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Impaired mitochondrial metabolism and mammary carcinogenesis

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Abstract

Mitochondrial oxidative metabolism plays a key role in meeting energetic demands of cells by oxidative phosphorylation (OxPhos). Here, we have briefly discussed (i) the dynamic relationship that exists among glycolysis, the tricarboxylic acid (TCA) cycle, and OxPhos; (ii) the evidence of impaired OxPhos (i.e. mitochondrial dysfunction) in breast cancer; (iii) the mechanisms by which mitochondrial dysfunction can predispose to cancer; and (iv) the effects of host and environmental factors that can negatively affect mitochondrial function. We propose that impaired OxPhos could increase susceptibility to breast cancer *via* suppression of the p53 pathway, which plays a critical role in preventing tumorigenesis. OxPhos is sensitive to a large number of factors intrinsic to the host (e.g. inflammation) as well as environmental exposures (e.g. pesticides, herbicides and other compounds). Polymorphisms in over 143 genes can also influence OxPhos system. Therefore, declining mitochondrial oxidative metabolism with age due to host and environmental exposures could be a common mechanism predisposing to cancer.

Keywords

Mitochondrial metabolism; Oxidative phosphorylation; OxPhos; inflammation; tumor suppressor p53; breast cancer

1. Introduction

Metabolism is central to cellular physiology. It provides energy in the form of adenosine 5′-triphosphate (ATP) and building blocks (other nucleotides, lipids, amino acids, etc.) for different cellular processes including cell growth, proliferation, and supporting functions of differentiated cells such as milk synthesis in lactating mammary glands [1,2]. The overall cellular metabolism relies on glycolysis, the tricarboxylic acid (TCA, also known as citric acid or Krebs) cycle, and oxidative phosphorylation (OxPhos). The reactions of glycolysis, the TCA cycle and OxPhos occur in the cytoplasm, mitochondrial matrix, and at mitochondrial inner membrane, respectively. Although occurring at distinct subcellular locations, these processes are interdependent (Fig. 1). Their relationship is tunable based on catabolic and anabolic demands of cells [3]. One of the key factors that regulate this relationship in real time is ATP-demand. In this review, we will briefly discuss their relationship and the role of impaired mitochondrial metabolism in breast cancer. Emphasis is

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given to OxPhos because it is susceptible to host and environmental factors that may contribute to a majority of breast cancers [4].

2. An overview of cellular metabolism

In a typical mammalian cell glucose is metabolized via glycolysis to generate pyruvate. Glycolysis produces 2 net ATP molecules per glucose using the reactions catalyzed by phosphoglycerate kinase and pyruvate kinase, which are [1, 3-bisphosphoglycerate + ADP ⇔ 3-phosphoglycerate + ATP] and [phosphoenolpyruvate + ADP + H^+ ⇔ pyruvate + ATP] respectively. This mode of ATP production is called substrate-level phosphorylation as it uses the chemical energy liberated from a substrate. In intact cells, this can be slowed down by a limitation in NAD^+ supply [5], because the reaction upstream to phosphoglycerate kinase is catalyzed by an NAD^+-dependent enzyme, glyceraldehyde-3-phosphate dehydrogenase [glyceraldehyde-3-phosphate + Pi + NAD^+ ⇔ 1,3-bisphosphoglycerate + NADH + H^+]. If the cytosolic NAD^+ pool is completely reduced to NADH by glyceraldehyde-3-phosphate dehydrogenase, the NAD^+ will become limiting. Therefore, NAD^+ must be regenerated in cytosol to permit continued operation of glycolysis. Cytosolic NAD^+ is regenerated using lactate dehydrogenase and NADH redox shuttles [6]. Lactate dehydrogenase reduces pyruvate into lactate by using an NADH [pyruvate + NADH + H^+ ⇔ lactate + NAD^+]. Conversion of glucose to lactate is favored when oxygen is limiting. However, even in the presence of excess oxygen nearly all mammalian cells convert a significant fraction of pyruvate into lactate. The fraction of pyruvate converted into lactate is higher in proliferative and tumor cells than in differentiated cells [7]. Apart from hypoxic conditions, genetic and drug-induced OxPhos deficiencies can also up-regulate pyruvate conversion into lactate. Therefore, release of lactate by cells provides a surrogate for real-time measurements of glycolysis [8].

OxPhos, the process of making ATP by consuming oxygen (i.e. respiration), is the major source of ATP in animal cells. It is carried out by five multimeric enzyme complexes (I–V) with the help of electron carriers (ubiquinone and cytochrome c) and electron donors (NADH and $FADH_2$). Complexes I to IV constitute the respiratory chain, which establishes H^+ gradient across the inner mitochondrial membrane. This gradient is established by Complexes I, III and IV, which pump out 4, 4, and 2 H^+ respectively while transferring electrons liberated from NADH and $FADH_2$ oxidations (Fig. 1). The H^+ gradient is also known as proton motive force (Δp) and has two components- the mitochondrial membrane potential ($\Delta\psi_m$) and pH gradient (ΔpH) [9]. The potential energy of Δp drives ATP synthesis by Complex V, a rotary motor that makes 3ATP/rotation while using 2.7H^+/ATP [10]. After accounting for the expense of inorganic phosphate (Pi) and adenine nucleotides exchange, the net cost of making 1 ATP molecule is thought to be 3.7 H^+ [10]. Thus, 10 NADH and 2 $FADH_2$ generated from complete oxidation of 1 glucose molecule can produce about 30 ATP molecules via OxPhos. However, in reality the actual number will be lower than the calculated value due to H^+ leak across the inner mitochondrial membrane.

