



# Pyruvate Kinase M2: a Metabolic Bug in Re-Wiring the Tumor Microenvironment

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## Abstract

Metabolic reprogramming is a newly emerged hallmark of cancer attaining a recent consideration as an essential factor for the progression and endurance of cancer cells. A prime event of this altered metabolism is increased glucose uptake and discharge of lactate into the cells surrounding constructing a favorable tumor niche. Several oncogenic factors help in promoting this consequence including, pyruvate kinase M2 (PKM2) a rate-limiting enzyme of glycolysis in tumor metabolism via exhibiting its low pyruvate kinase activity and nuclear moon-lightening functions to increase the synthesis of lactate and macromolecules for tumor proliferation. Not only its role in cancer cells but also its role in the tumor microenvironment cells has to be understood for developing the small molecules against it which is lacking with the literature till date. Therefore, in this present review, the role of PKM2 with respect to various tumor niche cells will be clarified. Further, it highlights the updated list of therapeutics targeting PKM2 pre-clinically and clinically with their added limitations. This upgraded understanding of PKM2 may provide a pace for the reader in developing chemotherapeutic strategies for better clinical survival with limited resistance.

**Keywords** Metabolic reprogramming · PKM2 · Tumor microenvironment · Aerobic glycolysis · Tumor metabolism

## Abbreviations

ANGPT2	Angiotensin II type 1 receptor antagonist	CD4	Cluster of differentiation 4
AKT	Serine/threonine-specific protein kinase	COX-2	Cyclooxygenase-2
ALCL	Anaplastic large cell lymphoma	CTGF	Connective tissue growth factor
ALT	Alanine aminotransferase	CXCL8	C-X-C motif chemokine 8
AMPK	5' AMP-activated protein kinase	DASA-58	Dihydro benzodioxin sulfonyl hexahydro diazepin sulfonyl benzenamine
ANGPTL4	Angiotensin-like 4	EGF	Epidermal growth factor
AP-1	Activator protein 1	EMT	Epithelial–mesenchymal transition
C/EBP $\beta$	CCAAT-enhancer binding protein $\beta$	ErbB3	Erb-B2 Receptor tyrosine kinase 3
		ERK-5	Extracellular-signal-regulated kinase 5
		FBP	Fructose biphosphate
		FGF	Fibroblast growth factor
		FGFR1	Fibroblast growth factor receptor 1
		GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
		GLUT-1	Glucose transporter -1
		HCT-116	Human colon cancer cell line
		HIF-1 $\alpha$	Hypoxia induced factor-1 alpha
		HK-2	Hexokinase-2
		HMGB1	High mobility group box-1
		IFN- $\gamma$	Interferon-gamma
		IL-10	Interleukin-10
		IL-13	Interleukin-13
		IL-1 $\beta$	Interleukin-1 $\beta$
		IL-4	Interleukin-4

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IL-6	Interleukin-6
JAK	Janus kinase
JNK	c-Jun N-terminal kinases
LDH	Lactate dehydrogenase
LDH-A	Lactate dehydrogenase-A
LOX	Lysyl oxidase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinases
MAP K-5	Mitogen activated protein kinase-5
MCT-1	Monocarboxylase transporter -1
MCT4	Monocarboxylate transporter 4
MDM2	Mouse double minute-2 homolog
miRNA-124	MicroRNA-124
MMP	Matrix metalloproteinase
MMP-2	Matrix metalloproteinase 2
mTOR	Mechanistic target of rapamycin
NF-kB	Nuclear factor kappa B
NK cells	Natural killer cells
OXPPOS	Oxidative phosphorylation
p53	Tumor protein p53
PD-1	Programmed cell death-1
PD-4	Programmed cell death-4
PDGF	Platelet-derived growth factor
PDGF-BB	Ligand for platelet derived growth factor receptor
PGAM1	Phosphoglycerate Mutase 1
PGE2	Prostaglandin E2
PI3K	Phosphoinositide-3-kinase
PIN1	Peptidyl-prolyl cis-trans isomerase
PTP1B	Tyrosine-protein phosphatase non-receptor type 1
ROS	Reactive oxygen species
RT-PCR	Real time-polymerase chain reaction
SAICAR	Succino 5-amino-4-imidazole-N-succinocarboxamide ribonucleotide
SDF-1	Stromal cell-derived factor 1
shRNA	Small hairpin ribonucleic acid
siRNA	Small interfering ribonucleic acid
SOD2	Superoxide dismutase 2
STAT3	Signal transducer and activator of transcription 3
TCA	Tricarboxylic acid
TCR	T cell receptor
TEPP-46	Thieno-pyrrole-pyridazine
TGF $\alpha$	Transforming growth factor alpha
TGF $\beta$	Transforming growth factor beta
TH-1	T helper cell-1
TIMP	Tissue inhibitor of metalloproteinases
TNF- $\alpha$	Tumor necrosis factor-alpha
TRAF-5	TNF-receptor associated factor-5
US28	G-protein coupled receptor homolog US28
VEGF	Vascular endothelial growth factor
vGPCR	Viral G protein-coupled receptor

## Introduction

Cancer, a dynamic disease which develops one in three people during lifetime and considered as a major health burden to the society across the worldwide. According to the National Cancer Institute (NIH), roughly 1.7 million new cases will be identified and 0.6 million people will perish with cancer in the USA [1]. Typically, throughout the progression of a disease, cancers become more variant due to their somatic evolutionary changes [2] and this is referred as tumor heterogeneity which was explained by a pathologist, Rudolf Virchow in 1800s [3]. Moreover, it has been considered as a key element for the failure of classical and modern therapeutics and offers multiple hindrances like chemotherapeutic resistance [4] cancer pharmacogenomics [5] and development of cancer stem-like cells [6]. It means the tumor shows the existence of divergent tumors cells in terms of genetic and phenotypic patterns within the subpopulations of same histopathological types of cancer in different patients or within tumors that show divergent biological behavior and metastatic potential [7, 8]. This heterogeneity may be spatial and temporal in which former describes the uneven distribution of cancers subclones in various sites of primary tumors due to genetic instability whereas the latter describes the variation in molecular markers expression in same sites of tumors over a period of time [9]. However, a new perspective of heterogeneity has been explained recently based on the metabolic demands of cancer cells termed as metabolic heterogeneity [10]. Clinical studies of various cancers like breast cancer, acute myeloid leukemia, and Barrett's esophagus showed a positive correlation with tumors heterogeneity and displayed a high risk for cancer progression [11–13]. Four inter-dependent drivers like epigenetic regulation, cellular differentiation hierarchies, gene expression stochasticity, and microenvironment have direct control of cancer cells resulting in the tumor heterogeneity [14]. Many researchers found a high spatial and metabolic heterogeneity under different microenvironments such as developing more vasculature in the hypoxic region [15], aberrant expression of glycolytic enzymes [16], lactate mediated acidification [17], and autonomous cell proliferation without stimuli [18]. Consequently, these relationships between cancer cells with its microenvironment might contribute the cancers cells to adopt various conditions existed within the tumor niche [19].

