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Exploring the link between Pyruvate Dehydrogenase Enzyme Complex (PDC) dysregulation and metabolic disorders: A concise literature review: A Mini Review

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Abstract

This mini review aims to provide a comprehensive and analytical exploration of the Pyruvate Dehydrogenase Enzyme Complex (PDC), shedding light on its structural characteristics, functional mechanisms, and its relevance to metabolic disorders. A key aspect discussed in this review is the inhibitory role of Pyruvate Dehydrogenase Kinase (PDK) on PDC activity, and the implications of this regulation on cellular metabolism. To begin with, the review delves into the intricate structure and function of the PDC, elucidating its pivotal role in converting pyruvate to acetyl-CoA. Furthermore, emphasis is placed on understanding the significance of PDC in cellular metabolism and the connection between its dysregulation and the onset of metabolic disorders. Notably, the review highlights the crucial role of PDK enzymes in the regulation of PDC activity. By inhibiting PDC, PDK plays a crucial role in modulating the flow of pyruvate metabolism. The review further explores the relationship between PDK expression and metabolic disorders, providing insights into how the upregulation of PDK1 is associated with conditions such as diabetes. Additionally, the review touches upon recent studies that have demonstrated the potential therapeutic benefits of inhibiting PDK activity in the treatment of metabolic diseases. Moreover, the review draws attention to the significance of PDC-targeting drugs and their potential as therapeutic options. By targeting the activity of PDC and its inhibitors, these drugs offer promising avenues for the treatment of metabolic disorders. The review sheds light on the potential therapeutic implications of pharmacological compounds, including the pyruvate analog dichloroacetate (DCA), which has shown promise in enhancing the survival rate of mice with septic hepatocytes. By providing a comprehensive overview of the structure, function, and regulatory mechanisms of the PDC complex, as well as its connection to metabolic disorders, this mini review offers valuable insights into the intricate interplay between PDC, PDK, and cellular metabolism. It underscores the potential of targeting PDC and its inhibitors as therapeutic strategies for metabolic diseases. Ultimately, this review seeks to contribute to the growing understanding of the complexities of the PDC complex and its implications in the development of effective therapeutic interventions for metabolic disorders.

KEYWORDS: Pyruvate dehydrogenase enzyme complex; pyruvate dehydrogenase kinase; tricarboxylic acid cycle; metabolic disorders; small molecules; inhibitors.

Introduction

The glycolytic pathway is a key metabolic process that converts glucose into pyruvate, which can either be metabolized into lactate or enter the tricarboxylic acid cycle (TCA) to produce energy. One of the critical reactions that diverts the pyruvate flux towards TCA cycle is the decarboxylation reactions catalyzed by the pyruvate dehydrogenase enzyme complex (PDC). PDC is a multienzyme complex that plays a crucial role in cellular metabolism by catalyzing the oxidative decarboxylation of pyruvate to generate Acetyl CoA. Before pyruvate can enter the TCA cycle, it must be transported into the mitochondria via specific pyruvate transporters, which assist

the enzyme in crossing the mitochondrial membrane. Once inside the mitochondria, pyruvate is oxidatively decarboxylated to Acetyl CoA by the PDC complex, as illustrated in Figure 1 [1].

The PDC complex consists of three enzymes: pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3) as shown in Figure 2 [1]. The PDC complex requires several cofactors, including thiamine pyrophosphate (TPP), lipolic acid, coenzyme A, FAD, and NAD. The PDC complex catalyzes the decarboxylation of pyruvate bound to TPP by pyruvate dehydrogenase, forming hydroxyethyl groups. These groups are then transported by TPP to dihydrolipoyl transacetylase, which transfers the acetyl group to CoA. The last two enzymes, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase, catalyze lipolic acid reactions, which exist in two forms: oxidized (lipoate) and reduced form dihydrolipoic acid (DHLA). Lipolic acid contains oxidized disulfide groups, and the carbon atom between nitrogen and sulfur atoms in the thiazole ring of TPP is highly acidic. Therefore, the H attached to it is easily separated, acting on the C atom of the carbonyl group. Lipolic acid is involved in reduction and oxidation and ensures the transport of acetyl groups before they are transferred to CoA. In the next reactions, while lipolic acid is reoxidized, a hydrogen atom and electrons are transferred to FAD and NAD+, respectively. The processes in this last step are catalyzed by dihydrolipoyl dehydrogenase. At the end of this, the lipolic acid returns to the reducing state again. The PDC complex has been extensively studied due to its critical role in cellular metabolism, and its structural, functional, and regulatory aspects have been investigated in numerous studies. Cellular metabolism is a complex network of biochemical reactions that convert nutrients into energy and other essential molecules. The Pyruvate Dehydrogenase Enzyme Complex (PDC) is a critical player in cellular metabolism, catalyzing the oxidative decarboxylation of pyruvate to generate Acetyl CoA, which can enter the TCA cycle to produce energy. Dysregulation of PDC activity has been linked to several metabolic diseases, including diabetes, obesity, and cancer [2-6]. Therefore, understanding the metabolic aspects of PDC is crucial for developing targeted therapies for these disorders. This review summarizes up-to-date information on the structure, function, metabolic context, expression of both PDC and PDK (1-4), and links to metabolic diseases. Understanding the PDC complex is crucial for developing targeted therapies for metabolic disorders. Overall, this review highlights the importance of the PDC in cellular metabolism and its potential as a therapeutic target for metabolic diseases.

Interlinking of PDC with Metabolic Diseases:

Dysregulation of PDC activity has been linked to several metabolic diseases, including diabetes, obesity, and cancer. In diabetes, PDC activity is reduced, leading to decreased glucose utilization and increased glucose production by the liver. This can lead to hyperglycemia, a hallmark of diabetes. In obesity, PDC activity is decreased in adipose tissue, leading to increased triglyceride synthesis and storage. This can contribute to the development of insulin resistance and type 2 diabetes. In cancer, PDC activity is often decreased, leading to increased reliance on glycolysis for energy production, known as the Warburg effect. This metabolic shift allows cancer cells to survive and proliferate in conditions of low oxygen availability [5,6]. Targeting PDC activity could be a potential therapeutic strategy for cancer treatment.