Other fuels such as fatty acids, ketone bodies and amino acids also support OxPhos.β-oxidation of fatty acids generates acetyl-CoA, NADH and $FADH_2$, which can support the TCA cycle and OxPhos (Fig. 1). β-oxidation derived $FADH_2$ feeds electrons to Complex III via an electron transferring flavoprotein-ubiquinone oxidoreductase [9]. Both the TCA cycle and β-oxidation are feedback inhibited by NADH buildup. Therefore, β-oxidation is not sustainable without functional Complex I. Ketone bodies such as acetoacetate and β-hydroxybutyrate also support the TCA cycle and OxPhos. Amino acids oxidation can also support OxPhos. Before entering the TCA cycle, amino acids are converted to pyruvate (Ala, Ser, Gly, Thr, Cys, Trp), acetyl-CoA (Leu, Ile, Lys, Phe, Tyr, Trp, Thr), α-ketoglutarate (Glu, Gln, Pro, His, Arg), succinyl-CoA (Met, Ile, Val), fumarate (Phe, Tyr),

or oxaloacetate (Asp, Asn) [3]. While all amino acids can enter TCA cycle, the oxidation of glutamine is a major contributor to OxPhos [11]. Clearly, the relative use of glucose, glutamine or other fuels will depend on catabolic and anabolic needs of cells [3]. For example, branched chain amino acid metabolism plays an important role in mammary epithelial cell physiology during lactation. The branched chain amino acids (Leu, Ile, Val) are metabolized to produce glutamate, glutamine, aspartate, alanine, and asparagine to be secreted in milk [12,13].

OxPhos is involved in a dynamic relationship with the TCA cycle and glycolysis. In real-time, inhibition of OxPhos with oligomycin up-regulates glycolysis and slows down the TCA cycle ([11,14,15]; unpublished data, C. Kim and N. Yadava). Oligomycin is a Complex V inhibitor. Respiratory chain defects result in auxotrophy for asparagine and CO_2 due to blockage of the TCA cycle [16–18]. The CO_2 auxotrophy is due to feedback inhibition of the NAD^+-dependent pyruvate and α-ketoglutarate dehydrogenases. The asparagine auxotrophy is thought to be due to a limitation in oxaloacetate production. The requirement of asparagine clearly underscores the interplay between anabolism and OxPhos via the TCA cycle. Although OxPhos is the major source of ATP production, cells with <8% respiratory activity can meet their total ATP demand by glycolysis alone. As long as glucose supply is not limiting even complete OxPhos deficiency does not impair growth and proliferation of cells [11,19]. In respiration-competent lung fibroblasts ~40% of ATP is derived from OxPhos supported by glucose and glutamine oxidations [11]. The contribution of OxPhos to cellular ATP pool is expected to vary with tissue type. When OxPhos is suppressed due to hypoxia or the presence of inhibitors, glutamine can undergo reductive metabolism without any ATP production [20,21]. Tumor cells use this pathway of reductive glutamine metabolism (carboxylation) to produce citrate for lipid synthesis [21]. Citrate breakdown by ATP-citrate lyase following export from mitochondria into the cytoplasm provides acetyl-CoA for fatty acid synthesis [citrate + ATP + CoA + $H_2O \Leftrightarrow$ acetyl-CoA + ADP + Pi + oxaloacetate]. ATP-citrate lyase inhibition suppresses tumor cell growth [22]. Citrate production in mitochondria is limited by oxaloacetate and acetyl-CoA availability (Fig. 1). However, citrate exit from the TCA cycle will limit oxaloacetate production. Therefore, anaplerosis (filling-in) of the TCA cycle with oxaloacetate will be required. Glutamine oxidation is a major contributor to oxaloacetate pool in tumor cells. This is clear from the elimination of glutamine dependence of tumor cells in the presence of glucose by pyruvate carboxylase over expression [23]. Pyruvate carboxylase converts pyruvate into oxaloacetate [pyruvate + HCO_3^- + ATP $\Leftrightarrow$ oxaloacetate + ADP + Pi]. Therefore, apart from glutamine dependence tumors may have pyruvate carboxylase up regulated [24]. The relative dependence on pyruvate carboxylase vs. glutamine metabolism for citrate synthesis may vary in different tumors.

Integrity of the OxPhos system is critical for optimal energy production, but functional assembly of OxPhos system is a very complicated process. Nuclear and mitochondrial genomes encode 45, 4, 11, 13, and 16 subunits of Complexes I, II, III, IV and V, respectively. The mitochondrial –(mt)DNA-encoded subunits are ND1-6 and ND-4L of Complex I; Cyt b of Complex III; COI-III of Complex IV; and ATP6 and ATP8 of Complex V. These 13 proteins are translated within mitochondria using a protein translation machinery similar to prokaryotes [25]. Thus, OxPhos system is vulnerable to antibiotics that inhibit bacterial protein synthesis. MtDNA also encodes 22 tRNAs and 2 rRNAs that constitute the mitochondrial protein synthesis system with nuclear-(n)DNA-encoded ribosomal proteins and other factors [25]. Between 2–10 assembly factors are required for the assembly of individual OxPhos complexes [26]. The final number of assembly factors for any given complex is not yet settled. The formation of super-complexes adds another layer of complexity to the OxPhos system. This also requires additional proteins such as

HIG2A [27,28]. Over 143 genes (89 structural proteins, 30 assembly factors, 22 tRNAs and 2 rRNAs) are directly involved in OxPhos system biogenesis [26].

