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Cell Signaling Pathway Proteins that Can Be Targeted to Keep in Check Proliferation in Cancer Cells: Scientific Review

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ABSTRACT

Cancer progression is primarily driven by uncontrolled cell proliferation, often resulting from dysregulated signaling and metabolic pathways. This review provides a comprehensive analysis of critical cell signaling pathway proteins that regulate cancer cell proliferation, highlighting potential therapeutic targets. It explores the role of proto-oncogenes, tumor suppressors, and key regulatory proteins such as p53, RB, cyclins, and CDKs in maintaining normal cell cycle progression and how their dysregulation promotes oncogenesis. The paper further delves into metabolic adaptations in cancer cells, including aerobic glycolysis and mitochondrial reprogramming, emphasizing enzymes like hexokinase, phosphofructokinase (PFK1), pyruvate kinase (PKM2), and lactate dehydrogenase (LDH) as central to the Warburg effect. Additionally, it discusses the significance of mitochondrial signaling in apoptosis, with a focus on the Bcl-2 family and the mitochondrial permeability transition pore (PTP) complex. The review integrates insights into protein import machinery (TOM and TIM complexes) and electron transport chain dynamics, underscoring their relevance to cancer cell survival and death. Emerging therapies such as targeted drugs, immunotherapies, and metabolic inhibitors are evaluated for their potential to selectively disrupt these pathways. The review advocates for a multi-targeted approach to cancer treatment that interferes with both proliferative signaling and metabolic support systems. This strategy may yield improved outcomes by halting tumor growth while preserving normal cellular function.

Key words: cell proliferation, cancer, proto-oncogenes, p53, RB protein, CDKs, glycolysis, hexokinase, PKM2, Warburg effect, mitochondria, apoptosis, signaling pathways, targeted therapy, metabolic reprogramming

INTRODUCTION

Cancer is a disease marked by the uncontrolled proliferation of transformed cells, which are subject to evolutionary processes governed by natural selection (Brown et al., 2023; Blackadar, 2016). Carcinogenesis can be attributed to a range of causative agents, including occupational and synthetic chemicals, pharmaceutical compounds, viral pathogens, and naturally occurring factors (Blackadar, 2016; Cavenee & White, 1995). Prolonged occupational exposure to carcinogens such as X-rays, benzo(a)pyrene found in coal tar, radium, and naphthylamine is associated with various human malignancies (Blackadar, 2016). Non-occupational carcinogens, notably long-term tobacco smoking, are strongly correlated with the development of lung cancer (Brown et al., 2023). Certain pharmaceutical agents, including high-dose phenacetin-containing analgesic mixtures, the immunosuppressive agent azathioprine used post-organ transplantation, and diethylstilbesterol administered during pregnancy, have been linked to different neoplasms across sexes and in the progeny of exposed individuals (Blackadar, 2016). Oncogenic viruses—such as the human immunodeficiency virus (HIV), human papillomavirus types 16 and 18, human T-cell leukemia virus, and hepatitis C virus—are also implicated in various cancer pathologies (Cavenee & White, 1995). Additionally, naturally occurring carcinogenic factors include endogenous hormones such as estrone, ultraviolet radiation, parasitic infections like *Schistosoma haematobium*, fungal toxins such as aflatoxin B1 produced by *Aspergillus flavus*, and certain bacterial species (Blackadar, 2016; Brown et al., 2023).

A healthy cell exhibits a defined morphology and maintains its position within the organized architecture of surrounding tissue (Israels & Israels, 2000; van den Heuvel, 2005). It responds to extracellular regulatory cues, initiating mitosis only when the cumulative effect of stimulatory and inhibitory signals supports proliferation (Blagosklonny & Pardee, 2000; Engeland, 2022). However, the replication process inherently carries the risk of genetic mutations—stochastic alterations that can disrupt the cellular regulatory framework (Cavenee & White, 1995; Weinberg, 1995). An initial mutation may result in a cell that, although morphologically indistinct from its neighbours, begins to engage in aberrant cell division due to reduced sensitivity to growth-regulating signals (Łukasik et al., 2021; Roussel, 1999). Over successive divisions, the accumulation of additional genetic aberrations may lead to a progeny cell that becomes increasingly autonomous—exhibiting hallmark features of malignancy (Bouska & Eischen, 2009; Engeland, 2022). These include the loss of normal shape and cell boundaries, insensitivity to anti-proliferative signals, and acquisition of autonomous replicative capacity (Weinberg, 1995; Cavenee & White, 1995). As this malignant mass enlarges, it can compress and compromise adjacent healthy tissues (Brown et al., 2023). More critically, it may acquire the capacity to breach anatomical boundaries and metastasize to distant organs, establishing secondary tumors (Blackadar, 2016). Malignant transformation typically requires the cooperative effect of multiple genetic mutations that collectively disable mechanisms controlling cellular proliferation, invasion, and motility (Cavenee & White, 1995; Roussel, 1999). Proto-oncogenes, which encode proteins integral to cell growth and division, play a key role in this process (Weinberg, 1995). These genes may encode membrane-bound receptors that bind to growth factors and initiate intracellular signalling cascades promoting mitosis, intracellular transducers that propagate these signals, or nuclear proteins that directly regulate cell cycle progression (Łukasik et al., 2021; Engeland, 2022). Mutational activation of proto-oncogenes converts them into oncogenes, driving neoplastic transformation and uncontrolled cell proliferation (Cavenee & White, 1995; Bouska & Eischen, 2009).

CELL PROLIFERATION

Cell proliferation is a biological process through which cells replicate via growth and division, resulting in an increased cellular population (van den Heuvel, 2005). In eukaryotic organisms, this process is tightly regulated by both extracellular environmental signals and the intrinsic differentiation status of the cells (Israels & Israels, 2000; Blagosklonny & Pardee, 2000). Cells that have exited the active cell cycle and remain in a non-dividing state are described as quiescent (van den Heuvel, 2005). Among these, terminally differentiated cells have permanently lost the capacity to proliferate (Junko & Campisi, 1995). However, certain quiescent cells retain the ability to re-enter the cell cycle and resume division upon receiving appropriate external stimuli (Blagosklonny & Pardee, 2000). Additionally, there are cell populations that exhibit continuous cycles of proliferation and differentiation (Junko & Campisi, 1995; Israels & Israels, 2000).

Regulation of cell proliferation primarily occurs at defined checkpoints within the cell cycle, with the first gap phase (G_1 phase) being a critical point of control (Weinberg, 1995; Engeland, 2022). During this phase, growth regulatory signals influence whether the cell proceeds to DNA synthesis (S phase) (Blagosklonny & Pardee, 2000; Łukasik et al., 2021). Entry into the S phase commits the cell to complete DNA replication (Israels & Israels, 2000). Subsequently, additional regulatory cues influence the progression through the second gap phase (G_2 phase) and determine whether the cell advances into mitosis (van den Heuvel, 2005; Engeland, 2022). Key regulators of these transitions include proto-oncogenes—genes that are essential for cell cycle progression during the G_1 and G_2 phases (Cavenee & White, 1995; Roussel, 1999). When proto-oncogenes become mutated or are aberrantly expressed, they may drive neoplastic transformation and uncontrolled proliferation (Bouska & Eischen, 2009; Weinberg, 1995). Mitogenic growth factors promote cell proliferation by inducing the expression of proto-oncogenes, while anti-proliferative or growth-inhibitory signals often function by repressing proto-oncogene expression and activity (Łukasik et al., 2021; Engeland, 2022).

CELL CYCLE

The cell cycle comprises distinct, sequential phases: G1 (Gap 1), the interval between mitosis (M phase) and DNA synthesis (S phase); G2 (Gap 2), the phase between the end of DNA replication and the onset of mitosis; and M phase, characterized by the assembly of bipolar mitotic spindles, segregation of sister chromatids, and cytokinesis (van den Heuvel, 2005; Israels & Israels, 2000). In G1, extracellular mitogens and growth factors influence cellular progression toward the S phase (Blagosklonny & Pardee, 2000). Upon successful completion of DNA replication in S phase, the cell advances to G2, where it prepares for mitosis (Łukasik et al., 2021). Crucially, the cycle is governed by checkpoints—strategically positioned in late G1 and at the G2/M transition—to assess DNA integrity and prevent propagation of damaged or mutated genomes (Engeland, 2022; Weinberg, 1995). Cells outside of the cycling state are in G0, a quiescent state that may be temporary or permanent (van den Heuvel, 2005). Entry back into the cycle from G0 is dependent on mitogenic signals, which initiate intracellular phosphorylation cascades that lead to the expression of cyclins (Israels & Israels, 2000). These cyclins associate with cyclin-dependent kinases (CDKs), key mediators of cell cycle progression (Łukasik et al., 2021; Blagosklonny & Pardee, 2000).

Cyclins function as regulatory subunits, while CDKs serve as their catalytic counterparts (Łukasik et al., 2021; Roussel, 1999). Once activated by phosphorylation, cyclin-CDK complexes act at specific stages of the cell cycle, phosphorylating target proteins to initiate DNA synthesis in late G1 and S, and mitotic spindle assembly in G2 and M (Weinberg, 1995). The periodic synthesis and targeted proteolysis of cyclins orchestrate the orderly transitions between phases (Blagosklonny & Pardee, 2000; van den Heuvel, 2005). Given the potential for genomic mutations to generate defective cell clones, stringent surveillance mechanisms are essential (Engeland, 2022). The most pivotal checkpoint is the G1 “restriction point” (R), which determines whether a cell proceeds with the cycle or halts (Blagosklonny & Pardee, 2000). Additional checkpoints in S phase and the G2/M transition detect DNA replication errors or damage, leading to repair, cell cycle arrest, or apoptosis depending on the extent of injury (Weinberg, 1995; Bouska & Eischen, 2009). Absence of mitogenic signalling prompts the cell to either re-enter quiescence, differentiate, or undergo apoptosis (Israels & Israels, 2000). The cycle is initiated by increased expression of cyclin D isoforms (D1, D2, D3), which complex with CDK4 and CDK6 to drive phosphorylation-dependent activation (Łukasik et al., 2021; Roussel, 1999).

