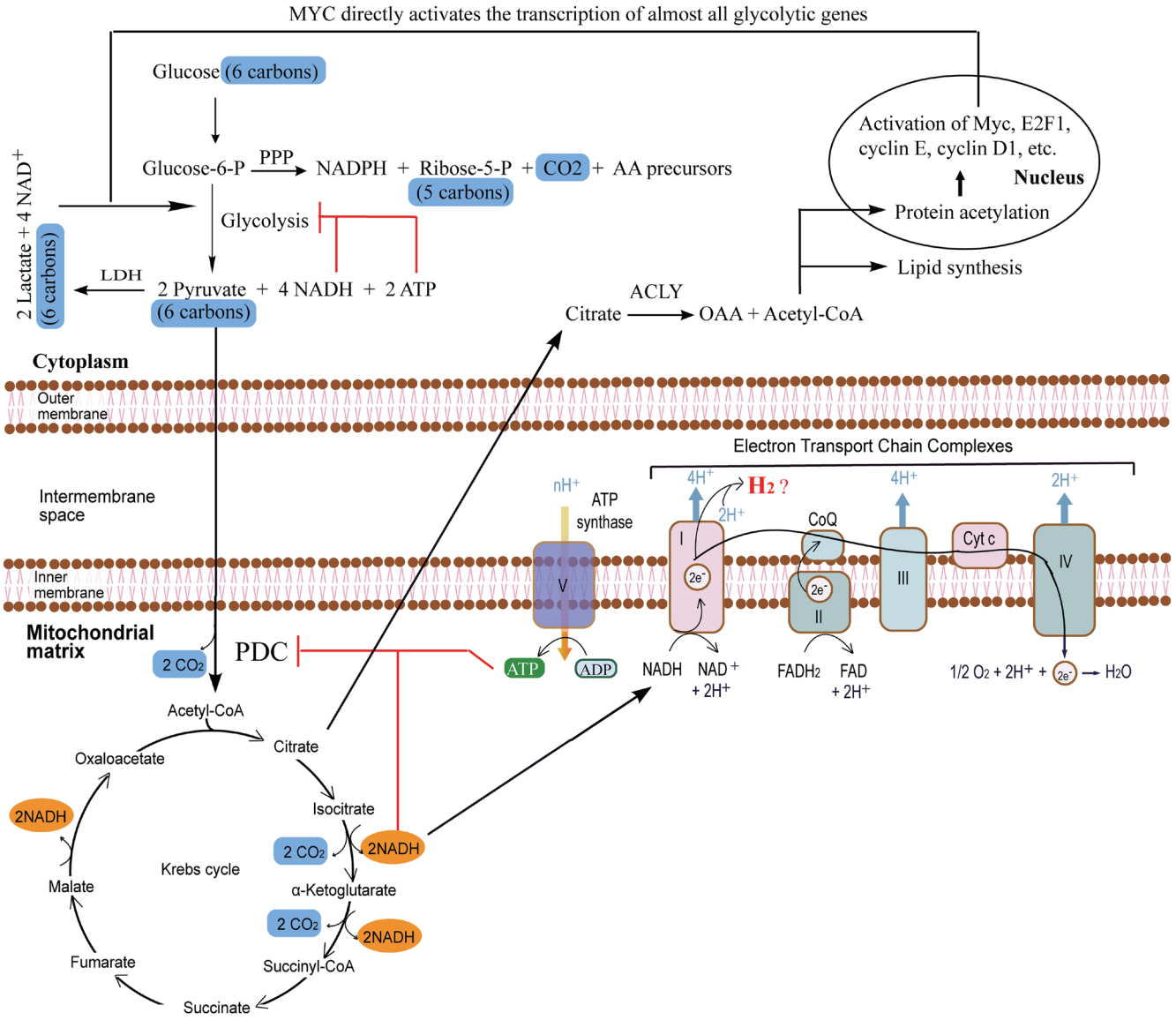

Fig. 1. Glucose metabolism and cancer formation. Under conditions of hypoxia, reduced OXPHOS, or low ATP demand, the pyruvate oxidation in mitochondria decreases. The accumulated pyruvate is reduced to lactate with regenerating NAD+ when cellular ATP demand is high, whereas the glucose metabolism diverts to PPP when the ATP demand is low. The PPP produces biomass precursors and NADPH for the syntheses of nucleic acids, proteins, and lipids, which lead to cell replications. On the other hand, the citrate is converted to OAA and acetyl-CoA that enhances protein acetylation. The acetylation of oncoproteins activates Myc, E2F1, cyclin E, cyclin D1, etc. If complex I possesses hydrogenase activity, the excessive equivalents of NADH could be released by the formation of molecular hydrogen. Thus, the PDC and Krebs cycle would be de-inhibited, resulting in complete oxidation of pyruvate to CO2 in mitochondria. Since the glucose carbons are completely converted to CO2, there is little biomass accumulation and the cell cannot replicate. Black arrow lines mean activation, red lines mean inhibition. AA, amino acid; OAA, oxaloacetate; ACLY, ATP citrate lyase; NAD, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; CoQ, coenzyme Q; Cyt C, cytochrome C.