Regulation of PDC Activity:

PDC activity is regulated by several mechanisms, including phosphorylation, allosteric regulation, and gene expression. Phosphorylation is a key mechanism for regulating PDC activity, where PDC kinase (PDK) phosphorylates and inactivates PDC [7]. Conversely, PDC phosphatase (PDP) dephosphorylates and activates PDC. The balance between PDK and PDP activity is critical for regulating PDC activity.

Pyruvate Dehydrogenase Enzyme Complex (PDC): Reaction Mechanism, Deficiencies, and Associated Diseases

The Pyruvate Dehydrogenase Enzyme Complex (PDC) plays a crucial role in the metabolism of carbohydrates. Pyruvate dehydrogenase (E1) is the first enzyme in the PDC complex, and its

activity is tightly regulated by phosphatases, which are activators, and kinases, which are inhibitors. The active reaction starts with the removal of a carbon atom from pyruvate, forming hydroxyethyl, which binds to TPP. Dihydrolipoamide S-acetyltransferase (E2) catalyzes the conversion of pyruvate to acetyl-CoA, and the TPP's hydroxyethyl group is transferred to an oxidized form of covalently bound lipoamide to form the reduced dihydrolipoamide-E2 complex. Acetyl-CoA is formed by the transfer of the acetyl group to free coenzyme A. The oxidation step occurs with the formation of lipoamide-E2, and this reaction produces NADH by flavoprotein dihydrolipoamide dehydrogenase (E3). As the connection between FAD and E3 begins, electron exchanges result in the production of NADH + H+. The components of this pathway are encoded by six genes, including PDHA1, PDHB, DLD, DLAT, PDHX, and PDP1. Mutations in these genes have been associated with PDC deficiencies, with about 75% of all mutations detected in the PDHA1 gene encoding the α subunit of the PDC-E1 (E1α). Additionally, mutations in other genes connected to this pathway may contribute to PDC deficiency. PDC deficiencies are subclassified as primary-specific or generalized, and secondary PDC deficiencies can occur. Pyruvate dehydrogenase phosphatase catalytic subunit gene (PDP1) is one of the primary-specific genes of PDC deficiency, and mutations in this gene cause a reduction in the activity of pyruvate dehydrogenase phosphatase (PDP). PDC deficiencies can cause metabolic syndrome and neurological disorders such as seizures and lethargy, which can be fatal. As the severity of PDC deficiency increases, the concentration of lactate in the blood increases, causing lactic acidosis, which can be fatal to newborns. Phenylbutyrate is a potential treatment for PDC deficiency, as it decreases the PDC-E1 subunit's phosphorylated inactive state in the brain by inhibiting pyruvate dehydrogenase kinase. Studies on human fibroblasts and zebrafish have demonstrated that phenylbutyrate is an effective treatment for PDC deficiency [7]. Reductions in PDC catalytic activity have been linked to mutations in the PDC-E1 subunit (V138M), which changes the direction of the thiazolium ring of the thiamine diphosphate (ThDP) coenzyme.

Decoding the Pyruvate Dehydrogenase Enzyme Complex (PDC): Composition, Structure, and the Impact of Phosphorylation on Protein Dynamics and Substrate-Enzyme Affinity

The PDC is composed of three enzymes, including pyruvate dehydrogenase (E1), dihydrolipoamide S-acetyltransferase (E2), and flavoprotein dihydrolipoamide dehydrogenase (E3). The central core of the PDC enzyme is E2 in bacteria and E3 binding protein (E3BP) in eukaryotes, which binds E3 to E2 in the core. The E2 subunit is bound to both E1 and E3 in bacteria, contributing to the structure and catalytic activity of both. Although the structure of the human E2/E3BP assembly is not yet known, the high-resolution structure of the E2 domains has been solved in Azotobacter vinelandii and Bacillus stearothermophilus crystal structures. Human E2 and E3BP have remarkably similar structures with three different types of domains, including subunit binding domains (SBD), flexible N-terminal lipoyl domains (LDs; two on E2 and one on E3BP), and a C-terminal domain (CTD) with Ala-Pro-rich linkers. Phosphorylation of PDC loops (loop A from 259-α to 282-α and loop B from 195-α to 205-α) is hypothesized to disrupt protein dynamics. Ser-264-α-P has a significant role in disturbing the substrate binding site, according to Seifert et al. Their work highlights that both Ser-264-α-P and Ser-271-α-P affect protein structure and dynamics. Although Ser-271-α-P does not affect pyruvate-PDC-E1 binding site, Ser-264-α-P affects substrate-enzyme affinity, causing a break in the H-bond as the interaction with His263-α exists [8]. However, further confirmation of the atomic structure of E1 in complex, as well as sophisticated computational data, is required to understand the PDC enzyme's atomic structure better.

Regulatory Roles of Pyruvate Dehydrogenase Complex and Pyruvate Dehydrogenase Kinase Enzymes in Metabolic Disorders: Insights into their Impact on Various Organs and the Potential Therapeutic Benefits of PDC Agonists

The Pyruvate Dehydrogenase Complex (PDC) and Pyruvate Dehydrogenase Kinase (PDK) enzymes are known to play regulatory roles in various metabolic disorders affecting different