These activated complexes phosphorylate the retinoblastoma (RB) protein, a key modulator of G1 progression (Weinberg, 1995; Engeland, 2022). RB is part of a "pocket protein" family that represses the E2F transcription factors (Roussel, 1999). In its hypophosphorylated state, RB binds and inactivates E2F, thereby blocking transcription of genes required for S phase entry (Weinberg, 1995). Phosphorylation by cyclin D-CDK4/6 complexes causes RB to release E2F, allowing transcription of cyclin E and other genes essential for progression through the restriction point (Łukasik et al., 2021). RB remains hyperphosphorylated throughout S, G2, and M phases and is only dephosphorylated after mitosis (Blagosklonny & Pardee, 2000). As G1 progresses, cyclin E accumulates and forms complexes with CDK2 to facilitate the G1/S transition (Israels & Israels, 2000). Subsequently, cyclin A is upregulated and associates first with CDK2 during S phase and then with CDK1 in late S, promoting continued DNA synthesis (van den Heuvel, 2005). At G2, checkpoint mechanisms respond to incomplete replication or DNA damage; repair is attempted, or the cell cycle is aborted (Engeland, 2022). High levels of cyclin A and B, in complex with CDK1 (also known as cdc2), drive the cell into mitosis (Weinberg, 1995; Łukasik et al., 2021).

Genomic surveillance is largely mediated by the tumor suppressor transcription factor p53 (Bouska & Eischen, 2009; Engeland, 2022). When DNA damage is detected, p53 inhibits cell cycle progression to allow time for repair by blocking RB phosphorylation (Roussel, 1999). Under normal conditions, p53 is present at low, nearly undetectable levels, maintained by its negative regulator MDM2 (murine double-minute 2), which downregulates p53 transcriptionally and targets it for nuclear export, ubiquitination, and proteasomal degradation (Bouska & Eischen, 2009). Upon DNA damage, p53 stabilizes through phosphorylation, escapes MDM2-mediated suppression, and accumulates, effectively doubling its half-life (Engeland, 2022). This enables p53 to bind to DNA response elements and transactivate target genes (Weinberg, 1995; Bouska & Eischen, 2009).

One key p53 target is the CDK inhibitor (CKI) p21, which inhibits CDKs 4, 6, and 2, halting RB phosphorylation and arresting the cell in G1 to facilitate repair (Engeland, 2022; Roussel, 1999). If damage is beyond repair, p53 induces apoptosis through upregulation of pro-apoptotic proteins such as Bax (Bouska & Eischen, 2009). Two major CKI families regulate the cell cycle (Łukasik et al., 2021). The Cip/Kip family, including p21 and p27, targets CDKs 4, 6, and 2 at multiple phases (Israels & Israels, 2000). The INK4 family includes members like p16^INK4a and p19^ARF, both transcribed from the INK4a gene (Roussel, 1999). p16^INK4a specifically inhibits CDK4/6, while p19^ARF inhibits MDM2, indirectly stabilizing p53 (Bouska & Eischen, 2009). In response to p53 activation, p21 is induced, inhibiting CDKs and preventing RB phosphorylation (Engeland, 2022). Collectively, these CKIs function as robust brakes on the cell cycle, ensuring genomic stability and preventing uncontrolled proliferation (Roussel, 1999; Weinberg, 1995).

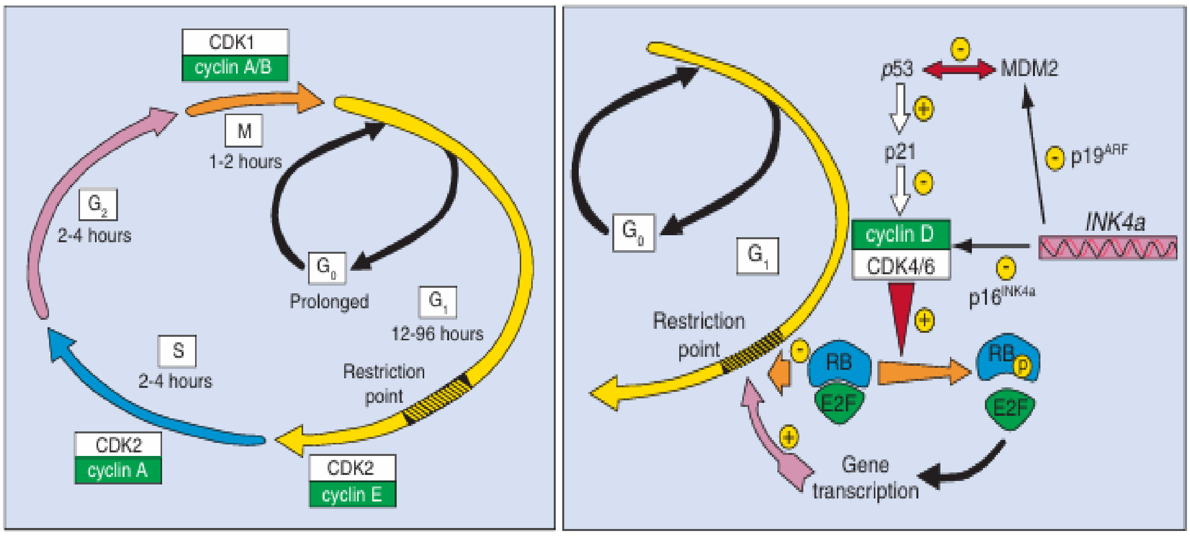

Figure 1: Cell cycle control pathways (Israels & Israels, 2000)

CELLULAR METABOLISM

The absorption and processing of nutrients are essential for sustaining fundamental cellular activities, including energy generation, the synthesis of cellular components, and the determination of cell fate (Finley, 2023; Bonora et al., 2012). The proliferation of cells depends on nutrient acquisition (Lunt & Vander Heiden, 2011). Growth factors play a crucial role: signaling through the PI3K/AKT pathway promotes the movement of nutrient transporters—responsible for bringing in key molecules like glucose, amino acids, and iron—to the cell membrane (Xue-bing Li et al., 2024). AKT (Protein Kinase B) further enhances glucose metabolism by directly phosphorylating hexokinase, an action that commits glucose to the glycolytic pathway (Wilson, 2003; Sebastian et al., 2000). Growth factor signals stimulate glucose utilization and promote aerobic glycolysis through various mechanisms, such as directly phosphorylating or modifying glycolytic enzymes through allosteric means (Mor et al., 2011; Yalcin et al., 2009). Notably, genetic mutations that result in the continuous activation of the PI3K/AKT signaling cascade are some of the most found mutations in human cancers (Altenberg & Greulich, 2004). Cancer-associated transcription factors like MYC and hypoxia-inducible factor (HIF) can stimulate the uptake and breakdown of nutrients by upregulating genes that support the intake and metabolism of crucial substances like glucose and glutamine (Minchenko et al., 2003; Finley, 2023).

Once nutrients are internalized, they are directed into a web of metabolic pathways that produce energy, reducing power, and molecular constituents needed for cellular growth (Bonora et al., 2012; Chandel, 2021). Electron carrier recycling is also essential (Liberti & Locasale, 2016). Studies suggest that the recycling of oxidized electron carriers—rather than merely the production of ATP—may be a limiting factor for cell proliferation (Adeva et al., 2014). All oxidative metabolic routes rely on a steady influx of electron acceptors to function, although the sensitivity of individual enzymes to fluctuations in these acceptors can vary (Finley, 2023; Scialò et al., 2017).

Glucose serves as the primary fuel for metabolic processes within the cell (Chandel, 2021; Wilson, 2003). Its breakdown follows three major stages—glycolysis, the tricarboxylic acid cycle (TCA or Krebs cycle), and oxidative phosphorylation—to generate ATP (Bonora et al., 2012). During glycolysis, glucose is transformed into pyruvate, yielding only a small amount of ATP (Chandel, 2021). The resulting pyruvate is then converted to acetyl coenzyme A (acetyl-CoA), which enters the TCA cycle and facilitates the creation of NADH (de Bari & Atlante, 2018). This NADH is subsequently utilized by the mitochondrial electron transport chain to establish a proton gradient across the inner mitochondrial membrane (Lenaz et al., 2006). These gradients power the mitochondrial ATP synthase, which generates large quantities of ATP (Bonora et al., 2012). It's also worth noting that acetyl-CoA can originate not only from glucose but also from the breakdown of lipids and proteins (Bonora et al., 2012; Finley, 2023).

GLYCOLYSIS

Glycolysis is the biochemical process in which a single glucose molecule is cleaved into two molecules of pyruvate, which is the final product of this metabolic pathway (Chandel, 2021; Altenberg & Greulich, 2004). When oxygen is present, pyruvate generally enters the mitochondria where it undergoes oxidative decarboxylation to form acetyl-CoA (de Bari & Atlante, 2018). However, under anaerobic conditions, in the absence of oxygen, pyruvate is instead reduced to form lactate (Liberti & Locasale, 2016; Adeva et al., 2014). This metabolic pathway comprises 10 sequential reactions that occur in the cytosol of the cell and result in a net gain of two ATP molecules, without the direct use of molecular oxygen (Chandel, 2021; Lunt & Vander Heiden, 2011).

The ten reactions of glycolysis are catalysed by ten distinct enzymes: hexokinase (HK) (Wilson, 2003; Sebastian et al., 2000), phosphoglucose isomerase (PGI), phosphofructokinase (PFK) (Webb et al., 2015; Mor et al., 2011), aldolase, triosephosphate isomerase (TPI), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PGM), enolase, and pyruvate kinase (PK) (Christofk et al., 2008; Yang & Lu, 2015).