3. Evidence for impaired metabolism in breast cancer

Metabolic reprogramming is a common feature of the majority of cancers. Increased glycolysis in cancer was suggested by Otto Warburg over 90 year ago [29,30]. Warburg proposed that an “irreversible injury” to the OxPhos system could lead to metabolic shift towards glycolysis (the *Warburg effect*) thereby causing cancer development [29]. His observations offered the premise for using 2-fluoro (^{18}F)-2′-deoxyglucose-positron emission tomography (FDG-PET) in cancer staging and monitoring [31,32]. The FDG signal is suggested to be proportional to the rate of glycolysis in viable cells and reports the relatively increased demand for glucose in tumors [33]. While the success of FDG-PET in diagnosis of primary breast cancers is variable, it has shown increased glucose uptake in metastatic breast cancers [34]. The redox scanning technique based on cryogenic NADH/flavoprotein fluorescence, which reports OxPhos activity in cells, has also revealed alterations in mitochondrial metabolism [35,36].

The Warburg hypothesis predicts that impaired OxPhos or mitochondrial dysfunction should be a common feature of cancer cells. To validate this hypothesis, several studies have examined mutations in mtDNA, which is relatively more vulnerable to damage than nuclear nDNA. A large number of mtDNA mutations in breast and other cancers have been identified [37–47]. Mutations in DNA polymerase-γ (POLG) that is involved in mtDNA replication result in reduced OxPhos activity due to mtDNA depletion/mutation in breast cancers [48]. The strongest support for the Warburg hypothesis has come from germ line mutations in Complex II genes [49–53]. Mutations in genes encoding Complex II subunits and assembly factor(s) predispose to hereditary paraganglioma/phaeochromocytoma syndrome [51–53]. Complex II germ line variants also modify breast and thyroid cancer risks in Cowden and Cowden-like syndrome [54]. A survey of COSMIC (catalogue of somatic mutations in cancers) database shows over 26 Complex I genes mutated in different cancers with ≤1% frequency (unpublished observations, N. Yadava). In ductal-breast carcinoma, somatic missense mutations have been found in several Complex I genes such as *Ndufa2, Ndufa3* and *Ndufa8*.

Functional analyses of OxPhos in cancers are relatively rare. Almost complete loss of Complex I (mostly due to mtDNA mutations) is associated with renal and thyroid tumors [43,55]. While mtDNA mutations in the regulatory regions are expected to result in multiple deficiencies of OxPhos, the amino acid altering mtDNA mutations affect Complex I more frequently (>50%) [46]. Mutations in ND4 (C12084T) and ND5 (A13966G) subunits affect Complex I function and influence metastatic properties of MDA-MB-231 breast cancer cell line [56]. The ND3 polymorphism (G10398A) associated with aggressive breast cancer in African-American women also alters Complex I activity [57]. A few other studies also suggest impaired OxPhos in breast cancers [58,59]. Expression of the nuclear-encoded NDUFS3 subunit of Complex I is associated with breast cancer invasiveness [60]. Its increased expression may be a compensatory response to OxPhos deficiency. This is not unexpected because the NDUFS3 subunit enters into Complex I assembly very early to facilitate additions of other subunits [61]. Reduced expression of the β-subunit of Complex V (β-F1-ATPase) in cancer is often accompanied by an increase in glyceraldehyde-3-phosphate dehydrogenase. A bioenergetic signature derived from the relative expressions of β-F1-ATPase and glyceraldehyde-3-phosphate dehydrogenase has high prognostic value for breast and other cancers [62,63]. Reduced β-F1-ATPase and increased inhibitory factor 1 (IF1) and uncoupling proteins (e.g. UCP2) levels suggest that OxPhos efficiency is compromised in breast cancer cells [64,65]. A few studies also suggest metabolic

interdependence between stromal fibroblasts and cancer cells. In this relationship fibroblasts provide nutrients (e.g. lactate) to tumor cells [66–68]. Whether metabolites shuttling is used for bioenergetic or other purposes remains to be clearly resolved. It is possible that limited NADH production in cancer cell mitochondria or a partial OxPhos deficiency force cells to switch their reliance on NADH redox shuttles. Lactate use via reversal of lactate dehydrogenase will support redox-shuttles by generating NADH. If respiration is primarily supported by the glycerol-3-phosphate shuttle (GPS) in cancer cells (Fig. 1), then substantial respiratory activity with less ATP yield is expected because only 6 H^+ are pumped out per $FADH_2$ oxidation vs. 10 per NADH oxidation.

4. Cancer development and altered metabolism

Cancer development is a multistep process involving over 4 rate-limiting stochastic events [69,70]. Somatic mutations, failures of negative regulatory feedback for proliferation and contact inhibition, senescence and the corruption of signaling pathways (an inhibitory pathway becoming promoter) are key events that occur at different stages of tumorigenesis. These events confer several hallmark capabilities in cancer cells. Six of these capabilities were described in the year 2000 by Hanahan and Weinberg [69]: (i) sustaining proliferative signaling, (ii) evading growth suppressors, (iii) resisting cell death, (iv) enabling replicative immortality, (v) inducing angiogenesis, and (vi) activating invasion and metastasis. Reprogramming of energy metabolism (vii) and evading immune destruction (viii) have been added to this list as emerging hallmark capabilities [70]. They are intricately linked and enabled by genomic instability, epigenetic modifications, and tumor-promoting inflammation [70]. Activation and suppression of signaling molecules such as B-Raf, Akt/PKB, mTOR, Ras, Myc, PTEN, pRB, p53, HIF1, NF-κB etc. are thought to result in acquisitions of the hallmark capabilities [70,71]. Whether modest changes in metabolism alone can alter multiple signaling pathways in otherwise normal cells and give them cancer hallmark capabilities remains to be elucidated.