Despite these heterogeneities, cancer cells even display some basic analogous characteristics of various cancers which are often described as “hallmarks of cancer” required for their growth and multiplication [20]. Amongst all, dysregulated cellular energetics has a starring role in supporting the tumor development by providing the desired necessities to the cells for fulfilling their metabolic demands by increasing aerobic glycolysis. This fulfillment gets accomplished by modifying the crucial metabolic enzymes (Hexokinase II, PKM2, LDH-

A, and enolase, etc.) [21, 22], transporters (GLUT1, MCT-1, and MCT-4 etc.) [23] involved in different biosynthetic pathways through various oncogenic stimuli (HIF-1 $\alpha$ , c-Myc, PI3K/mTOR signaling) [24, 25]. Amongst all, PKM2 has a major role in shifting the tumor towards the aerobic glycolysis and even remodels the tumor microenvironment (TME) cells towards the tumor development, invasion, and metastasis. Lately, the studies on cancer cell metabolism greatly expanded the understanding of cancer pathology and even revealed the presence of a widespread metabolic heterogeneity in tumors compared to spatial and temporal heterogeneities [10]. This disclosure exhilarated the researchers to focus more on recognizing the root cause of the phenotype and even the genetic anomalies facilitating these progressions in the TME. Even though the aerobic glycolysis is understood in many aspects, yet the role of a glycolytic enzyme PKM2 which catalyzes the last step of glycolysis has not been understood properly with respect to its role in various cells of TME. In addition, a study even suggested the differential requirement of PKM2 among the TME population [26] and has a positive correlation with cancer growth, metastasis, and resistance [27, 28]. Therefore, this contemporary review in detail highlights the role of PKM2 in the metabolic reprogramming of different cell population involved with the TME. Further, we discussed the preclinical and clinical studies with respect to PKM2 and the limitations included by aiming its activation or inhibition for modulating the metabolic conditions of cancer cells.

## Glycolysis and its Metabolic Reprogramming

In both prokaryotes and eukaryotes, glycolysis is a central metabolic pathway for the energy generation and anabolism with coupled TCA [29]. In the 1920s, Dr. Otto Warburg and his colleagues observed an alteration in the glycolysis of the tumor. Huge amounts of glucose were consumed by the cancer cells in comparison to normal cells, and the consumed glucose was further fermented to lactate in aerobic conditions characterizing it as aerobic glycolysis or Warburg effect [30–32]. For the detailed process of aerobic glycolysis and the upregulated enzymes involved with it were mentioned in Fig. 1. Uptake of glucose is considered as a rate-limiting step of glycolysis in both cancer and fast dividing cells. Its uptake above the threshold level gradually increases aerobic glycolysis and slightly decreases mitochondrial respiration. This relationship between metabolic switching from mitochondrial oxidation to aerobic glycolysis improves the cancer cell proliferation and progression. Even the dysfunction of mitochondrial respiration and dysregulated glycolysis promoted the transformation of cells to form tumor [33].

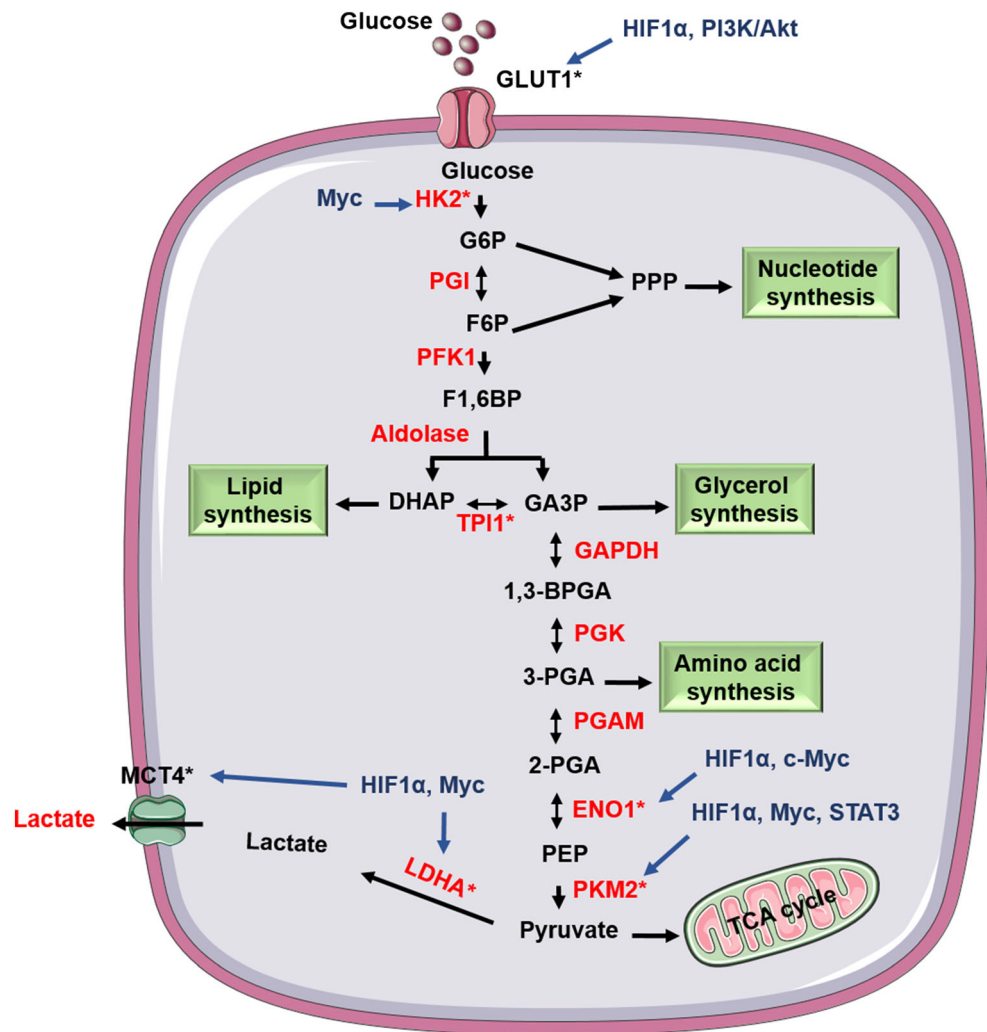
In the conditions of malignancies, increased expression of GLUT1 occurs due to the different signaling molecules (HIF-1 $\alpha$ , c-Myc) preparing the cell to uptake more glucose. A study

displayed the involvement of transporter (GLUT1) in the drug resistance of EGFR inhibitors (Gefitinib and Erlotinib) by increasing the glucose metabolism in lung cancer suggesting that glucose uptake by GLUT1 is a critical factor for the EGFR resistance [34]. Even the enzymes entailed with this process are upregulated and are modified post-translationally [35–37] by which the intermediates of the pathway like glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), glyceraldehyde-3-phosphate (G3P), dihydroxyacetone phosphate (DHAP) are fluxed for the biosynthesis of various macromolecules (nucleic acids, fatty acids, phospholipids, amino acids, and sphingolipids) and maintains the redox system against ROS in cancer cells by making NADPH [38]. Recently, researchers are more interested to target the increased expression of various signaling molecules ( $\beta$ -catenin, c-Myc, HIF-1 $\alpha$ , STAT), glycolytic pathway enzymes (HK, PKM2, PDK1, enolase, LDH) and glucose transporters (GLUT1, GLUT2) using siRNAs, small molecules treatment and formulation based strategies. From the above-mentioned proteins, PKM2 has a major role in assisting the progression of cancer by shifting the cellular metabolism to aerobic glycolysis by its nuclear moonlighting functions [39, 40].

## PKM2

In the glycolytic pathway, a rate restrictive enzyme termed as pyruvate kinase (PK) catalyzes the phospho-transfer reaction of phosphoenolpyruvate (PEP) to pyruvate with the generation of one molecule of ATP. In mammals, it exists in four isoforms namely PKM1 in high energy demanding tissues (skeletal muscle, heart, and brain), PKM2 (proliferating more cells and most cancer cells), PKL (liver and intestine), and PKR (erythrocytes) [41]. PKL and PKR isoforms are encoded from the gene PKLR, whereas PKM1 and PKM2 isoforms are encoded from a mutually exclusive exon of a gene PKM through alternative splicing [42–44]. Heterogeneous nuclear ribonucleoproteins (hnRNPs) - hnRNPA1 and hnRNPA2 controls this alternative splicing of PKM gene and inclusion of either the exon 9 or exon 10 into the pyruvate kinase mRNA results in PKM1, a constitutively active tetramer, and PKM2 respectively. Low expression of hnRNPs allows, exon 9 recognition by splicing machinery and favors the exon 10 inclusion into the PKM mRNA resulting in the upregulated PKM2 protein expression. Apart from hnRNPs some oncogenic factors like HIF-1 $\alpha$  and c-Myc were reported to enhance the splicing of PKM2 [45, 46]. In addition, both the PKM isoforms possess a pyruvate kinase activity towards the PEP. But, due to the presence of 22 amino acid residues difference in mRNA of PKM2 exon 10 with respect to PKM1 exon 9, it offers an FBP binding pocket and letting it dependent on the FBP for the allosteric regulation and induction of tetrameric PKM2 [47]. Moreover, PKM2 exists in tetrameric and dimeric forms and the ratio of these isoforms in the cancer cells