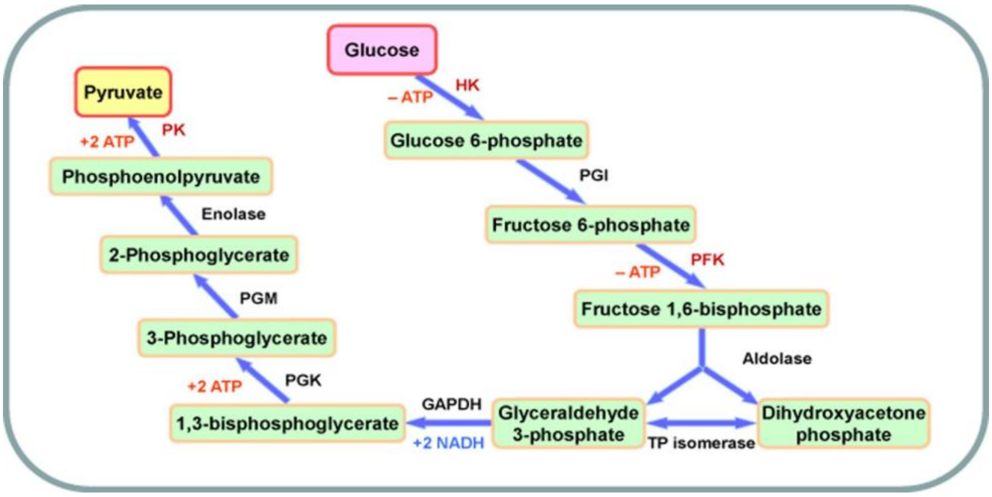

Figure 2: Schematic diagram of glycolysis (Xue-bing Li et al., 2024)

There is an important link between glycolysis and the tricarboxylic acid (TCA) cycle, where pyruvate produced from glycolysis is converted inside the mitochondria into acetyl-CoA and carbon dioxide. This acetyl-CoA then enters the TCA cycle—also known as the citric acid cycle or Krebs cycle—where it undergoes complete oxidation into carbon dioxide and water, resulting in the generation of a significantly greater amount of energy than that produced during glycolysis alone (Li et al., 2024).

AEROBIC GLYCOLYSIS

Aerobic glycolysis refers to the process where glucose is converted into lactate despite the availability of adequate oxygen to support its full oxidation through the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (Lunt & Vander Heiden, 2011; Liberti & Locasale, 2016). This phenomenon is commonly observed in cancerous cells as well as in certain normal cell types (Finley, 2023). Many proliferating animal cells exhibit this type of metabolism, where most of the glucose taken up by the cell is transformed into lactate, and only a minor portion undergoes complete oxidation to carbon dioxide (Yalcin et al., 2009; Altenberg & Greulich, 2004). The ATP generated through aerobic glycolysis clearly plays a vital role in supporting basic cellular activities and contributes significantly to the biosynthetic needs of some proliferative cells (Lunt & Vander Heiden, 2011). Cell proliferation involves creating new daughter cells, which requires the duplication of all cellular materials (Bonora et al., 2012; Finley, 2023).

HEXOKINASE

Glucose is a vital metabolite for all living organisms, and its initial step in metabolism involves phosphorylation by the enzyme hexokinase (HK; E.C. 2.7.1.1) to produce glucose-6-phosphate (G6P) (Wilson, 2003; Katzen & Schimke, 1965). This molecule can then enter multiple metabolic pathways, including glycolysis to generate ATP, the pentose phosphate pathway to produce NADPH and sugar phosphates, and the synthesis of glycogen, which acts as a storage form of energy (Wilson, 2003; Clarke & Sokoloff, 1999). Hexokinase catalyses the reaction: glucose + ATP → G6P + ADP. In mammals, four different isozymes of hexokinase (HK I-IV) exist, each varying in their tissue and subcellular distribution (Wilson, 2003; Polakis & Wilson, 1985). HK IV, also known as glucokinase, is liver-specific and has a high Km for glucose (Wilson, 2003). The other three isozymes, with molecular weights of about 100 kDa, have a low Km for glucose (Katzen & Schimke, 1965). Types I and II are found in nearly all tissues, while type IV is exclusively found in the liver, where all four types coexist (Wilson, 2003). Hexokinase in the brain is primarily of type I, whereas in the epididymal fat pad, type II predominates (Clarke & Sokoloff, 1999). In heart muscle, both types I and II are present in equal amounts, while skeletal muscle primarily contains type II hexokinase activity (Katzen & Schimke, 1965). The transcriptional regulation of these isozymes allows their selective expression in specific tissues and dictates their involvement in various metabolic processes (Wilson et al., 1985). These isozymes differ in their affinity for substrates, including glucose and ATP, and their inhibition by glucose-6-phosphate (G6P) and phosphate (Wilson, 2003).

The HK I isozyme contains a hydrophobic N-terminal sequence that enables it to bind to the voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane, where it can preferentially use ATP generated from oxidative phosphorylation (Polakis & Wilson, 1985; Wilson, 2003). This property is shared by HK II, while some HK III isozymes are associated with the nuclear membrane, although they are also present in the cytoplasm (Wilson et al., 1985).

The increased phosphate-to-phosphate ratio would stimulate type I hexokinase activity, suggesting that it primarily plays a catabolic role, directing glucose into glycolysis to generate ATP (Wilson, 2003). The widespread expression of the type I isozyme is consistent with this function, as glycolysis is essential in nearly all mammalian tissues (Clarke & Sokoloff, 1999). The type I isozyme binds to mitochondria via interaction with porin, a protein that forms channels in the outer mitochondrial membrane through which metabolites are transported (Polakis & Wilson, 1985). Studies have indicated that the type I isozyme selectively uses intramitochondrial ATP as its substrate when bound to actively phosphorylating mitochondria, facilitating coordination between glycolysis and mitochondrial oxidative stages (Wilson, 2003). This ensures that glucose metabolism aligns with cellular energy demands and prevents excessive lactate production (Clarke & Sokoloff, 1999).

Furthermore, type I isozyme is highly expressed in the brain, which is heavily reliant on glycolytic glucose metabolism to meet its high energy demands (Clarke & Sokoloff, 1999). In contrast, type II isozyme is more limited in expression and is primarily found in insulinsensitive tissues like skeletal muscle and adipose tissue (Katzen & Schimke, 1965; Wilson, 2003). It has been suggested that the type II isozyme's response to glucose-6-phosphate and phosphate better suits it for anabolic roles, such as supplying G6P for glycogen resynthesis during skeletal muscle recovery after contraction (Wilson, 2003; Sebastian et al., 2000).

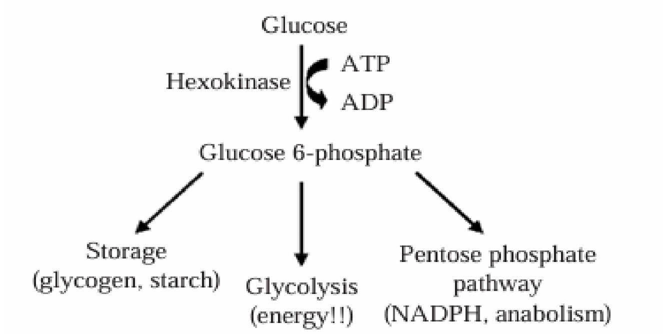

Figure 3: Phosphorylation, catalyzed by hexokinase, is the initial step in common pathways of Glucose metabolism (Wilson, 2003)

PHOSPHOFRUCTOKINASE 1 (PFK1)

Phosphofructokinase 1 (PFK-1) is a key regulatory enzyme that governs glucose flow through the glycolytic pathway (Goldhammer & Paradies, 1979; Webb et al., 2015). As a rate-limiting enzyme, PFK-1 catalyses the conversion of fructose 6-phosphate (F6P) and ATP into fructose-1,6-bisphosphate (F-1,6-P) and ADP, marking the commitment of glucose to glycolysis (F6P + ATP → F-1,6-P + ADP) (Wang et al., 2024; Hers & Van Schaftingen, 1982). This reaction is crucial and tightly regulated. One of the most powerful activators of PFK-1 is fructose 2,6-bisphosphate (F2,6BP), which is produced and broken down by a group of bifunctional enzymes - 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB) (Sakai et al., 2004; Wu et al., 2006). The cellular levels of F2,6BP depend on glycolytic flux and can boost PFK-1 activity even in the presence of ATP (Hers & Van Schaftingen, 1982; Rider et al., 2004). The relationship between F2,6BP levels, the enzymes involved in its synthesis and degradation, and PFK-1 activity has significant implications for various cellular metabolic processes and systemic metabolic conditions (Yalcin et al., 2009). TIGAR, a newly discovered F2,6BPase, may also contribute to the regulation of metabolic pathways in the cell (Mor et al., 2011; Bensaad et al., 2006).

PFK-1 is encoded by three genes: PFK-M (muscle), PFK-L (liver), and PFK-P (platelets), each producing a different isoform (Webb et al., 2015; Ismail & Hussain, 2017). The enzyme functions as a tetramer (Webb et al., 2015). Mutations that impair PFK-1 activity lead to glycogen storage disease type VII, also known as Tarui disease (García et al., 2009). Mice lacking muscle PFK-1 exhibit reduced fat stores (Webb et al., 2015). Without functional PFK-1, glucose cannot be fully committed to glycolysis and is instead directed to glycogen storage, causing glycogen accumulation (García et al., 2009; Atsumi et al., 2005).