transformation. In hypoxia, reduced mitochondrial capacity for OXPHOS, or low ATP demand, the electron transport chain (ETC) is unable to oxidize as much amount of NADH as that produced by pyruvate dehydrogenase complex (PDC) and tricarboxylic acid (TCA) cycle. In these situations, the hydrogenase activity of complex I could transfer electrons from NADH to proton to form molecular hydrogen and release excessive reducing equivalents of NADH. In case of losing the hydrogenase activity, the NADH/NAD^+ ratio is increased, which inhibits PDC and TCA cycle, resulting in reduced entry of pyruvate into mitochondria. The accumulated pyruvate in cytoplasm is reduced to lactate by lactate dehydrogenase (LDH) with regenerating NAD^+ that accelerates glycolysis. The activation of glycolysis may also be accompanied by an increase in pentose phosphate pathway (PPP) activity for biosynthesis.[6] On the other hand, the mitochondrial citrate can be transported to cytoplasm to restore oxaloacetate (OAA) and acetyl-CoA, which serves de novo lipid synthesis and enhances protein acetylation that activates oncoproteins.[7,8] Taken together, these metabolic alterations lead to sustained biomass syntheses and cell proliferations (Fig. 1). Thus, decreasing NADH/NAD^+ ra-

tio, recovering hydrogenase activity or increasing ATP consumption may reverse the transformation.

Similarities of complex I with hydrogenases

Hydrogenases exist in bacteria, archaea, and some eukarya.[9] It is unknown whether hydrogenases exist in human cells. The prototype complex I from prokaryotes may be viewed as a combination of 14 subunits, constituting a peripheral and a membrane arm. The peripheral arm is composed of seven subunits (NuoB to G and NuoI). The membrane arm is also constituted by seven subunits (NuoA, H, J to N).[10] It has long been recognized that complex I is closely related with group 4 hydrogenases. These hydrogenases are membrane-bound enzymes, which receive electrons from cytoplasmic donors and reduce protons to hydrogen. The simplest known functional group 4 hydrogenases are the energy-converting hydrogenase (Ech). They are constituted by six subunits EchA to F. The membrane subunits EchA and EchB are homologous to NuoL and AuoH respectively, whilst the peripheral subunits EchC, D, E and F are homologous to subunits NuoB, C, D and I, respectively.[1] In addition, the most closely related to complex I are the formate hydrogenlyases (FHL) of *Escherichia coli:* a seven subunit FHL-1 and a ten subunit FHL-2. Nine subunits of FHL-2 are homologous to 10 subunits of complex I.[11] Moreover, the quinone binding site in complex I appears to correspond to the NiFe active site in hydrogenase.[12] Besides prokaryotes, the bovine mitochondrial complex I was also related to bacterial NAD^+-reducing hydrogenase. The protein sequences of the 51-, 24-, and 75-kDa subunits of bovine mitochondrial complex I all have homologues in the soluble NAD^+-reducing hydrogenase from *A. eutrophus*.[2]

Hydrogenase activity of mitochondrial complex I in higher plants

It was reported that higher plants can produce H_2 during seed's germination or from tissue lysis.[13] The photosystem in chloroplasts had been shown to have H_2 evolution activity.[14] Recently, Zhang *et al*.[3] have reported that a hydrogenase activity existed in higher plants mitochondria and was closely related to complex I. Hypoxia could simultaneously promote H_2 evolution and succinate accumulation. The authors suggest that increased succinate and NADH are the driving force of the mitochondrial H_2 production. The H_2 production at least in plants is to adapt to the metabolic stress such as succinate accumulation, increased reduction of Q-pool and NADH/NAD+ ratio caused by hypoxia in order to maintain a more effective redox homeostasis regulation.[3] It is interesting to note that exogeneous H2 could greatly reduce the oxidative damage in animal and human body,[15] although the actual effect of oxidative stress in cancer formation was disputed.[16]