organs such as the liver, brain, heart, kidney, and pancreas. In mice with liver damage caused by hepatotoxins, the activation of metabolic enzymes such as PDC and lactate dehydrogenase (LDH) leads to an increase in acetyl-CoA and lactate levels, respectively, resulting in an increase in histone H3 hyper-acetylation and gene damage response. Mice with acute liver failure have longer lifespans in the presence of LDH inhibitors. However, the expression of pyruvate dehydrogenase kinase 1 (PDK1) is suppressed, while the survival rate of mice with septic hepatocytes increases when the PDC enzyme is stimulated with the pyruvate analog dichloroacetate (DCA). Glucose serves as the brain's primary fuel source, and glucose oxidation impairment has been noted as a 50% decrease in PDC enzyme, which leads to a fall in glutamate (an excitatory neurotransmitter) levels. The heart attempts to produce energy during stress by utilizing glucose, lactate, and ketones. Additionally, the generation of lactate and the enzyme pyruvate dehydrogenase kinase isoform 2 (PDK2), as well as phosphorylated PDC form, are all enhanced during heart stress. During cardiac arrest, the activity of Pyruvate Dehydrogenase Kinase Isoform 4 (PDK4) is increased, but PDC enzyme activation and PDK4 deactivation occur during therapeutic hypothermia. Lean mice with cardiac PDHA1 gene deletion or ablation develop a phenotype similar to cardiomyopathy, suggesting that PDC agonists may lessen the effects of cardiomyopathy while boosting glucose oxidation. In late-stage heart failure, studies have discovered a decrease in PDK4 expression and an increase in PDC protein expression (E1 subunit) to encourage glucose oxidation to compensate for the diminished energy source. DCA increases the level of acetylcarnitine, which serves as an acetyl group donor during chronic hypoxia, demonstrating the usefulness of PDC in improving heart function. Rats with nephritis treated with Huangkuisiwufang (HKSWF), a Chinese herbal remedy, show improved kidney function and PDC enzyme levels as pyruvate content interacts with ischemic renal injury and nephrotoxic stress [6-9].

Mitochondrial Dysfunction in Diabetes: Role of PDC and FAO in T2D

The disruption of normal mitochondrial metabolic pathways and a decrease in ATP synthesis due to mutations in pancreatic cells caused by diabetes or high blood sugar levels have been reported. Diabetes alters the expression of several genes and proteins, with some upregulated (such as PDK1) and some downregulated (such as pyruvate carboxylase), leading to reduced pyruvate flux into the TCA cycle. T2DM mice with hyperglycemia and insulin resistance (IR) were found to have decreased expression of the (PDC-E1(E1α)) PDHA1 protein in their pancreatic tissues using an immunofluorescence approach. The decrease in PDHA1 expression caused by cellular dysfunction in β-cells, a hallmark of islet enlargement, results in decreased insulin production and impaired glucose tolerance. PDC plays a crucial role in maintaining glucose homeostasis in both the fed and fast states, and the absence of carbohydrates increases fatty acid oxidation (FAO) and inactivates PDC, which is required for the glucose oxidation process. Increased FAO could be a marker of insulin resistance (IR), which could lead to type 2 diabetes (T2D). High levels of acetyl-CoA synthesis due to fatty acid oxidation may lead to PDC inactivation, as explained by the Randle cycle (glucose-fatty acid cycle) [6,8,9]. Fatty acids increase the amount of diacylglycerol (DAG) in the liver, which stimulates protein kinaseCε (PKCε), inhibits the insulin receptor, decreases glycogen production, and stimulates gluconeogenesis. IR decreases glucose absorption, impairs the removal of insulin-stimulated glucose, and affects other aspects of metabolism, including insulin signaling, glucose transport, and metabolism. In obese patients, ranolazine, an antianginal drug, increases pyruvate dehydrogenase complex (PDC) activity and decreases the burden of non-alcoholic fatty liver disease (NAFLD) and T2D.

Targeting Pyruvate Dehydrogenase Kinase 1 (PDK1) as a Therapeutic Strategy for Cancer: Mechanisms and Inhibitors

The Warburg effect is a phenomenon in which cancer cells shift from mitochondrial oxidative phosphorylation (OXPHOS) to cytoplasmic aerobic glycolysis. While Warburg originally proposed that this was due to suppression of mitochondrial phosphorylation, other researchers

have suggested that aerobic glycolysis is actually at its peak in cancer cells. This shift occurs when pyruvate, the by-product of glycolysis, is used in the citric acid cycle (TCA cycle) to complete glucose oxidation in the presence of oxygen. However, pyruvate production in cancer cells results in lactate regardless of oxygen presence. Lactate dehydrogenase enzyme (LDH) converts pyruvate to lactate, which plays an essential role in cell proliferation, maximizes acidity, increases the number of blood vessels, and suppresses immunity. Targeting LDH can prevent cell proliferation.

Cancer cells modulate normal metabolic pathways through an increase in the expression of pyruvate dehydrogenase kinase enzymes (PDKs), which contribute to the proliferation and metastasis of cancerous cells. PDK1 phosphorylates serine residues (293, 300, and 232) of the pyruvate dehydrogenase E1-α subunit to inhibit pyruvate dehydrogenase complex (PDC) activity. Different types of cancer cells express PDK1, including non-small cell lung cancer (NSCLC), which promotes aerobic glycolysis that supports increased lactate build-up. PDK1 is also significantly expressed in breast cancer stem cells (BCSC), and cancer cell proliferation may be affected in case of PDK1 inhibition. H19, a hypoxia-related long non-coding RNA (lncRNA), is expressed at higher levels in BCSC and is a crucial step in BCSC glycolysis. Its inhibition has been reported to reduce the expression of PDK1, lactate generation, glucose absorption and ATP level. Aspirin inhibits H19 and PDK1 in vitro and in vivo, preventing cancer cell glycolysis and stemness. Several PDK1 inhibitors have been identified to induce apoptosis and slow the development of cancer cells both in vitro and in vivo. DAP (2,2-dichloroacetophenone) is a PDK1 inhibitor, which has been shown to induce apoptosis and slow the development of acute myeloid leukemia (AML) cells. Dichloroacetate (DCA), another PDK inhibitor, prevents HepG2 and HepG3B liver cancer cells from proliferating and causes cell cycle arrest at the G2/M stage as well as apoptosis. Inhibiting PDK1 restores the regular oxidative phosphorylation pathway, reduces p-PDC expression, lactate production levels, and tumor growth, all of which have been demonstrated in vivo. Additionally, DCA restores the stability of reactive oxygen species (ROS), but N-acetyl-L-cysteine (NAC), a non-specific antioxidant, reduces ROS and improves HepG2 cell viability. JX06, a novel PDK1 inhibitor, prevents glycolysis and induces death in cells with multiple myeloma. Further investigation revealed that the JX06 and metformin (Met) combination suppresses the growth of the Endometrial cancer (EC) cell line Ishikawa culture under long-term high glucose level (IshikawaHG). JX06 nanoparticle form (JX06-NPs) demonstrated a more potent growth-inhibitory effect on ShikawaHG. Shikonin (SK) derivatives also target PDK1, preventing the MDA-MB-231 breast cancer cell line from proliferating by inhibiting tumor glycolysis, inducing apoptosis, and binding to PDK1. The organic arsenic molecule (Aa-Z2) increases reactive oxygen species (ROS), which in turn promotes osteosarcoma (OS) cell death, G2/M cycle arrest, and autophagy. It also changes the metabolism of glucose from aerobic glycolysis to glucose oxidation by downregulating the production of both PDK1 and phosphorylated E1α subunit of PDC (p-PDC-E1α). The expression of both phosphorylated form of PDK1 and PDC-E1(E1α) subunit(PDC component) have been increased regarding to elevation of BRCA1-associated protein 1 (BAP1) (a tumor suppressor gene) mutant Uveal Melanoma (UM), eliminates BAP1, which are significantly associated with patients' poor prognoses. DCA has been found to prevent the formation of tumors, and in combination with other drugs such as diclofenac or telaglenastat (CB-839), both lowering glycolysis and inhibiting the TCA cycle in melanoma cells. Further investigation has found that Engulfment and cell motility 1 (ELMO1), a crucial component of tumor cells, plays a role in regulating the levels of phosphorylation of PDK1 in colorectal cancer cells. Deletion of ELMO1 leads to lower levels of PDK1 phosphorylation in SW480 and DLD1 colorectal cancer cells [10-12].