PFK-1 activity is linked to cellular metabolism and physiology, with its levels increasing in response to growth signals and heightened glycolysis in proliferating cells (Moon et al., 2010; Yalcin et al., 2009). In cancer cells, PFK-1 activity is often elevated due to oncogene activation or HIF1α-induced signalling (Yalcin et al., 2009; Minchenko et al., 2003). The enzyme is inhibited by lactate, a byproduct of glycolysis, which causes the tetramer to dissociate into dimers, reducing enzymatic activity and providing negative feedback to regulate glycolytic rates (Mor et al., 2011). PFK-1 is also inhibited by ATP and activated by AMP, allowing the enzyme to respond to the cell's energy status and prevent excessive glucose degradation when ATP is abundant (Goldhammer & Paradies, 1979; Mor et al., 2011). Additionally, PFK-1 is regulated by other metabolites from pathways downstream of glycolysis

(Almeida et al., 2010). For instance, citrate from the TCA cycle and long-chain fatty acids can inhibit PFK-1 activity (Mor et al., 2011; Rider et al., 2004).

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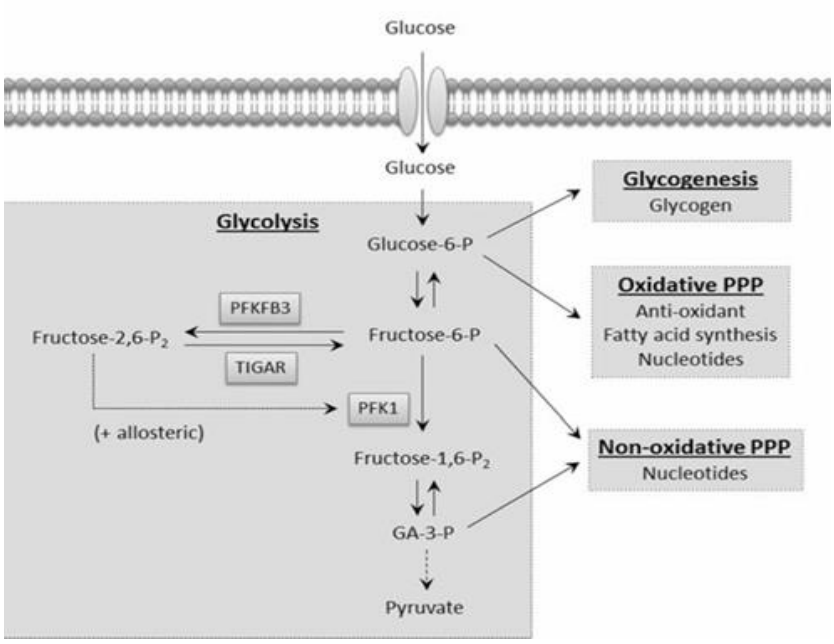

Figure 4

FRUCTOSE-2,6-BISPHOSPHATE IN CELL METABOLISM (F2,6BP)

Fructose-2,6-bisphosphate (F2,6BP) is produced by phosphofructokinase-2 (PFK-2), which uses fructose 6-phosphate (derived from glucose 6-phosphate) as a substrate, the same substrate used by PFK-1 (Mor et al., 2011; Hers & Van Schaftingen, 1982). The levels of F2,6BP in cells are determined by a balance between its synthesis by PFK-2 and its hydrolysis to fructose 6-phosphate by fructose-2,6-bisphosphatase (F2,6BPase) (Rider et al., 2004; Wu et al., 2006). F2,6BP plays a critical role in stimulating glycolysis in the presence of glucose in various tissues, including the liver and kidney (Sakai et al., 2004). In the liver, F2,6BP helps coordinate glucose utilization in glycolysis with its regeneration via gluconeogenesis (Wu et al., 2006). Since the reaction catalysed by PFK-1 is irreversible, the conversion of fructose-1,6-bisphosphate (F1,6BP) to fructose 6-phosphate is carried out by F1,6BPase, which is a key enzyme in gluconeogenesis (Hers & Van Schaftingen, 1982). F2,6BP regulates the interaction between glycolysis and gluconeogenesis in the liver by activating PFK-1 and inhibiting F1,6BPase (Sakai et al., 2004; Mor et al., 2011).

The enzymes in the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Pfkfb) family (Pfkfb1, Pfkfb2, Pfkfb3, and Pfkfb4) control the synthesis and degradation of F2,6BP through their kinase and phosphatase activities, respectively (Güzel et al., 2023; Minchenko et al., 2003). Most of these isoenzymes have both kinase and phosphatase activities, except for PFKB3, which primarily functions as a kinase (Caro et al., 2003; Yalcin et al., 2009). PFKFB expression is tissue-specific, with PFKFB1, PFKFB2, and PFKFB4 being expressed in the liver, muscle, heart, and testes, respectively, and PFKFB3 being predominantly found in the placenta (Yalcin et al., 2009; Atsumi et al., 2005).

PFK-2 activity is also regulated by hormones (Rider et al., 2004). Insulin signalling enhances PFK-2 activity, leading to increased F2,6BP levels and boosting the glycolytic rate (Moon et al., 2010). Other hormonal signals, such as adrenaline and androgens, also activate the kinase function of different PFK-2 isoforms, increasing F2,6BP levels (Atsumi et al., 2005;

Rider et al., 2004). This hormonal regulation helps cells respond to increased glucose by enhancing glycolysis (Yalcin et al., 2009). Elevated levels of PFKFB3, which increase F2,6BP and glycolysis, are involved in adipocyte lipogenesis and triglyceride synthesis (Atsumi et al., 2005). Therefore, F2,6BP plays a crucial role in adapting various tissues to changes in metabolic demands, which can influence systemic metabolic conditions (Mor et al., 2011; Almeida et al., 2010).

The inducible isoform of PFKB3 is continually degraded through ubiquitination in resting cells (Almeida et al., 2010). Just before the G1-to-S transition, the E3 ubiquitin ligase APC/C-Cdh1 is inactivated, leading to increased PFKFB3 levels and enhanced glycolysis required for cell proliferation (Mor et al., 2011). PFKFB3 is degraded by both APC/C-Cdh1 in G1 and by the SCF complex during the S-phase, and its activity is thus confined to a specific phase of the cell cycle (Almeida et al., 2010). This regulation helps coordinate metabolic processes with cell cycle progression, ensuring glycolysis peaks at the appropriate time (Mor et al., 2011). These findings suggest that proper timing of PFK-2 activity and glycolysis is crucial for controlling cell cycle progression and cell proliferation (Yalcin et al., 2009; Rider et al., 2004).

PYRUVATE KINASE

Pyruvate kinase (PK) governs the final and rate-limiting step of glycolysis by facilitating the transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, producing ATP and pyruvate (Altenberg & Greulich, 2004; Yamada & Noguchi, 1999). Based on cellular metabolic needs, PK is expressed as four distinct isoforms—L, R, M1, and M2—in mammalian tissues, each exhibiting unique regulatory behaviours (Zanella et al., 2007). Their expression is largely influenced by developmental stages, nutritional status, and hormonal signals (Yamada et al., 1999). M1 is predominantly found in skeletal muscle, heart, and brain, while M2 is present in early embryonic and rapidly dividing tissues (Christofk et al., 2008). Over time, M2 is gradually replaced by tissue-specific isoforms, though it remains the main variant in the lung, kidney, spleen, adipose tissue, leukocytes, and platelets (Zanella et al., 2007). Notably, M2 is re-expressed in various tumors, replacing native isoforms and serving as a potential cancer marker (Christofk et al., 2008; Bluemlein et al., 2011). The L isoform is mainly localized in the liver, but is also found in the renal cortex, small intestine, and pancreatic β-cells (Zanella et al., 2007), whereas the R isoform is specific to red blood cells (Yamada et al., 1999). Each isoform demonstrates unique kinetic responses suited to their tissue environment (Zanella et al., 2007). Except for M1, all PK isoforms display sigmoidal kinetics for PEP, are activated by fructose 1,6-bisphosphate (FBP), and inhibited by ATP (Dombrauckas et al., 2005). In humans, two genes—PK-M and PK-LR—encode the four isoforms (Noguchi et al., 1986). PK-M gives rise to M1 and M2 through mutually exclusive mRNA splicing (Noguchi et al., 1986), whereas PK-LR gives rise to L and R isoforms via tissue-specific promoters (Yamada et al., 1999).

PKM1 is broadly expressed in healthy tissues, but PKM2 is also found in normal tissues like lung, liver, colon, thyroid, bladder, and kidney (Bluemlein et al., 2011). Beyond its glycolytic role, PKM2 has additional cellular functions (Yang et al., 2012). It serves as a cytosolic receptor for thyroid hormone and, under mitogenic and oncogenic signals, translocates into the nucleus (Yang et al., 2015). There, PKM2 acts as both a protein kinase and transcriptional co-activator, influencing histone phosphorylation, gene expression, and transitions through the G1-S phase—central elements of the Warburg effect (Yang et al., 2012; Luo et al., 2011). PKM2 also phosphorylates key regulators of the cell cycle such as Bub3 (for chromatid segregation and mitotic checkpoint control) and myosin light chain 2 (MLC2), facilitating cytokinesis and tumor cell proliferation (Yang et al., 2015; Gao et al., 2012).

Because PKM2 catalyses glycolysis's final step, it is modulated by metabolic intermediates (Chaneton et al., 2012). FBP activates PKM2 allosterically by enhancing its tetramer formation, which is more catalytically active than the dimeric form (Dombrauckas et al., 2005). Serine activates PKM2 independently of FBP, ensuring efficient glucose utilization in serine-rich conditions (Chaneton et al., 2012). When serine is scarce, PKM2 activity drops, redirecting glucose carbon toward serine biosynthesis to restore balance (Keller et al., 2012). Another modulator, SAICAR—a purine biosynthesis intermediate—also enhances PKM2 activity independently of FBP, particularly during glucose starvation, promoting cancer cell survival via increased glucose and glutamine uptake (Keller et al., 2012). PKM2 is also subject to post-translational modifications (Hitosugi et al., 2009). FGFR1 phosphorylates PKM2 at Y105, inhibiting FBP binding and promoting the less active dimer form (Hitosugi et al., 2009). This suppression of pyruvate kinase activity boosts PKM2's function as a protein kinase, including phosphorylation of STAT3 (Gao et al., 2012). Expression of a PKM2 Y105F mutant reduces tumorigenesis and cell growth (Hitosugi et al., 2009).