**Fig. 1** Aerobic glycolysis in cancer cells: Illustration represents that cancer cells undergo aerobic glycolysis by inflowing the glucose molecules and converts the produced pyruvate to lactate instead of allowing it to enter TCA cycle in the aerobic conditions. Oncogenic proteins involved with the tumorigenesis upregulate many enzymes and transporters (indicated by \*) of glycolysis further supporting this process



depends on the influence of different oncogenic proteins and has no fixed value. The dimeric form has the less affinity towards its substrate PEP at physiological conditions ( $K_m$ , 0.46mM) and acts as an active nuclear protein kinase whereas the tetrameric form has a high affinity toward PEP ( $K_m$ , 0.03mM) [48]. Post-translational modifications of PKM2 with signaling molecules (EGF,  $\beta$ -catenin, and FGFR), glucose and ROS mediate the phosphorylation at Y105 [49], acetylation at K305, and oxidation at C358 respectively promoting the dimeric form instead of a tetrameric form [50]. While, in-built molecules like serine, FBP and SAICAR were recognized allosteric PKM2 activators. Recently, small molecules like DASA-58 and TEPP-46 were found to be the reported synthetic PKM2 activators which act by steadying or stabilizing the tetrameric form [51].

This peculiar enzyme has both canonical and non-canonical roles in both glycolysis and cancer development respectively. It executes the canonical role by upholding the metabolic program of cancer cells. PKM2 contains a nuclear localization sequence (NLS) similar to importin  $\alpha$ -5, by which

it takes the assistance of importin  $\alpha$ -5 for its translocation to the nucleus [52]. In addition, in the nucleus, this dimer PKM2 possess the protein kinase activity and phosphorylates STAT3 at Y705 [53] resulting in the upregulation of N-cadherin and metalloproteases like MMP-2, MMP-9 promoting colorectal cancer (CRC) and metastasis [54]. Further, by acting as a transcription factor, it phosphorylates histone at threonine 11 (T11) and upregulates the cell cycle proliferation markers (cyclin D and c-Myc) promoting brain tumorigenesis [55]. Interaction with the phosphotyrosine-containing proteins and JMJD5 [56] inhibits the pyruvate kinase activity of PKM2 and supports the accumulation of the glycolytic metabolite. Apart from this PKM2 also upregulates expression of various tumor proliferation ( $\beta$ -catenin) and metastasis markers (E-cadherin, N cadherin and vimentin). So, these findings indicated that PKM2 has both protein kinase and pyruvate kinase role. Butyrate (short-chain fatty acid) found in the gut lumen at higher concentration decreases glycolytic intermediates and nucleotide synthesis in HCT-116 cells by inhibiting Warburg effect due to dephosphorylation and tetramerization activating



PKM2 [57]. Many researchers reported that small molecules activating PKM2 tetrameric form attenuated PKM2 mediated tumor growth and metastasis, although some reports suggested that inhibition of PKM2 with its inhibitors like shikonin and lapachol inhibits tumor growth [58, 59]. Structural studies revealed that these small molecules bind at the subunit interface of PKM2 site but not at the endogenous activator FBP binding sites [60]. However, understanding the mechanism of PKM2 in the metabolic reprogramming of cells (cancer cells and TME cells) is an important criterion for targeting it in anticipation to inhibit the cancer progression.

### The Interplay between PKM2 and the Metabolic Reprogramming of Tumor Niche

Metabolic reprogramming is one of the newly emerged hallmarks of cancer for the fast and continuous proliferation of a cancer cell. It is an important feature in satisfying the biosynthetic and redox requirement of a cell by reprogramming the important biosynthetic cycles like glycolysis, OXPHOS, pentose phosphate pathway, and glutaminolysis [61, 62]. Signaling molecules (Akt,  $\beta$ -catenin, Nrf2, c-Myc, Ras, and HIF-1 $\alpha$ ) along with the specific isoforms of metabolic enzymes (dimeric PKM2, hexokinase, and LDH) can enhance the reprogramming of cells in tumor niche in accordance to their needs [63].

### Tumor Microenvironment (TME)

Formation of the tumor niche involves, the cancer cell communication with its surrounding cells necessary for the metastasis and resistance to cancer therapeutics. It acts like an ocean with embedded tumor cells encircled by extracellular matrix and stromal cells. It consists of non-malignant (TME cells) and malignant (cancer) cells and exists a bidirectional interaction between these cell components creating an environment known as TME, which partakes a role in tumor development and metastasis of cancer in all stages of carcinogenesis. Consistency in these interactions is maintained by paracrine and juxtacrine signalings involving different pathways like Notch, Foxp3, TGF $\beta$ , and IFN- $\gamma$  [64, 65]. Early stages of tumorigenesis show an inflammatory microenvironment with a co-operative effect of COX-2/PGE<sub>2</sub> and TLR/MyD88 pathways [66]. Hypoxia, altered pH, condensed extracellular matrix and immunosuppression are the features of TME which helped in developing different strategies like physical based targeting (thermal), pulsed based targeting, permeability based targeting, and chemical based targeting based on pH and hypoxia for targeting TME. Further, nano-therapeutic targeting (nanomedicines, nanoparticles, nanocarriers and ultra-small gold nanosatellite bearing nanoparticles), molecular-based

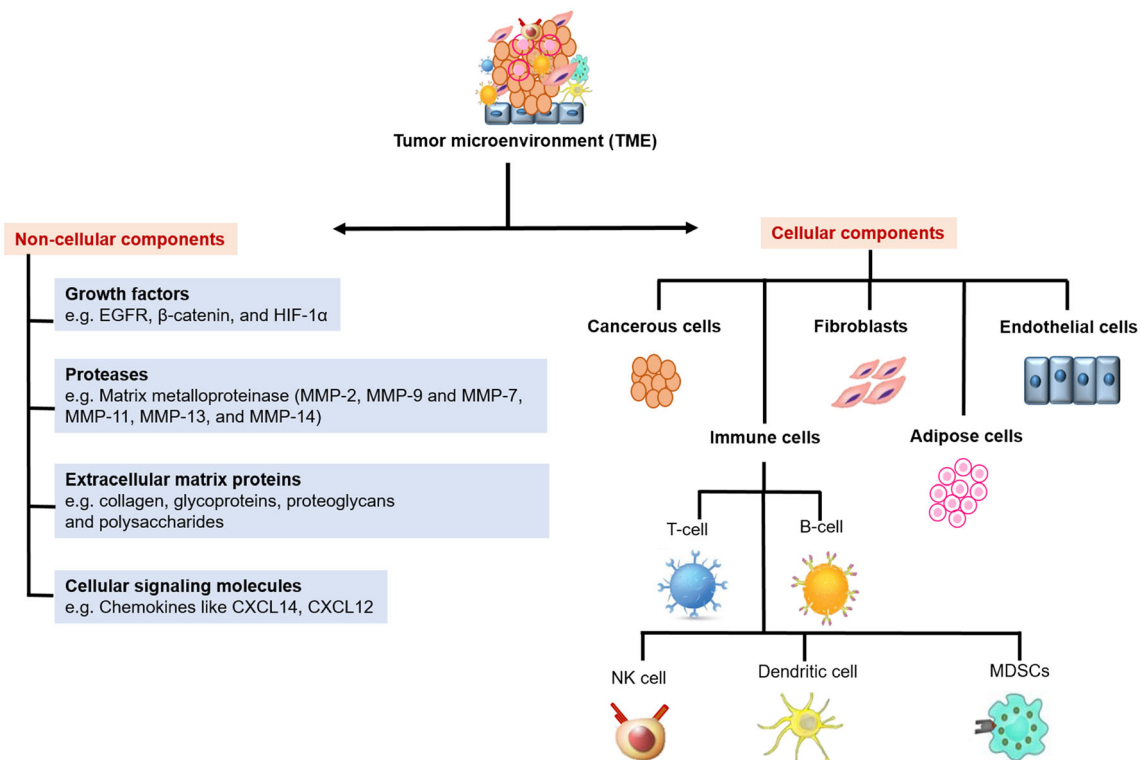
targeting (siRNA, anti-PD-1, anti-VEGF, anti-HIF $\alpha$ ) and cancer vaccines were developed focusing the tumor niche [67–69]. TME consist of different types of components namely non-cellular and cellular components surrounded by stromal cells Fig. 2 [70, 71].