Forkhead box M1 (FOXM1) is a transcription factor that increases the expression of PDK1 in nasopharyngeal carcinoma (NPC) cells. PDK1 phosphorylation results in the inhibition of glycolysis and the development of NPC. Increasing FOXM1 expression is linked to poor prognosis in head and neck cancer, suggesting a possible therapeutic target.

Capsaicin, an anti-tumor compound, inhibits the growth of lung cancer cells by reducing the accumulation of the protein Hypoxia-inducible factor (HIF)-1, which raises intracellular oxygen levels, and the expression of its target genes, including PDK1 and glucose transporter 1 (GLUT1).
Celastrol is a compound that targets both PDK1 and HIF-1, causing inhibition of glycolysis-related enzymes lactate dehydrogenase A (LDHA), glucose transporter 1 (GLUT1), and hexokinase 2 (HK2). This indicates its vital role as neuroprotection after cerebral ischemia-reperfusion (I/R) injury.
Methyl-CpG binding protein 2 (MeCP2) stimulates PDK1 expression, which reduces the effects of cisplatin on gastric cancer (GC). PDK1 inhibition, on the other hand, reverses MeCP2's stimulatory actions, indicating a possible therapeutic target.
PDK1 levels in human hepatocellular carcinoma (HCC) cells have been shown to be higher than normal cells, leading to lower overall survival rates in patients. Decreased activity of PDK1 induces oxidative phosphorylation, an increase in mitochondrial reactive oxygen species (mtROS), and a decrease in mitochondrial membrane potential (MMP) in HCC cells. Dicoumarol (DIC) treatment prevents PDK1 from phosphorylating the Ser232 position on PDC in SNU-449 and SNU-387 cells, and the addition of Oxaliplatin (OXA) increases the synergistic efficiency of treatment on tumor cancer cells both in vitro and in vivo.
In chemoresistant KRAS mutant colorectal cancer cell lines, such as SW480 and DLD1 cancer cells with the KRAS mutation, 5mM of the water-soluble vitamin C inhibits PDK1 (G12V and G13D, respectively). A recent study found that vitamin C can block the HIF1α subunit of Hypoxia-inducible factor 1 (HIF-1), which supports the transcription of genes involved in cancer, glucose metabolism, and other processes, by increasing proteasome degradation. Overall, these findings suggest that targeting PDK1 could be a promising therapeutic strategy for multiple types of cancer [8,14].

The Role of Pyruvate Dehydrogenase Kinase 2 (PDK2) in Cancer: Mechanisms and Inhibitors

Pyruvate dehydrogenase kinase 2 (PDK2) is expressed in various organs, particularly in the liver and kidney, and is found to be significantly lower in expression in hepatocellular carcinoma (HCC) cells compared to healthy cells. PDK2 also serves as a prognostic tool as it correlates with various clinical factors in patients, including radiation therapy and vital status. In the case of Fusarium head blight (FHB), a disease caused by the fungus Fusarium graminearum, the absence of the FgPDK2 gene resulted in changes in the morphological features of the species. In chemoresistant ovarian cancer, increased PDK2 protein levels are linked to lower progression-free survival (PFS). In vitro and in vivo studies on PDK2 knockdown chemoresistant ovarian cancer cells have shown a decrease in cell growth, malignancy, glycolysis, and lactate generation, while enhancing cisplatin sensitivity. Knocking down either PDK1 or PDK2 in glioblastoma (GB) is found to reduce tumor burden and improve survival rates. While PDK1 is associated with hypoxic regions, PDK2 is linked to invasive tumors in GB. DCA has been shown to prevent GB invasion and growth while increasing intracellular ROS when tested in vitro. Although PDK2 is strongly associated with head and neck cancer (HNC) stages and grades, as well as age under 55, inhibition of both PDK1 and PDK2 has been found to enhance the susceptibility of HNC cancer stem cells (CSCs) to cisplatin and gemcitabine. In addition, PDK2 knockdown has been found to stimulate ATP production while inhibiting lactate synthesis, HNC cell tumor sphere formation, stemness, and multidrug resistance gene expression. In response to transforming growth factor beta 1 (TGF-1), which enhances the Warburg effect, inhibition of both PDK1 and PDK2 has been found to inhibit HNC cell migration [11-44].