Besides its glycolytic duties, PKM2's nuclear functions influence gene transcription and cell division (Yang et al., 2011). Nuclear PKM2 binds to c-Src–phosphorylated β-catenin at Y333 and enhances β-catenin's transcriptional activity (Yang et al., 2011). PKM2 then phosphorylates histone H3 at T11 using PEP, prompting HDAC3 dissociation from the promoters of β-catenin targets such as CCND1 and MYC, enabling acetylation at H3K9 (Yang et al., 2012). This modification is essential for EGFR-driven gene activation, cell growth, and oncogenesis (Lu, 2012). PKM2 also acts on tyrosine residues, including phosphorylation of STAT3 at Y705, which boosts STAT3's transcriptional activity and enhances MEK5 gene transcription (Gao et al., 2012).

PKM2 also modulates cellular metabolism through its non-glycolytic functions (Luo et al., 2011). Upon EGFR and PDGFR stimulation, PKM2 enters the nucleus, activates β-catenin, and drives c-Myc expression (Yang et al., 2011). This upregulation promotes GLUT1 and LDHA expression and enhances PKM2 transcription via PTB-dependent feedback (Yang et al., 2015). These changes increase glucose uptake and lactate production, supporting tumor progression (Christofk et al., 2008). PKM2 regulates HIF1α activity to reshape cancer metabolism (Luo et al., 2011). PKM2, upon hydroxylation by PHD3, binds HIF1α, facilitating its attachment to hypoxia response elements and the recruitment of coactivator p300 (Luo et al., 2011). This boosts HIF1α-dependent gene transcription, thereby amplifying glycolysis in cancerous cells (Yang et al., 2011; Luo et al., 2011).

LACTATE DEHYDROGENASE

LDH primary role involves catalysing the reversible conversion between lactate and pyruvate, simultaneously facilitating the reduction of NAD^+ to NADH and vice versa (Schumann et al., 2002). Where oxygen is scarce or absent, the production of ATP through oxidative phosphorylation is hindered (Passarella et al., 2018). LDH expression is increased during such stress to meet cellular energy demands (Adeva et al., 2014). However, the lactate formed from anaerobic glucose metabolism is metabolically inert in most tissues except the liver (Liberti et al., 2016). It is released into the bloodstream and transported to the liver, where LDH reverses the reaction, converting lactate back to pyruvate in the Cori cycle (Drent et al., 1999).

During intense physical activity, muscle oxygen supply diminishes, and LDH catalyses the conversion of pyruvate to lactic acid (Liang et al., 2016). In red blood cells, where mitochondria are absent, pyruvate cannot undergo further oxidation, remaining in the cytoplasm and eventually converting to lactate (de Bari et al., 2018). This reaction also oxidizes NADH to NAD^+, which is essential for maintaining the NAD^+ pool required during glycolysis

(Chinnery et al., 2003). Anaerobic glycolysis generates only 2 ATP per glucose molecule, significantly less than the 36 ATP produced by oxidative phosphorylation (Faou et al., 2012).

In cancer cells, the activity of LDH, particularly the LDHA isoform, is altered compared to normal cells (Anderson et al., 1981). Cancerous cells utilize LDH to support aerobic glycolysis, a phenomenon where glycolysis and lactate production persist even in the presence of oxygen—a process known as the Warburg effect (Kroemer, 2003). This metabolic switch benefits cancer cells by avoiding reactive oxygen species generated by the electron transport chain while preserving metabolic intermediates for biosynthesis of nucleic acids and lipids, supporting rapid growth (van Gurp et al., 2003).

LDH plays a significant role in gluconeogenesis and nucleotide metabolism (Lemasters et al., 1998). LDH resides in the cytoplasm and is abundant in tissues such as the liver, kidney, and muscle, while red blood cells contain moderate levels (Li et al., 2000). The enzyme exists in five isomeric forms, each composed of four subunits—either muscle-type (M) or heart-type (H) (Rapaport, 2005). These isoforms, known as LDH-1 to LDH-5, are distributed differently across tissues (Bykov et al., 2020).

Although LDH is primarily cytoplasmic, studies have confirmed the presence of a mitochondrial variant, mL-LDH, in species such as yeast, plants, and animals (Young et al., 2003). This L-lactate-specific enzyme utilizes transporters—L-lactate/H symporters and L-lactate/pyruvate or L-lactate/oxaloacetate antiporters—to move lactate into the mitochondria, where it is oxidized back to pyruvate (Meisinger et al., 2001). In cancer cells, where energy demands are elevated, mitochondrial reprogramming occurs, and mL-LDH may contribute to enhanced oxidative phosphorylation (Shoubridge, 2001).

MITOCHONDRIAL SIGNALING

Mitochondria are dynamic, double-membrane-bound organelles that play an essential role in energy metabolism, cellular signalling, and programmed cell death (Kuwana et al., 2002). As a sub-compartment of the eukaryotic cell, mitochondria possess a unique structure comprising an outer membrane, an intermembrane space, an inner membrane, and a matrix (Schinder et al., 1996). The inner mitochondrial membrane houses the respiratory chain, a group of five enzyme complexes (complexes I to V) that are integral to mitochondrial oxidative metabolism (Söllner et al., 1989). These complexes are arranged sequentially and functionally cooperate to perform oxidative phosphorylation (OXPHOS), the primary process by which cells generate adenosine triphosphate (ATP) (Neupert, 1997).

The respiratory chain begins with complex I (NADH: ubiquinone oxidoreductase), the largest of the five, composed of over 40 polypeptide subunits (Scheufler et al., 2000). Complex II (succinate dehydrogenase), which also participates in the tricarboxylic acid (TCA) cycle, acts as a secondary entry point for electrons (Pearl & Prodromou, 2006). Reduced cofactors such as NADH and $FADH_2$, produced during the intermediary metabolism of carbohydrates, fats, and proteins, donate electrons to complexes I and II respectively (Agarraberes & Dice, 2001). These electrons are transferred along the respiratory chain via mobile electron carriers—ubiquinone (also known as coenzyme Q10) and cytochrome c—through complexes III and IV, before ultimately reducing molecular oxygen to water (Fan et al., 2011).

Within this system, energy is released through redox reactions involving prosthetic groups (Saitoh et al., 2007). Iron–sulphur clusters in complexes I, II, and III, and heme-containing cytochromes in complex IV and cytochrome c facilitate these electron transfers (Perry et al., 2006). This process is tightly coupled to the translocation of protons (H^+) across the inner mitochondrial membrane by complexes I, III, and IV, thereby establishing an electrochemical proton gradient (Yano et al., 2000). This gradient, which generates a membrane potential and a pH difference across the inner membrane, constitutes the proton-motive force used by complex V (ATP synthase) to synthesize ATP from ADP and inorganic phosphate (Bausewein et al., 2017). This entire series of reactions constitutes oxidative phosphorylation, a process crucial to cellular survival and function (Wang et al., 2020).

Mitochondria are protein-rich organelles, containing approximately 25% of the cell's proteins, or about 1500 proteins in mammalian cells (Sayyed & Mahalakshmi, 2022). These proteins originate from two distinct genetic systems: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) (Marzo et al., 1998). The human mitochondrial genome encodes 13 polypeptides involved in oxidative phosphorylation, along with 2 ribosomal RNAs and 22 transfer RNAs necessary for mitochondrial protein synthesis (Adam et al., 1999). Most mitochondrial proteins, however, are nuclear encoded (Roise & Schatz, 1988). These proteins are synthesized in the cytoplasm and contain specific mitochondrial targeting sequences that direct their import via the translocase of the outer membrane (TOM) and the translocase of the inner membrane (TIM) complexes (von Heijne, 1986). Once imported, the targeting sequences are cleaved, and the proteins are assembled into their respective respiratory complexes by nuclear-encoded chaperones and assembly factors (Curran et al., 2002).

Beyond energy metabolism, mitochondria also serve as central hubs in regulating apoptosis, a programmed cell death mechanism crucial for maintaining cellular homeostasis (Kovermann et al., 2002). One of the earliest and most critical steps in the mitochondrial apoptotic pathway is the permeabilization of the outer mitochondrial membrane, leading to the release of several pro-apoptotic proteins from the intermembrane space into the cytosol (Koehler et al., 2000). Among these are cytochrome c, Smac/DIABLO, apoptosis-inducing factor (AIF), and endonuclease G (Hobbs et al., 2001). Once in the cytosol, cytochrome c binds to apoptotic protease activating factor-1 (Apaf-1), promoting the formation of the apoptosome complex, which recruits and activates initiator caspase-9 (Bohnert et al., 2010). This activation triggers a cascade involving effector caspases such as caspase-3, culminating in the systematic dismantling of cellular components (Glick et al., 1992).

Smac/DIABLO contributes to apoptosis by inhibiting inhibitor of apoptosis proteins (IAPs), thereby removing repression from caspases (Rojo et al., 1999). Meanwhile, AIF and endonuclease G translocate to the nucleus, where they mediate large-scale DNA fragmentation independently of caspases (Geissler et al., 2000). The regulation of mitochondrial membrane permeability is largely governed by the Bcl-2 family of proteins (Truscott et al., 2001). This family includes both pro-apoptotic members (e.g., Bax, Bak, Bid, and the BH3-only proteins such as Bad and Bid) and anti-apoptotic members (e.g., Bcl-2 and Bcl-xL) (Bauer et al., 1996). The delicate balance between these opposing factions determines cell fate (Jensen & Johnson, 1999).