Apart from a secondary role, impaired mitochondrial metabolism may also play a primary role in cancer development. Observations that mtDNA mutation/depletion enhance tumorigenicity and metastatic potential have confirmed the secondary role of mitochondrial dysfunction [56,72,73]. Assuming that metabolic alterations are a consequence of transformation, the majority of studies have focused on the effects of tumor suppressors and oncogenes on cellular metabolism to explain the Warburg effect [74–78]. This subject has been discussed in several reviews [7,79,80]. It is noted that while tumor suppressors promote oxidative metabolism, the oncogenes favor glycolysis, and they have opposing effects on glycolysis and OxPhos. Altered functions of tumor suppressors and oncogenes reprogram the metabolism of transformed cells to meet their anabolic demands [7,79,80]. The idea that altered metabolism may play a primary role in cancer development is gaining momentum [81]. Alterations in activities of enzymes such as pyruvate kinase (PKM2) and isocitrate dehydrogenase (IDH1, 2), and others are thought to enhance cellular transformation. The associations of heritable polymorphisms with the risks of breast and other cancers suggest that mitochondrial dysfunction could play a causative role in tumorigenesis [44,45,54,82]. Two recent studies suggest that mitochondrial dysfunction can predispose to tumorigenesis. One is based on maternal transmission of mtDNA mutations originated in mice due to haploinsufficiency of mitochondrial helicase mSuv3 [83]. The other is based on a Complex I deficient model due to a mutation in the mtDNA-encoded ND6 subunit [84]. However, it remains to be determined whether a primary OxPhos defect can predispose to mammary tumorigenesis. The observations of mtDNA and p53 mutations together in the majority of breast and other cancers support a causal link between mitochondrial dysfunction and cancer development [45,85,86].

Mitochondrial dysfunction may promote cancer development by conferring hallmark capabilities: (i) by suppressing p53 and PTEN function [87,88], (ii) activating AKT1 and HIF1α pathways [87,89–91], (iii) inducing extracellular matrix remodeling for invasion [73,92], (iv) providing resistance to cell death [88,93], (v) shifting metabolism towards glycolysis [94], and controlling inflammation [95]. Mitochondrial proteins such as SIRT3 (a protein deacetylase), CHCHD4 (a component of disulfide relay system regulating electron transport to cytochrome c), and NDUFA4L2 (a hypoxia inducible Complex I inhibitor) are clearly implicated in regulating both tumorigenesis and cellular respiration [96–98]. Impairments of the OxPhos system are linked with changes in cellular redox status, reactive oxygen species (ROS) levels, bioenergetics and ionic homeostasis. These changes in cellular physiology are linked with regulations of different pathways including p53 and AKT [54,87,99]. Both p53 and AKT are involved in early events of mammary epithelial cell transformation [100,101].

There is a possibility that declining OxPhos function could play a causative role in increased cancer incidence with age. The Yadava laboratory has shown that OxPhos deficiency can suppress p53 expression/function in several cell types [88]. The p53 suppression by OxPhos deficiency is a reversible phenomenon. Ablation of Complex I provides protection against γ-radiation induced apoptosis when glucose is not limiting in different cells including a mouse mammary epithelial cell line TM40A (unpublished, S. Compton and N. Yadava) [88]. The suppression of p53 is not limited to Complex I deficiency as inhibition of mitochondrial protein synthesis and Complex II-deficiency also result in reduced p53 expression/function (unpublished, C. Kim and N. Yadava, [88]). In mice the mtDNA mutation rate is linked to premature aging and p53 function declines with age [102,103]. Mitochondrial dysfunction is suggested to play a causative role in aging and other diseases including cancer [104]. As noted above, there is evidence for predisposition to tumorigenesis by mtDNA mutations in mice [83,84]. Therefore, there is a possibility that functional decline of p53 mediated by reduced mitochondrial function could predispose to cancer [88,103]. Even modest impairments of mitochondrial function can significantly suppress p53 function [88]. In humans, germ line mutations in the *TP53* gene result in Li-Fraumeni syndrome, which is characterized by a strong predisposition to a spectrum of cancers with breast cancer being the most common in women [105]. Furthermore, studies with p53 knockout mice have clearly demonstrated the critical role p53 plays in tumor prevention [106–108].