PDK3 in Cancer: Prognostic Marker and Target for Inhibition with DCA, Artemisinin, and Hordenine

Pyruvate dehydrogenase kinase isoform 3 (PDK3) has a high expression level, 27-fold higher in cholangiocarcinoma (CCA) tissue, and is considered a prognostic marker. As the level of PDK3

increases, it is substantially correlated with shorter patient survival times. Furthermore, overexpression of PDK3 enhances glycolysis, reduces PDC expression, and increases heat shock factor 1 (HSF1) expression in chemoresistant cancer cells, while PDK3 inhibition restores mitochondrial function. When the chemotherapy medication cisplatin (DDP) is combined with DCA, cancer cells are prevented from growing and are forced to undergo apoptosis. Molecular docking studies have confirmed that artemisinin (AMS), an anti-malarial medicine, has a high affinity for PDK3 and can reduce its activity. Knockdown of circRNF13, which is involved in hypoxia-induced tumor development and metastasis in pancreatic cancer (PC), decreases PDK3 protein expression, and this effect can be countered by the miR-654-3p inhibitor. PDK2 and PDK3 are found to be overexpressed in breast cancer cells (MDA-MB-231 and MDA-MB-468) compared to the non-cancerous cell line MCF10A (human mammary epithelial cell line), and both enzymes have a significant effect on the proliferation of cancer cells. Reduction of both enzymes results in a decrease in tumor size in xenograft mouse models. Hordenine, a phenylethylamine alkaloid, is known to control PDK3 by tightly binding to it, resulting in a decrease in PDK3 activity. In summary, PDK3 overexpression in CCA tissue is a prognostic marker that correlates with shorter patient survival times. Inhibition of PDK3 or a combination of DDP and DCA results in the prevention of cancer cell growth and apoptosis. Artemisinin has been found to have a high affinity for PDK3, and circRNF13 knockdown decreases PDK3 protein expression. Moreover, PDK2 and PDK3 overexpression in breast cancer cells promotes the proliferation of cancer cells. Hordenine can control PDK3 by tightly binding to it, resulting in a decrease in PDK3 activity [33-53].

PDK4: A Multifaceted Enzyme with Diverse Roles in Health and Disease, from Metabolic Regulation to Cancer Biomarker

Discovering the diverse roles of Pyruvate Dehydrogenase Kinase isoform 4 in metabolic regulation, disease pathophysiology, and cancer treatment strategies

Pyruvate Dehydrogenase Kinase isoform 4 (PDK4) is located in various organs such as the liver, pancreatic islets, skeletal muscle, heart, and kidney. Its reduction in skeletal muscle leads to a decrease in glucogenic substances such as lactate, pyruvate, and alanine amino acid, in the liver. PDK4 expression triggers the production of glucose and hepatic gluconeogenesis by increasing cAMP [54-65]. Downregulation or inhibition of PDK4 has been reported to reduce the activity of glucagon-mediated gluconeogenic genes. Moreover, decreased levels of PDK4 prevent the process of fatty acid oxidation (FAO), and it may also play a role in the pathophysiology of coronary artery disease (CAD) by stimulating CD14+ monocyte activation in CAD patients. In breast cancer, PDK4 is highly expressed and has been studied as a potential treatment biomarker. Its downregulation has been verified by a tumor suppressor technique (miR-221), and its high expression is strongly correlated with the occurrence and progression of gastric cancer (GC) as well as tumor grade and stage, inducing S-phase and poor prognosis. Immune cells such as CD4+T cells, dendritic and B cells, and macrophages are strongly associated with PDK4. PDK4 suppression causes G0/G1 cell-cycle arrest, reduces the capacity of GC cells to invade, migrate, and results in a rise in the overall survival (OS) rate as the transcriptional level falls. PDK4 overexpression has also been linked to the induction of S-phase transition, growth, proliferation, and progression of GC malignancies. So, PDK4 plays a crucial role in various organs, including the liver, skeletal muscle, and heart. Its downregulation or inhibition reduces the activity of glucagon-mediated gluconeogenic genes and prevents the process of fatty acid oxidation. PDK4 also has a potential role in the pathophysiology of coronary artery disease and has been studied as a potential treatment biomarker in breast and gastric cancer. Its overexpression is strongly correlated with the occurrence and progression of gastric cancer and is associated with poor prognosis and S-phase induction. Moreover, PDK4 expression has been shown to increase in glioma cells that are resistant to tozasertib, a compound with anticancer properties [51-74]. However, cryptotanshinone (CPT) has been found to effectively inhibit the expression of PDK4

