Spring 5-14-2021

Beyond Glycolysis: Pyruvate Kinase M2 Regulates Cancer Cell Glutaminolysis And Macrophage Polarization

Guangda Peng

BEYOND GLYCOLYSIS: PYRUVATE KINASE M2 REGULATES CANCER CELL GLUTAMINOLYSIS AND MACROPHAGE POLARIZATION

by

GUANGDA PENG

Under the Direction of Zhi-Ren Liu, PhD

ABSTRACT

Cancer cells alter their nutrition metabolism to cope the stressful environment. One important metabolism adjustment is that cancer cells often activate glutaminolysis in response to the reduced carbon from glucose entering into TCA cycle due to inactivation of several enzyme activity in glycolysis. An important question is how the tumor cells coordinate the changes of glycolysis and glutaminolysis. In this report, we demonstrate that the pyruvate kinase inactive

dimer PKM2 facilitates activation of glutaminolysis. Our experiments show that the dimer PKM2 plays a role in upregulation of mitochondrial glutaminase I. Growth stimulations activate activity of PKM2 in regulation of glutaminolysis. PKM2 dimer regulates the glutaminase expression by controlling the IRES-dependent c-myc translation. Growth stimulations promote PKM2 and c-myc IRES interaction. Our study reveals an important linker that coordinates the adjustments of the metabolism pathways in cancer cells.

Cancer controls its immunity by educating its microenvironment, including regulation of polarization of cancer associated macrophages (CAMs). It is well documented that cancer cells release PKM2 to facilitate tumor progression. We report here that extracellular PKM2 (EcPKM2) modulates cancer immunity by facilitating M2 macrophage in tumors. EcPKM2 interacts with integrin $\alpha_v\beta_3$ on macrophage to activate the integrin-PI3K signal axis. Activation of PI3K by EcPKM2 suppresses PTEN expression, which subsequently upregulates arginase-1 (Arg-1) expression in macrophage. Our studies uncover a novel and important mechanism for modulation of cancer immunity. More importantly, an antibody disrupting the interaction between EcPKM2 and integrin $\alpha_v\beta_3$ is effective in converting M2 macrophages to M1 macrophages in tumors, suggesting a new therapeutic strategy and target for cancer immunotherapies. Combination of the PKM2 antibody with checkpoint blockades and chemotherapeutics provides synergistically enhanced treatment effects.

INDEX WORDS: Pyruvate kinase M2, Glutaminolysis, C-myc, IRES, Macrophage, Polarization

BEYOND GLYCOLYSIS: PYRUVATE KINASE M2 REGULATES CANCER CELL GLUTAMINOLYSIS AND MACROPHAGE POLARIZATION

by

GUANGDA PENG

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

in the College of Arts and Sciences

Georgia State University

2021

BEYOND GLYCOLYSIS: PYRUVATE KINASE M2 REGULATES CANCER CELL GLUTAMINOLYSIS AND MACROPHAGE POLARIZATION

by

GUANGDA PENG

Committee Chair: Zhi-Ren Liu

Committee: Timothy Denning

Didier Merlin

Electronic Version Approved:

Office of Graduate Services

College of Arts and Sciences

Georgia State University

May 2021

DEDICATION

I would like to dedicate my dissertation to my family members. Without their sacrifices and support, I will not be able to have the chance to continue my study this far. Thank you for your love and care for all these years. I will always love you all.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, Dr. Zhi-Ren Liu, for his guidance and support during my Ph.D. study. He encouraged me and gave me a lot of valuable advices on my dissertation. I want to thank my committee members Dr. Timothy Denning and Dr. Didier Merlin, both of whom provided me many great suggestions and comments. I must give my special thanks to Dr. Jenny Yang because she motivated and helped me a lot. I need to thank Dr. Liangwei Li for his fundamental work and his help on my project. I also want to thank Dr. Vu Ngo for his helping with the flow cytometry experiments. Besides, I would like to thank all lab members from Dr.Zhi-Ren Liu's group for all the help and suggestions.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS V

1 INTRODUCTION 1

1.1 Cell Metabolism 1

1.2 Glycolysis and Warburg Effect 2

1.3 Glutaminolysis 3

1.4 Pyruvate Kinase 5

1.4.1 Pyruvate kinase muscle isozyme: PKM1 and PKM2 6

1.4.2 PKM2 expression regulation 7

1.4.3 PKM2 dimer and tetramer 7

1.4.4 The role of PKM2 in cancer cells 8

1.4.5 PKM2's functions and subcellular locations 9

1.5 C-Myc and GLS 13

1.6 Translation-protein synthesis 14

1.6.1 Cap-dependent and Cap-independent initiation translation 14

1.6.2 The internal ribosome entry site (IRES) translation 14

1.7 Cancer 15

1.7.1 Causes and statistics 15

1.7.2 Breast cancer and Triple-negative breast cancer (TNBC) 17

1.7.3 Lung cancer 17

1.7.4 Melanoma *18*

1.8 Cancer microenvironment **18**

1.8.1 Cancer microenvironment overview *18*

1.8.2 Immune cells in cancer microenvironment *19*

1.8.2.1 Innate immunity and adaptive immunity 19

1.8.2.2 Macrophages and macrophage polarity 20

1.8.2.3 T lymphocytes 22

1.8.2.4 Myeloid-derived suppressor cells 22

1.8.2.5 Neutrophils 23

1.8.3 Non-immune cells in cancer microenvironment *23*

1.8.4 Cytokines in cancer microenvironment *25*

1.8.5 Extracellular matrix *26*

1.9 Cancer therapy **27**

1.9.1 Cancer treatment types *27*

1.9.2 Immunotherapy *27*

1.10 Integrin **28**

1.10.1 Integrin family *28*

1.10.2 αvβ3 integrin *30*

1.11 The focuses in this study **30**

2 PYRUVATE KINASE M2 COORDINATES METABOLISM SWITCH BETWEEN GLYCOLYSIS AND GLUTAMINOLYSIS IN CANCER CELLS 33

2.1 Abstract 33

2.2 Introduction 33

2.3 Results 34

2.3.1 PKM2 dimer facilitates glutaminolysis in cancer cells 34

2.3.2 PKM2 dimer facilitates glutaminolysis by regulation of c-myc IRES-dependent translation. 35

2.3.3 PKM2 facilitates hnRNP L and hnRNP K interacting with c-Myc IRES 37

2.3.4 PKM2 dimer confers cancer cells glutamine addiction. 38

2.4 Discussion 39

2.5 Limitations of the Study 41

3 EXTRACELLULAR PKM2 MODULATES CANCER IMMUNITY BY REGULATING MACROPHAGE POLARITY 58

3.1 Abstract 58

3.2 Introduction 58

3.3 Results 60

3.3.1 EcPKM2 in patient tumors and secretion of PKM2 by cultured cancer cells 60

3.3.2 EcPKM2 interacts with integrin αvβ3 on macrophage and promotes M2 macrophage. 61

3.3.3 EcPKM2 increased M2 macrophages and decreased M1 macrophages in tumors 62

3.3.4 EcPKM2 activates integrin-PI3K signaling axis and upregulates Arg-1 in macrophage 64

3.3.5 PKM2 Ab synergistically enhances the efficacy of checkpoint blockades. 65

3.3.6 PKM2 Ab synergistically enhances efficacy of cancer chemotherapeutics. 66

3.4 Discussion 66

4 CONCLUSIONS AND DISCUSSIONS 83

4.1 The unique role of PKM2 in regulation of glutaminolysis 84

4.2 Could dimeric PKM2 regulate IRES-dependent translation of other genes? 86

4.3 Could dimeric PKM2 regulate glutaminolysis in non-cancer cell types? 87

4.4 What are the possible roles of PKM2 in IRES-dependent translation of c-Myc? 88

4.5 What is implicated by EcPKM2 modulating macrophage polarity? 89

5 METHODS AND MATERIALS 91

5.1 Reagents, Antibodies, and Cells 91

5.2 Primers 93

5.3 Cell culture 93

5.4 Construction of PKM2 and mutant expression, bicistronic, and HA-myc expression vectors 94

5.5 Glutamine consumption measurement 94

5.6 Biotinylation of c-Myc 5'-UTR 94

5.7 Western blot..... 95

5.8 RNA-pulldown assay and PKM2 immunoprecipitation..... 96

5.9 Identification of c-MYC IRES interacting proteins by Mass Spectrometry 97

5.10 Mass spectrometry data quantitative analysis 97

5.11 Animal model and treatment. 97

5.12 Flow cytometry. 98

5.13 Hematoxylin and eosin (H&E) staining 99

5.14 Immunohistochemistry staining. 99

5.15 Statistical calculations..... 100

APPENDIX: ECPKM2 REGULATES LUNG FIBROSIS DEVELOPMENT IN MICE BY MODULATING M2 MACROPHAGE POLARIZATION. 101

Appendix 1. EcPKM2 is involved in lung fibrosis progression..... 101

Appendix 2. EcPKM2 regulates the development of bleomycin-induced lung fibrosis in mice by modulating M2 macrophage polarization. 103

REFERENCES..... 105

LIST OF FIGURES

Figure 1 Dimer PKM2 regulates glutamine metabolism. 43

Figure 2 PKM2 regulates c-myc expression via translational control. 45

Figure 3 PKM2 Regulates c-myc Expression by IRES-Dependent Translation 47

Figure 4 PKM2 Mediates Cancer Cell Glutamine Addiction under Growth Stimulation 49

Figure 5 Expression, pyruvate kinase activity, and dimer/tetramer status of PKM2 TM mutant. 51

Figure 6 PKM2 regulates c-myc and GLS expression and glutamine consumption in breast cancer M4A4 cells. 53

Figure 7 PKM2 interacts with hnRNP L/K and c-Myc IRES. 55

Figure 8 Schematic diagram of the proposed mechanisms of extracellular PKM2 promoting lung fibrosis progression. 57

Figure 9 High level of EcPKM2 expressed in cancer. 70

Figure 10 Macrophage expresses αvβ3, EcPKM2 interacts with αvβ3 on macrophage. 72

