Role of pyruvate kinase M2 in regulating sepsis (Review)

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Abstract. Glycolysis occurs in all living organisms as a form of energy supply. Pyruvate kinase M2 (PKM2) is one of the rate-limiting enzymes in the glycolytic process. PKM2 is considered to serve an important role in several terminal diseases, including sepsis. However, to the best of our knowledge, the specific mechanistic role of PKM2 in sepsis remains to be systematically summarised. Therefore, the present review aims to summarise the roles of PKM2 in sepsis progression. In addition, potential treatment strategies for patients with sepsis are discussed. The present review hopes to lay the groundwork for studying the role of PKM2 and developing therapeutic strategies against metabolic disorders that occur during sepsis.

Contents

1. Introduction

Sepsis is a multiorgan dysfunction caused by the response of the host organism to an infection due to the invasion of pathogenic microorganisms, such as bacteria and fungi (1). It is one of the leading causes of mortality in severely ill patients worldwide, sepsis has a mortality rate of up to 30%, ~0.2-3 per 1,000 individuals are affected by sepsis yearly in the developed world, resulting in about a million cases per year in the United States (2). Over the past decade, there has been a steady increase in the incidence and mortality of sepsis; overall, the incidence of sepsis is increasing by 8-13% per year in the United States (3). In addition, sepsis treatment is costly, which places a significant financial burden on both the health system and families of the patient (4). The progression of sepsis is closely associated with changes (polarization of macrophages, infiltration of neutrophils, etc.) in immune cells such as neutrophils, T cells and macrophages and the homeostasis is ensured by means of macrophage polarization and neutrophil infiltration (5-7). Previous studies on sepsis have focused on the status of immune cells, including neutrophil infiltration, macrophage polarization and lymphocyte deletion, but it is currently under consideration that glycolytic enhancement is one of the most common features of sepsis-related metabolic disorders; therefore, the metabolic disorders of sepsis deserve more attention (8,9).

Glycolysis is a 10-step metabolic pathway that produces pyruvate and two molecules of adenosine triphosphate (ATP) (Fig. 1). In proliferative cells, energy is typically only supplied through glycolysis (10). In the 1920s, Warburg (10) discovered that tumor cells exhibit an increase in the rate of glucose uptake and lactate accumulation even in the presence of adequate oxygen availability and fully functioning mitochondria, a phenomenon today known as the 'Warburg effect' (11,12). Glycolysis occurs in all cells of the body. Notably, glycolysis is crucial for maintaining immune function in macrophages (13,14). The Warburg effect has previously been reported to be important for patients with sepsis, it is affected by various metabolic disorders such as lactic acid metabolism (15,16). Pyruvate kinase (PK) is a kinase that catalyzes the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP during glycolysis (Fig. 1) (17,18). Since PK is the last rate-limiting enzyme in glycolysis, it would be prudent to hypothesize that PK will also likely serve an important role in metabolic disorders caused by sepsis. However, the mechanistic role of PK in sepsis remains unclear. The present review therefore summarizes the role of PK and discusses potentially

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viable treatment strategies for patients with sepsis treated by targeting PK through glycolytic or non-glycolytic pathways.

2. Methods

The present study presents an up-to-date literature review covering the years 2010-2024 on the role of PKM2 in sepsis, immune cells and targeting therapy. The literature search was performed using PubMed (https://pubmed.ncbi.nlm.nih.gov/?db=PubMed) and Google Scholar (https://scholar.google.com.hk/?hl=zh-CN). A limited number of studies antecedent to 2010 would also be included in the evaluations if they contained information that could support the up-to-date study results. The key words used for the search were 'PKM2', 'sepsis', 'glycolysis', 'macrophage', 'T cell', 'NK cell' and 'B cell'. The studies discussing the effects of PKM2 in sepsis and its potential as a therapeutic target were included. Of these, studies not related to the immune and metabolic effects of sepsis were excluded. The present review aimed to determine whether PKM2 also has a therapeutic target effect similar to that observed in tumors, providing novel ideas for future sepsis research.