both in vitro and in vivo. Upon receiving CPT, the epithelial-mesenchymal transition, which is a crucial factor in the invasion and metastasis of bladder cancer, is affected by a reduction in N-cadherin expression as a result of a decrease in PDK4. In a recent study, PDK4 mRNA levels were found to be two times higher in T3 bladder cancer tissues than in normal tissues. Reduction in PDK4 decreases the amount of cancer cells invading and proliferating and halts bladder cancer tumor growth. Inhibition of PDK4 dramatically elevates p-ERK expression while reducing p-SRC and p-JNK levels. In prostate cancer (PC), PDK4 is a promising prognostic marker, and the clinically relevant androgen receptor miR-32 is adversely correlated with low expression of PDK4 in PC patients. Low expression of PDK4 induces PC cells to be metabolically active and results in shorter or poorer recurrence-free survival of PC patients, particularly those with high levels of the tumor's Gleason score (>7). However, increasing PDK4 expression decreases glycolysis rate, mitochondrial respiration, and cell proliferation in PC cells. In summary, PDK4 expression has been found to increase in glioma cells that are resistant to tozasertib. Cryptotanshinone (CPT) effectively inhibits PDK4 expression and affects the epithelial-mesenchymal transition in bladder cancer [19,56-77]. In T3 bladder cancer tissues, PDK4 mRNA levels are two times higher than in normal tissues, and its reduction decreases cancer cell invasion, proliferation, and tumor growth. PDK4 is a promising prognostic marker for PC, and its low expression induces PC cells to be metabolically active and results in poorer recurrence-free survival in PC patients, particularly those with high Gleason scores. Increasing PDK4 expression decreases glycolysis rate, mitochondrial respiration, and cell proliferation in PC cells . The metabolism of pyruvate can lead to the production of fatty acids, lipid peroxidation, and ferroptosis, which can cause membrane rupture. The activation of pyruvate oxidation via the PDC pathway requires the inhibition of PDK4 by using erastin or sulfasalazine in the presence of glucose, but these agents are ineffective in the absence of glucose. Inhibitors of PDK4, such as anthraquinone derivatives, can prevent the phosphorylation of specific sites on the PDC-E1α subunit, leading to improved insulin resistance and anti-diabetic effects. PDK4 inhibitors have also been shown to phosphorylate the protein p53, which is necessary for apoptotic cell death, making them potential anti-cancer agents. Celastrol has been found to dramatically reduce obesity and insulin resistance by increasing PDC activity and reducing PDK4 expression. Geniposide, an iridoid glycoside compound, has been reported to inhibit the activity of PDK4 and upregulate PDC in skeletal muscle, making it a potential therapeutic agent for maintaining glucose homeostasis in skeletal muscle [77-81]. The deficiency of farnesoid X receptor (FXR) has been linked to impaired lipid and glucose metabolism in the liver, and the elevation of PDK4 in FXR-null mice increases gluconeogenesis, while its reduction alleviates lipid accumulation in hepatocytes, increases glucose oxidation, and inhibits fatty acid oxidation. Retinoic acid has been shown to elevate PDK4 mRNA levels in the hearts of female mice, and the retinoic acid receptor pan-antagonist BMS493 can inhibit this effect by dephosphorylating the PDC-E1α subunit at specific regulatory sites, leading to increased PDC activity. Finally, a complex of 1,2,4-amino-triazine derivatives has been found to suppress the activity of PDK1 and PDK4 enzymes while retaining a strong antiproliferative action against pancreatic cancer cells [82-106].

To sum up, extensive scientific research has established a clear link between the dysfunction of pyruvate dehydrogenase complex (PDC), a vital multienzyme complex responsible for glucose metabolism, and various metabolic disorders. This comprehensive study aims to delve deeper into the intricate connections between PDC, its inhibitors, and metabolic illnesses. Additionally, it seeks to explore the role of specific pharmacological compounds that are commercially available, either as inducers or inhibitors, in regulating the activity of both PDC and its inhibitors. By gaining a better understanding of these connections and employing controlled modulation of PDC and its inhibitors, it becomes possible to maintain normal metabolic functions and prevent the

development of metabolic diseases. The insights presented in this review highlight the potential of manipulating PDC activity and its inhibitors as a means to preserve overall metabolic health [89].

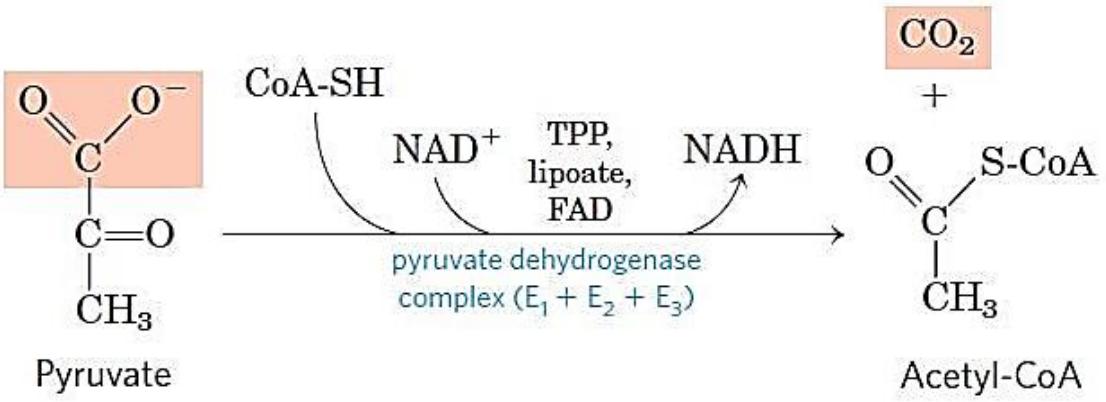
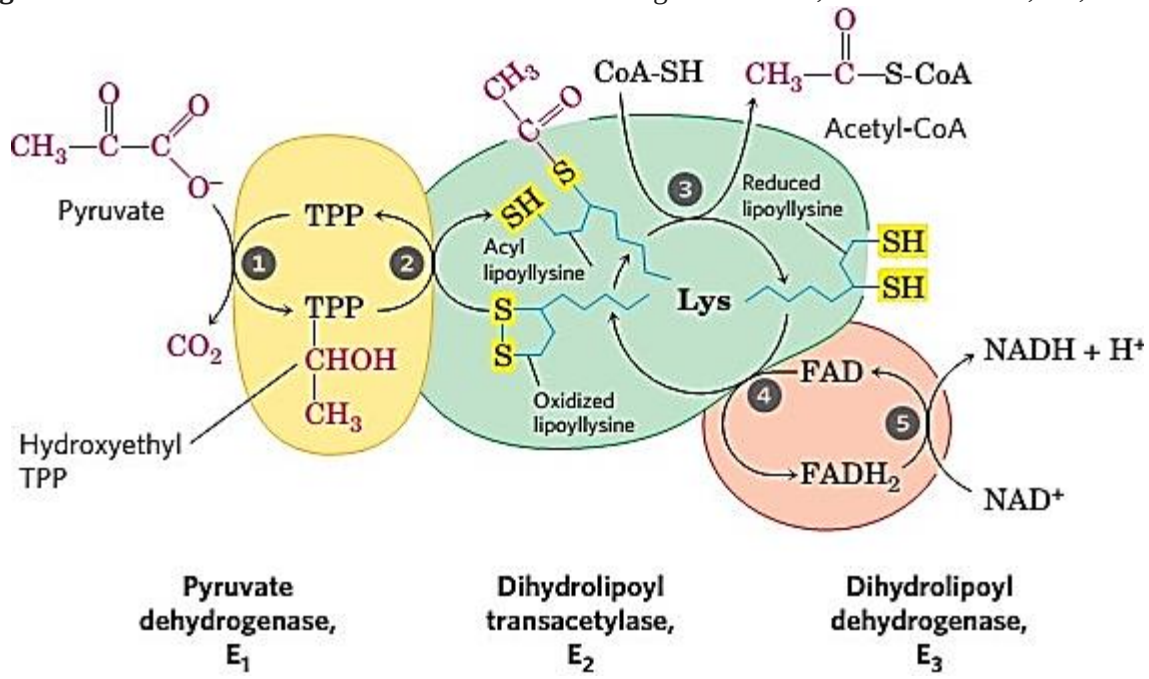