Implications of complex I hydrogenase activity in glucose metabolism

Glucose is converted to pyruvate through glycolysis, most of which is transferred to mitochondria under normal oxygen condition and is oxidized to acetyl-CoA and CO_2 by PDC, with reduction of NAD^+ to NADH. The acetyl-CoA then enter into TCA cycle in which it is oxidized to CO_2 and reduces NAD^+ to NADH. The NADH is oxidized by complex I to regenerate NAD^+. Electrons from NADH and FADH2 pass through the ETC to oxygen to generate a proton gradient across the mitochondrial membrane . This gradient is utilized by the ATP synthase to produce ATP to meet cellular energy demand (Fig. 1).[17–19]

In hypoxia, reduced capacity for OXPHOS or resting state of cells (low ATP demand), the ETC is unable to oxidize as much amount of NADH as that produced by PDC and TCA cycle. The increase in NADH/NAD^+ ratio inhibits PDC and all the regulatory enzymes in the TCA cycle.[20,21] The inhibition of pyruvate oxidation results in reduced entry of pyruvate into mitochondria. Hence the accumulated pyruvate in cytoplasm is reduced to lactate by LDH, regenerating NAD^+ and accelerating glycolysis since it requires NAD^+ to keep function. If complex I possesses a hydrogenase activity the excessive NADH could transfer electrons to protons to form molecular hydrogen and reduce NADH/NAD^+ ratio, providing the redox potential of H^+/H_2 is higher than that of NAD^+/NADH and that of UQ/UQH_2 (electrons flow from low potential to high one). Consequently, the excessive reducing equivalents of NADH are released as a form of molecular hydrogen. Without constrain of high ratio of NADH/NAD^+, the pyruvate oxidation and TCA cycle can keep functions, even in hypoxia or low capacity for OXPHOS (Fig. 1).

Loss of complex I hydrogenase activity may lead to cancer formation

Cancer cells may lose the hydrogenase activity due to complex I gene mutations or epigenetic alterations. Loss of it could increase NADH/NAD^+ ratio, inhibit PDK and TCA cycle, upregulate glycolysis and PPP, enhance lipid synthesis, and activate oncogenes via protein acetylation.

Upregulation of glycolysis and PPP

Increase in NADH/NAD^+ ratio

Under hypoxia the NADH/NAD^+ ratio in the mitochondria often increases owing to slowing of electron transport and consequent decrease in the rate of NADH oxidation.[3,22] It was also observed that several cell lines from cholangiocellular carcinoma and hepatocellular carcinoma showed significantly high NADH levels compared to normal hepatocytes.[23]

Inhibition of PDC and TCA cycle by NADH

The increase in NADH/NAD^+ ratio inhibits PDC and Krebs cycle (especially NADH producing reactions) activities.[20,21] PDC catalyzes the rate-limiting oxidative decarboxylation of pyruvate into acetyl-CoA with reduction of NAD^+ into NADH. The PDC activity is regulated mainly by pyruvate dehydrogenase kinases (PDKs). PDC phosphorylation by PDK inactivates its activity. The PDKs regulate PDC activity by responding to diverse allosteric modulators. Increase in NADH/NAD ratios is strong allosteric activators of PDKs, shutting down PDC activity.[21] The regulation of the TCA cycle and its constant feedback with OXPHOS is critical to keep the cells in a stable state. NADH inhibits all the regulatory enzymes in the TCA cycle. Thus, when electron flux through ETC slows down, NADH accumulates and the TCA cycle shuts down consequently (Fig. 1).[20]