Figure 11 PKM2 can induce macrophage into M2 type. 74

Figure 12 PKM2 can induce macrophages into M2 type in 4T1 mouse model. 76

Figure 13 PKM2 Ab can reduce M2 macrophage level in 4T1 mouse model. 78

Figure 14 PKM2 and PKM2 Ab also work in mouse models of melanoma and lung cancer. 80

Figure 15 PKM2 induces macrophages to M2 type through αvβ3, PI3K and PTEN. 82

LIST OF TABLES

Table 1 Reagents, antibodies, and cell lines 91

Table 2 primer sequences..... 93

1 INTRODUCTION

1.1 Cell Metabolism

Cancer cells reprogram their nutrition metabolism to meet their high bioenergetic and biosynthetic demands in support of their rapid growth and continuous proliferation (Phan, Yeung, & Lee, 2014). Both glycolysis and glutaminolysis are altered in cancer cells (Akins, Nielson, & Le, 2018). Glycolysis is a universal pathway used by all living cells for energy production, which can occur in both aerobic and anaerobic states. However, about a century ago, Otto Warburg first discovered that the glycolysis rate is 200 times higher in cancer cells compared to healthy cells; and cancer cells predominantly undergo anaerobic glycolysis even whether oxygen is present (Akins et al., 2018; Warburg, 1924). This phenomenon is later termed the Warburg effect. After its discovery, scientists have come up with many theories for why cancer cells switch to inefficient anaerobic glycolysis. One widely accepted theory is that the Warburg effect allows cancer cells to maintain large pools of intermediates which provide building blocks for synthesis of nucleotides, fatty acids and amino acids, assisting the rapid proliferation and growth of cancer cells (Liberti & Locasale, 2016).

Warburg effect limits the entry of pyruvate into the TCA cycle in cancer cells. To compensate for the reduction in carbon coming from glycolysis, cancer cells switch to the glutaminolysis which utilizes glutamine to replenish the TCA cycle (Anderson, Mucka, Kern, & Feng, 2018). In addition, glutaminolysis also provides reducing power by converting glutamate to glutathione (GSH), the most abundant antioxidant in mammalian cells in handling oxidative stress (Anderson et al., 2018). Elevated glutaminolysis has been recognized as a critical hallmark of cancer (Cluntun, Lukey, Cerione, & Locasale, 2017). Cancer patients have much lower concentrations of glutamine in their blood circulation compared to healthy people (Cluntun et al.,

2017). It has been shown that the reduction in glutamine consumption can restrain the cancer progression to some extent (Jiang, Srivastava, & Zhang, 2019). Glutamine, the most abundant amino acid in blood circulation, is beneficial for cancer cells as it supplies a great amount of carbon and nitrogen for macromolecule biosynthesis and maintenance of redox balance (Alberghina & Gaglio, 2014; Kodama et al., 2020; Souba, 1993). By this means, with increased glutamine converted via glutaminolysis, cancer cells display enhanced cell proliferation rate and reduced cell death (Matés, Pérez-Gómez, de Castro, Asenjo, & Márquez, 2002).

Glutaminolysis as well as glycolysis in cancer cells have been extensively studied, but rare attention has been put on how the cancer cells coordinate these two metabolic pathways. Finding out how the cancer cells coordinate these two pathways might be critical for finding cures for cancer.

1.2 Glycolysis and Warburg Effect

Glycolysis is one of the most important metabolic pathways in most kinds of organisms. Due to the wide occurrence of glycolysis in different species, we know it is an ancient metabolic pathway (Fothergill-Gilmore & Michels, 1993). It was first studied in the nineteenth century; however, it took scientists around 100 years to fully understand how it works in normal cells. Glycolysis converts glucose into pyruvate and generates free energy which can be used to form energy molecules ATP and NADH (X. B. Li, Gu, & Zhou, 2015). A sequence of 10 enzyme related reactions is involved in glycolysis, including Hexokinase, Phosphoglucoisomerase, Phosphofructokinase, Aldolase, Phosphotriose isomerase, Glyceraldehyde 3-phosphate dehydrogenase, Phosphoglycerate kinase, Phosphoglycerate mutase, Enolase and Pyruvate kinase (X. B. Li et al., 2015).. Therefore, a lot of intermediates are produced during the reactions, including glucose-6-phosphate (PPP, glycogen metabolism), Fructose 6-phosphate (PPP),

Fructose 1,6-bisphosphate, Glyceraldehyde 3-phosphate, Dihydroxyacetone phosphate, 1,3-Bisphosphoglyceric acid, 3-Phosphoglyceric acid, 2-Phosphoglyceric acid, Phosphoenolpyruvate, and pyruvate. The intermediates produced at each step can be used for other reactions by leaving glycolysis and entering different metabolic pathways (X. B. Li et al., 2015; Vaupel, Schmidberger, & Mayer, 2019).

The glycolysis metabolic pathway is an oxygen-independent pathway. In most cases, the glycolysis occurs in cytosol, and it normally can be divided into 2 phases, the investment phase and the pay-off phase (Lunt & Vander Heiden, 2011). The investment phase involves ATPs consumption, but the pay-off phase has ATP production. Overall, the glycolysis will produce the net energy ATPs along with NADH molecules and pyruvates (Lunt & Vander Heiden, 2011). Cells rely on glycolysis to get sufficient energy for survival. In cancer cells, glucose metabolism is altered (DeBerardinis & Chandel, 2016; Pavlova & Thompson, 2016). Though it consumes more glucose molecules, less amount of pyruvate enters the TCA cycle (Liberti & Locasale, 2016). This phenomenon has been reported and evidence has shown that it is because some enzymes are altered. One well-studied mechanism is that, in cancer cells, pyruvate kinase switches from high enzymatic tetramer form to lower enzymatic dimer form, which leads to a decrease in production of pyruvates (W. Yang & Lu, 2015). The advantage of such metabolic alteration in cancer cells is that cancer cells use this mechanism to synthesize more building blocks for their proliferation (Vander Heiden, Cantley, & Thompson, 2009).

1.3 Glutaminolysis

In human blood circulation, there are a lot of different amino acids, cells can uptake these amino acids and are used for different purposes. Glutamine is the most abundant amino acid in the

blood circulation (J. Zhang, Pavlova, & Thompson, 2017). Glutamine can come from various dietary sources, especially the protein-rich foods like beef, chicken, fish, eggs, and beans etc.

Once up-taken by the cells, Glutamine can be lysed to glutamate, aspartate, CO2, pyruvate, and citrate. This process is called glutaminolysis (McKeehan, 1982). The whole process involves some steps from the citric acid cycle and the malate-aspartate shuttle. Glutaminolysis is an important energy source for cells, especially in highly proliferating cells like lymphocytes, thymocytes, adipocytes and cancer cells (Song, Wei, Lu, Li, & Chen, 2017; Lifeng Yang, Venneti, & Nagrath, 2017). In the cancer cells, due to the Warburg effects, the carbon source coming from glucose which enters into the TCA cycle is much reduced (Jin, Alesi, & Kang, 2016). Though this change can increase the building blocks for cell proliferation purposes, the energy from glucose is reduced. To overcome this disadvantage, the cancer cells utilize glutamine as one of the major energy sources (Jin, Alesi, & Kang, 2016). High concentration of extracellular glutamine is beneficial for the tumor growth (Cluntun et al., 2017).

The glutaminolysis can produce ATPs in many steps (McKeehan, 1982). It can generate one ATP from direct phosphorylating GDP; then it can also give two ATPs by oxidating FADH2; lastly, it can produce three ATPs from α-ketoglutarate dehydrogenase reaction (McKeehan, 1982).

There are a lot of advantages for cancer cells to use glutaminolysis. Firstly, as we said, since the glutamine is the most abundant amino acid in the blood circulation, cancer cells have a big energy pool (Chen et al., 2019; Newsholme et al., 2003). Secondly, the degradation products of glutamine can be used as sources of biosynthesis, for instance, glutamate and aspartate are precursors for nucleic acid synthesis (Cruzat, Macedo Rogero, Noel Keane, Curi, & Newsholme, 2018). Thirdly, in the cancer cells, there are a lot of reactive oxygen species, and glutaminolysis is insensitive to the high ROS in cancer cells (Kumari, Badana, G, G, & Malla, 2018; Panieri &

Santoro, 2016). Fourthly, glutaminolysis is beneficial for the synthesis of fatty acids and cholesterol (Phan et al., 2014). Lastly, since glutaminolysis can produce glutamate and fatty acids, and studies have shown that glutamate and fatty acids are immunosuppressive, by releasing the produced glutamate and fatty acids, cancer cells can protect themselves from immune attack (Boodhoo, Kamble, Sharif, & Behboudi, 2020; Z. Wang et al., 2020).

1.4 Pyruvate Kinase

Pyruvate kinase is the enzyme catalyzing the conversion of phosphoenolpyruvate (PEP) to pyruvate, the last step of glycolysis (W. J. Israelsen & Vander Heiden, 2015). It is a rate-limiting step and produces one molecule of ATP. The pyruvate can further be broken down to carbon dioxide to make more ATP molecules in aerobic respiration when oxygen is present through subsequent steps of pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation, or it can be converted to lactic acid or ethanol when oxygen is no available(W. J. Israelsen & Vander Heiden, 2015).

Pyruvate kinase has four isoforms L, R, M1 and M2. These four isoforms are tissue specific (Gupta & Bamezai, 2010). The L isoform is expressed in liver tissue while R isoform is specific in erythrocytes, both of which are encoded by the same gene PKLR. There are two distinct conformational states of PKL and PKR: the R state and L state (Mattevi, Bolognesi, & Valentini, 1996). The R state is an active form with a high affinity for the substrate while the T-state is a less active form with the low affinity for the substrate. Therefore, the R-state plays a role in glycolysis, but not the T-state.