Figure 1. Illustration of the biological process of pyruvate dehydrogenase (PDC) along with five coenzymes (thiamine pyrophosphate (TPP), lipolic acid, coenzyme A, FAD, and NAD) and the enzymes pyruvate dehydrogenase (E1) (EC 1.2.4.1), dihydrolipoyl transacetylase (E2) (EC 2.3.1.12), and dihydrolipoyl dehydrogenase (E3) (EC 1.8.1.4). Entering the tricarboxylic acid cycle (TCA) is made simpler by the process, which changes pyruvate to acetyl-COA[1].

Figure 2. Three PDC subunits are needed for the biological reaction; the first subunit, E1, has



thiamine diphosphate cofactor (TPP) covalently attached to pyruvate during the decarboxylation step, which is followed by CO2 generation. The oxidized lipoamide cofactor in E2 is where the

connected complex is attached. After being linked to CoA, it then creates acetyl-CoA. Dihydrolipoamide is oxidized in step E3 to yield NADH + H+[1].

Illuminating the Intricacies: Advancing Knowledge on the Interactions between Pyruvate Dehydrogenase Complex, its Inhibitors, and Metabolic Disorders through Systematic Database Analysis

To investigate the intricate relationships between the pyruvate dehydrogenase complex (PDC), its inhibitors, and metabolic disorders, a systematic review approach was adopted. The review process involved a meticulous examination of electronic databases, namely PubMed, Google Scholar, and ScienceDirect, to identify and retrieve pertinent articles related to the research topic. These databases were selected for their extensive coverage of scientific literature across diverse disciplines. PubMed, widely recognized for its specialization in biomedical and life sciences, played a pivotal role in capturing relevant articles concerning the PDC complex. By searching this database, the research team accessed a rich array of scholarly publications, including research studies, reviews, and clinical trials, offering valuable insights into the interplay between PDC, its inhibitors, and metabolic disorders. Google Scholar, a comprehensive academic search engine, proved instrumental in broadening the scope of the literature search. Its vast repository of scholarly resources encompassed articles from various scientific domains, enabling the exploration of multidisciplinary perspectives on the subject matter. This ensured a holistic understanding of the connections between PDC, its inhibitors, and metabolic disorders. Moreover, ScienceDirect, a renowned platform for scientific literature, served as an additional electronic database to further enhance the comprehensiveness of the review. By accessing this resource, the research team obtained access to a multitude of high-quality articles dedicated to elucidating the intricate mechanisms underlying PDC dysfunction and its implications for metabolic disorders. By systematically searching and incorporating findings from these authoritative electronic databases, the review article encompassed a diverse range of studies, ensuring a robust analysis of the connections between PDC, its inhibitors, and metabolic disorders. The utilization of these databases guaranteed a rigorous and comprehensive exploration of the existing literature, providing valuable insights into the complex interrelationships within this research domain.

Selective Screening for Rigorous Inclusion: Unveiling the Optimal Studies on the Connections between Pyruvate Dehydrogenase Complex, its Inhibitors, and Metabolic Disorders

A meticulous screening process was implemented to ensure that only the most relevant and high-quality studies were included in this research. The screening criteria encompassed three key aspects: relevance to the research topic, study design quality, and publication date. By employing these stringent criteria, the aim was to uphold the scientific rigor and timeliness of the included literature. To eliminate bias and enhance objectivity, the screening process was conducted independently by two expert reviewers. Their expertise in the field ensured a comprehensive evaluation from multiple perspectives, leading to more robust and reliable study selection. In cases where discrepancies arose between the reviewers, consensus was reached through thorough discussion and deliberation. During the screening process, a comprehensive examination was performed on the titles, abstracts, and full texts of the identified articles. The reviewers meticulously assessed whether each study met the predefined criteria for relevance to the research topic. Additionally, the study design quality was evaluated to ensure that only scientifically rigorous and valid investigations were considered. Furthermore, a specific publication date range was applied to include the most recent findings and exclude outdated research. By implementing this systematic and rigorous screening process, this research article guarantees the inclusion of studies that not only directly address the connections between the pyruvate dehydrogenase complex (PDC), its inhibitors, and metabolic disorders but also adhere to rigorous scientific standards. The independent review process, coupled with consensus-building, fosters a

comprehensive and unbiased selection of studies, enhancing the validity and reliability of the research findings.

Systematic Categorization: Enhancing Precision and Quality in Article Selection for the Review

To ensure the precision and quality of the articles included in the review, a systematic categorization process was employed. The categorization was primarily based on two key criteria: relevance to the research topic and the study design's quality. By categorizing the articles according to these factors, the review aimed to include only highly pertinent and scientifically rigorous studies, thereby enhancing the overall validity and reliability of the findings. During the categorization process, each included article was thoroughly assessed for its alignment with the research topic. This involved scrutinizing the article's content, methodology, and objectives to determine its direct relevance to the connections between the pyruvate dehydrogenase complex (PDC), its inhibitors, and metabolic disorders. Articles that demonstrated clear and substantial relevance to the research topic were classified as "relevant". Furthermore, the categorization process took into account the quality of the study design. Rigorous assessment was conducted to evaluate the methodology, data collection procedures, and statistical analysis employed in each study. Studies that adhered to robust scientific standards and exhibited a high level of methodological rigor were classified as "high-quality". By systematically categorizing the included articles based on relevance and study design quality, the review ensured that only the most pertinent and scientifically robust studies were incorporated. This categorization process enhances the precision and accuracy of the literature selected for analysis, ensuring that the review encompasses a comprehensive and reliable assessment of the connections between PDC, its inhibitors, and metabolic disorders.