3. General characteristics of PKs

PK catalyzes the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP, rendering it the final enzyme in the entire glycolytic process (19). This enzyme was termed PK because it was supposed to directly catalyze pyruvate phosphorylation to promote glycolysis. There are four different currently known isoenzymes, namely L, R, M1 and M2, each of which has distinct specific kinetic properties necessary to accommodate the metabolic requirements of the cells and organs they reside in (20). These four isozymes of PK are expressed in vertebrates as follows: L is mainly expressed in the liver; R mainly in erythrocytes; M1 mainly in the muscle and brain tissues; and M2 in mainly in the early fetal tissue and most adult tissues (21). The L and R isozymes are expressed by the gene *PKLR*, whereas the M1 and M2 isozymes are expressed by the gene *PKM2* (22). PKR is characterized by high substrate affinity and promotes the glycolytic pathway by catalyzing pyruvate phosphorylation. By contrast, PKL serves an opposite role on PKR, causing the phosphorylation of pyruvate kinase and inhibition of glycolysis (23). PKM serves a key role in metabolic disorders as a result of a variety of malignant diseases like liver cancer, glioma and lung cancer (24,25).

The *PKM* gene consists of 12 exons and 11 introns (26). In the M-gene products PKM1 and PKM2, PKM1 contains exon 9, whilst PKM2 contains exons that differ by only 23 amino acids within a 56-amino acid stretch (aa 378-434) at the carboxy terminus (27). Structurally, a human PKM2 monomer consists of 531 amino acids and is a single chain divided into the A, B and C domains. The difference in amino acid sequence between PKM1 and PKM2 allows PKM2 to be allosterically regulated by fructose 1,6-bisphosphate and to form dimers and tetramers, whilst PKM1 can only form tetramers (28). The tetramers and dimers of PK mediate different functions; dimers and tetramers play different roles through different activities. PKM2 is known to exist as a dimer in tumor cells and to promote tumorigenesis through multiple mechanisms (29). This process has been studied, and PKM2 can affect tumor cell angiogenesis and apoptosis, while mediating drug resistance or affecting tumors through other pathways (29,30). The existence of PKM2 monomer is less, and there are few reports. Therefore, the tetramer and dimer conversion in PKM2 in tumors may also imply its involvement in sepsis metabolism.

4. Regulation of PK structural change

It has been frequently reported that tumor cells preferentially express PKM2, leading to the metabolic reprogramming towards the glycolysis process (31,32). By contrast, PKM2 has been previously observed to mediate a number of metabolic changes in sepsis, mostly in immune cells (18). The expression of PK isomers is tissue-specific, which suggests that the expression of different isomers meets different metabolic needs (28). Therefore, PKM2 can be regulated through structural alterations in tumors or sepsis. Understanding the dimer and tetramer of PKM2 facilitates the understanding of PKM2 and its use as a therapeutic target.

PKM2 can mainly exist as a dimer or tetramer (33), with the latter showing higher activity (34). The PKM2 dimer/tetramer ratio is regulated by a variety of factors, including metabolic intermediates, micheliolide (MCL), M239 and dihydropyrimidinase like 2 (35). The ratio between the two forms decides whether the carbon from glucose would be directed into the biosynthetic process or be used for glycolytic ATP production (36). These two forms of conversion are not only pivotal for glycolysis but also have important implications in various diseases, including tumors; it shows the importance of this structural change in understanding PKM2 (27). Notably, compared with the high activity of the tetramer, the low-activity PKM2 dimer serves to promote the conversion of PEP to pyruvate, resulting in enhanced glycolysis. Therefore, maintaining the tetramer form of PKM2 is also one of the strategies for targeting glycolysis (maintaining the tetramer of PKM2) in disease treatment, which can be applied in tumors like M239 for the treatment of liver cancer (36). There have been attempts to correct metabolic disorders caused by structural changes in PKM2 using MCL. MCL selectively activates PKM2 through covalent bonds to cysteine 424 (C424), thereby promoting tetramer formation and ultimately inhibiting the nuclear translocation of PKM2 and inhibiting as a protein kinase and cotranscription factor after nuclear heterotopia (36).