M1 and M2 isoform are alternative splicing products of the same PKM gene which contains 12 exons and 11 introns (Morgan et al., 2013). The M1 isoform is dominantly present in muscle

and brain tissues while PKM2 isoform is usually found in early fetal tissue. The PKM gene M1 isoform has exon 9 while M2 isoform has exon 10 (M. Chen, Zhang, & Manley, 2010). Therefore, the sequences of M1 and M2 are almost the same except for the part which is encoded by the different exon. Interestingly, PKM2 has non-metabolic roles and is also found in fast-growing cells like tumor cells due to its non-metabolic roles (Dong et al., 2016). It is reported that PKM2 is intensively involved in tissue repair and regeneration (Y. Zhang, L. Li, Y. Liu, & Z.-R. Liu, 2016).

1.4.1 Pyruvate kinase muscle isozyme: PKM1 and PKM2

Both PKM1 and PKM2 have tetrameric form which consists of four identical subunits (or monomers). Each monomer contains A, B, C, and N-terminal structural domain (P. Wang, Sun, Zhu, & Xu, 2015). The monomer dimerizes to form dimer, which further dimerizes to form tetramer. The dimer-dimer interaction interface is localized at the C domain on the monomer, which happens to be at the site of the differential sequence between PKM1 and PKM2. Therefore, tetramer formation of PKM1 and PKM2 is different, PKM1 is always present as a tetramer while PKM2 can be in the form of tetramer or dimer (Wong, Ojo, Yan, & Tang, 2015). In addition, the difference in sequence also has an impact on allosteric regulation of two isoforms. Domain C is where allosteric activator FBP binds. FBP can directly bind with PKM2, leading to the conformational change of the enzyme, thus the enzymatic activity of pyruvate kinase is enhanced (Gui, Lewis, & Vander Heiden, 2013). While FBP does not bind PKM1 so it does not affect PKM1 activity. FBP is the intermediate product in the glycolytic pathway, and it serves as a feedforward stimulator by regulating pyruvate kinase (Gui et al., 2013). In addition to GBP, PKM2 can be activated by serine and succinylaminoimidazolecarboxamide ribose-50 phosphate SDH succinate dehydrogenase (SAICAR)(Keller, Doctor, Dwyer, & Lee, 2014). There are also inhibitors which suppress enzymatic activity of PKM2, such as L-Cysteine, pyruvate, P-tyrosine, phenylalanine,

alanine, adenosine triphosphate, and thyroid hormone T3 (Z. Li, Yang, & Li, 2014). PKM2 activity also can be regulated by post-translational modification, such as phosphorylation, acetylation and oxidation, which reduce the activity PKM2 (Chaneton & Gottlieb, 2012). Another difference between PKM1 and PKM2 is energy production and intermediate utilization. PKM1 constitutively exists as the active tetramer to provide ATP for cells (Dayton, Jacks, & Vander Heiden, 2016). However, PKM2, when in active tetrameric form, mainly produce ATP; when in dimeric form, it accumulates glycolytic intermediates for the biosynthesis of different biomolecules such as amino acids, phospholipids and nucleotides (Méndez-Lucas et al., 2017).

1.4.2 PKM2 expression regulation

The alternative splicing can be regulated by splicing factor family heterogenous ribonucleotide proteins (hnRNPs) including hnRNP 1 (or PTB), hnRNPA1, hnRNPA2 (Clower et al., 2010; Israelsen et al., 2013). The hnRNPs enter the nucleus and release exon 10 by binding to exon 9 and promote PKM2 expression. The 3 splicing factors are under the regulation of c-myc (David, Chen, Assanah, Canoll, & Manley, 2010). During hypoxia conditions, the HIF1-α upregulating PKM2 isoform through increasing hRNPs (Williams et al., 2018). Insulin is also reported to up-regulate the expression of M2 isoform (Iqbal et al., 2013). Studies show that EGF signaling leads to enhanced PKM2 expression via up-regulating PTB (W. Yang, Xia, Cao, et al., 2012). In addition, recent studies have revealed that PKM2 expression is also closely controlled by microRNAs (miRNA) (Z. Zhang et al., 2019).

1.4.3 PKM2 dimer and tetramer

The PKM2 has tetramer and dimer, which are composed of the same monomer, however, they are distinct in biological functions (P. Wang et al., 2015). The tetramer PKM2 is an active form of pyruvate kinase and regulates the glycolysis process, while the dimer PKM2 has very low

enzymatic activity (Dong et al., 2016). The dimer PKM2 and tetramer PK2 can switch between each other when interacting with various activators and inhibitors. The conversion from dimer to tetramer is considered as activation of PKM2 enzyme while the reversal process is considered as inhibition of PKM2 enzyme (P. Wang et al., 2015). For example, the high level of FBP can stimulate tetramerization of PKM2 and stabilize tetramer PKM2. When the level of FBP is low, PKM2 tetramer will dissociate to dimmer PKM2.

PKM2 is present in various cellular locations including cell cytosol, nucleus and extracellular space and perform many non-glycolytic roles at different subcellular locations (William J. Israelsen et al., 2013). When PKM2 enters the nucleus, it can modify gene regulation (X. Gao, H. Wang, J. J. Yang, X. Liu, & Z.-R. Liu, 2012). When it attaches to the outer membrane of mitochondrial, it can regulate mitochondrial function (Liang et al., 2017). When it can exist in the endoplasmic reticulum, it releases endoplasmic reticulum stress (Z. Z. Li, Wang, Liu, Li, & Wang, 2020). When it is secreted in extracellular space, it acts as a ligand and triggers intracellular signal pathways. Moreover, PKM2 can also be modified with phosphorylation, acetylation and other proteins, adding complexity of the function of PKM2 (Weiwei Yang et al., 2012).

1.4.4 The role of PKM2 in cancer cells

An important molecular signature of cancer development and progression is that a shift in expression of isoenzymes of pyruvate kinase in tumor of almost all types. The tissue specific isoform (L, R, or M1) disappears, while PKM2 is expressed in cancer cells in replacement (Boros, Cascante, & Lee, 2002; Elbers et al., 1991; Hacker, Steinberg, & Bannasch, 1998). Interestingly, PKM2 is converted to an inactive dimer form from an active tetramer in response to growth stimulation (Gao et al., 2013; X. Gao, H. Wang, J. J. Yang, X. Liu, & Z. R. Liu, 2012; Mazurek, Boschek, Hugo, & Eigenbrodt, 2005). As a critical metabolic enzyme, PKM2 in cancer cells

switches to an inactive dimer form that limits pyruvate entering into TCA cycle, by which more precursors are saved for biosynthesis (Anastasiou et al., 2012). PKM2 in cancer cells has also been found to act as a transcriptional regulator in the nucleus (Dong et al., 2016). For example, our lab has reported that PKM2 localizes to the cancer cell nucleus and function as an active protein kinase which can activates transcription of MEK5 (X. Gao, H. Wang, J. J. Yang, X. Liu, & Z.-R. Liu, 2012). PKM2 has also been reported as an extracellular signaling communicator, involved in many diseases including cancer (Dong et al., 2016; Hsu et al., 2016). Our lab has demonstrated that extracellular PKM2 facilitates wound healing (Y. Zhang, Li, Liu, & Liu, 2016). Extracellular PKM2 increases the angiogenesis by promoting endothelial cell proliferation and migration, thus promotes cancer progression (L. Li, Zhang, Qiao, Yang, & Liu, 2014). Though more and more investigations have been conducted to understand the function of extracellular PKM2 in diseases, so for it is not clear whether PKM2 can interact with immune cells and its potential role in the immune environment modulation.

1.4.5 PKM2's functions and subcellular locations

PKM2 in the cytoplasm primarily acts as a metabolic enzyme in glycolysis. The cancer cells express a high level of PKM2 (Y.-h. Li et al., 2018). Tumor cells prefer the inactive dimer form of PKM2, rather than its enzymatic active tetramer form because the low enzymatic activity of dimer PKM2 blocks the normal flow of glycolysis, allowing those glycolytic intermediates accumulated and converted to biomolecules to support fast growth of cancer cells (Dong et al., 2016). This phenomenon is discovered along with the discovery of the Warburg effect by German biologist Warburg in the early twentieth century (Vander Heiden et al., 2009). Warburg observed that, even when surrounded with a high level of oxygen, tumor cells still chose glycolysis not oxidative phosphorylation (Liberti & Locasale, 2016). Such aerobic glycolysis is later termed the

Warburg effect. The key game changer, dimeric form of PKM2, is also called Tumor M2-PK (Rajala, Rajala, Kooker, Wang, & Anderson, 2016). The tumor M2-PK plays vital roles in cancer progression (Zahra, Dey, Ashish, Mishra, & Pandey, 2020). Tumor cells can increase the transcription of PKM2, for instance through hypoxia-inducible factor HIF1- α (Semenza, 2013). Tumor cells can also increase dimer PKM2 post-translationally (Weibo Luo & Semenza, 2012; Prakasam, Iqbal, Bamezai, & Mazurek, 2018). Dimerization of PKM2 is elevated in tumor cells through the interaction between PKM2 and various oncoproteins such as A-Raf (Mazurek, Drexler, Troppmair, Eigenbrodt, & Rapp, 2007). Cytoplasmic PKM2 also acts as a signaling modulator by interacting with other partner proteins, such as A-Raf and fibroblast growth factor receptor 1 (FGFR 1) (Jing et al., 2017; Mazurek et al., 2007). These interacting partners drive conformation change of PKM2 and promote the dimer/tetramer switch of PKM2, on the other hand, PKM2 also changes the catalytic kinetics, substrate affinity and cytoplasmic location of those interacting partners, affecting signal transduction. The anti-apoptotic protein Bcl2 is another PKM2 interacting partner. Under oxidative stress, PKM2 translocates to mitochondria and binds with Bcl2, preventing it from degradation (Liang et al., 2017).