Upregulation of aerobic glycolysis and PPP

Pyruvate, as an end product of glycolysis, can be converted to lactate by LDH, whose activity is regulated by the relative concentrations of its substrates. The increased levels of pyruvate and NADH activate LDH to produce lactate and regenerate NAD^+.[24,25] The NAD^+ is required for the activation of glyceraldehyde phosphate dehydrogenase (GAPDH), one of key enzymes of glycolysis. However, the enhanced glycolysis itself is insufficient to promote cell replication.[6,26] Glycolysis provides cells with ATP production, whereas PPP produces ribose-5-phosphate, amino acid precursors[27] and NADPH for the syntheses of nucleic acids, proteins and lipids. When a cell needs more ATP pyruvate is reduced to lactate with NAD^+ regeneration, so that the glycolysis can keep function to supply the cell with ATP. This is the cases that skeletal muscles produce lactate while exercising, and that most people with mitochondrial diseases such as MELAS have a lactic acidosis, but no higher incidence of cancers.[26] In this situation, the cells cannot replicate due to lack of biomass precursors. When a cell has sufficient ATP for maintenance of its structures and functions, the increased ATP level inhibits glycolysis,[28] and the glucose and glycolytic intermediates are diverted to PPP to promote biomass syntheses and cell replications.[6] Thus, the glucose metabolism may oscillate between glycolysis and PPP according to cellular energy demand. This hypothesis is supported by that the PPP, together with glycolysis, coordinates glucose flux and supports the cellular biogenesis of macromolecules and energy production.[6] The glycolysis provides cells with energy, while PPP supplies precursors for biomass syntheses. If the hypoxia or reduced OXPHOS capacity persist the cancer cells could adapt metabolically to these environments and alter epigenetics (activation of oncogenes) in favor of glycolysis and PPP. After the epigenetics have been changed, cancer cells could proliferate continuously even under normal oxygen condition (aerobic glycolysis, so called Warburg effect) (Fig. 1).[4]

In perspective of central carbon metabolism, glucose can be converted to metabolic precursors that are used to generate the entire biomass of the cell.[27] When pyruvate is not oxidized completely to CO_2 in mitochondria or reduced to lactate in cytosol, the glucose metabolism is shunted to PPP in which only 1/6 of glucose carbons are converted to CO_2 and rest to biomass precursors (Fig. 1). As the biomass accumulates, the internal energy (U, it depends on the amount of substance it contains) of a cell increase. When the internal energy doubles owing to the accumulations of biomass, the cell should divide into two in order to accommodate the internal energy level at a physiological range. Therefore, cancer cells duplicate uncontrollably.

Citrate, acetyl-CoA, and protein acetylation in cancer cells

In addition to upregulation of aerobic glycolysis and PPP, the intermediate metabolites of TCA cycle play important roles in cell growth and proliferation as well.[20] Citrate is synthesized from acetyl-CoA and oxaloacetate (OAA) in the first step of TCA cycle and can be transferred to cytosol via the citrate/isocitrate carrier (CIC) and be converted back to acetyl-CoA and oxaloacetate by ATP citrate lyase (ACLY). Acetyl-CoA is a vital building block for the endogenous biosynthesis of fatty acids and cholesterol. The increased fatty acid synthesis in cancer cells enhances membrane biogenesis in rapidly proliferating cancer cells.[29] On the other hand, the increase in acetyl-CoA levels has been associated with alterations in acetylation of both global histone and non-histone substrates including oncoproteins.[8,30–33]

It was reported that CIC mRNA levels were increased in various cancer cell lines and human tumors, and CIC levels were found elevated in many tumor cell lines.[34] In addition, ACLY is shown to be upregulated in cancer cells, and inhibition of ACLY suppresses proliferation of certain types of cancer cells.[30,35–37] Moreover, distinctive elevation of ACLY expression and activity was observed in colon, breast, lung, stomach, bladder, prostate, and liver tumors.[30,38–40]

Acetyl-CoA represents a critical metabolic signal for growth and proliferation. Upon entry into growth, intracellular acetyl-CoA levels increase substantially.[41] Cancer cells often manifest alterations of acetylation patterns and levels.[8] Acetylation levels are regulated by a balance in the activities of acetyltransferases and deacetylases. It has been shown that lysine acetyltransferases (KAT) play important roles in cellular differentiation and embryo development,[42] and are involved in oncogenesis.[8] MYC plays a key role in the regulation of aerobic glycolysis and directly activates the transcription of almost all glycolytic genes through binding the classical E-box sequence (CACGTG). Glucose transporter SLC2A1 is one of MYC targets, and upregulated by MYC to enhance glucose uptake.[43,44] The acetylation of Myc by GCN5 (general control non-derepressible 5) acetyltransferase stabilizes the Myc protein. Myc often acts in concert with another transcription factor important in the regulation of cell growth, E2F1. In small-cell lung cancer, E2F1 recruits GCN5 to acetylate H3K9, facilitating transcription of the *E2F1,* cyclin E, and cyclin D1 target genes,[45] all of which promote cell proliferation and tumor growth. In addition, it was shown that acetylation of ACLY stabilizes the protein by blocking lysine ubiquitination, resulting in elevated ACLY protein levels, increased lipid biosynthesis and enhanced cell proliferation.[46]

In summary, citrate can travel from mitochondria to cytoplasm, where it is converted into acetyl-CoA and OAA. The Acetyl-CoA is capable of promoting lipid synthesis and protein acetylation that activates growth related genes and proteins including oncoproteins.