PKM2 has received increased attention in tumor research due to its special structural regulation (the conversion of tetramers and dimers), although its mechanism in sepsis remains unclear. Based on its crucial role in the regulatory process in glycolysis, it could therefore be hypothesized that targeting this process would be viable for treating sepsis. A previous study has reported that PKM2 oligomers can enter the nucleus, bind to the hypoxia-inducible factor (HIF)-1α and signal transducer and activator of transcription (STAT3), bind to the IL-1β promoter, downregulate IL-1β, upregulate IL-10 and regulate hypoxic injury and inflammation (37). In addition, epidermal growth factor receptor activation has been documented to

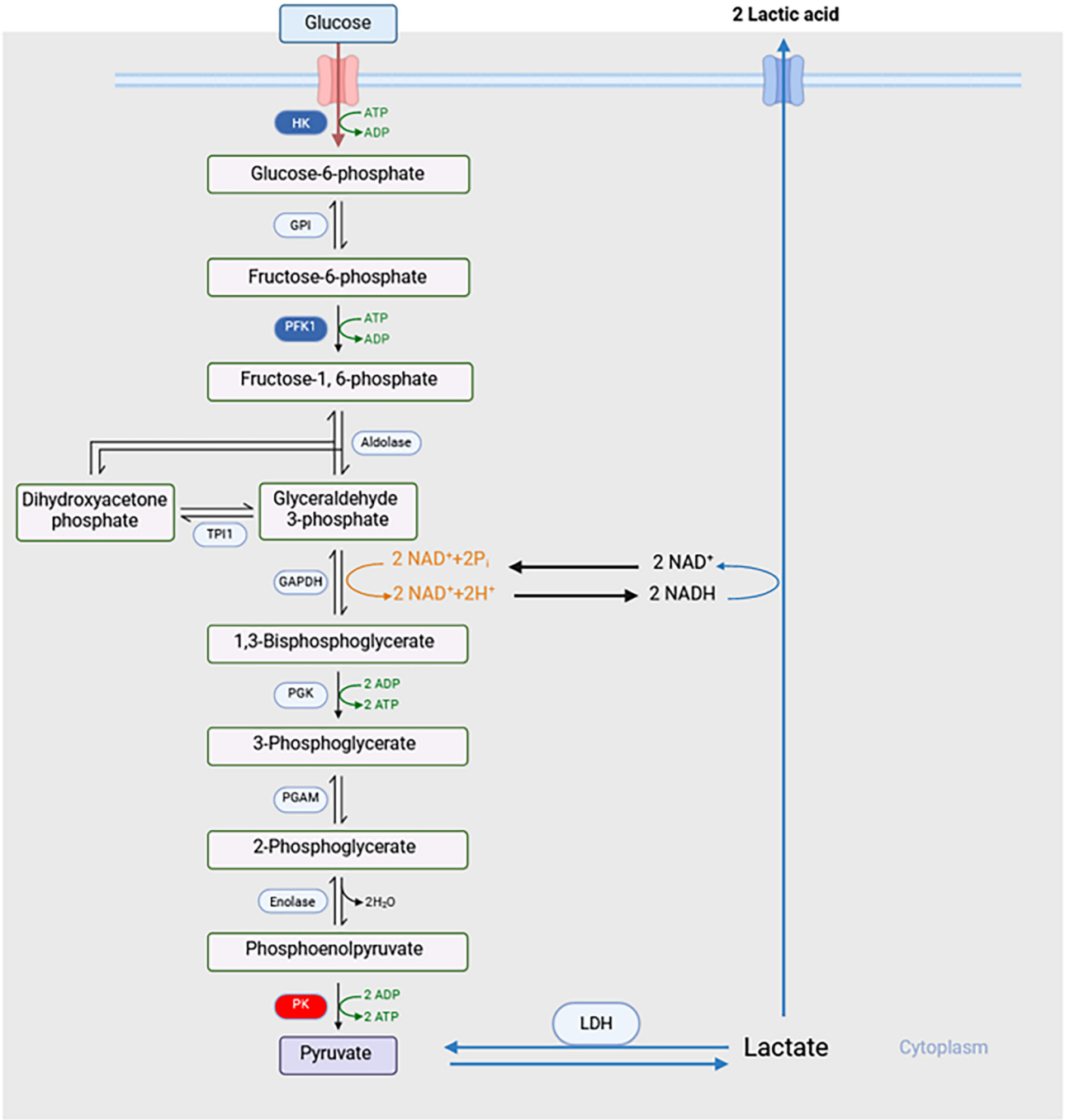

Figure 1. Overview of glycolytic pathways. Rate-limiting enzymes are marked in blue, and PKs are marked in red. HK, hexokinase; GPI, Glucose-6-phosphate isomerase; PFK1, phosphofructokinase 1; TPI1, triose phosphate isomerase 1; PGK, phosphoglycerate kinase; PGAM, phosphoglycerate mutase; PK, pyruvate kinase; LDH, lactate dehydrogenase.

promote the ERK1/2-dependent phosphorylation of PKM2 S37 and peptidyl-prolyl cis-trans isomerase-catalyzed PKM2 cis-trans isomerization, which binds to the input protein α5, leading to nuclear PKM2 translocation and promoting the Warburg effect in glioblastoma cells (38). PKM2 dimers can also mediate non-glycolytic functions affecting inflammation. It has been reported that PKM2 can exist in an oligomeric form in monocytes and macrophages, where it promotes IL-6 and IL-1β production, resulting in a proinflammatory effect (39). Even in the absence of disease, the low catalytic activity of the PKM2 dimer leads to the accumulation of intermediate products in the cell. As a result, a large number of acidic intermediates such as phosphoenolpyruvate accumulate in the cell, resulting in an acid-base imbalance, eventually leading to metabolic disorders (40). These aforementioned previous studies suggest that PKM2 can serve an important role in sepsis by converting into the dimer form and promoting inflammation through multiple pathways.

The regulatory properties of structural changes in PKM2 have been previously studied in the context of cancer therapy, providing a novel avenue for the treatment of sepsis. At present, the following two approaches have been adapted by cancer cells to control PKM2 function: i) Impeding PKM2 nuclear translocation through inhibition of the PKM2 dimer form; and