PKM2 contains nuclear localization sequence (NLS). It can enter the nucleus and act as a protein kinase and transcriptional regulator (Xueliang Gao et al., 2012). When PKM2 is in tetramer form, the NLS site is buried inside and is not exposed, while in dimeric form, the NSL site is exposed, allowing nucleus entrance of PKM2. Mitogen-activated protein kinase 1 (MAPK1) phosphorylates dimer PKM2 and results in conformational change of PKM2 so that PKM2 can enter into nucleus and regulate gene expression (W. Yang, Zheng, et al., 2012). Once entering the nucleus, PKM2 acts as a transcriptional co-activator and participates in gene regulation to promote cell growth and proliferation (W. Luo et al., 2011; Matsuda et al., 2016). PKM2 increases the

transcription of β-catenin targets (W. Yang, Xia, Cao, et al., 2012). After binding to Y333-phsphorylated-β-catenin, PKM2 is recruited to nucleosomes to phosphorylate histone H3, leading to enhancement in histone H3 acetylation. This acetylation of histone H3 activates the transcription of β-catenin target genes, such as cyclin D (W. Yang, Xia, Cao, et al., 2012; W. Yang, Xia, Hawke, et al., 2012). PKM2 is also demonstrated to enhance transcription of mitogen-activated protein kinase 5 (MEK5) by directly phosphorylating signal transducer and activator of transcription 3 (STAT3) (Xueliang Gao et al., 2012). PKM2 is also shown to promote tumor cells growth by phosphorylating extracellular signal-regulated kinase 1 and 2 (ERK l and ERK 2) (W. Yang, Zheng, et al., 2012). In addition, PKM2 can regulate PD-L1 expression in tumor cells and immune cells like macrophages (Long et al., 2020; Palsson-McDermott et al., 2017). Moreover, PKM2 executes its transcription regulation in a phosphorylation-independent manner. For example, PKM2 is reported to bind with the transcription factor Oct4 and inhibit Oct4-mediated transcription in cell stemness (Morfouace et al., 2014). PKM2 can interact with NF-κB and HIF-1α in the nucleus and activate the expression of VEGF-A, resulting in increased level of VEGF-A in blood circulation and sequential enhancement of tumor angiogenesis (Azoitei et al., 2016). However, the role of PKM2 as a transcription regulator has been questioned by studies of PKM2 knockout cells (Hosios, Fiske, Gui, & Vander Heiden, 2015).

PKM2 serves as a signaling communicator when present extracellularly. It has been found that PKM2 is present in exosomes, suggesting its role in conducting cell-cell communication and extracellular signaling. PKM2 is first identified PKM2 in B-cell exosomes (Buschow et al., 2010). Later more studies report the presence of PKM2 in exosomes which is released by various cancer cells (Wan et al., 2019). The information is accessible in public extracellular vesicle databases like ExoCarta and EVpedia. The presence of PKM2 in exosomes indicates that PKM2 could play a

role in cell-cell communication, carrying messages from host cells to recipient cells. Besides, cancer cells of various type have been shown to secrete PKM2. High levels of PKM2 are found in the blood circulation of cancer patients (Wong, De Melo, & Tang, 2013). PKM2 in blood circulation promotes tumor growth by enhancing endothelial cells migration and proliferation, promotes angiogenesis (L. Li et al., 2014). PKM2 enhances colon cancer cell migration via PI3K/AKT and Wnt/β-catenin signal pathways (P. Yang et al., 2015). PKM2 increases breast cancer cell proliferation via activating epidermal growth factor receptor (EGFR) (Hsu et al., 2016). Extracellular PKM2 is shown to mediate tumorigenesis of colon cancer cells by up-regulating claudin through EGFR-PKC-claudin-1 pathway (H. Kim et al., 2020). A recent study elucidates that secreted PKM2 promotes lung cancer metastasis via the integrin β1/FAK/PI3K signaling pathway (C. Wang et al., 2020). In addition to cancer cells, our lab has demonstrated that neutrophils secrets PKM2 at would sites to promote angiogenesis and accelerate wound healing process (Y. Zhang et al., 2016), suggesting the implication of extracellular PKM2 in inflammation regulation. It is not clear whether the extracellular PKM2 functions as free PKM2 on its own or a vesicle wrapped PKM2. The big molecular weight of free PKM2 makes it hard to enter the cell readily. Therefore, the effect of PKM2 has to be through binding with cell surface receptors like EGFR or integrin β1. Vesicle wrapped PKM2 is likely to be endocytosed by the cell, which makes no difference in its effect as what intracellular PKM2 does. It is not known yet which form of extracellular PKM2 plays the role outside the cells or it may be both forms.

Compared to what we have already known about PKM2, what we don't know might account for the majority of the PKM2 knowledge kingdom. What's more, there are debates even about the current knowledge of PKM2. The PKM2-knockout mice, contrast to researchers' expectation of suppressing tumor development, do not show tumor growth prevention (Dayton et

al., 2016). This study challenges our traditional impression on the tumor promoting role of PKM2, which might indicate that PKM2 may not be required for tumor development (Israelsen et al., 2013). Moreover, PKM2 knockout mice are susceptible to develop hepatocellular carcinoma spontaneously likely due to the metabolism imbalance (Dayton et al., 2016).

1.5 C-Myc and GLS

c-Myc is an oncogene which is highly expressed in various cancers (Chi V. Dang, 2012; Miller, Thomas, Islam, Muench, & Sedoris, 2012). c-Myc can be activated by various mitogenic factors such as EGF. Highly expressed c-Myc can increase the expression of many other genes, many of which are involved in cell proliferation (C. V. Dang, 1999). C-Myc protein can activate expression of many pro-proliferative genes through enhancer box sequences(E-boxes) and recruiting histone acetyltransferases (HATs) (Poole & van Riggelen, 2017). In addition, c-Myc can also control DNA replication, contributing to DNA amplification in cancer cells (Dominguez-Sola & Gautier, 2014). By modifying the expression of its target genes, Myc activation promotes cell proliferation, cell growth, differentiation and stem cell self-renewal (Chi V. Dang, 2012; Murphy, Wilson, & Trumpp, 2005).

Glutamine is the most abundant amino acids in the serum. Increased glutaminolysis is a hallmark of cancer and is recognized as a key metabolic change in cancer cells (Lifeng Yang et al., 2017). Glutaminase is the critical enzyme that converts glutamine to glutamate. Glutaminase is an amidohydrolase enzyme that generates glutamate from glutamine. Glutaminase has two isoenzymes, GLS and GLS2. GLS and GLS2 have opposing roles in tumorigenesis (Márquez et al., 2017; Saha et al., 2019). GLS correlates with tumor growth rate and malignancy, and is regulated by the oncoprotein c-Myc, whereas GLS2 tends to have tumor suppressive features, and is regulated by p53 (M. H. Kim & Kim, 2013; Masisi et al., 2020).

1.6 Translation-protein synthesis

Protein translation is the process that ribosomes synthesize proteins based on mRNA information (Orphanides & Reinberg, 2002). It is an important step of gene expression. The protein translation process includes three steps: initiation, elongation and termination. Initiation is the process that ribosome assembles around the target mRNA; Elongation is the process that an amino acid chain is generated by tRNA; termination is the process that ribosome releases the formed amino acid chain (Orphanides & Reinberg, 2002). In prokaryotes, translation occurs in the cytoplasm. However, in eukaryotes, translation occurs in the cytosol or across the endoplasmic reticulum (ER) membrane, the translated protein will be released into the ER (Harding, Zhang, & Ron, 1999).

1.6.1 Cap-dependent and Cap-independent initiation translation

Based on the difference in initiation process, the protein translation in eukaryotes can be divided in two ways. One is Cap-dependent initiation translation; the other is Cap-independent initiation translation (Richter & Sonenberg, 2005; Svitkin, Ovchinnikov, Dreyfuss, & Sonenberg, 1996). Initiation of translation usually starts with certain key proteins, which are called initiation factors. The initiation factors need to interact with mRNA 5' end to start the initiation by scanning the start codon. Initiation factor eIF4E is the mRNA 5' cap-binding protein (Richter & Sonenberg, 2005). Binding of the cap by eIF4E is often considered the rate-limiting step of cap-dependent initiation, and the concentration of eIF4E is a regulatory nexus of translational control (Richter & Sonenberg, 2005).

1.6.2 The internal ribosome entry site (IRES) translation

Except the Cap-dependent initiation translation, there are other Cap-independent initiation translation ways in eukaryotes (Sonenberg & Hinnebusch, 2009). One best example of Cap-

independent translation is the internal ribosome entry site (IRES) translation (Hellen & Sarnow, 2001; Komar & Hatzoglou, 2011). Unlike cap-dependent translation, IRES translation dose not require mRNA 5' cap to initiate the scanning of start codon from the mRNA. The ribosome can localize to the start site by direct binding to the initiation factors. This method of translation is important in circumstances where the translation of specific mRNAs is required during cellular stress, when overall translation is reduced. In cancer cells, the IRES translations are important for expression of many proteins like c-Myc (Silvera, Formenti, & Schneider, 2010; Sriram, Bohlen, & Teleman, 2018).

1.7 Cancer

1.7.1 Causes and statistics

Cancer is a name describing a group of disease which is caused by abnormal cells in the body that divide unstoppably and may invade distal tissues/organs. Cancer can occur at almost any organ/tissue in the human body (Bailar & Gornik, 1997).

Normally, the growth of cells in the human body is under tight control. Cells only divide when it is necessary. And when cells grow old or get injured, they undergo cell death. However, if cells lose control of their growth, they become cancerous. Cancers usually are caused by a series of mutations (Hanahan & Weinberg, 2000).

When cells become cancerous, there are six essential alterations in cell physiology. Cancer cells have self-sufficient growth signals to divide, usually through the activation of oncogenes such as ras or myc (Levine & Puzio-Kuter, 2010). Cancer cells are resistant to growth-inhibitory signals usually via inactivating tumor suppressor genes, such as Rb, that normally inhibit growth (Hinds & Weinberg, 1994; Levine & Puzio-Kuter, 2010). Cancer cells can escape programmed cell death via inhibiting apoptosis pathways (Lowe & Lin, 2000). Cancer cells become immortal by gaining

an uncontrolled replication ability via activating specific gene pathways. Cancer cells manage to draw out their own blood vessel for nutrients supply. Cancer cells are able to undergo epithelial-mesenchymal transition and migrate to other organs and re-colonize at metastatic sites (Brabletz et al., 2005).