Activation of PKM2 in TME cells results in different physiological functions like reversed metabolic shift, increased OXPHOS, reduced lactate production, inhibition of tumor growth [72], and even provides immunity depending on the cell specificity [73]. Till date many reviews suggested the role of PKM2 with respect to the cancer cells [74–76], therefore in the next sections of the review, we will focus on discussing the importance of PKM2 in TME cells for their modulation and their maintenance Fig. 3.

### T cells

T cell plays an important role in cell-based immunity against the pathogen. Naive T cell requires low energy requirement to prevent cells from atrophy and to keep cell survival and migration into circulation. However, activated T cell has high metabolic demand for rapid proliferation, differentiation and massive synthesis of cytokines and interleukins. The purpose of activated T cells to increase metabolic demand is either for its self-development or to erect the pathogens. To achieve this high metabolic demand, T-cell subtypes like TH1, TH2, and TH17 starts the massive influx of nutrients like glucose and amino acids via increased expression of GLUT1 and glycolytic enzymes shifting the cell to aerobic glycolysis. In controversy, regulatory T-cells downregulate GLUT1 increasing fatty acid oxidation. This phenomenon of metabolic shifting was even observed in CD4+ inflammatory T-cells but not with the CD4+ regulatory cells [77]. Moreover, aerobic shifting initiated by T-cell receptor (TCR) was sustained by cytokines like interleukin-2 and PEP concentration [78, 79]. But, TME shows glucose limiting condition which suppresses the tumouricidal function of T cell, compromising TCR-induced proliferation and growth increasing the expression of effectors molecules such as IFN- $\gamma$ .

Many preclinical studies have shown the relation of PKM2 with the T-cell activity. Programmed death ligand 1 (PD-L1), a transmembrane protein present on the normal and cancer cells interacts with PD-1 checkpoint receptor existing on T-cells. This interaction plays a crucial role in autoimmunity and cancer by acting as “off-switch”. It means this interaction halts the T-cell activity towards the PD-L1 representing cells [80]. A report suggested that PKM2 dimer upregulates the PD-L1 expression by binding to the PD-L1 promoter region, and either the RNA silencing of PKM2 or treatment with TEPP-46 (PKM2 activator) inhibits LPS induced PD-L1 expression showing the significant role of PKM2 in limiting the T-cell response against tumors [81]. Even the increased expression of c-Myc proteins also resulted in modulating T inflammatory



**Fig. 2** Components of tumor microenvironment: Illustration represents the cellular and non-cellular composition of the tumor microenvironment

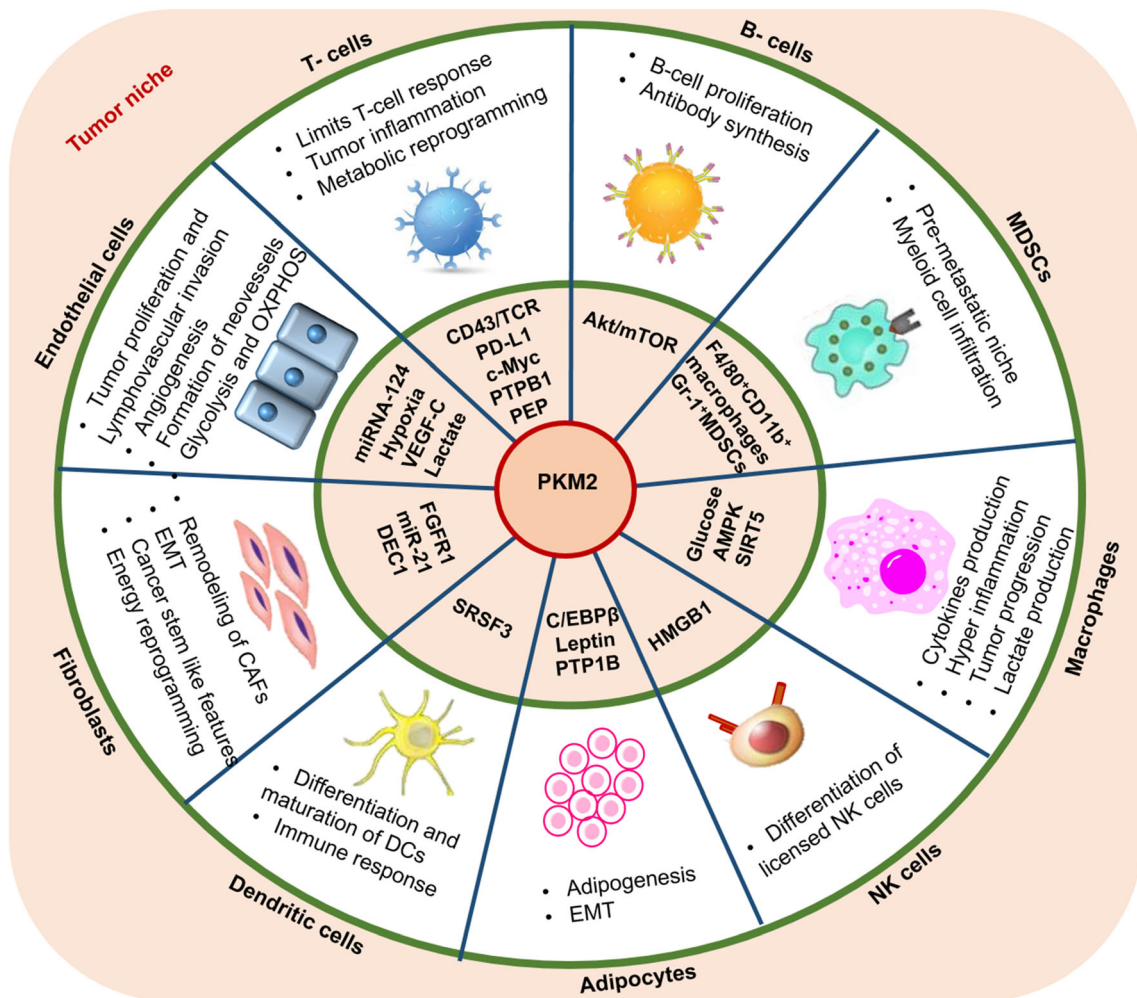
cell metabolic reprogramming contributing to the inflammation of tumor [82, 83]. c-Myc signaling has a role in post-transcriptional modification levels of PKM2 where it controls the splicing pattern by upregulating hnRNPAI (primary transcript) expression resulting in the high expression of PKM2 in glioma cells [84]. In controversy to the above study, the haploid efficiency of a transcriptional repressor of c-Myc gene, FUSE-binding protein (FBP)-interacting repressor (FIR) resulted in the alternative splicing of PKM2 by inhibiting hnRNPAI expression [85]. Apart from these signaling molecules, some co-stimulatory molecules found on T-cell surface like CD43 also affect PKM2 expression and proliferation, activation and migration of T cells [86]. CD43 and TCR co-signaling stimulates the phosphorylation of Y105 of PKM2 and Y705 of Signal transducer and activator of transcription 3 (STAT3) transducer molecules resulting in the activation of MEK5/ERK5 pathway which activates nuclear localization kappa B (NF-κB), Myc as well as Bcl-2-associated death promoter (*BAD*) phosphorylation promoting the survival [87]. Abnormal expression of this protein is also observed in some non-hematologic neoplasms such as lung, colon, and salivary gland cancers [86]. Even the siRNA silencing of CD43 increased the vulnerability of breast and lung cancer cells to NK cells and apoptosis [88, 89].