Comprehensive Data Extraction: Unveiling Key Insights into the Pyruvate Dehydrogenase Complex

A meticulous and comprehensive data extraction process was employed to unravel crucial insights into the pyruvate dehydrogenase complex (PDC). By synthesizing information from the included articles, the review aimed to provide a comprehensive understanding of various facets related to the PDC complex.

The data extraction process encompassed several essential aspects. Firstly, the structural organization of the PDC complex was thoroughly examined, with a focus on extracting information about its composition, arrangement, and spatial interactions between the enzymes involved. This allowed for a deeper comprehension of the complex's structural intricacies and how they contribute to its overall functionality. Secondly, the mechanisms underlying the enzymatic activity of the PDC complex were carefully analyzed. By extracting pertinent details, such as the catalytic steps involved in the conversion of pyruvate to acetyl-CoA, the review aimed to shed light on the molecular processes and kinetics of PDC activity. Furthermore, the data extraction process sought to uncover the diverse regulatory mechanisms that modulate PDC activity. Information regarding the various factors, signaling pathways, and metabolites that influence the complex's functionality was gathered. This enabled a comprehensive understanding of the regulatory networks that fine-tune PDC activity in response to cellular and metabolic demands. The role of post-translational modifications in regulating PDC activity was another crucial aspect explored during the data extraction process. The review aimed to extract relevant details about modifications such as phosphorylation, acetylation, and others, elucidating their impact on PDC functionality and metabolic regulation. Lastly, the implications of PDC dysfunction in the context of metabolic disorders were thoroughly examined through the data extraction process. By extracting information from the articles, the review aimed to identify and synthesize the evidence linking PDC dysfunction to various metabolic disorders, thereby highlighting the clinical relevance of understanding the complex.

Through this comprehensive data extraction process, the review synthesized key insights into the pyruvate dehydrogenase complex, encompassing its structural organization, enzymatic mechanisms, regulatory processes, post-translational modifications, and disease implications. By gathering and analyzing these essential details, the review offers a comprehensive understanding of the PDC complex and its significance in metabolic processes and associated disorders.

Robust Assessment of Study Quality: Ensuring Validity through the Cochrane Risk of Bias Tool

To uphold the validity and reliability of the review findings, a rigorous assessment of the quality of the included articles was carried out using the widely recognized Cochrane risk of bias tool. This tool provided a comprehensive framework for evaluating potential biases within the selected studies, thereby ensuring the credibility of the information presented. During the quality assessment process, each included article underwent meticulous scrutiny to identify any potential sources of bias that could impact the internal validity of the findings. The Cochrane risk of bias tool facilitated a systematic evaluation of key domains, such as randomization, allocation concealment, blinding, data completeness, selective reporting, and other factors known to introduce bias. By employing this established tool, the reviewers were able to objectively assess the risk of bias in each study and assign a quality score accordingly. This rigorous evaluation helped identify any methodological limitations, inadequate reporting, or other factors that could potentially compromise the reliability of the results. The application of the Cochrane risk of bias tool in the quality assessment process was essential for ensuring the robustness of the review. By systematically evaluating the included articles, the review authors could accurately gauge the strengths and weaknesses of the individual studies, thereby enhancing the overall validity and trustworthiness of the findings. Through this thorough quality assessment, the review article aimed to provide readers with confidence in the credibility of the synthesized evidence. By identifying and minimizing potential sources of bias, the review ensured that the information presented was based on high-quality studies and could be relied upon for making informed conclusions and recommendations. In conclusion, the utilization of the Cochrane risk of bias tool in the quality assessment process served as a robust method for evaluating the validity of the included articles. This systematic evaluation enhanced the overall rigor and credibility of the review, ensuring that the information presented was based on reliable evidence and could be trusted by readers and researchers alike.

Integrated Data Synthesis: Unveiling a Holistic Perspective of the Pyruvate Dehydrogenase Complex

The extracted information from the included articles underwent a comprehensive data synthesis process to provide a holistic and in-depth understanding of the pyruvate dehydrogenase complex (PDC). This synthesis involved the integration of the extracted data points, enabling the review to present a comprehensive overview of the structural, functional, and regulatory aspects of the PDC complex, along with its implications in metabolic disorders. Initially, the extracted information was organized and summarized in a tabular format. This tabular representation facilitated the systematic organization of key findings from each article, allowing for a clear overview of the research landscape surrounding the PDC complex. Subsequently, the synthesized data from the tabular format was analyzed and integrated to form a cohesive narrative review. By synthesizing the information, the review aimed to provide a comprehensive understanding of the interrelationships between various aspects of the PDC complex, such as its structural components, enzymatic activity, and regulatory mechanisms. This integrative approach allowed for a more nuanced and holistic perspective on the complex and its role in cellular metabolism and disease. Moreover, the narrative review placed particular emphasis on articles that provided valuable insights into the structural organization, functional mechanisms, and regulatory controls of the PDC complex. By highlighting these aspects, the review aimed to uncover the intricate workings

of the complex and elucidate its relevance in the context of metabolic disorders. The systematic literature search and rigorous screening process ensured the inclusion of high-quality studies in the review. The selection of studies was guided by predetermined criteria, ensuring that only relevant and reliable articles were considered. This approach helped maintain the integrity and credibility of the synthesized data. Additionally, the use of the Cochrane risk of bias tool enhanced the reliability of the review by systematically assessing the risk of bias in the included studies. This quality assessment process further ensured that the information presented in the review was based on sound and trustworthy evidence. By integrating and synthesizing the extracted information, the review provided a comprehensive overview of the PDC complex and its role in cellular metabolism and disease. This holistic understanding contributes to advancing knowledge in the field and provides a foundation for future research and therapeutic interventions targeting metabolic disorders associated with PDC dysfunction.

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