Either according to the kind of origin fluid or tissue, or according to the origin location in the body, cancer can be classified into subtypes. There are five broad categories of cancer:

Carcinoma is a type of cancer found in epithelial tissue which covers surfaces of organs (Fass, 2008). For example, a cancer of the surface of the skin is called a carcinoma. Many Carcinomas account for 80-90% of all cancer cases (de Haes & van Knippenberg, 1985). Sarcoma is a type of cancer that grows from connective tissues, such as cartilage, fat, muscle, tendons, and bones, for instance osteosarcoma (bone) (Lee et al., 2003). Lymphoma is a type of cancer that originates in the nodes or glands of the lymphatic system (Wybran & Fudenberg, 1973). Lymphomas are further classified into two categories: Hodgkin's lymphoma and non-Hodgkin's lymphoma. Leukemia is a type of cancer that occurs in cells from the bone marrow, such as acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphocytic leukemia, and chronic lymphocytic leukemia (Appelbaum et al., 2006). Myeloma is a type of cancer that grows in the plasma cells of bone marrow (Rajkumar, Gertz, Kyle, & Greipp, 2002).

Cancer is the second leading cause of death globally; and is responsible for about 10 million deaths per year. Globally, about 1 in 6 deaths is due to cancer. Worldwide, an estimated 19.3 million new cancer and almost 10.0 million cancer deaths occurred in 2020 (Sung et al., 2021). The most common are breast cancer, lung and bronchus cancer and prostate cancer. According to CDC, approximately 39.5% of men and women will be diagnosed with cancer at some point during their lifetimes based on 2015–2017 data.

1.7.2 Breast cancer and Triple-negative breast cancer (TNBC)

Breast cancer is a type of cancer in which cells in the breast grow out of control. Depending on which part of the breast, there are different types of breast cancer (Harris, Lippman, Veronesi, & Willett, 1992). A breast is made up of three main parts: lobules where milk is produced, ducts which are tubes that carry milk to the nipple, and connective tissue which is made of fibrous and fatty tissue. Commonly, breast cancer occurs at the ducts or lobules. Breast cancer easily metastasizes to other parts of the body, such as the lung (Harris et al., 1992).

Triple-negative breast cancer (TNBC) is a type of cancer which only accounts for 10-20% of patients (Yin, Duan, Bian, & Yu, 2020). TNBC lacks estrogen receptors, progesterone receptors and HER2 proteins on its surface. HER2 is a protein that fuels breast cancer growth. Though rare, TNBC has a tendency to grow and spread more quickly than other types of breast cancer, moreover, it is more difficult to treat because hormonal therapy for breast cancer is not effective (De Laurentiis et al., 2010).

1.7.3 Lung cancer

Lung cancer is a type of cancer that occurs in the lung. It is the leading cause of cancer death among all types of cancer (Bade & Dela Cruz, 2020). There are two major types, small cell lung cancer and non-small cell lung cancer, with non-small lung cancer being the most common lung cancer. These two types of cancer grow differently and are treated differently. The main risk factors for lung cancers are smoking. Tobacco smoking causes about 9 out of 10 cases of lung cancer in men and about 8 out of 10 cases in women (Alberg, Brock, Ford, Samet, & Spivack, 2013). Exposure to secondhand smoke also increases the risks of lung cancer occurrence (Asomaning et al., 2008).

1.7.4 Melanoma

Melanoma is a type of cancer that occurs in pigment producing cells in skin called melanocytes (Rastrelli, Tropea, Rossi, & Alaibac, 2014). Though not the most common skin cancer, melanoma is a serious form of cancer and very dangerous because it can spread to other organs rapidly if not treated at an early stage (Damsky, Rosenbaum, & Bosenberg, 2010). Once spreading, it can be difficult to treat, and the outcome would be very bad. Risk factors for melanoma include overexposure to the sun, having fair skin, and a family history of melanoma (Rastrelli et al., 2014).

1.8 Cancer microenvironment

1.8.1 Cancer microenvironment overview

The cancer microenvironment, or tumor microenvironment (TME), refers to the environment surrounding cancer cells, which includes both cell compartments such as fibroblasts, immune cells, endothelial cells and non-cell compartments such as extracellular proteins produced by all of the cells present in the tumor (Whiteside, 2008). It has been well appreciated that cancers are not criminals by themselves, they recruit a group of surrounding cells and educate them to become accomplices in support of cancer cell growth and metastasis (Whiteside, 2008). There are dynamic interactions between malignant and non-cancer cells form the TME. The TME affects the therapeutic response and resistance of the tumor; and recently there are emerging therapies targeting specific components of the environment (Balkwill, Capasso, & Hagemann, 2012). But, so far the crosstalk and relationships between different kinds of compartment in the TME is still not fully understood and our further investigations may lead to establish effective new treatments.

1.8.2 Immune cells in cancer microenvironment

1.8.2.1 Innate immunity and adaptive immunity

The immune system refers to special organs, cells and chemicals in the human body that are used to fight infection. The immune system includes white blood cells, antibodies, the complement system, the lymphatic system, the spleen, the thymus, and the bone marrow.

The immune system is typically divided into two categories: innate immune and adaptive immune system (Medzhitov & Janeway Jr, 1998). Innate immunity is a non-specific defense mechanism which acts as the first line of defense. It responds to the pathogen invasion immediately but is not specific (Medzhitov & Janeway Jr, 1998). Innate immunity includes physical barriers such as skin, chemicals in the blood, and immune system cells such as neutrophils, macrophages and NK cells (Medzhitov & Janeway Jr, 1998). Adaptive immunity is an antigen-specific defense mechanism. It comes to play later than innate immunity due to a more complex process to produce antigen-specific T cells and antibodies (Bonilla & Oettgen, 2010). Firstly, the antigen first must be recognized and presented by antigen-presenting cells such as dendritic cells and macrophages. After antigen presentation, T cells and B cells undergo clonal selection and expansion to generate antigen-specific cytotoxic T cells and antibodies. Adaptive immunity also has an advantage of a long-term memory mechanism that makes future responses against a specific antigen fast and efficient (Bonilla & Oettgen, 2010).

Cells in the innate immunity include macrophages, neutrophils, eosinophils, basophils, mast cells, and dendritic cells. Cells in the adaptive immunity include B cells and T cells, including helper T cells and suppressor T cells (Bonilla & Oettgen, 2010).

1.8.2.2 Macrophages and macrophage polarity

Macrophage is a type of white blood cell which belongs to innate immunity that engulfs and digests foreign pathogens, damaged or old self-cells and cell debris, a process called phagocytosis (Gordon, 2007). Macrophages do not phagocytose normal body cells due to specific proteins expressed on the cell surface.

Depending on its location, macrophages have specific names in different organs (Gordon & Plüddemann, 2017). Monocytes in bone marrow /blood; Kupffer cells in liver; Alveolar macrophages or dust cells in pulmonary alveoli; Microglia in central nervous system; Hofbauer cells in placenta; Intraglomerular mesangial cells in kidney; Osteoclasts in bone.

The major function of macrophage is phagocytosis (Hirayama, Iida, & Nakase, 2017). Macrophages are professional phagocytes. In innate immunity, neutrophils are first defenders against pathogen, macrophages then come to place to remove dying or dead cells and cellular debris. macrophages are also involved in adaptive immunity. Macrophages are responsible for antigen-presentation after digesting a pathogen. In addition, macrophages produce a wide range of enzymes, complement proteins, and cytokines to regulate recruitment and activation of lymphocytes in adaptive immunity (Arango Duque & Descoteaux, 2014).

Macrophages have various activated subtypes, mainly M1 and M2 (Martinez & Gordon, 2014). M1 is also referred to as classically activated macrophages, while M2 is also known as alternatively activated macrophages (Martinez & Gordon, 2014; Rőszer, 2015). M1 and M2 can convert between each other. M1 macrophages are usually activated by pathogen substances such as LPS and pro-inflammatory cytokines such as IFN-gamma. They secret pro-inflammatory cytokines such as IFN-gamma and are predominantly responsible for phagocytosis of pathogens (Martinez & Gordon, 2014). However, M2 are commonly activated by anti-inflammatory

cytokines such as TGF-beta1, IL4, they are believed to suppress disruptive pro-inflammatory response and help constructive processes like wound healing and tissue repair (Martinez & Gordon, 2014). M2 macrophages produce abundant anti-inflammatory cytokines like IL-10 and TGF-beta. TAMs are more like M2 phenotype and assist tumor cells to suppress immune response (Noy & Pollard, 2014).

Macrophages in the tumor microenvironment are called Tumor-associated macrophages (TAMs). TAMs are abundant in most humans (Qian & Pollard, 2010). An abundance of TAMs in the TME is associated with poor patient outcome (Bingle, Brown, & Lewis, 2002). Macrophages are major contributors to tumor angiogenesis (Lin et al., 2006; Zumsteg & Christofori, 2009). Transcriptional profiling of TAMs shows enhanced transcripts that encode angiogenic molecules in TAMs (Ojalvo, Whittaker, Condeelis, & Pollard, 2010). TAMs are predominant cytokine producers in TME. They are a major source of TGF-β which can fuel persistent activity of CAFs and promote immunosuppression. TAMs also interact with other types of immune cells in the TME to inhibit immune response against tumor cells and promote the immunosuppressive microenvironment (Nakamura & Smyth, 2020).

TAMs are recognized to be alternative M2 phenotype as they display anti-inflammatory effects (Najafi et al., 2019). TAMs play a pro-tumoral role in tumor development by facilitating multiple steps including cancer cell survival, proliferation, stemness, and invasiveness along with angiogenesis and immunosuppression (Najafi et al., 2019). It has been elucidated that macrophages that infiltrate tumor tissues are driven by tumor-derived and T cell-derived cytokines to acquire a polarized M2 phenotype (Hao et al., 2012).