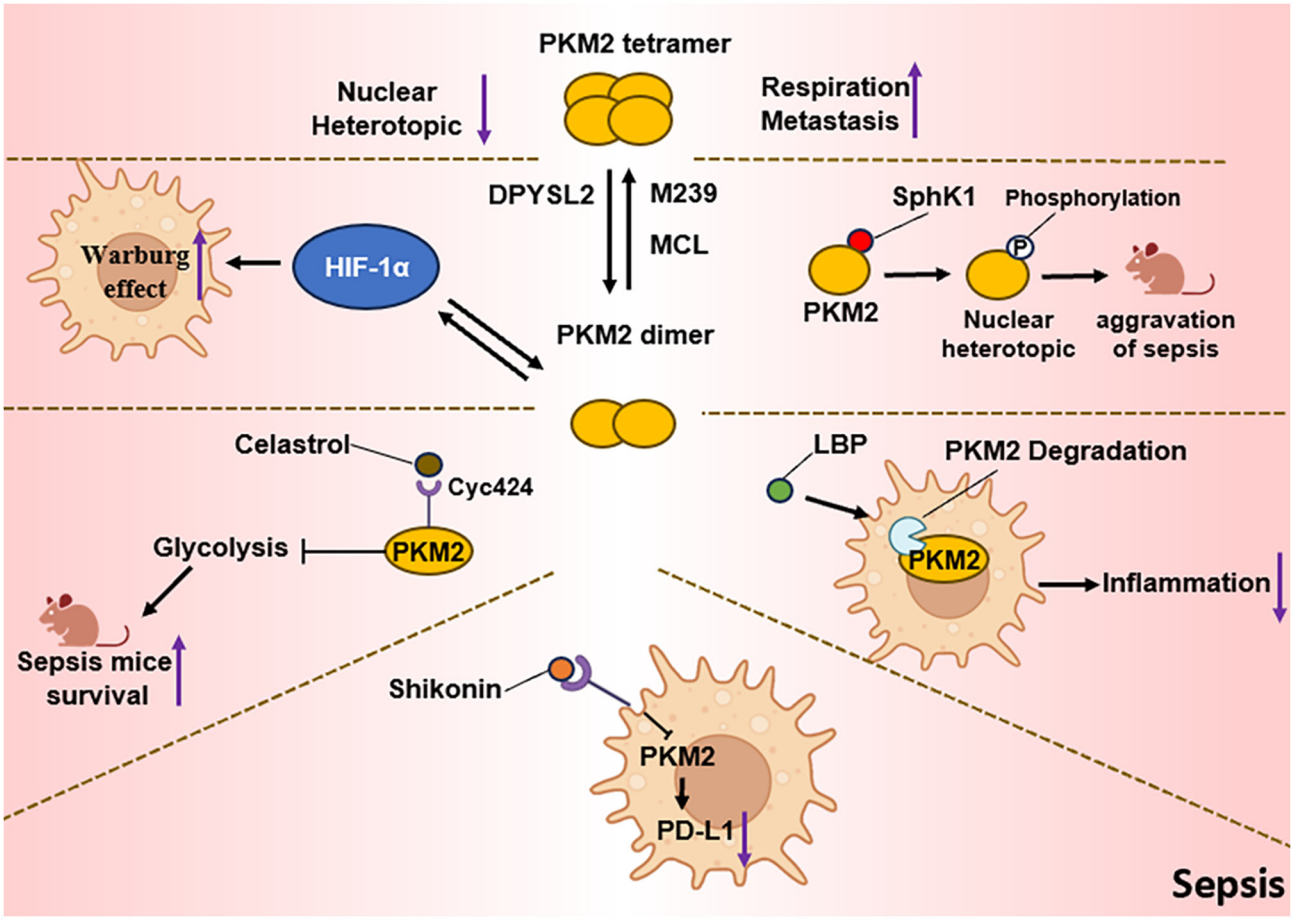

Figure 2. Overview of the roles of PKM2 in sepsis development. PKM2 is critical for regulating sepsis via macrophage metabolism and other effects. PKM2, pyruvate kinase M2; HIF-1α, hypoxia-inducible factor 1α; DPYSL2, dihydropyrimidinase-related protein 2; MCL, myeloid cell leukemia sequence 1; SphK1, sphingosine kinase 1; LBP, lipopolysaccharide binding protein; PD-L1, programmed death ligand 1.

ii) activation of PKM2 tetramer form, thereby maintaining its normal function of converting PEP into pyruvate (41). In conclusion, regulating the PKM2 structure may also be a potential target for the treatment of sepsis.

5. PKM2 regulates immune cell-mediated sepsis

The Warburg effect is common in activated immune cells (42). During approximately the same period, the Warburg effect was first observed, the same phenomenon was found in white blood cells (43). A number of reports have previously suggested that the rapid rate of proliferation in immune cells after activation is the reason for their need for such metabolic changes (44,45). During sepsis, the initial inflammatory response is typically driven by innate immune cells, such as neutrophils, monocytes and macrophages (5). Activation of immune cells serve an important role in the development of sepsis, which will be summarized in this section.