Nuclear factor of activated T cells (NFAT1), a member of transcription factors family act as a tumor repressor by inhibiting the growth and differentiation of cancer cells [90].

Moreover, the substrate of PKM2, PEP was involved in the activation of calcium-dependent NFAT1 signaling by repressing the calcium uptake in the endoplasmic reticulum by Sarco/ER  $\text{Ca}^{2+}$ -ATPase (SERCA) in T-cell. So, glucose deficient TME may diminish the anti-tumor activity of T-cell by downregulating NFAT1 signaling [91]. Even, NFAT1 was found to regulate MDM2 oncogene *in-vitro* and *in-vivo* in MCF-7 and MDA-MB-231 cells in which JapA showed inhibitory activity [92]. Interacting proteins of PKM2, like PTPB1, promotes cell proliferation and colony formation in ALCL cells by facilitating pY105 of PKM2 and nuclear STAT3 activation [93]. Further, a sulfur-containing amino acid homocysteine found to regulate the T-cell glycolytic reprogramming by upregulating PKM2 expression through PI3K/AKT/mTOR signaling in the conditions of hyperhomocysteinemia mediated inflammation in Apo<sup>-/-</sup> mice (apolipoprotein E-deficient mice) promoting atherosclerosis [94]. Moreover, high serum levels of homocysteine show a positive correlation with risk of cancer [95]. So, an additional study is required to know the possibility of homocysteine in dysregulating PKM2 levels of T-cells within TME in promoting the inflammation induced cancer progression.

### B cells

B-cells are the part of the immune system and are the subtypes of lymphocytes, which gets differentiated into plasma cells



**Fig. 3** Regulation of TME cells in the tumor microenvironment: Illustration represents the regulatory molecules modulated by PKM2 in different TME cells. This modulation helps in their activation and progression

and memory cells. Plasma cells finally get differentiated into antibodies and memory cells that help in keeping a track record of attacked antigens. These are derived from bone marrow stem cells and contribute a major role in immune response and immune system related disease such as autoimmunity and alloimmunity [96]. B-cell infiltration is common in draining of lymph nodes and lymphoid structures and also in the prognosis of some cancers like breast and ovarian cancers which are associated with TME [97].

A study disclosed the role of PKM2 in B-cell activation, in which homocysteine upregulated the expression of enzymes involved in both glycolysis and oxidative phosphorylation activating B-cell by shifting the glycolysis towards pentose pathway. PKM2 inhibitor like shikonin or knockdown of PKM2 attenuated Hcy mediated metabolic changes, B-cell proliferation and antibody synthesis (IgM & IgG) *in-vitro* and *in-vivo* implies the role of PKM2 in B-cell activation. The in-depth analysis showed the involvement of Akt dependent mTOR signaling, whereas the treatment with mTOR inhibitor (rapamycin) and shikonin attenuated Hcy induced

metabolic changes and ultimately diminished atherosclerotic lesion [98]. These findings conclude that PKM2 activation is involved with the B-cell activation which is necessary for the immune response.

### Macrophages

Macrophages are considered as a protective and pathogenic driver for an immune response [99]. Their activation is classified as classic vs. alternative or also M1 and M2. M1 stimuli e.g. Granulocyte-macrophage colony-stimulating factor (GM-CSF), Toll-like receptors (TLRs), IFN- $\gamma$ , LPS or TNF that show Th1 dependent response whereas M2 stimuli e.g. macrophage colony-stimulating factor (M-CSF), IL-4, IL-10, IL-13, and glucocorticoids show Th2 dependent response [100]. They perform wide range of functions involving some receptors and different molecules like TLR (Toll-like receptors, intracellular pattern recognition receptors), nitric oxide (NO), reactive oxygen species (ROS), various types of inflammatory

markers like TNF $\alpha$ , cytokines, chemokines, tissue-damaging proteases, and different types of interferon [101].

In macrophages, PKM2 act as a critical modulator for the production of cytokines as well as inflammatory markers. An endotoxin named lipopolysaccharide (LPS), which is a *Toll-like receptors 4 (TLR4) agonist* activates M1 macrophages by increasing the three-fold expression of PKM2. These activated macrophages induce the inhibition of HIF-1 $\alpha$  and modulate the differentiation of T-helper cells. Activators of PKM2 like DASA-58 and TEPP-46 promote tetramer form, inhibits the IL-1 $\beta$  production and boost up the production of IL-10 *in-vivo* [102, 103]. Already studies had proved that over-production of ROS by glucose metabolism has a positive correlation with various types of pathologies like diabetes, cancer and neurodegenerative diseases. Glucose metabolism increases the cellular ROS by means of several mechanisms like glucose autoxidation, polyol pathway, and glycation [104]. Overconsumption of glucose drives up the glucose-ROS-PKM2-STAT3 pathway which led to the increased expression of various cytokines (IL-6, IL-1 $\beta$ , TNF- $\alpha$ ). Thus, ultimately resulting in the hyper-inflammatory macrophages leading to the host damage [105]. So, the glucose-rich tumor niche can even activate the macrophages in the TME and may encourage the tumor inflammation by releasing cytokines promoting tumor progression. 5' AMP-activated protein kinase or AMPK (5' adenosine monophosphate-activated protein kinase) is an enzyme that has a role in energy metabolism, inflammation, and cell homeostasis. Like p53 and liver 9 kinase B1 (LKB1), activation of AMPK was also closely related to tumor suppressor function [106]. However, deficiency of AMPK upregulates PKM2 expression which results in the release of HMGB1 and lactate production in macrophages. But, administration of the shikonin reduces the lactate production and HMGB1 release [107]. Even, AMPK shows the protective effect by modulating PKM2 expression. Plumbagin plant-derived naphthoquinone also showed the similar actions of PKM2 inhibitors decreasing the lactate production and pro-inflammatory cytokines expression in lipopolysaccharide (LPS) activated macrophages [108].

Apart from oxidation, acetylation, and phosphorylation of PKM2 dimeric form, succinylation is another factor that promotes PKM2 dimer transition. SIRT5 overexpression predicts the poor survival of non-small cell lung cancer patients [109]. In LPS activated macrophages, PKM2 act as a substrate for SIRT5 and regulates the hyper succinylation and inhibit the PKM2 activity by upregulating its transition from tetrameric to dimeric form. It promotes PKM2 nuclear accumulation where it complexes with HIF1 $\alpha$  and promotes transcription of inflammatory cytokines like IL-1 $\beta$ . But, PKM2 activators like TEPP-46 attenuates IL-1 $\beta$  production and macrophages activation in SIRT5 deficient mice [110] indicating the role of nuclear PKM2 in the activation

of macrophages. Further, the  $\omega$ -Alkynyl arachidonic acid treatment decreased the expression of macrophages M1 biomarkers (TNF- $\alpha$ , CXCL10, iNOS, and IL-6) and displayed a cardio protective effect by upregulating PKM2 expression in a mouse model of myocardial infarction. But downregulation of nuclear expression of PKM2 in LPS activated macrophages was observed. [111]. Thus, a further investigation is needed to understand the effect of  $\omega$ -alkynyl arachidonic acid on the nuclear accumulation of PKM2 in cancer cells as its nuclear accumulation has a major oncogenic role in cancer progression. Altogether, PKM2 acts as a pivotal regulator in cytokines production, tumor inflammation and macrophage activation involved with the maintenance of TME.