Bax and Bak oligomerize in the outer mitochondrial membrane to form pores, facilitating the release of cytochrome c and other intermembrane proteins (Gakh et al., 2002). Conversely, Bcl-2 and Bcl-xL bind to and inhibit these pro-apoptotic proteins, maintaining mitochondrial integrity (Rehling et al., 2001). The activity of BH3-only proteins is particularly important in initiating the apoptotic cascade. These proteins are typically activated in response to stress signals, and they promote apoptosis by neutralizing anti-apoptotic Bcl-2 proteins or directly activating Bax and Bak (Yin & O'Neill, 2021).

A pivotal structure in this regulation is the mitochondrial permeability transition (PT) pore, a multiprotein complex situated at contact sites between the inner and outer membranes (Lenaz et al., 2006). The PT pore acts as a conduit for molecules up to 1.5 kDa in size and plays a central role in mitochondrial-mediated apoptosis (Scialò et al., 2017). Opening of the PT pore results in the loss of mitochondrial membrane potential, osmotic imbalance, and mitochondrial swelling, ultimately leading to membrane rupture and the release of apoptogenic factors (Hu et al., 2021). This process is driven by elevated mitochondrial Ca^{2+} levels, increased production of reactive oxygen species (ROS), the presence of free fatty acids, and exposure to pro-oxidants such as nitric oxide (Cobley, 2020). Notably, ROS are predominantly generated by complexes I and III of the respiratory chain (Scialò et al., 2017).

The PT pore comprises several key components including the voltage-dependent anion channel (VDAC) on the outer membrane, the adenine nucleotide translocator (ANT) on the inner membrane, and cyclophilin D in the matrix (Söllner et al., 1989). Bcl-2 family proteins also interact with PT pore components to modulate their function (Neupert, 1997). For instance, Bcl-2 prevents the association of Bax with mitochondria and blocks the release of cytochrome c and AIF (Scheufler et al., 2000). The interaction between Bcl-2 family members and VDAC is also crucial in modulating mitochondrial outer membrane permeability and regulating apoptosis (Pearl & Prodromou, 2006).

Interestingly, the degree and duration of PT pore opening determine whether a cell undergoes apoptosis or necrosis (Agarraberes & Dice, 2001). Transient opening of the pore allows the release of apoptogenic factors while preserving enough mitochondrial function and ATP production to support the energy-dependent steps of apoptosis (Fan et al., 2011). In contrast, prolonged or complete opening leads to irreversible mitochondrial depolarization, energy collapse, and necrotic cell death (Saitoh et al., 2007). This duality highlights the role of mitochondria as integrators of cellular fate in response to various metabolic and environmental stimuli (Perry et al., 2006).

In addition to apoptosis regulation, mitochondria also serve as platforms for integrating diverse intracellular signalling pathways (Yano et al., 2000). Several proteins involved in cell signalling are known to translocate to mitochondria, where they either retain their original functions or acquire new roles (Bausewein et al., 2017). For instance, kinases such as protein kinase C (PKC), p38 MAPK, and transcription factors such as nuclear receptor TR3 (also known as Nur77) can translocate to the mitochondria in response to stress signals (Wang et al., 2020). TR3 is of particular interest; upon activation by retinoids, calcium signalling, or phorbol esters, TR3 translocates from the nucleus to the mitochondria, where it binds to Bcl-2 and induces conformational changes that convert Bcl-2 from an anti-apoptotic to a pro-apoptotic protein, thereby promoting cytochrome c release and apoptosis (Sayyed & Mahalakshmi, 2022).

TOM PROTEINS OF THE OUTER MITOCHONDRIAL MEMBRANE

The translocase of the outer mitochondrial membrane (TOM complex) is a multi-subunit protein assembly responsible for importing precursor proteins into mitochondrial compartments and facilitating the integration of proteins into the outer mitochondrial membrane (von Heijne, 1986). These peptides are initially produced in cytosol, and cytosolic chaperones (holdases) maintain the import-targeted polypeptides in a transport-ready state while also preventing their aggregation (Curran et al., 2002). Chaperones like Hsp40 and Hsp70 aid in guiding these nascent polypeptides to the outer mitochondrial membrane (OMM), where the mitochondrial import machinery directs them either to the OMM or into the mitochondrial intermembrane space (IMS) (Kovermann et al., 2002). Once synthesized on cytosolic polysomes, the targeting signals of preproteins are identified by specific receptors on the mitochondrial surface (Koehler et al., 2000). These preproteins are transported across the OMM via a general import pore (GIP) and subsequently transferred to internal mitochondrial compartments (Hobbs et al., 2001). Among the TOM components, three proteins act as receptors for preproteins: Tom20 and Tom70 serve as initial receptors for proteins bearing N-terminal and internal targeting sequences, respectively, while Tom22 operates as the central receptor and is linked to the channel-forming subunit Tom40 (Bohnert et al., 2010). The GIP complex includes Tom22, Tom40, and three smaller TOM proteins—Tom5, Tom6, and Tom7 (Glick et al., 1992). Tom5 facilitates the transition of preproteins from Tom22 to Tom40, whereas Tom6 and Tom7 play roles in the assembly and maintenance of the GIP complex (Rojo et al., 1999).

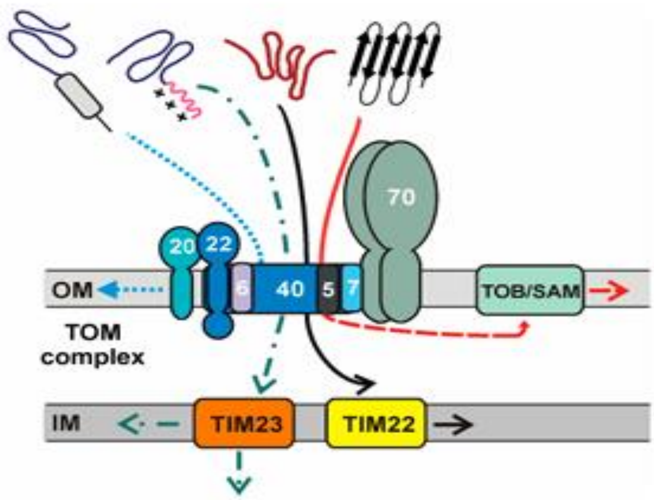

Figure 5: Diagram (Rapaport, 2005)

THE TOM COMPLEX AND PROTEIN IMPORT

The TOM complex governs the passage of mitochondrial precursor proteins (Rapaport, 2005). After traversing the OM, preproteins with presequences are processed by the Tim23 complex located in the inner membrane (IM) (Jensen et al., 2002). Polytopic inner membrane proteins are inserted through the Tim22 complex (Curran et al., 2002). β-barrel precursors are imported via the TOM complex and later integrated into the OM through the TOB-SAM complex (Sayyed et al., 2022).

The TOM complex consists of seven subunits: the principal receptors Tom20 and Tom70, as well as the core TOM complex composed of Tom5, Tom6, Tom7, Tom22, and Tom40 (Bausewein et al., 2017). Tom40 features a 19-stranded β-barrel structure with closure facilitated by parallel hydrogen bonds like those in porins (Wang et al., 2020). The surface of Tom40 facing the IMS carries a positive charge along the dimer interface (Yano et al., 2000). The cytosolic side contains a negatively charged pore-lining region (Perry et al., 2006). Tom40 subunits do not share a large interface but are stabilized by the binding of two Tom22 receptors (Fan et al., 2011). The TOM core complex (TOM-CC) is stabilized by two copies each of Tom22, Tom5, Tom6, and Tom7 (Saitoh et al., 2007). The transmembrane helices of Tom5, Tom6, and Tom7 are peripheral and situated at specific locations around each Tom40 pore (Söllner et al., 1989). Tom22 serves as the central receptor associated with Tom40-CC (Neupert, 1997). Tom20 and Tom70 are the primary receptors linked to Tom40 (Young et al., 2003).

PROTEIN RECOGNITION AND IMPORT MECHANISMS

Proteins containing classical N-terminal targeting signals are recognized by Tom20 during mitochondrial import (Pearl & Prodromou, 2006). Inner membrane proteins with internal targeting signals are directed through the Tom70 pathway (Agarraberes & Dice, 2001). Tom70 and Tom20 can functionally interact in importing presequence-containing mitochondrial proteins (Scheufler et al., 2000). Cytosolic factors including Hsp70 preserve proteins in an import-competent state (Faou & Hoogenraad, 2012). Mammalian cells utilize both Hsp70 and Hsp90 to transfer preproteins to the TOM complex (Young et al., 2001). Tom70 functions as both a docking site for chaperones and a receptor for internal targeting signals (Rehling et al., 2001).

TRANSLOCASE OF THE INNER MEMBRANE (TIM23 COMPLEX)

The TIM23 complex facilitates protein movement into the mitochondrial matrix (von Heijne, 1986). The complex consists of Tim23p, Tim17p, Tim44p, and mtHsp70p (Bauer et al., 1996). Tim23p and Tim17p form the translocation channel (Truscott et al., 2001). An ATP-powered motor generates the force required for protein import (Lohret et al., 1997). Tim23p's function is inhibited by mitochondrial presequence peptides (Truscott et al., 2001). The C-terminal half of Tim23p forms a channel that no longer reacts to presequences (Bauer et al., 1996). The N terminus of Tim23p binds to mitochondrial presequences (Kovermann et al., 2002).