During the occurrence and development of sepsis, monocytes differentiate into macrophages and migrate to the site of infection under the stimulation of various inflammatory substances like pathogens, damaged cells or irritants (46). Fibrinogen-like protein 2 has been previously shown to target PKM2 and directly exacerbate alcoholic liver injury by downregulating macrophage glycolytic reprogramming (47) (Fig. 2). In addition, hypoxic exosomal PKM2 has been observed to induce M2 polarization in macrophages by activating the 5'AMP-activated protein kinase pathway and aggravating lung cancer (48), although not in sepsis, regulation of macrophages by PKM2 also provides insights into sepsis. In macrophages, the recombinant Treponema pallidum protein Tp47 can activate the nucleotide-binding oligomerization domain-like receptor family protein 3 inflammasome through PKM2-dependent glycolysis and induce phagocytosis (49,50). Sepsis is very closely related to immune cells, the effect of PKM2 on macrophages suggests its role. Digoxin can also activate the PKM2/HIF-1α axis, reduce HIF-1α axis-sustained inflammasome activity in macrophages and ameliorate mouse hepatitis (Fig. 2) (51). The long non-coding RNA HIF-1α inhibitor at the transcriptional level has been previously found to inhibit lactate production as a result of miR-106 induction and facilitate PKM2 oligomerization, which polarizes macrophages towards an M2-like anti-inflammatory phenotype and contributes to immune escape (mostly in macrophages) *in vivo* (52). The metabolic regulation of macrophages by PKM2 serves an important role in the regulation of macrophage polarization and other functions like inhibiting glycolysis in macrophages and regulating PD-L1, which may offer potential treatment ideas for sepsis.

T cells can also serve important roles in sepsis and inflammation (53). The hyperactivation of T cells can mediate immune disorders in patients with sepsis, where previous studies have sought to utilize this characteristic for treatment. T-cell metabolism is regulated by targeting reactive oxygen species, key substances for T-cell activation, and the metabolite citrate (54). Activated T cells also notably produce energy for proliferation using the Warburg effect (55). Previous studies have demonstrated that pharmacological activation of PKM2 or maintaining its tetramer state may limit T-cell activity in treating inflammation and autoimmunity caused by PKM2 hyperactivation (56,57). PKM2 is a critical non-metabolic regulator that can influence Th17 cell differentiation and mediate functions in autoimmune-mediated inflammation (58). PKM2 has been previously reported to play an important role in the pathogenesis of non-alcoholic fatty liver disease through Th17 cell glycolysis in the conditionally steatogenic liver microenvironment (59). The CoA that is generated by the breakdown of vitamin B5 has been previously observed to bind to PKM2, preventing its phosphorylation and nuclear translocation to inhibit glycolysis, STAT3 phosphorylation and Th17-cell differentiation (60). These aforementioned previous findings suggest that PKM2 can affect metabolism by regulating the glycolytic ability of T cells, which is also one of the ideas for sepsis treatment.

The role of PKM2 in other immune cells may also be noteworthy. In natural killer (NK) cells, PKM2 mainly exists as a monomer and tetramer, which functions through metabolic regulation, not transcriptional regulation (61). Silencing PKM2 was found to disable NK cell activation (61). PKM2 can also regulate the activation by enhancing IL-12p35 expression and metabolic function of dendritic cells through HIF-1α-dependent pathways or by reprogramming the expression of metabolic genes such as PKM2 (62,63). PKM2 is required to support metabolic reprogramming (an increase in both oxidative phosphorylation and glycolysis) for homocysteine-induced B-cell activation and function both *in vivo* and *in vitro*, where the shikonin compound can reverse this process and inhibit the proliferation of B-cells (64). PKM2-dependent glycolysis is crucial for the activation of various immune cells. The occurrence of sepsis is closely associated with the hyperactivation of immune cells and cytokine storms (33). The role of PKM2 in immune cells therefore provides novel ideas for the treatment of sepsis.