### Dendritic Cells

Dendritic (DC) cell is a bone marrow-derived leukocytes, as well as a potent antigen-presenting cell type. Paul Langerhans in 1968, was the first person in observing DC cell in human skin. Major subtypes in human DC includes myeloid DCs (e.g. Dectin1 and Dectin2), *Plasmacytoid DCs* (e.g. *CD304 (neurophilin)*, *CD123 (IL-3R)*) and *monocytes related* (e.g. *CD<sup>+</sup>16 monocyte*, *CD16 negative and inflammatory DC*) [112]. These are mainly involved in antigen presenting and processing of antigen. These cells are also found in the TME but are not sufficient to stimulate an immune response due to their disturbed function by hypoxia and inflammation in TME [113].

Aqueous extract of *Artemisia iwayomogi* (AIP1) (a member of the Compositae family) inhibited the differentiation and maturation of bone marrow-derived dendritic cells that consequences the suppression of immunological responses. Proteomic analysis and RT-PCR profiling revealed that AIP1 treatment downregulated the expression of various proteins like PKM2, TRAF 5-like protein, coactosin-like protein 1, and allogeneic stimulation ability of T cell by dendritic cell (CLP1) in BALB/c mice [114]. Thus, this drug can have the potential in reversing the aerobic glycolysis by targeting PKM2. SRSF3 is a Splicing factor encoded by *SFRS3* gene was found to aberrantly overexpress in various cancers like ovarian cancer, triple-negative breast cancer and malignancy of mammary epithelial cells [115]. Treatment with trichostatin A, a broad range of histone deacetylase possesses immunomodulatory and protective function by upregulating the expression of SRSF3-PKM2-dependent glycolytic pathway in DC2.4 cells. Silencing of PKM2 by shRNA effects the cytokines expression and lactate production but trichostatin re-treatment restores partially the DC cell function [116]. This shows that PKM2 is involved in the immune response and the proper functioning of DC during antigen processing.



### Myeloid-Derived Suppressor Cells (MDSCs)

Myeloid-derived suppressor cell is a myeloid originated type of cell and are considered to be a potent suppressor of the various function of T-cell as well as macrophages. These are the population of immune cells which are classified under the inhibitory immune cells influencing the development of T regulatory cell and inhibit CD8<sup>+</sup> T-cell activation by controlling nitric oxide synthase in arginase dependent manner [117].

Wei-R et al., studied the non-canonical role of PKM2 in MDSCs in hepatocellular carcinoma in which PKM2 upregulates the recruitment of Gr-1<sup>+</sup>CD11b<sup>+</sup> granulocytic MDSCs, F4/80<sup>+</sup>CD11b<sup>+</sup> macrophages, and Ly6C<sup>+</sup>CD11b<sup>+</sup> monocytic MDSCs in the TME. High levels of MDSC in bone marrow, peripheral blood and spleen have a role in the formation of a pre-metastatic niche in pulmonary foci. [118]. This shows that PKM2 has a role in designing the metastatic niche. Concanavalin-A induced immune-dependent liver injury involving TNF and IL-6 pathways triggering liver proliferation [119]. A similar study demonstrated the role of PKM2 in immune-mediated liver injury induced by Concanavalin A in mice. There was a significant correlation with increased plasma levels of PKM2, ALT, IL-1 $\beta$ , and IL-6 and hepatic myeloid cell infiltration and necrosis which was reversed by TEPP-46 [120]. This further gives an additional role to dimeric PKM2 in inducing liver injury.

### NK Cells

NK cell is a type of lymphocytes derived from a common progenitor cell as B and T cells. NK cells are part of the innate immune system which spontaneously responded to cytolytic effect toward cancer and virus-infected cells. Activation of NK cell results in the release of various cytokines (IFN- $\gamma$ , TNF- $\alpha$ , GM-CSF) and chemokines (CCL1-5 and CXCL8) [121]. In Humans, NK cells functionally categories according to the expression of CD56 and CD16 marker. CD56<sup>low</sup>CD16<sup>+</sup> NK cell type kill by means of produce cytokines by specific manners of recognition of target cells while CD56<sup>hi</sup>CD16<sup>-</sup> NK cells type (major NK cell subtype of peripheral lymphoid organs) produce a large number of cytokines (IFN- $\gamma$ ), TNF and granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulation by pro-inflammatory cytokines and attain cytotoxicity for a sustained period [122]. Innate cytotoxic lymphocytes or natural killer cells also infiltrate towards TME.

NK cell functionally exists as licensed and unlicensed NK cells based on the presence of MHC-1 molecule. Licensed NK cell in which cell-specific Ly49 receptor interaction exists with self MHC-1 while unlicensed NK cell lacks Ly49 receptor and show impairment in MHC-1 cell recognition [123]. PKM2 is a crucial enzyme which gets upregulated during the

differentiation of licensed NK cells and cross-links with KIR (killer cell immunoglobulin-like receptor) increasing the expression of phosphorylated metabolic modulators like p38- $\alpha$  and AMPK $\alpha$ . Moreover, licensed NK cell showed high metabolic reprogramming in glycolysis and OXPHOS dependent glutaminolysis while the unlicensed NK cells are exclusively reliant on OXPHOS for cytolytic function [124]. Evolutionary, HMGB1 protein was conserved as a regulator for cell death and survival process. It is a nuclear chromatin protein [125] derived from the NK cells was found to be an allosteric inhibitor of tetramer PKM2 by competing with its FBP binding sites thus blocking the Warburg effect in cancer cells. But, by amassing the glycolysis through dimer PKM2 and glutaminolysis, tumor cells can acquire resistance to HMGB1 induced metabolic cell death [126].

### Fibroblasts

Cancer-associated fibroblasts (CAFs) in tumor environment has a very well familiar role in tumorigenesis which includes tumor initiation and progression, metastasis, stimulate angiogenesis, and chemotherapy resistance [127]. These include matrix components (collagen I-II, fibronectin, tenascin C and periostin), enzymes (MMP, LOX, and TIMPs), cytokines, growth factor receptors (GF $\beta$ , VEGF, PGE2, CTGF, SDF-1), and cytoplasmic proteins (desmin, vimentin,  $\alpha$ -SMA). Recently, a new phenotypic marker of CAF like caveolin-1 (Cav-1) and MCT4 were documented [128, 129]. Normally, myofibroblasts get activated in the wound healing process. But, in TME, residential fibroblasts gets differentiated to myofibroblasts known as CAFs by direct or indirect signaling crosstalk between fibroblast and cancerous cells using paracrine signals. Signaling molecules that involved in activation of fibroblast include various cytokines like TGF $\beta$ , IL-1 $\beta$  [130]. Activated myofibroblasts show deregulated MMPs and increased the level of ROS that increases high chances of organs fibrosis and development of tumorigenesis and malignancy [131].

Primary culture of MCKH (mammary carcinoma from KH) cells shows the similar properties of CAFs like EMT, cancer stem cell-like features and also displayed many upregulated glycolytic enzyme genes including GAPDH, LDH, PGAM1, HIF1- $\alpha$ , and PKM2. This enrichment of CAF features suggested to occur via the dependence of FGF signaling [132]. US28 signaling activates HIF-1 $\alpha$ /PKM2 axis, in turn promoting proliferation, angiogenesis, and energy reprogramming in cancer cells. It upregulated the expression of many proteins including VEGF, HIF1 $\alpha$  and many glycolytic enzymes like GAPDH, PKM2 in fibroblasts and glioblastoma cells [133]. FGFR1 expression also has a positive correlation with the p-PKM2 and p-LDHA which are having the most suitable diagnostic potential in thyroid malignancies in thyroglobulin negative thyroid cancer [134]. A

contradictory study displayed that PKM1 expression in fibroblasts induced inflammation and increase lactate production (~1.5) fold higher than PKM2 containing fibroblast when co-injected with MDA-MB-231 in a xenograft model. PKM2 expressed fibroblasts triggered pseudo-starvation in stromal cells with NF- $\kappa$ B dependent upregulation of Beclin-1 and Cathepsin-B. They also found the loss of Cav-1 expression, which is a marker of autophagy and stromal glycolysis [135]. Dysregulated expression of caveolin-1 was reported to be associated with various cancers due to the aberrant activation of JAK/STAT, JNK, estrogen and src signalings in pancreatic cancer [136]. It is even reported that acute loss of caveolin-1 results in mitochondrial dysfunction, increased ROS, and stimulation of aerobic glycolysis. Oxidative stress in fibroblasts is capable of genetic instability and creates an oncogenic/mutagenic effect to reprogramming fibroblast. Treatment of fibroblasts with anti-oxidants likes N-acetylcysteine and quercetin) or NO inhibitors (L-NAME) reversed the aggressive behavior of CAFs in MCF-7 breast cancer cells [137].