The relationship between p53 and metabolism is quite complicated. While it has been observed that basal levels of p53 promote mitochondrial metabolism, p53 activation following telomere dysfunction reduces mitochondrial biogenesis [76,109]. p53 is also reversibly suppressed by OxPhos deficiency [88]. The accepted view is that p53 suppresses glycolysis and promotes oxidative metabolism in proliferating cells (Fig. 2A) [110]. Positive regulation of TIGAR is primarily associated with negative regulation of glycolysis [77]. P53 promotes OxPhos by modulating Complex IV biogenesis [76], mtDNA copy number [111] and mtDNA maintenance [112]. Therefore p53 function loss is thought to cause a metabolic shift toward glycolysis (Fig. 2B). Suppressing p53 in the presence of OxPhos deficiency may not be in the best interest of cells because it may result in bioenergetic crises. From this point of view, a negative regulation of p53 by mitochondrial dysfunction in not unexpected [88]. It is possible that p53 and mitochondrial function act together to maintain cellular homeostasis and when their coordination is lost pathological consequences result. Alterations in p53 can also affect other signaling pathways such as AKT and NF-κB, which make mammary epithelial cells susceptible to oncogenic transformation [100,101,113,114]. Alterations in the AKT and NF-κB pathways can influence cellular metabolism and cell fate [115–117].

P53 primarily functions as a transcription factor that regulates genes implicated in a variety of cellular processes such as cell cycle arrest, DNA repair, apoptosis, senescence, autophagy, metabolism, oxidative/redox stress, angiogenesis and many more relevant to normal physiology and pathology [118]. P53 prevents cell proliferation when conditions are not favorable such as in the presence of acute DNA damage or oncogene activation [119]. In addition to acting as a transcription factor, p53 protein also acts directly to regulate cell fate [120–122]. This regulation is tissue- and stimulus-specific [123]. It remains to be explored whether variation in mitochondrial metabolism influences cell-specific properties of p53.

Mitochondrial dysfunction may result in genetic and epigenetic changes. Complexes I, II and III produce reactive oxygen species (ROS) when electron transfer is impaired [124–126]. Hypoxia, OxPhos deficiencies, high Δp, NADH/NAD^+ and ubiquinone (reduced/oxidized) influence ROS homeostasis [127]. Increased ROS production due to OxPhos deficiency can result in genomic instability [128]. Additionally, alterations in the dTTP nucleotide pool due to OxPhos deficiency can also cause genomic instability [129]. Mitochondrial proteins such as Sirt3, CHCHD4 and NDUFA4L2 directly link OxPhos activity with hypoxic response, which plays an important role in cancer development [96–98,130]. ROS are thought to play major role in breast tumorigenesis. This is supported by a study showing that catalase expression in mitochondria can suppress invasive breast cancers in mice [131]. Catalase is an enzyme that degrades hydrogen peroxide (H_2O_2). Oxidative stress, particularly in stromal fibroblasts, is suggested to drive breast cancer development [132]. It is associated with the loss of caveolin-1 (Cav-1) in stromal fibroblasts [133,134]. Cav-1 deficiency is associated with cholesterol localization to mitochondria, which results in impairment of OxPhos and oxidative stress [135–137]. Hypoxia and OxPhos deficiency induce reductive glutamine metabolism that provides growth advantages [20,21,81]. The relationship between mitochondrial function and epigenetic changes have been recently described [47,81,138]. DNA methylation and histone acetylation that impart epigenetic influences are linked with mitochondrial metabolism [139,140].

5. Host and environmental factors affecting mitochondrial metabolism

Genetic alterations, polymorphisms and stochastic changes in expression of over 143 genes may exert influence on mitochondrial function [26,141]. nDNA and mtDNA polymorphisms are linked with breast cancer risk [44,45,54,142]. While polymorphic variations can directly affect the function of a protein, they are expected to differ in severity based on interactions between nDNA- and mtDNA-encoded subunits of OxPhos complexes. This possibility is clearly exemplified by mutational analysis of the MWFE (or NDUFA1) subunit of Complex I, which interacts with mtDNA-encoded proteins [19,143].

In addition to genetic factors, inflammation can play a significant role in suppressing mitochondrial metabolism. Thus, chronic inflammation very often associated with obesity/metabolic syndrome may facilitate cancer initiation and progression by suppressing OxPhos [144,145]. Inflammation can occur through localized secretion of factors such as TNFα, Wnt and leptin, which impair OxPhos [146–150]. Inflammation and oxidative stress are linked with TNFα-mediated damage to OxPhos system in ob/ob mice via protein nitration [151]. Often Complexes I, III and IV are affected by inflammatory cytokines. The Yadava Laboratory has found that low levels of TNFα (10 ng/ml) that do not induce cell death can reduce the levels of NDUFS3 subunit of Complex I in HEK293 cells (Fig. 3A). In primary human mammary epithelial cells (HMECs), TNFα exposure causes 30–40% reduction in Complex I-dependent respiration (Fig. 3B). The effect of TNFα could be seen even with 1 hr exposure suggesting that mitochondrial metabolism of HMECs is highly susceptible to inflammatory cytokines. Apart from TNFα, local macrophages can also secrete Wnts that promote epithelial-to-mesenchymal transition (EMT) and stem cell like behavior in breast

cancer cells [152,153]. Interestingly, exposure to Wnts has also been shown to inhibit OxPhos and enhance glycolysis through the down-regulation of Complex IV subunits [150]. Thus, inflammation may influence cancer progression though alterations of mitochondrial metabolism.