1.8.2.3 T lymphocytes

Various types of T cells infiltrate into the tumor areas as an adaptive immune response to clear out uncontrolled cancer cells. Cytotoxic CD8+ memory T cells, after presented antigen by antigen presenting cells in lymphoid sites, are capable of killing tumor cells. They migrate to the TME. Cytotoxic CD8+ T cells are strongly associated with a good prognosis (Fridman, Pagès, Sautès-Fridman, & Galon, 2012). CD4+ T helper 1 (TH1) cells produce the cytokines such as interleukin-2 (IL-2) and interferon gamma (IFN-γ) to support CD8+ T cells. To escape the immune surveillance, cancer cells use various strategies to block infiltrated cytotoxic cells, including overexpressing inhibitory ligand PDL-1 and CTLA on cell surface, and building physical fences by secreting massive extracellular matrices (Alsaab et al., 2017). In addition, Cancer cells turn a certain type of T cell in their favor, which is the immunosuppressive T regulatory cells (Tregs), characterized by expression of FOXP3 and CD25 (Hsieh, Lee, & Lio, 2012). By production of IL-10 and transforming growth factor beta (TGF-β) and cell-mediated contact through CTLA4, T reg cells inhibit Cytotoxic T cells' recognition and clearance of tumor cells (Campbell & Koch, 2011). There are several other T cells whose anti-tumor roles are under debates, such as CD4+ TH2 cells which produce IL-4, IL-5 and IL-13 and TH17 cells which release IL-17A, IL-17F, IL-21 and IL-22 (Kennedy & Celis, 2008).

1.8.2.4 Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a population of inhibitory immune cells originating from myeloid lineage in the TME (Gabrilovich, Ostrand-Rosenberg, & Bronte, 2012; Sica & Bronte, 2007). Depending on their progenitors, MDSCs have two major subtypes: monocytic and neutrophilic MDSCs. They are not terminated differentiated phenotypes and they can further differentiate into TAMs (Kusmartsev, Nagaraj, & Gabrilovich, 2005). MDSCs inhibit

CD8+ T cell activation through the expression of nitric oxide synthase 2 (NOS2) and arginase (ARG1) (Bronte, Serafini, Mazzoni, Segal, & Zanovello, 2003). They also promote immunosuppressive TME by inducing the development of Tregs (Huang et al., 2006) and the polarization of macrophages to a TAM-like phenotype (Sinha, Clements, Bunt, Albelda, & Ostrand-Rosenberg, 2007).

1.8.2.5 Neutrophils

Neutrophiles in the TME are termed as tumor-associated neutrophils (TANs). It has been shown that TANs promote primary tumor growth in mouse cancer models (Pekarek, Starr, Toledano, & Schreiber, 1995) by enhancing angiogenesis (Shojaei, Singh, Thompson, & Ferrara, 2008), promoting extracellular matrix degradation and immune suppression (De Larco, Wuertz, & Furcht, 2004; Youn & Gabrilovich, 2010). However, their pro-tumorigenic role is still controversial. TANs are reported to actively eliminate tumor cells (Granot et al., 2011), and perform an anti-tumor effect by inhibition of TGF-β1 (Fridlender et al., 2009).

1.8.3 Non-immune cells in cancer microenvironment

Myofibroblasts are predominant cells which are responsible for extracellular matrix production and contractility. They are differentiated from tissue residential fibroblasts to assist tissue repair when tissues are injured (B. Li & Wang, 2011). However, under pathological conditions, the persistent activated myofibroblasts can also cause organ fibrosis (Desmoulière, Guyot, & Gabbiani, 2004; Radisky, Kenny, & Bissell, 2007). Fibroblasts are found abundant in many TMEs. Myofibroblasts in the TME are termed as cancer-associated fibroblasts (CAFs) (Sugimoto, Mundel, Kieran, & Kalluri, 2006). They are heterogeneous populations derived from various precursor cells including resident fibroblasts, adipocytes, endothelial cells, smooth muscle

cells, myoepithelial cells, or mesenchymal stem cells (Brittan et al., 2002; Spaeth et al., 2009; Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002; Willis, duBois, & Borok, 2006).

CAFs produce massive extracellular matrix, which forms dense desmoplastic stroma which is shown to inhibit cytotoxic T cell infiltration and dampen the accessibility of chemotherapy (X. Chen & Song, 2019). In addition, CAFs have a potent secretion ability and they can secrete various growth factors to support tumor cell growth, such as the EGF family members hepatocyte growth factor (HGF), fibroblast growth factor (FGFs) and insulin-like growth factor 1 (IGF1) (Brittan et al., 2002). TGF-β from CAF induces epithelial–mesenchymal transition (EMT) in malignant cells, contributing to the metastasis of cancer, and also involved the immune-suppressive microenvironment (Erez, Truitt, Olson, Arron, & Hanahan, 2010). CAF produce chemokines such as CCL2 and CXCL12 that chemo-attract other types of stromal cells to the TME (Orimo et al., 2005). CAF also secretes cytokines to regulate immune suppression. FAP-positive CAFs in TME are reported as important mediators of immune suppression (Kraman et al., 2010).

To meet their nutrient requirement, cancer cells induce formation of new vessels sprouting from existing vasculature, which process is called angiogenesis. During this process, endothelial cells and their associated pericytes are stimulated through abundant chemokines, growth factors in the TME including VEGFs, FGFs, platelet-derived growth factors (PDGFs) (Carmeliet & Jain, 2011). The angiogenic vasculature is abnormal in almost every aspect of its structure and function (Jain, 2005). For example, vessel lumen of angiogenic vessels is uneven, unmatured and leaky. The leakiness of the vessels leads to a high interstitial fluid pressure which causes unevenness of blood flow, oxygenation, nutrient and drug distribution in the TME. This, in turn, increases hypoxia and facilitates metastasis. VEGF is the predominant angiogenic factor in the TME and is

produced by both malignant cells and inflammatory leukocytes; however, advanced tumors can produce a range of other angiogenic factors (Carmeliet & Jain, 2011).

1.8.4 Cytokines in cancer microenvironment

Cytokines are potent signal small molecules that are major mediators of cell-to-cell communication. Stromal cells in the TME, such as CAFs and TAMs, secrete a great number of cytokines and facilities tumor cell proliferation, survival, migration and invasion (Landskron, De la Fuente, Thuwajit, Thuwajit, & Hermoso, 2014). Tumor Necrosis Factor (TNF-α) is an essential pro-inflammatory cytokine implicated in carcinogenesis. TNF-α-deficient mice are protected against the tetradecanoyl-phorbol-13-acetate- (TPA-) induced skin cancer. Interleukin 6 (IL-6) is also a proinflammatory cytokine with a typical pro-tumorigenic effect (Fisher, Appenheimer, & Evans, 2014). Serum IL-6 levels have been detected elevated in patients with cancers. IL-6 promotes tumor cell proliferation and prevents their apoptosis, by activating the JAK/STAT signaling (Johnson, O'Keefe, & Grandis, 2018). TGF- β is a powerful pleiotropic cytokine, with immune-suppressing and anti-inflammatory properties, majorly produced by immune cells and CAFs (Li Yang, Pang, & Moses, 2010). Three isoforms of TGF- β have been identified: TGF-β1, TGF-β2, and TGF-β3. TGFβs bind to TGFβ receptors and activate phosphorylation of SMAD2 and SMAD3, which translocate into the nucleus once activated, where they induce gene expression. The role of TGFβ in cancer is controversial. TGF-β suppresses tumor formation through upregulating cyclin-dependent kinase inhibitor (CKI) p21 and downregulating c-Myc (Donovan & Slingerland, 2000). While TGF-β is also reported to enhance invasion and metastasis by inducing epithelial-mesenchymal transition (EMT) (Wendt, Allington, & Schiemann, 2009). Interleukin 10 (IL-10) is a commonly known anti-inflammatory cytokine. In the TME, IL-10 is secreted by tumor cells and TAMs (Sato et al., 2011). The role of IL-10 in cancer is also complex.

IL-10 acts as an anti-tumor cytokine by inhibiting NF-κB signaling therefore downregulates proinflammatory cytokine expression (Murray, 2005). IL-10-deficient murine models are prone to bacteria-induced inflammation (S. C. Kim et al., 2005). IL-10 can dampen antigen presentation and suppress functions of dendritic cells and macrophages (Iyer & Cheng, 2012). Elevated IL-10 levels are associated with poor prognosis in diffuse B cell lymphoma (Gupta et al., 2012).

1.8.5 Extracellular matrix

The extracellular matrix is the non-cellular component of the TME which are critical for cell–cell communication, cell adhesion and cell proliferation in the TME (Winkler, Abisoye-Ogunniyan, Metcalf, & Werb, 2020). The ECM is mainly secreted by CAFs. It includes various components including collagens, glycoproteins (fibronectin and laminins), proteoglycans, and polysaccharides (Franco, Shaw, Strand, & Hayward, 2010).

Cancer cells, CAFs and TAMs modulate together ECM within the TME through deposition and cross-linking of ECM via enzymes of the lysyl oxidase (LOX) and transglutaminase families, particularly LOX-1, LOXL-2, and transglutaminase-2. ECM contributes to EMT of cancer cells and facilitates cancer cell migration (Poltavets, Kochetkova, Pitson, & Samuel, 2018). In breast cancer cells, physical signal from ECM stiffness stimulates EMT by promoting TWIST1 translocalization into the nucleus (Wei et al., 2015). An excessive accumulation of dense and rigid ECM serves as a barrier to prevent the entrance of chemotherapy into TME (Henke, Nandigama, & Ergün, 2020). The increased hypoxia stress also activates anti-apoptotic and drug resistance pathways in cancer cells.