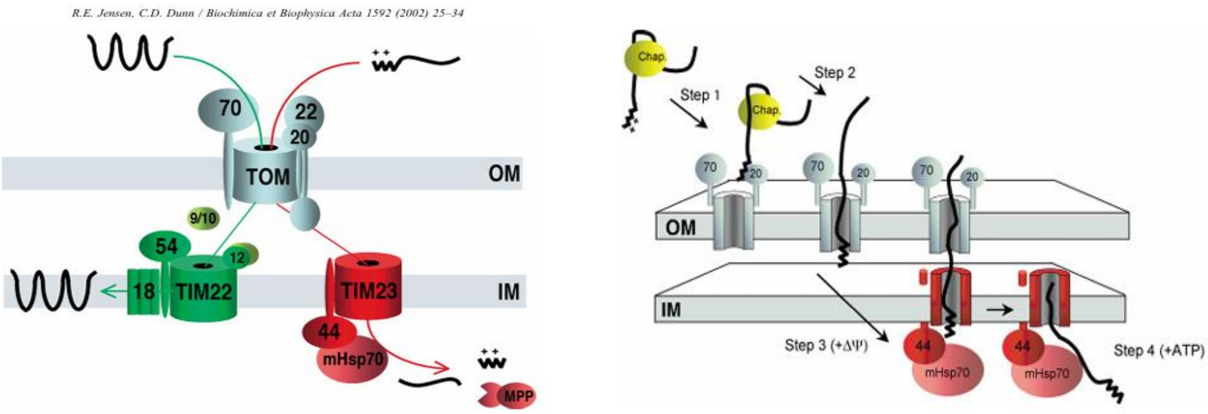

Figure 6

PROTEIN IMPORT MECHANISMS

By either a pulling or a trapping mechanism, the tethered mtHsp70p facilitates the movement of the precursor through the TIM23 translocon in an ATP-dependent manner (Jensen & Dunn, 1999). Since the mitochondrial matrix is negatively charged, the potential may facilitate import through the TIM23 translocon by attracting and 'electrophoresing' the positively charged presequence (von Heijne, 1986). During or after import, the presequence is cleaved off by a matrix-localized processing peptidase called mitochondrial processing peptidase (MPP) (Gakh et al., 2002).

Although the TOM and TIM23 machinery can act independently, it is likely that both complexes will cooperate during the import of precursor proteins (Jensen et al., 2002). Supporting this idea, the N-terminus of Tim23p has been shown to protrude through the OM into the cytosol. This configuration links the mitochondrial OM and IM and has been proposed to increase the efficiency of import, facilitating the transfer of preproteins from the TOM complex to the TIM23 complex (Rehling et al., 2001). This connection of OM and IM by Tim23p would potentially form a type of contact site between the mitochondrial membranes (Koehler et al., 2000).

TIM22 TRANSLOCON

The TIM22 translocon is believed to be specialized in importing polytopic inner membrane (IM) proteins, such as carrier proteins and certain membrane-integrated components of the TIM translocons (Kovermann et al., 2002). Like Tim23p and Tim17p, Tim22p possesses four transmembrane (TM) domains and is situated in the IM with both its N- and C-terminal regions facing the mitochondrial intermembrane space (IMS) (Curran et al., 2002). Tim22p has been demonstrated to form a sizeable aqueous pore (Koehler et al., 2000). When fully open, the channel is broad enough to permit the passage of two tightly folded alpha-helices (Adam et al., 1999). Unlike the TIM23 pathway, protein insertion via the TIM22 complex does not depend on ATP or matrix-localized chaperones but is instead powered entirely by the membrane potential across the IM (Hobbs et al., 2001).

Further components of the TIM22 complex have been uncovered through genetic and biochemical investigations (Jensen et al., 2002). Among these, Tim54p and Tim18p are recognized as integral membrane proteins (Koehler et al., 2000). Tim54p features a single TM domain near its N-terminal end, with a substantial C-terminal portion extending into the IMS (Adam et al., 1999). Tim54 interacts with Mmm1p—a mitochondrial outer membrane (OM) protein that maintains mitochondrial DNA (mtDNA) stability and connects to mtDNA nucleoids—Tim54p suggests potential cooperation between the OM and TIM22 machinery (Hobbs et al., 2001). Mmm1p is found in mitochondrial contact sites, which are zones where the OM and IM are linked, implying that such contact may aid the TIM22 complex in importing polytopic IM proteins (Rehling et al., 2001).

ELECTRON TRANSPORT CHAIN COMPLEXES

The mitochondrial electron transport chain (ETC) is situated within the cristae of the mitochondria. It gathers electrons from NADH or $FADH_2$ and transfers them through a succession of electron carriers embedded in multiprotein respiratory assemblies—complexes I to IV—ultimately to molecular oxygen (Yin & O'Neill, 2021). This transfer creates an electrochemical gradient used by the F_1F_0-ATP synthase (complex V) in the mitochondrial inner membrane to produce ATP (Lenaz et al., 2006). This entire mechanism is referred to as oxidative phosphorylation (OXPHOS) (Scialò et al., 2017). All OXPHOS complexes are multi-subunit transmembrane structures (Hu et al., 2021). The ETC is predominantly fuelled by NADH and $FADH_2$, which are produced in the tricarboxylic acid (TCA) cycle and through β-oxidation, though other metabolic pathways also contribute (Kotlyar & Vinogradov, 1990). Electron flow between complexes is facilitated by mobile carriers, namely Coenzyme Q (CoQ) and cytochrome c (cyto c) (Vinogradov, 1998).

COMPLEX I

Complex I, also known as NADH: ubiquinone oxidoreductase, Type I NADH dehydrogenase, or CI, is the first and largest component of the mitochondrial ETC (Lenaz et al., 2006). It facilitates the transfer of two electrons from NADH to CoQ, concurrently translocating four protons across the inner mitochondrial membrane into the intermembrane space (Scialò et al., 2017). During OXPHOS, the ETC is a major site of mitochondrial ROS production, which are the predominant source of free radicals (Hu et al., 2021). Although ROS can cause oxidative damage—particularly during ischemia-reperfusion (IR) events (Kotlyar & Vinogradov, 1990)—they also play a crucial signalling role (Vinogradov, 1998). Complex I is a significant source of ROS, especially during Reverse Electron Transport (RET) (Lenaz et al., 2006). While forward electron transfer can also produce superoxide, the levels are typically minimal (Scialò et al., 2017). Notably, mitochondrial complex I in several organisms displays a regulatory transition between an inactive D-form and an active A-form (Kotlyar & Vinogradov, 1990). In the absence of substrates, the enzyme shifts to the D-form, which can revert to the fully active A-form during catalytic turnover (Vinogradov, 1998). This A/D transition is functionally significant in vivo (Lenaz et al., 2006).

The architecture of complex I includes 14 central subunits responsible for its core bioenergetic functions (Scialò et al., 2017). These central components consist of seven hydrophilic and seven hydrophobic polypeptides (Hu et al., 2021). In most eukaryotes, the hydrophobic subunits are encoded by mitochondrial DNA (Kotlyar & Vinogradov, 1990). The hydrophilic subunits host the redox-active groups, including a single flavin mononucleotide (FMN) and eight traditional iron-sulfur clusters (Vinogradov, 1998).

COMPLEX II

Succinate dehydrogenase (SDH), also known as succinate-CoQ reductase or respiratory complex II, holds a unique dual role in mitochondrial metabolism, serving as a component of both the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) (Sun et al., 2005). Unlike other ETC complexes, complex II does not contribute directly to proton pumping across the mitochondrial membrane but plays a crucial role in funnelling electrons into the chain. It catalyses the oxidation of succinate to fumarate in the TCA cycle, a reaction that simultaneously reduces ubiquinone (CoQ) to ubiquinol ($CoQH_2$), facilitating electron flow through the respiratory chain (Yoshikawa et al., 1998).

Complex II is a tetrameric enzyme composed of four subunits: SDHA, SDHB, SDHC, and SDHD (Crofts, 2004). The SDHA subunit houses the flavin adenine dinucleotide (FAD) prosthetic group necessary for succinate oxidation, while SDHB contains three iron-sulfur clusters that mediate electron transfer to the membrane-bound SDHC and SDHD subunits (Senior, 1973). Although complex II does not pump protons, its activity is critical for maintaining ETC function and mitochondrial energy homeostasis (Sena et al., 2013). Disruption of SDH activity leads to the accumulation of succinate, which acts as an oncometabolite, inhibiting α-ketoglutarate-dependent dioxygenases and contributing to the stabilization of hypoxia-inducible factors (HIFs) (Inigo et al., 2021).

COMPLEX III

The mitochondrial cytochrome bc_1 complex (complex III), also referred to as ubiquinol-cytochrome c oxidoreductase, constitutes the third complex of the mitochondrial ETC and plays a central role in cellular bioenergetics (Schwerzmann et al., 1986). It catalyses the transfer of electrons from ubiquinol ($CoQH_2$) to cytochrome c (Cyt c) while concurrently pumping protons from the mitochondrial matrix into the intermembrane space (Al-Khallaf et al., 2017). This proton translocation is essential for maintaining the proton motive force necessary for ATP synthesis at complex V (Ramachandran et al., 1980).

A notable aspect of complex III function is the Q cycle, a mechanism that ensures efficient electron transfer and proton pumping through two distinct binding sites for ubiquinone. Complex III is also a significant source of mitochondrial reactive oxygen species (ROS), especially under conditions where the Q cycle is impaired (Koh et al., 2004). Electron leakage during the transfer from ubiquinol to cytochrome c can result in the partial reduction of oxygen to form superoxide anion (O_2^-) (Menendez et al., 2007). Unlike complex I, which largely releases ROS into the mitochondrial matrix, complex III generates ROS in both the matrix and the intermembrane space (Santos et al., 2012).

COMPLEX IV

Cytochrome c oxidase (COX), also known as complex IV, is the terminal enzyme complex of the mitochondrial ETC and is responsible for the final step in oxidative phosphorylation (Selak et al., 2004). It catalyses the transfer of electrons from reduced cytochrome c to molecular oxygen (O_2), the ultimate electron acceptor, converting it into water (H_2O) (Tomlinson et al., 2002). This highly regulated process is coupled with proton translocation from the mitochondrial matrix to the intermembrane space, thereby sustaining the electrochemical gradient required for ATP synthesis by complex V (Pollard et al., 2005).