A reciprocal relationship may exist between mitochondrial metabolism and inflammation. As noted above, while inflammatory cytokines can suppress mitochondrial function, there is a possibility that mitochondrial dysfunction can alter immune response. Immune cells carefully modulate their metabolism during development and activation, which often involves extensive proliferation. It is suggested that during proliferation metabolism of T-lymphocytes switches toward glycolysis [154]. Switching back from glycolysis to OxPhos supported by β-oxidation is linked with establishment of the memory T-cells [155]. During the contraction phase, the T-cells that cannot utilize OxPhos fail to form memory T-cells. Other immune cells, such as dendritic cells, also exhibit a similar switch to glycolysis upon toll-like receptor (TLR)-induced maturation [156]. Mitochondrial function also affects cytokine production from macrophages in response to lipopolysaccharides (LPS)-stimulation [157], and positively modulates MHC class I antigens expression on the cell surface [158]. Thus, diminished OxPhos activity in tumor cells may protect them by reducing MHC class I expression. MtDNA-variants are known to affect transplantation outcomes [159,160]. Additionally mitochondria-derived peptides may result in an inflammatory response [161,162]. P53 and NF-κB pathways that regulate inflammatory and autoimmune responses may also exert their influence on the immune response via altering cellular metabolism [76,77,117,163]. Taken together there is sufficient evidence to suggest that mitochondrial metabolism may play an important role in regulating immune response. Thus, it is possible that inflammation-induced mitochondrial dysfunction can sustain inflammation by altering properties of local immune cells and protect tumor cells by immune avoidance. Although, a transient exposure to inflammatory cytokine IL-6 is found sufficient to promote MCF10A cells transformation with 100% efficiency, the role of impaired oxidative metabolism in this process remains to be explored [113].

The effect of hormones on mitochondria provides a possible link by which endocrine disrupting chemicals can influence the outcome of breast cancer and other diseases[164]. Of particular interest are estrogens that regulate glycolysis, the TCA cycle and OxPhos in a sex-specific specific manner [165]. Estrogens regulate mitochondrial biogenesis and function both directly and indirectly [166]. Humans are exposed to a host of estrogenic compounds that occur naturally (e.g. isoflavones enriched in soy products). In addition, industrial contaminants (e.g. BPA) and agricultural chemicals (e.g. atrazine, permethrins) have been shown to have estrogenic activities. These compounds interact with a complex estrogen signaling pathway composed of 2 isoforms of estrogen receptors (ERα and ERβ) that act as transcription factors in the nucleus. These receptors form homo- and hetero-dimers, which bind promoters of target genes to either enhance or inhibit transcription depending on the presence of co-activators or co-repressors [167,168]. Estrogenic ligands show selectivity in binding to ERα and ERβ resulting in differences in transcriptional responses, effects on target genes and cellular behavior [169–171]. However, these estrogen receptors can also localize to membranes and activate distinct signaling cascades referred to as “non-genomic” signaling. Functional estrogen receptors are also found in mitochondria [165,172,173]. The effects of estrogenic compounds vary greatly in tissues depending on the complement of receptors.

Lipophilic compounds of natural and synthetic origins, to which humans are often exposed, can cause mitochondrial dysfunction [174]. Agricultural chemicals such as pesticides are known to inhibit OxPhos [175]. Use of rotenone, a common pesticide, is linked with Parkinson’s disease in rodents and humans [175,176]. Pyrethrins are commonly used to

control insects. While these compounds break down quickly, residues can persist when used in poorly ventilated areas. Following treatment with insecticides, pyrethrins are detectable in cows' milk [177]. Pyrethroids such as permethrin and cyhalothrin were shown to inhibit Complex I activity at picomolar levels [178]. Atrazine, a common herbicide, has estrogenic activity, and thus, it may also have significant consequences for mammary gland development. Although in utero exposure can alter mammary gland development in neonates by increasing the number of terminal end buds, this effect was transient [179]. Nonetheless, long-term exposure may have implications for breast cancer risk in women through its presumed estrogenic effects [180,181]. Atrazine's herbicidal activity is due to its inhibitory action on photosystem Complex II in chloroplasts, which can also inhibit OxPhos [182]. As modest OxPhos-deficiency results in a near complete abrogation of the tumor suppressor p53 activity (unpublished data C. Kim and N. Yadava; [88]), the oncogenic effects of environmental contaminants may be related to their inhibitory effects on mitochondrial function.

6. Conclusion

Glycolysis, the TCA cycle and OxPhos are engaged in a dynamic relationship, which is primarily controlled by ATP demand (Fig. 1). Normal cells switch their metabolism from glycolysis toward OxPhos and *vice versa* based on their energetic needs and fuel availability. Glycolysis can alone supply sufficient ATP via substrate level phosphorylation for cellular growth and proliferation[11]. Thus, OxPhos is not essential as long as glucose entry into glycolysis is not limited and the ATP-demand does not exceed the capacity of glycolysis [11,183]. In proliferative and tumor cells, increased demands for nucleotides such as NADPH and lipids synthesis are thought to favor a metabolic switch toward glycolysis resulting in the Warburg effect [7,30]. The modification of breast cancer risk by genetic polymorphisms affecting the OxPhos system suggests that impaired mitochondrial metabolism could play a causative role in breast cancer development [44,45,54,142]. Several observations with cell lines showing promotion of cancer hallmark capabilities indicate that mitochondrial dysfunction could play a primary role in tumorigenesis. Two studies show that mice bearing mtDNA mutations are indeed predisposed to tumorigenesis [83,84]. The vulnerability of the OxPhos system to host factors (e.g. age, inflammatory cytokines) and environmental factors (e.g. pesticides, natural and synthesis compounds) may increase the susceptibility to breast cancer. We propose that the suppression of p53 by mitochondrial dysfunction could play a key role in enhanced breast cancer susceptibility [88].