Discussion

The soluble hydrogenase consists of four different subunits, α, β, γ, and δ. The αγ dimer is an NADH oxidoreductase, whereas the other two subunits are concerned with the hydrogenase activity. The analysis of amino acid sequences showed that the similarity fell only in NADH oxidoreductase subunits α and γ of soluble hydrogenase and the 51-, 24-, and 75-kDa subunits of bovine mitochondrial complex I, but no similarity was observed between the hydrogenase subunits (β and δ) and bovine complex I.[2] Theoretically, this observation lowers the probability that human complex I possesses hydrogenase activity. Since eukayotic complex I contains as many as 45 subunits, the actions of a few subunits are yet to elucidated, it could not be excluded that human complex I have the hydrogenase activity under certain conditions.

Several theories of cancer transformation have been proposed, but even the most prominent and accepted model (mutation theory) is confronted by a growing amount of experimental data and arguments that could either not explained by the model, or that contradicted this model.[47] The mutation theory cannot explain the facts that there exist of cancers without mutations,[48] and that the normal tissues can show massive genetic changes including changes in cancer-initiating and cancer-driving genes.[49] The Warburg's hypothesis[4] of the disturbance of OXPHOS was unable to explain the fact that hereditary mitochondrial diseases are not associated with

an increased rate of cancer formation, even when most mitochondria are affected.[50] In addition, these two models contradicted each other. However, the hydrogenase hypothesis could account for the effects of the OXPHOS defect, aerobic glycolysis, and oncogene activation in cancer formation. This hypothesis may provide a united framework that combines previous different models.

If human mitochondria indeed possess hydrogenase activity, the excessive reducing equivalents of NADH can be released by the hydrogenase activity via the formation of molecular hydrogen. Thus, the releasing of excessive equivalents of NADH could prevent the cell malignant transformation when the cells are in hypoxia, ETC defect or low ATP consumption. Reducing NADH/NAD^+ ratio, recovering the hydrogenase activity or increasing ATP consumption would reverse the transformation and provide a novel approach to combat cancers. If the hydrogenase hypothesis is confirmed by experiment, we could use genetic engineering techniques to correct the mitochondrial gene mutations or import exogenous NADH oxidizing hydrogenase in order to reduce NADH/NAD^+ ratio and keep TCA cycle functioning. It was reported that physical activity reduces the risk of developing invasive cancer by 10 to 20%, and the relative risk of all-cause mortality of cancer survivors by as high as 49%.[51] The underlying mechanism of the relationship between physical activity and cancer risk can be explained by the hydrogenase hypothesis. Since physical activity consumes a large amount ATP, which can inhibit electron flux through ETC, the OXPHOS activity is increased and the cytosol pyruvate is reduced to lactate that is transported to outside of cells. Thus, there are no glucose carbons accumulated to support biomass synthesis and cell proliferations. For this reason, the government health authority would launch a campaign to promote a more physical activity to prevent cancer development in vast populations.

Future direction

First, the hydrogenase activity of human mitochondrial complex I must be falsified or confirmed by experiments. If the complex I indeed possesses hydrogenase activity, we need study how the hydrogenase activity of complex I influences PDC, TCA cycle, and OXPHOS in mitochondria; and glycolysis and PPP in cytoplasm. Second, it is of importance to detect whether cancer cell complex I loses the hydrogenase activity and elucidate how it is lost in respect of gene mutations or epigenetic alteration. Third, preceding discussion indicates that the direction of metabolic pathway is mainly controlled thermodynamically, rather than kinetically, since enzymes only increase reaction speed. Thus, future study on metabolic pathways would pay more attention to how concentrations of substrates and products alter the pathway direction.

Conclusion

The complex I of human mitochondria may possess hydrogenase activity. Under conditions of hypoxia, low OXPHOS capacity, or low ATP demand, the hydrogenase activity could release excessive reducing equivalents of NADH by transferring electrons from NADH to proton to form molecular hydrogen. Loss of the hydrogenase activity would inhibit PDC and TCA cycle due to increased NADH/NAD^+ ratio in low OXPHOS capacity. Reduced activities of PDC and TCA cycle leads to upregulation of glycolysis and PPP, resulting in cancer formation. Conversely, restoring the hydrogenase activity, lowing NADH/NAD^+ ratio and/or increasing ATP demand may stop the proliferations of cancer cells.

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Conflict of interest

None.

Author contributions

Contributed to study concept, drafting of the manuscript, critical revision of the manuscript (FL).

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