A study found that cancer-associated fibroblasts have the ability to transfer high quantity proteins (PKM2, SOD2, thio0redoxin, malate dehydrogenase, and galectin-1) and lipids to human prostate cancer cells (DU145) through ectosome mediated delivery. But, a reverse transfer from cancer cell to fibroblast is negligible or absent and even the rate of protein transfer depends upon the donor cell and recipient cell line co-culture ratio [138]. miR-21 is a single-stranded micro-RNA (miRNAs) in human encode by *MIR21* gene present on chromosome number 17q23.2. Various experimental data show that overexpression of miR-21 is a feature of pathological cell growth or cell stress which target various survival signaling pathway like STAT3, MAPK, AKT, and AP-1 [139]. miR-21 mediated remodeling of CAFs metabolic changes was also reported in the pancreatic cancer cell. CAF showed high expressions of LHDA, PKM2, and miR-21 during the co-culture of CAFs with human pancreatic cancer cells (BxPc-3). Treatment (miR-21 inhibitor) of CAFs and co-culture showed a significant reduction in the expressions of PKM2, LDHA, and MCT [140]. Similarly, a study report suggested miR-21 regulation of PTBP1 in the fibroblasts of pulmonary arterial walls. Knockdown of PTBP1, treatment with PKM2 activator (TEPP-46) and histone deacetylase inhibitors reversed glycolytic phenotypes and decrease cell proliferation [141]. An additional study is needed to explore the role of PTBP1 knockdown to target PKM2 during angiogenesis. Another miRNA named miR-205 regulates development, apoptosis, and cancer. It is found that miR-205 has the ability to suppress EMT by directly targeting VEGF-A and ErbB3 in breast cancer [142]. CAFs upregulate PKM2 and allows its nuclear accumulation of PKM2 that form complex with HIF1 $\alpha$  that recruits transcriptional Chondrocytes-1 (DEC1). Chondrocytes-1 (DEC1) that downregulate the

expression of miR205. Treatment with metformin and DASA-58 decreased the metastatic potential of the prostate cancer cell in SCID mice [143]. These all findings suggest the role of PKM2 in promoting glycolysis, proliferation, and remodeling of CAFs.

### Endothelial Cells

Endothelial cells are specialized types of cells that form the inner lining of blood vessels and play a crucial role in many physiological processes including to regulate vasomotor tone, blood cells, and WBCs trafficking, to uphold blood fluidity, angiogenesis, and both innate and adaptive immunity [144]. Vascular endothelial cells are the main key players in cellular homeostasis, normal tracking of leukocytes and in tumor development. In normal and cancer cells, soluble factors like chemokines, platelets derived factor, vascular endothelial factor (VEGF), EGF, and HIF1- $\alpha$  are important to provoke the endothelial proliferation, differentiation into neovascularisation process [145, 146].

Hypoxia-induced PKM2 expression in tumor results in the activation of HIF1- $\alpha$  and NF- $\kappa$ B signaling subsequently triggering the secretion of VEGF-A factor. Deletion of PKM2 impaired tumor proliferation, induced apoptosis and decrease blood vessel formation in *in-vitro* (pancreatic cancer cell lines) and *in-vivo* (tumor xenograft on chicken chorioallantoic membranes) [147]. Even the circulating PKM2 stimulates angiogenesis, proliferation, migration and cell-ECM junctions in endothelial cells of colon xenograft cells (SW620) [146]. A positive correlation was observed with PKM2 upregulation and aberrant expression of VEGF-C in breast cancer. The study confirmed that knock-down of PKM2 downregulates the expression of VEGF-C and inhibit proliferation, metastasis and lymphovascular invasion [148]. Further, analysis of 65 patients with lung adenocarcinoma found a significant relationship between PKM2 expression and proliferation, invasion and metastasis. siRNA mediated knock-down of PKM2 result in decreased glucose uptake (25%), ATP formation (20%), synthesis of fatty acids and down-regulated MMP-2 and VEGF expressions in A549 cell lines and patient specimens [149]. PKM2/HIF-1/vGPCR signaling pathway promotes the angiogenesis and tumor growth in Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8). PKM2 controls the paracrine angiogenesis (VEGR dependent) and proliferation through vGPCR in KSHV infection of an endothelial cell. Upregulation of HIF-1 results in increased expression of angiogenic promoting factors like VEGF, PDGF, TGF $\alpha$ , TGF $\beta$ , ANGPT2, and ANGPTL4 [150].

A study found that TCM treated with lymphatic endothelial cells (LEC) promotes the growth of tumor *in-vitro* and *in-vivo* by releasing the excessive amount of EGF and PDGF-BB. PDGF-BB, in turn, stimulates the pericyte infiltration and angiogenesis in breast cancer [151]. Even the accumulation of the end product of glycolysis, i.e. lactate, due to the low

enzyme activity of PKM2 contributes to the formation of neovessels by the inhibition of prolyl hydroxylase 2, activation of HIF-1-NF- $\kappa$ B signaling pathway and stimulation of interleukin (IL-8/CXCL8) synthesis which enhances the endothelial proliferation and maturation of mature blood vessels [146]. miR-143, a short non-coding mRNA regulates various genes and has a role in various cancers like oesophageal squamous cell carcinoma, gastric cancer, and gall bladder and acts as a tumor suppressor [152]. Its overexpression results in a significant decrease of glycolytic enzymes like PKM2, LDHA, and HK2 in atherosclerotic plaque forming endothelial cell lines [153]. Similarly, miRNA-124 was found to be a tumor suppressor by acting through diverse mechanisms in various cancers like breast, bone and colorectal cancer [154]. It was examined that, there exists a positive correlation between miRNA-124 and a splicing factor, polypyrimidine-tract-binding protein (PTBP1) which is a PKM2 alternative splicing factor. They found that overexpression of miRNA-124 silences the PTBP1 expression resulting in the restoration of dysregulated glycolysis and mitochondrial respiration [155]. These suggest that PKM2 is involved with the proliferation and metastasis of endothelial cells.

### Adipocytes

Adipocytes are mainly involved in storing the energy as fats and triglycerides in lipid droplets. In mammals, two types of Adipocytes are present i.e. brown adipocytes which have a large number of mitochondria and involved in maintaining the body temperature while white adipocytes have large lipid droplets and are mainly involved in the storage of fats. A proper balance between brown and white fat is required for maintaining the energy homeostasis [156]. Adipocytes play a role in the development of some cancers like intra-abdominal tumors by means of providing homing to cancer cells, by releasing adipokines which includes interleukins-8 (IL-8) and provide direct transfer of fatty acids to cancer cells. Obesity is considered as one of the risk factors for breast cancer development by chronic low-grade inflammation, macrophages recruitment and upregulation of several signaling pathways like AMPK, p53 and HIF, etc. [157].