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Abbreviations

NAD^+ and NADH	oxidized and reduced nicotinamide adenine dinucleotides, respectively
$NADP^+$ and NADPH	phosphorylated forms of NAD^+ and NADPH, respectively
ADP	adenosine diphosphate
ATP	adenosine triphosphate
Pi	inorganic phosphate
FAD and FADH2	oxidized and reduced flavin adenine dinucleotides, respectively

nDNA	nuclear-DNA
mtDNA	mitochondrial-DNA
OxPhos	oxidative phosphorylation
TCA	tricarboxylic acid
CoA	coenzyme A

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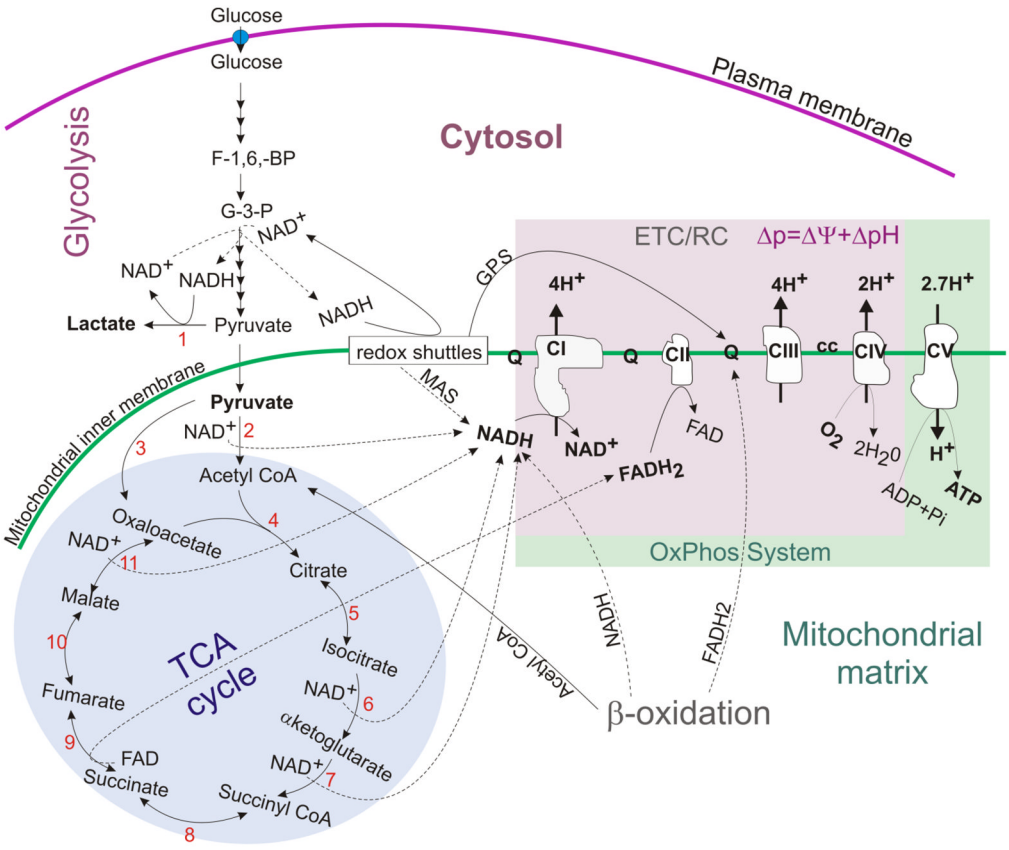

Figure 1. An overview of cellular metabolism showing the links among glycolysis, the TCA cycle and OxPhos

Glycolysis is linked with the TCA cycle and OxPhos via pyruvate and redox shuttles (MAS, GPS). CI-V: Complexes I–V; ETC/RC: electron transport chain/respiratory chain made up by C-IV; OxPhos system: oxidative phosphorylation system made by CI-V (or RC + CV). Ubiquinone (Q) and cytochrome c (cc) are electron carriers; MAS: malate-aspartate redox shuttle; GPS: glycerol-3-phosphate redox shuttle. Numbers indicate selected enzymes- 1: lactate dehydrogenase (LDH); 2: pyruvate dehydrogenase (PDH); 3: pyruvate carboxylase (PC)- an anaplerotic enzyme; 4: citrate synthase (CS); 5: aconitase; 6: isocitrate dehydrogenase (IDH3: NAD^+-dependent shown; IDH1, 2: $NADP^+$-dependent not shown); 7: α-ketoglutarate dehydrogenase (KGDH); 8: succinyl-CoA synthase (SCS: ATP, GTP-dependent); 9: succinate dehydrogenase (SDH or Complex II); 10: fumarate hydratase or fumarase (FH); 11: malate dehydrogenase (MDH). Broken thin lines indicate reactions generating electron donors (NADH and $FADH_2$) and the solid thin lines indicate reactions oxidizing NADH and $FADH_2$.