Unlike complexes I and III, which are major contributors to ROS production, complex IV is relatively insulated from electron leak and does not generate significant amounts of ROS (Sudarshan et al., 2009). Complex IV is a large, multi-subunit complex composed of 13 subunits in mammals, including core catalytic components such as COX1, COX2, and COX3, which are encoded by mitochondrial DNA. Interestingly, complex IV also plays a pivotal role in apoptosis (Crofts, 2004). The release of cytochrome c from the intermembrane space into the cytosol, a hallmark of intrinsic apoptotic signalling, depends on mitochondrial membrane integrity and is indirectly influenced by complex IV function (Yoshikawa et al., 1998).

COMPLEX V

Mitochondrial Complex V, also known as ATP synthase or F_1F_0-ATPase, represents the terminal enzyme of oxidative phosphorylation and is responsible for the synthesis of ATP from ADP and inorganic phosphate (Pi) (Senior, 1973). This process is powered by the proton motive force generated by the upstream electron transport chain complexes (Sena et al., 2013). ATP synthase is reversible in situ and catalyses the overall reaction: ADP + Pi + nH^+ (in) → ATP + H_2O + nH^+ (out) (Inigo et al., 2021).

The structure of Complex V in animal cells comprises at least 11 different polypeptide subunits (Al-Khallaf et al., 2017). Ten of these are considered essential for the catalytic activity of the enzyme, while one subunit, known as the ATPase inhibitor peptide, plays a regulatory role (Ramachandran et al., 1980). This inhibitor peptide has been identified as a key modulator of the enzyme's ATP hydrolytic function. Specifically, it binds to F_1-ATPase to inhibit its ability to hydrolyse ATP under certain conditions, such as during ischemia or other metabolic stress when conserving ATP is vital (Koh et al., 2004).

TCA CYCLE

The tricarboxylic acid (TCA) cycle, also known as the citric acid cycle or Krebs cycle, serves as the central hub of cellular metabolism, orchestrating the oxidative breakdown of carbohydrates, fats, and proteins into usable energy (Menendez et al., 2007). The TCA cycle enables the controlled combustion of nutrients, with each turn of the cycle regenerating its key intermediates to allow the oxidation of an unlimited number of molecules (Santos et al., 2012). However, intermediates of the TCA cycle are often siphoned off to support biosynthetic processes (a phenomenon known as cataplerosis), necessitating their replenishment through anaplerotic pathways (Selak et al., 2004).

Certain cancers exhibit mutations in key TCA cycle enzymes such as isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarate hydratase (FH) (Tomlinson et al., 2002). These mutations disrupt normal metabolic flux through the cycle, leading to the accumulation of oncometabolites and contributing to tumorigenesis (Pollard et al., 2005). The IDH family, consisting of NADP-dependent IDH1 and IDH2, and NAD-dependent IDH3, plays a particularly complex role (Sudarshan et al., 2009). While IDH3 functions within the mitochondria to catalyse the irreversible oxidative decarboxylation of isocitrate to α-ketoglutarate (2KG), IDH1 and IDH2 also participate in cytosolic and mitochondrial NADPH production, respectively (Sun et al., 2005).

In cancer cells, where anabolic demand is elevated and oxygen may be limited, the NADH/NAD^+ ratio becomes skewed (Yoshikawa et al., 1998). This halts the TCA cycle and shifts citrate generated by IDH2-mediated reductive carboxylation toward the cytosol (Senior, 1973). There, it supports the biosynthesis of fatty acids, phospholipids, and cholesterol, essential for rapid cell proliferation (Schwerzmann et al., 1986). Glutamine metabolism also contributes to this anabolic reprogramming, as glutamine-derived 2KG serves as a substrate for reductive carboxylation via both mitochondrial IDH2 and cytosolic IDH1 (Sena et al., 2013).

Additional TCA enzymes implicated in cancer include SDH and FH (Inigo et al., 2021). SDH serves a dual function, linking the TCA cycle and ETC by oxidizing succinate to fumarate and transferring electrons to ubiquinone (Al-Khallaf et al., 2017). Mutations in SDH subunits B, C, and D are more frequently associated with tumors than those in subunit A (Ramachandran et al., 1980). Similarly, loss-of-function mutations in FH are linked to hereditary leiomyomatosis and renal cell carcinoma (HLRCC). These mutations result in fumarate accumulation, excessive ROS production, and aberrant HIF-1α stabilization, which together contribute to a tumor-promoting microenvironment (Koh et al., 2004).

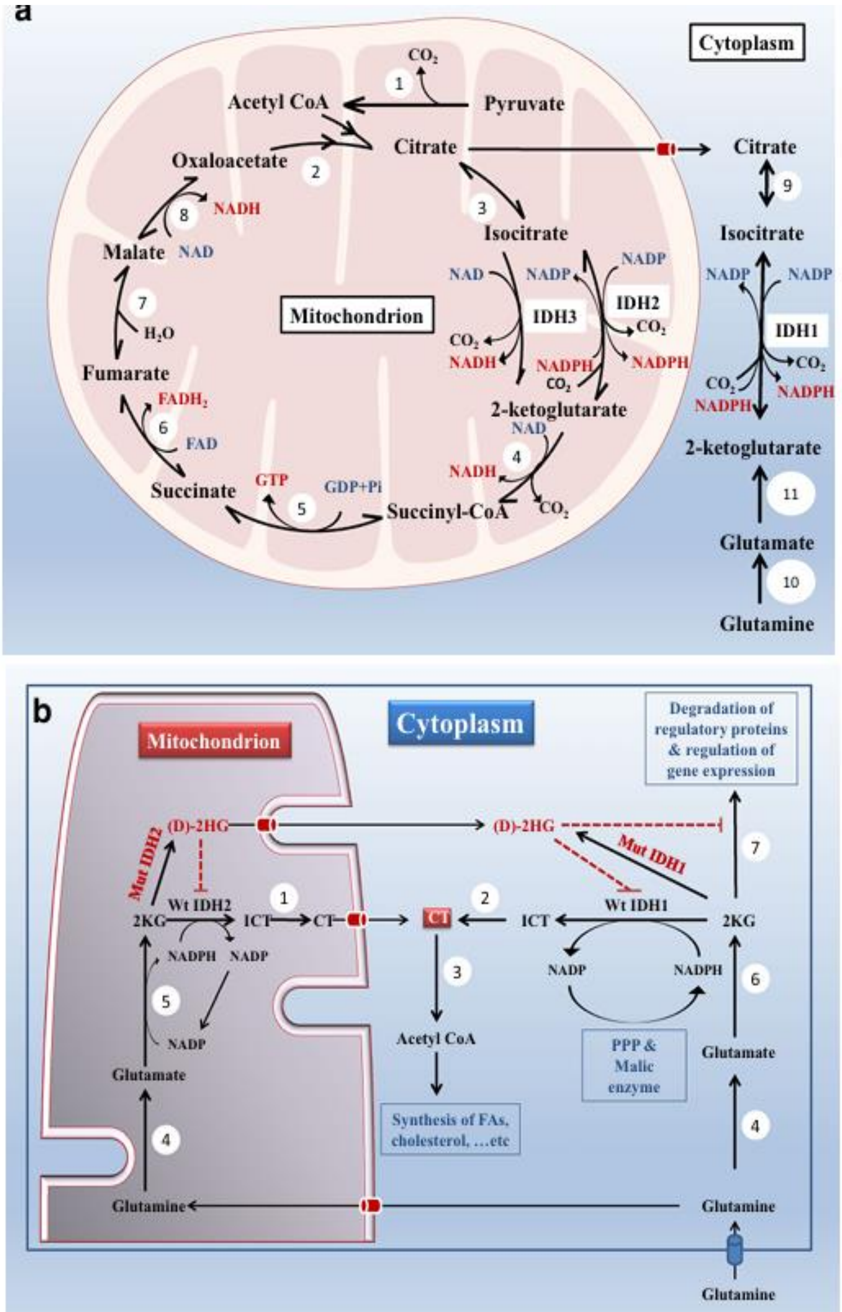

Figure 7 (Al-Khallaf et al., 2017)

CONCLUSION

Traditional cancer treatments—surgery, radiation, and chemotherapy—remain foundational, but advances in targeted drug therapies, immunotherapy, gene editing, and antioxidants have ushered in more precise, individualized care (Debela et al., 2021). These modern strategies aim to destroy malignant cells while minimizing harm to healthy tissues. Prevention efforts have also expanded, including SERMs for breast cancer, finasteride for prostate cancer, and vaccines for HPV and HBV (Umar et al., 2012). Additionally, dietary and lifestyle interventions play a major role in reducing cancer risk (Doll & Peto, 1981).

This review has underscored the significance of cellular signalling proteins and metabolic regulators in cancer proliferation. Therapeutic targeting of proto-oncogenes, growth factor receptors, cyclins, CDKs, and metabolic enzymes like hexokinase and pyruvate kinase can disrupt the cell cycle and reduce tumor growth. Key interventions include preventing pRb phosphorylation and enhancing tumor suppressors by inhibiting MDM2. Cyclin-dependent kinase inhibitors also present promising targets for halting proliferation (Doll & Peto, 1981).

On a metabolic level, inhibiting the PI3K/AKT pathway and enzymes involved in glycolysis and the TCA cycle can deprive cancer cells of the energy and substrates needed for rapid growth. Similarly, disrupting mitochondrial biogenesis, protein import machinery (TOM/TIM complexes), and ATP production can compromise tumor viability (Doll & Peto, 1981). Apoptosis induction remains a crucial strategy, particularly by activating proteins like Bax and modulating the BCL-2 family to promote cytochrome c release and caspase activation (Debela et al., 2021). Precision is critical—selective targeting ensures cancer cells are eradicated while preserving healthy tissues. In sum, a multifaceted approach integrating conventional and targeted therapies, with a focus on signalling and metabolic pathways, holds promise for more effective and potentially curative cancer treatments.

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