Proteomic data by 8-plexi TRAQ-2DLC-MS/MS analysis shows the involvement of 549 proteins in adipogenesis. Among them, PKM2, C/EBP $\beta$ , and PIN1 are found to have a link with the PI3K/AKT pathway. But, knockdown of C/EBP $\beta$  downregulated the PKM2 expression at RNA and protein level displaying the role of PKM2 in adipogenesis [158]. Leptin promotes the EMT of breast cancer cells by targeting the PI3K/AKT pathway via PKM2 over-expression. But, leptin-induced PKM2 expression and metastasis markers were abolished by PI3K/AKT inhibitor LY294002 and PKM2 knockdown *in-vitro* and *in-vivo* [159]. Overexpression of PTP1B, a member of protein tyrosine phosphatase (PTP)

family was observed in 70% of TNBC cases [160]. Reports suggest that PKM2 acts as a novel substrate for PTP1B, and downregulated PTP1B expression increased Tyr105 phosphorylation of PKM2 in *in-vivo* and cultured adipocytes [161]. All these findings strengthen the role of PKM2 in adipogenesis.

### Therapeutics Targeting PKM2

In recent years, apart from other glycolytic enzymes (LDH, hexokinase, and enolase), PKM2 comes out as an emerging and potential target for anticancer therapy. In fact cancer cells utilize more amounts of glucose and due to the existence of dimeric PKM2, it accumulates the upstream substrates of glycolysis, activating one carbon metabolism a feature that assists the cancer progression [162]. As discussed earlier, even the nuclear PKM2 controls and modulates various signaling pathway mediators (STAT3, histone H3, and HIF-1 $\alpha$ ) in various diseases like cancer, inflammatory disease, and diabetes. Still, a study lacked the evidence in displaying the protein kinase activity of PKM2 [163]. mTOR is a central factor in the warburg effect in inducing PKM2 expression [164]. Further, activation of PKM2 with the small molecules induced serine auxotrophy by allowing the cells not to get adapt with the nutrient stress [165]. So, PKM2 targeting shows a synergistic effect in cancer treatment by means of suppressing PKM2 dependent cancer proliferation and metastasis as well as in decreasing the macromolecules synthesis and lactate production. While some contradictory studies reported that PKM2 deletion accelerated mammary tumors formation in Brca1-drive model of breast cancer by disrupting PKM1 expression [166]. Even knockdown of PKM2 resulted in modest inhibition of tumor growth [167], developed hepatic cancer, metabolic stress [168] and soft tissue sarcoma [169] whereas the nuclear PKM2 induced the cell death in a caspase-independent manner [170] indicating PKM2 is not needed primarily for cancer proliferation [171]. Besides, its requirement is not needed in APC lost colon cancer model [172]. Based on all these studies, various drugs have been developed and recognized in stabilizing or promoting the PKM2 in its tetramer form as well as to inhibit the PKM2 activity directly. But, the drugs inhibiting PKM2 expression directly may have limitations, particularly the functional consequences associated with kinase inhibition and may even give other possible drawbacks. Firstly, there will be a suppressed immune response in the TME, as the immune cells (T-cell, B-cell) needs PKM2 for their activation and proliferation. Secondly, it influences the glycolytic pathway globally which may hamper the processes in which PKM2 plays a major role. So, it is desired to develop PKM2 activators rather than its inhibitors to limit these drawbacks. Some of the reported drugs targeting

**Table 1** Drugs targeting PKM2 at the preclinical and clinical stage

S.no	Drugs	Consequence	Condition	Signaling molecules effected	Outcomes and the status of the study	References
<b>Preclinical studies</b>						
1	Chaetoglobosin k	Inhibition	Cancer	PI-3K, AKT, mTOR	Inhibits cell growth, cell cycle arrest, and induces apoptosis	[173]
2	ZINCC08383544 (1,5,2-H-pyrrole-dione)	Inhibition	Lung cancer	PKM2	Anti-proliferative	[174]
3	4-hydroxy-thiozolidine-2-thione derivatives	Activation	Lung cancer and prostate cancer	PKM2	Arrests the G2/M Phase, Anti-proliferative activity	[175]
4	Naphthoquinone derivatives	Inhibition	Lung cancer	PKM2	Anti-proliferative effect, upregulation of Glut-1 and LDH-A	[176]
5	Lapachol	Inhibition	Melanoma cancer cell lines	PKM2	Promoted apoptosis and anti-proliferative	[59]
6	Pyridin-3-ylmethyl carbamodithioic esters	Activation	Human colon cancer cell lines	PKM2	Potent anti-proliferative	[177]
7	Shikonin	Inhibition	Breast cancer and lung cancer cell lines	PKM2	Promoted apoptosis and anti-proliferative	[178]
8	DASA-58	Activator	Lung cancer	PKM2	Anti-proliferative	[51]
9	TEPP-46	Activator	Colon carcinoma cells	PKM2	Inhibits cell growth and cell cycle	[179]
10	1-(sulfonyl)-5-(arylsulfonyl) indoline	Activator	Tumor cells	PKM2	Decreased cellular proliferation	[180]
11	2-((1 <i>H</i> -benzo[ <i>d</i> ]imidazo[1- <i>y</i> ]methyl)-4 <i>H</i> -pyrido[1,2- <i>d</i> ]pyrimidin-4-ones	Activator	Tumor cells	PKM2	Inhibits tumor growth	[181]
12	3-(trifluoromethyl)-1 <i>H</i> -pyrazole-5-carboxamide	Activator	Tumor cells	PKM2	Inhibits cell growth	[182]
13	ML265	Activator	Tumor cells	PKM2	Inhibits cell proliferation and cell growth	[183]
14	ML285	Activator	Tumor cell	PKM2	Inhibits cell growth	[184]
15	PA-12	Activator	Lung cancer	PKM2	Inhibits cell proliferation	[185]
<b>Clinical studies</b>						
1	TLN-232/CAP-232	Inhibition	Melanoma	PKM2	Phase II trials metastatic renal cell carcinoma but trails stopped due to legal reasons in 2010	[186]
2	Digoxin	Inhibition	Inflammatory response	PKM2	In future	NCT03559868
3	Fluorine F 18 DASA-23	Activation	Intracranial Neoplasm and Glioblastoma Malignant Mesothelioma	PKM2	In future	NCT03539731
				PKM2	In the future for the diagnostic purpose	NCT03558932



PKM2 at preclinical and clinical setup are discussed in Table 1.

It is clear from Table 1 that several molecules having an activator or inhibitory action on PKM2 have been reported. Some of them being more drug like and promising have moved to clinical phases while several others could not be taken further either because of poor drug likeness or lack of *in vivo* efficacies. Some metal based (2, Table 1), phenolics (4, 5, 7, 1, Table 1), and heterocyclics (6, 8–15, Table 1) are known to cause PKM2 inhibition and in terminating tumor progression. Some of the molecules like 8 and 13 which are heterocyclics have more likelihood to cross the valley of death because of better drug like features and it will be interesting to find them in the list of clinical candidates in near future. The clinical candidates have been successful in several ways and their future promise to lessen the burden of cancer is yet to be elucidated [187, 188].

## Concluding Remarks

In diverse cancers, PKM2 is known to act as a gatekeeper in regulating the tumor metabolism, proliferation, and its niche maintenance. Till date, the clear cut role of PKM2 involvement in the TME cells is not established appropriately. So, a comprehensive discussion on the role of PKM2 in the TME cells was clarified in this review. As this enzyme is a key metabolic conduit in the metabolism and proliferation of various body cells including immune cells, aiming it creates a major challenge. In this context, targeting PKM2 with activators rather than its inhibitors may provide a therapeutic effect with limited resistance, lesser immune suppression by consequently avoiding the functional consequences associated with kinase inhibitors. Further, these activators can be used in combination with the existed anti-cancer agents to achieve a synergistic effect inhibiting the tumor growth and proliferation. In addition, its involvement with the inflammatory cells was discussed in this report which may provide a new application for the PKM2 activators in drug repurposing. The upcoming approaches and investigations should be to improve the molecular studies on PKM2 with respect to its role in tumor niche cells and to understand the effect of therapeutics against PKM2 in developing resistance and immune suppression. For all these whys and wherefores, PKM2 signifies as a potential target in metabolic reprogramming of tumor.

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare no conflict of interest.

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