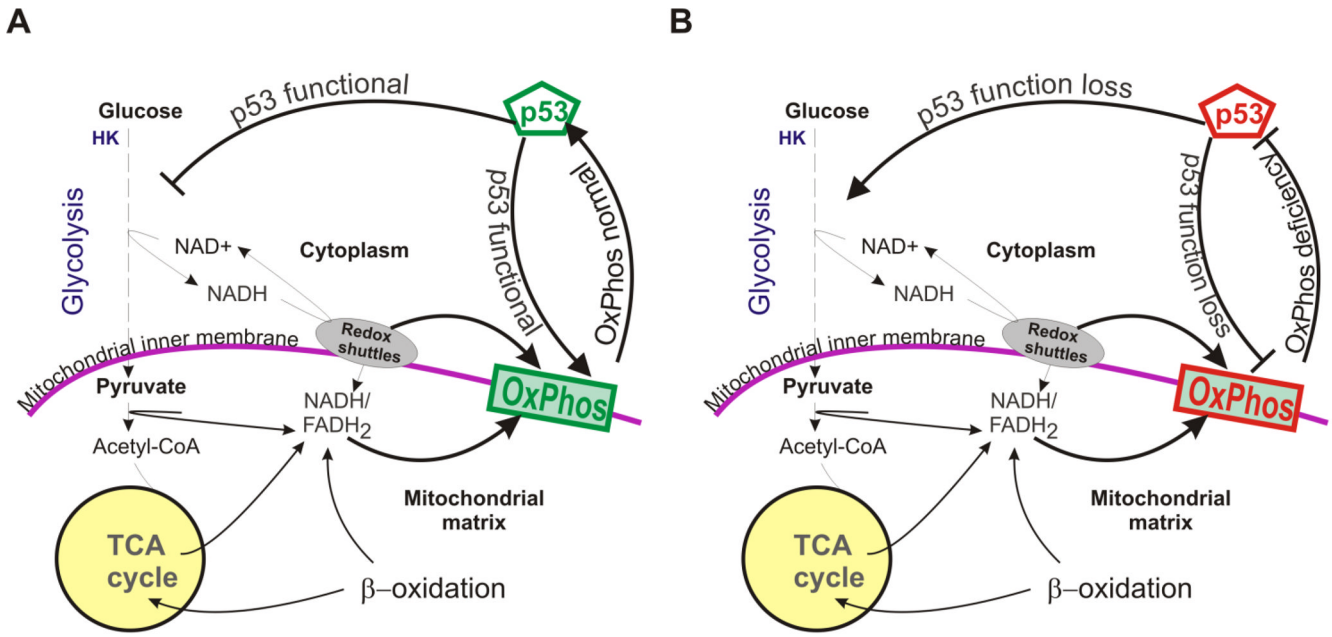

Figure 2. Relationship between the tumor suppressor p53 and metabolism

A) p53's relationship with glycolysis and OxPhos in normal cells. **B)** p53's relationship with glycolysis and OxPhos in cells with OxPhos deficiency. Because p53 is suppressed by OxPhos deficiency, its negative and positive effects on glycolysis and OxPhos would be removed.

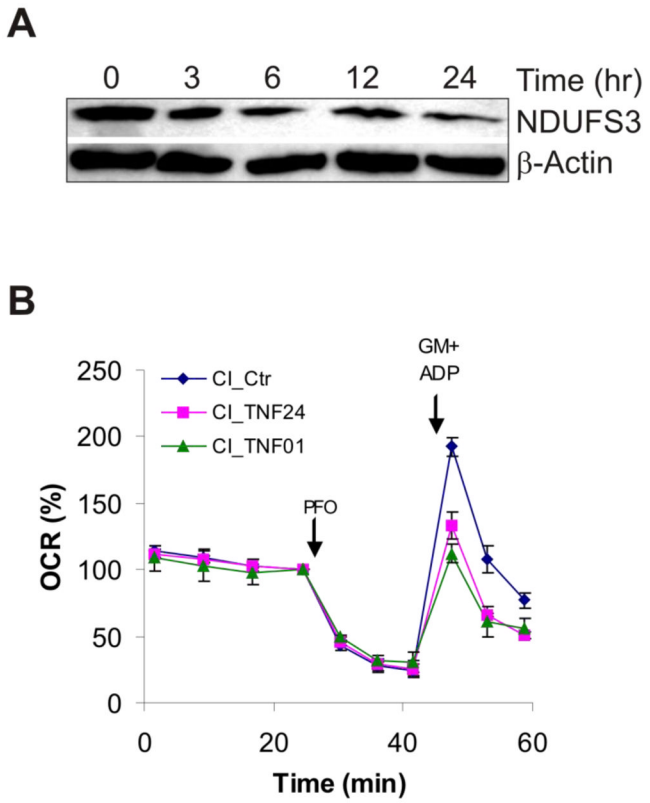

Figure 3. Effect of inflammatory cytokine TNFα on Complex I (CI)

A) Effect of TNFα (10 ng/ml) on CI subunit NDUFS3 expression in human HEK293 cells. **(B)** Effect of TNFα (10 ng/ml) on CI function in human mammary epithelial cells (HMECs). Primary HMECs (passage 3, 50,000/well) were permeabilized with 1 nM PFO (perfringolysin-O) and then glutamate and malate (10 mM each) were added with 1 mM ADP (GM+ADP) to measure CI-dependent oxygen consumption rates (OCR). Relative OCR is shown after setting the basal rate before PFO addition to 100%. OCR was measured using a 24-well Extracellular Flux (XF24) Analyzer from Seahorse Bioscience (Billerica, MA). CI_Ctr, CI_TNF01, and CI_TNF24 show CI-dependent respiration in control, 1 hr and 24 hr TNFα-treated HMECs. Respiration rates were measured in a Ca^{2+}-free buffer. The measurement of respiration in PFO-permeabilized cells is a novel method (unpublished, C. Kim, A. Heuck and N. Yadava).