6. Other direct or indirect roles of PKM2 in sepsis

PKM2 can also regulate sepsis development in several other manners. Total PKM2 is considered to be an indicator of sepsis diagnosis and prognosis (65). PKM2 can interact with HIF-1α and activate the HIF-1α-dependent transcription of enzymes necessary for aerobic glycolysis in macrophages, promoting the Warburg effect to exacerbate sepsis (66). In a mouse model of sepsis, sphingosine kinase 1 was found to directly bind to PKM2, resulting in nuclear heterotopic and PKM2 phosphorylation, aggravating sepsis (Fig. 2) (67). Research on the mechanism of PKM2 has expanded the understanding of the metabolic regulation of sepsis.

There have also been attempts to target sepsis with PKM2 in recent years. The chemical compound Celastrol can bind to Cys424 of PKM2, inhibiting the enzyme and suppressing aerobic glycolysis, improving survival in an animal model of sepsis (Fig. 2) (68). Capsaicin has also been documented to directly bind to and inhibit PKM2 and lactate dehydrogenase A to suppress the Warburg effect in inflammatory macrophages (69). Similarly, *Lycium barbarum* polysaccharide has been observed to inhibit lipopolysaccharide-induced inflammation by altering the glycolysis and the differentiation of macrophages by triggering PKM2 degradation (Fig. 2) (70). Shikonin can also regulate PKM2 by inhibiting the expression of programmed death-ligand 1 in macrophages to control the development of sepsis (Fig. 2) (71). In addition, PKM2 was previously found to regulate the function of platelets by PI3K/glycogen synthase kinase 3 signaling in humans and mice (72-74), which serve a role in sepsis and arterial thrombosis (75).

7. Conclusion

PK is the last rate-limiting enzyme in the glycolytic pathway. Metabolic disorders are found in malignant proliferating cells and depend on glycolysis as a means of obtaining energy (76). In malignant diseases, energy consumption is high, but energy utilization is low, which harms the patient. The metabolic characteristics of tumors can be detected at an early stage of tumorigenesis and tumors are also considered to be a class of metabolic diseases (76). Therefore, several kinases involved in glycolytic metabolism like hexokinase (HK)2, phosphofructokinase (PFKM) and lactate dehydrogenase were also considered to be oncogenes and were used as targets for the treatment of tumors (77,78). HK2 has been the most studied and plays an important role in promoting glycolysis (79). PFKM is also one of the rate-limiting enzymes in the glycolytic pathway and is considered as a therapeutic target (80). The role of the glycolytic pathway in disease is promising.

Sepsis is characterized by the hyperactivation of immune cells and proliferating immune cells are similar to tumor cells, in that both depend on the Warburg effect for energy (42). The presence of various factors suggests that the Warburg effect is also important in sepsis (15). Current research suggests that sepsis occurs when immune cells undergo metabolic reprogramming, leading to excessive inflammation and immunosuppression. At the same time, the interaction of the metabolic and immune systems further limits treatment (15). The present review summarized the latest research progress on the role of PK in sepsis and the regulatory effect of the conversion of tetrameric and dimeric PK structures on glycolysis. Consistent with its role in tumors, the PKM2 subtype serves an important role in sepsis and has the greatest potential as a therapeutic target (Fig. 2). By targeting the Warburg effect in immune cells, several studies have reported that PKM2 is important for the hyperactivation of macrophages, T cells and NK cells. In particular, macrophages serve an important role in sepsis and warrant attention (Fig. 2). Several studies have used drugs to promote structural changes in PKM2 or directly regulate sepsis through the downstream HIF-1α pathway (35,80). However, the majority of these studies involved *in vitro* and *in vivo* experiments. Further investigation in this area is warranted.

Metabolic disorders and even the Warburg effect have been involved in an increasing number of diseases in recent years (81). The present review summarized the mechanism of

PKM2 in sepsis and discussed its potential as a therapeutic target, which may promote the understanding of the metabolic aspects of sepsis. The present review provides a basis for studying the mechanism of PKM2 and developing therapeutic strategies for metabolic disorders, including sepsis.

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Availability of data and materials

Not applicable.

Authors' contributions

XC designed and conceived the present review. YH, JT, QX, ZF, RL, MY, JZ and XC wrote the draft. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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