1.9 Cancer therapy

1.9.1 Cancer treatment types

Cancer therapy is the treatment of cancer in a patient. Most common cancer therapies include surgery which doctors cut out tissue with cancer cells; chemotherapy where medicines are used to kill cancer cells; radiation therapy where high-energy rays are applied locally to kill cancer cells (Sawyers, 2004). Other cancer therapy includes hormone therapy where hormones are suppressed to avoid cancer cell growth; immunotherapy which is an emerging therapy in recent years where immune system are manipulated to kill cancer cells; Stem cell transplant or bone marrow transplant for blood cancer and cancer in lymph nodes where bone marrow cells are replaced (Sawyers, 2004).

Chemotherapeutic drugs usually target DNA replication or protein synthesis processes during cell division because cancer cells are highly proliferating cells (Chabner & Roberts, 2005). However, chemotherapy works throughout the whole body, which means it also can affect some fast-growing healthy cells, like those of the skin, hair, intestines and bone marrow (Chabner & Roberts, 2005). Therefore, chemotherapy causes side effects. Compared to chemotherapy, immunotherapy is a new and promising therapy which does not cause strong side effects because it aims to make use of your own immune system to fight cancer.

1.9.2 Immunotherapy

Over the past decade, immune therapy has been advancing fast. So far, immune therapy includes monoclonal antibodies, immune adjuvants, and vaccines against oncogenic viruses (Dougan & Dranoff, 2009). The 2018 Nobel Prize in Physiology or Medicine was awarded to the discovery of the programmed death molecule-1 (PD-1) on T cells which give rise to the immune therapeutic strategy of PD-1/PDL-1 antibodies. Advances in our understanding of interactions

between tumors and the immune cells have led to many novel investigational therapies and continue to drive more potent therapeutics. Great attention has been put on the immune cells in the TME such as TAM, MDSC as they play an essential role to modulate the activity of cytotoxic T cells (Couzin-Frankel, 2013). Novel approaches of immunotherapy strive to augment antitumor immune responses by enhancing cytotoxic T cells, blocking immunosuppressive functions of TAM and MDSC. The future of immunotherapy for cancer is likely to combine many of these approaches to generate more effective treatments (Bashir & Wilson, 2019).

Various types of immunotherapy are available: Immune checkpoint inhibitors which are drugs that block immune checkpoints (Darvin, Toor, Sasidharan Nair, & Elkord, 2018). The immune checkpoints are a part of normal regulation of the immune system to prevent immune response from going out of control. However, cancer cells overexpress some immune checkpoints to inhibit the normal function of cytotoxic T cells (Darvin et al., 2018). By blocking these immune checkpoints, the cytotoxic T cells are more active in killing cancer cells. T-cell transfer therapy, which is a strategy to boost T cells *in vitro* and deliver these modified T cells to the same patient's body to fight cancer (Dudley & Rosenberg, 2003). It requires the *in vitro* selections and expansion of cancer cells isolated from patients, and later transfer of these cells into the body of the same patient through a needle via a vein (Rosenberg, Restifo, Yang, Morgan, & Dudley, 2008). Monoclonal antibodies, which are immune system proteins created to bind to specific targets on cancer cells to enhance antigen presentation of cancer cells (Scott, Allison, & Wolchok, 2012).

1.10 Integrin

1.10.1 Integrin family

The integrins are a superfamily of transmembrane proteins serving as primary cell adhesion receptors. They bind to the extracellular matrix and cell-surface ligands that are responsible to

transfer the outside signal inside or the alternative way. They are critical for sensing physical and chemical signals from matrices and conduct communication with surrounding cells (Takada, Ye, & Simon, 2007). Integrins are involved in multiple critical cellular processes such as cell survival, proliferation, cell migration and cell transformation (Kumar, 1998) (Wozniak, Modzelewska, Kwong, & Keely, 2004). Integrins are heterodimers, composed of two subunits: α subunit and β subunit. There are 18 α subunits and 8 β subunits which make 25 different combinations of integrin heterodimers. The amino acid sequence Arginine-Glycine-Aspartic acid (RGD) is a conserved sequence that is found in the interaction site of many extracellular matrix proteins which bind to integrins (Horton, 1997). Integrins physically link with the actin cytoskeleton through cell-matrix adhesion complexes termed "focal adhesions" (FAs) (Sun, Guo, & Fässler, 2016). Focal adhesions are membrane-associated multi-protein complexes that transmit outside signals inside cells (Schiller & Fassler, 2013). Focal adhesions proteins include focal adhesion kinase (FAK), paxillin, vinculin and talin (Stutchbury, Atherton, Tsang, Wang, & Ballestrem, 2017). Ligand binding activates integrins, followed by the assembly of focal adhesion complex, leading to oligomerization and trans-autophosphorylation of tyrosine kinase FAK. Phosphorylated FAK can bind with and activate PI3K, leading to activation of proliferation and survival pathways. A common proliferation pathway induced by integrins such as αvβ3 is the RAS-ERK signal pathway (Hood , Frausto , Kiosses , Schwartz , & Cheresh 2003). Integrin αvβ3 is reported to enhance signaling of insulin and insulin-like growth factor receptors by binding with insulin receptor substrate (IRS)-1 (Zheng & Clemmons, 1998). Integrins are also involved in cell survival. Integrins provide a constitutive survival signal to cells via the PI3K-AKT pathway (X. Wu, Cai, Zuo, & Li, 2019).

1.10.2 αvβ3 integrin

The αvβ3 integrin, also known as vitronectin receptor, has been reported essential for angiogenesis and cancer malignancy (Eliceiri & Cheresh, 2000). Besides vitronectin, αvβ3 can also interact with other extracellular proteins such as fibronectin and fibrinogen. The interaction between integrin αvβ3 and extracellular matrix molecules is demonstrated to be involved in the inflammatory response, tissue repair and remodeling, and bone resorption (Horton, 1997). The evidence also indicates αvβ3 play a role in regulating cell proliferation, differentiation, migration and apoptosis (Horton, 1997; Kumar, 1998). The integrin is intensively studied as a therapeutic target since upregulation of αvβ3 is found in many disease conditions especially angiogenesis during tumor progression and bone diseases such as osteoporosis and bone metastasis (Goodman & Picard, 2012; Z. Liu, Wang, & Chen, 2008). However, unexpectedly, β3 integrin knockout mice display marked increases in angiogenesis (Reynolds et al., 2002), tumor growth (Reynolds et al., 2002; Robinson, Reynolds, Wyder, Hicklin, & Hodivala-Dilke, 2004), elevated fibrosis (Friman et al., 2012), lung inflammation (Weng et al., 2003) and wound healing responses (Weis & Cheresh, 2011), probably due to compensatory effect of increased VEGFR2. Inhibition of αvβ3 through Cilengitide, a specific αvβ3(αvβ5) inhibitor, aggravates liver fibrosis (Patsenker et al., 2009).

1.11 The focuses in this study

Due to high demand of biomaterials for fast proliferation, cancer cells inactivate several enzymes in glycolysis to create a large pool of intermediates. Thus, cancer cells switch to glutaminolysis for energy supply. However, it is not clear how cancer cells fine-tune the regulation between glycolysis and glutaminolysis. In our study, we elucidate that dimer PKM2 plays a role

in the coordination of glycolysis and glutaminolysis. Upon growth factor stimulation, PKM2 dimer regulates the expression of mitochondrial glutaminase I, which is a key enzyme in glutaminolysis. Further investigations on the mechanism show that dimer PKM2 controls glutaminase expression by interacting with IRES complex and modulate IRES-dependent c-myc translation. Our study reveals dimer PKM2 is an important linker that coordinates the adjustments of the metabolism pathways in cancer cells.

Cancer cells not only adjust their inner metabolic pathways, but they also modulate the surrounding microenvironment. One of the well-recognized examples is that cancer cells regulate polarization of macrophages in its microenvironment, the TAM, in their favor. Although there are growing studies on the mechanism how cancer cells educate TAM, it has not been fully understood yet.

It is well documented that cancer cells release PKM2 to facilitate tumor progression. We have performed in vitro and in vivo studies to investigate whether extracellular PKM2 (EcPKM2) promotes cancer progression by facilitating TAM regulation in tumors. We demonstrate that EcPKM2 interacts with integrin αvβ3 on macrophage, which leads to the activation of integrin-PI3K signal pathway. We further show that integrin-PI3K signaling triggered by EcPKM2 reduces PTEN expression, which subsequently upregulates arginase-1 (Arg-1) expression in macrophages.

Disruption of EcPKM2 and integrin $\alpha_v\beta_3$ interaction by a house made PKM2 antibody abolishes the effects of EcPKM2 on promoting M2 macrophage and increasing M1 macrophage population in tumors, which suggests a new strategy and target in cancer immunotherapy. Furthermore, cancer immunotherapy is powerful but has limitations in clinical application due to immunosuppressive cancer microenvironment. TAMs are important effector cells modulating

immune suppression. We added the PKM2 antibody treatment to checkpoint inhibitors and chemotherapeutics and observed synergistic cancer treatment effects.

Our studies uncover a novel and important mechanism for modulation of cancer immunity. More importantly, combination of the PKM2 antibody with checkpoint blockades and chemotherapeutics provides novel cancer treatment strategies.

2 PYRUVATE KINASE M2 COORDINATES METABOLISM SWITCH BETWEEN GLYCOLYSIS AND GLUTAMINOLYSIS IN CANCER CELLS

2.1 Abstract

Cancer cells alter their nutrition metabolism to cope the stressful environment. One important metabolism adjustment is that cancer cells often activate glutaminolysis in response to the reduced carbon from glucose entering into TCA cycle due to inactivation of several enzyme activity in glycolysis. An important question is how the tumor cells coordinate the changes of glycolysis and glutaminolysis. In this report, we demonstrate that the pyruvate kinase inactive dimer PKM2 facilitates activation of glutaminolysis. Our experiments show that the dimer PKM2 plays a role in upregulation of mitochondrial glutaminase I. Growth stimulations activate activity of PKM2 in regulation of glutaminolysis. PKM2 dimer regulates the glutaminase expression by controlling the IRES-dependent c-myc translation. Growth stimulations promote PKM2 and c-myc IRES interaction. Our study reveals an important linker that coordinates the adjustments of the metabolism pathways in cancer cells.

2.2 Introduction

An important molecular signature of cancer development and progression is that a shift in expression of isoenzymes of pyruvate kinase occurs to the tumor of almost all types. The tissue specific isoform (L, R, or M1) disappears. In replacement, PKM2 is expressed in cancer cells (Boros, Cascante, & Lee, 2002; Elbers et al., 1991; Hacker, Steinberg, & Bannasch, 1998). Interestingly, PKM2 is converted to a pyruvate kinase inactivation dimer form from the more pyruvate kinase active tetramer in response to growth stimulation(Gao et al., 2013; Xueliang Gao et al., 2012; Mazurek, Boschek, Hugo, & Eigenbrodt, 2005). It is believed that the inactive dimeric PKM2 actually provides a metabolic advantage for supplying precursors for biosynthesis

(Ferguson and Rathmell, 2008; Hitosugi et al., 2009; Mazurek, 2007). The dilemma is that it is not sufficient to meet the needs of both energy and biosynthesis intermediates for the rapid growth solely by adjustment of glycolysis. Tumor cells often turn to glutaminolysis, a metabolism pathway which uses another abundant nutrition source glutamine (Cairns et al., 2011; Chen and Russo, 2012; Dang, 2010; DeBerardinis and Chandel, 2016; Yang et al., 2017). Indeed, it is well documented that glutaminolysis is upregulated in cancer cells of many types (DeBerardinis et al., 2007). Metabolism of glutamine, in addition to providing carbon frame for biosynthesis and TCA cycle intermediates, also provides reducing power by directly converting to glutathione (GSH), which is the most abundant antioxidant in mammalian cells in handling oxidative stress (Cairns et al., 2011; Gorrini et al., 2013; Lamonte et al., 2013). An important question is how cancer cells coordinate the activities of glycolysis and glutaminolysis to meet their growth needs.

2.3 Results

2.3.1 PKM2 dimer facilitates glutaminolysis in cancer cells

Since conversion of tetramer PKM2 to dimer PKM2 inactivates the last step of glycolysis 4, 5, 18, which consequently affects the metabolism of glucose, glucose and glutamine are two most abundant nutrition sources, we asked whether PKM2 also plays a role in the glutamine metabolism. Thus, we measured the glutamine consumption in PKM2 knockdown SW480 cells. Interestingly, PKM2 knockdown (Fig. S1A) largely reduced the glutamine consumption. Exogenous expression of wild-type PKM2 restored the glutamine metabolism, while expression of PKM1 could not restore the glutamine consumption in the cells (Fig. 1A), suggesting a potential role of PKM2 in glutamine metabolism. It is demonstrated that growth stimulation activates glutaminolysis 19-21. We examined the glutamine consumption in SW480 cells under EGF stimulation. The growth factor treatment increased the cell growth by over 3 folds (Fig. S1B). In

consistent, glutamine consumption increased almost 3 folds. Knockdown PKM2 abolished the effects of the growth factor on increase of the glutamine consumption (Fig. 1B). We previously reported that growth stimulation increased the dimer PKM2 in cancer cells 4, 5. Thus, we question whether increase in dimer PKM2 upregulated glutamine consumption. We generated a PKM2 mutant with triple mutations (R399E, K422A, N523A, Ref to as TM mutant thereafter). Size-exclusion chromatography analyses indicated that the recombinant TM mutant mainly existed as a dimer (Fig. S1C). We then asked if expression of this PKM2 TM mutant would increase glutamine consumption. Clearly, exogenous expression of the TM mutant in cancer cells (Fig. S1D) increased glutamine consumption by over 2 fold compared to that of expression wildtype PKM2 (Fig. lC). PKM2 activator increases pyruvate kinase activity of the enzyme by converting dimer to a tetramer 22. We tested whether treatment of SW480 cells by the PKM2 activator would affect glutamine metabolism in the cells. It was evident that glutamine consumption decreased 4 folds by the PKM2 activator (Fig. 1D). The results suggest that the dimer PKM2 facilitates glutaminolysis under growth stimulations.

2.3.2 PKM2 dimer facilitates glutaminolysis by regulation of c-myc IRES-dependent translation.

We then sought to determine how the dimer PKM2 affected glutamine metabolism. Mitochondrial glutaminase I (GLS-I) is the enzyme that catalyzes the first step of glutaminolysis, and is a key enzyme in regulation of glutamine metabolism 23-26. We therefore investigated whether knockdown of PKM2 affected the GLS-1 expression. Immunoblot analyses of mitochondrial extracts prepared from SW480 cells in which PKM2 was knocked down showed that the GLS-1 was downregulated upon PKM2 knockdown (Fig. 1E). Exogenous expression of wt PKM2 could restore the GLS-1 expression, but PKM1 could not (Fig. 1F). Expression of the

TM mutant in SW480 cells increased GLS-1, while treatment of the cells by the PKM2 activator decreased GLS-1 in the cells (Fig. 1G), suggesting that the pyruvate kinase inactive PKM2 dimer played a role in regulation of GLS-1 expression. It is known that c-myc regulates glutaminolysis by controlling GLS-1 expression 21, 24, 27. We reasoned whether dimer PKM2 regulates glutaminolysis via regulation of c-myc expression. Knockdown of PKM2 downregulated c-myc expression. Expression of PKM2 but not PKM1 could restore c-myc expression in PKM2 knockdown cells (Fig. 1F). Expression of the TM mutant increased c-myc expression (Fig. 1G). Treatment cells with PKM2 activator decreased c-myc expression (Fig. 2A and 1G). The results suggest that PKM2 dimer regulates glutaminolysis by controlling c-myc expression. We asked how PKM2 dimer regulated c-myc expression. RT-PCR analyses of C-MYC pre-mRNA and mRNA in the PKM2 knockdown cells showed that PKM2 knockdown did not affect C-MYC pre-mRNA and mature mRNA levels (Fig. 2B), suggesting PKM2 knockdown did not affect C-MYC mRNA transcription and processing. We speculated that PKM2 dimer regulated c-myc expression at the translation level. Two pathways contribute to c-myc protein synthesis, canonical cap-dependent translation and internal ribosome entry site (IRES) dependent translation. The IRES-dependent c-myc translation is a pathway that is implicated in oncogenesis, particularly, the IRES-dependent c-myc translation remains active during apoptosis induction and G2/M transition of cell cycle when the cap-dependent translation is largely suppressed 28-32, suggesting a potential role of the IRES-dependent c-myc translation in cope with various stresses. Cap-analog m7G is an inhibitor that specifically inhibits cap-dependent translation with no effects on IRES-dependent translation, while CHX inhibits all protein synthesis (Fig. 2C). Treatment of cells with CHX completely abrogated c-myc expression in cells with or without expressing the TM mutant, while m7G only partially inhibited c-myc expression. The inhibitory effects of m7G in the TM mutant

expressing cells is less than that in cells without TM mutant expression (Fig. 2D), suggesting that dimer PKM2 might regulate c-myc expression by IRES-dependent translation. To test this conjecture, bicistronic expression vectors were constructed containing ORFs of HA-actin and GFP separated by c-myc IRES or Idh LDH 5'-UTR (Fig. 3A). The vectors were transfected into SW480 cells. Expression of GFP was monitored to assay IRES-dependent translation. Knockdown of PKM2 abrogated GFP expression (Fig. 3B). To further test the role of PKM2 in regulating c-myc IRES-dependent translation, we probed the interaction of PKM2 with c-myc mRNA by RNA-immunoprecipitation (RIP). Clearly, PKM2 interacted with c-myc mRNA in the IRES region (Fig. 3 C & D & E). Furthermore, we isolated a complex assembled on the c-myc IRES. Protein contents in the isolated complex were identified by MALDI-tof/tof. Evidently, PKM2 was presented in the isolated c-myc IRES complex (Fig. 3F). We concluded from our experiments that PKM2 regulates c-myc expression by IRES-dependent translation, and PKM2 interacts with the c-myc IRES complex.

2.3.3 PKM2 facilitates hnRNP L and hnRNP K interacting with c-Myc IRES

How PKM2 regulates c-myc IRES-dependent translation is an open question. To elucidate the possible mechanism, we carried out co-immunoprecipitation attempting to find PKM2 interacting partners. Interestingly, hnRNP L co-immunoprecipitated with PKM2 in SW480 extracts (Fig. S3A). The co-immunoprecipitation was RNA independent (Fig. S3B), which excluded a possibility that the co-IP was due to precipitation of large RNP complexes. The co-IP was verified by using anti-hnRNP L antibody (Fig. S3C). MALDI-tof/tof analyses of the coprecipitates with c-myc IRES complex also revealed that PKM2, hnRNP L, and hnRNP K are present in the c-Myc IRES complex (see Fig. 3F). In consistent, hnRNP L interacted with c-myc IRES by RIP (see Fig. 3 C&D). It is well known that hnRNP K and hnRNP L functions in the

cmyc IRES-dependent translation by modulating the IRES-RNA structure (Godet et al., 2019; Vaklavas et al., 2015). Knockdown of hnRNP L reduced cellular GLS-1 levels and decreased glutamine consumption, while exogenous expression of c-myc could restore the GLS-1 levels and glutamine consumption (see Fig. 4 C&D), suggesting that hnRNP L plays a role in regulation of glutaminolysis by IRES-dependent c-myc expression. We reasoned whether PKM2 regulated c-myc IRES-dependent translation by controlling the hnRNP L/K and c-myc IRES interaction. The hnRNP L interaction with c-myc IRES. However, knockdown of PKM2 reduced the interaction of hnRNP L with the c-Myc IRES (Fig. S3D), and expression of the TM mutant in hnRNP L knockdown cells could not rescue the effects of hnRNP L knockdown on c-myc expression (Fig. S3E). Interestingly, knockdown of hnRNP L/hnRNP K also abrogates the interaction of PKM2 with c-myc IRES (see Fig. 3E). It is possible that PKM2 and hnRNP L/hnRNP K cooperatively interact with the c-MYC IRES-RNA. Thus, our experiments support a mechanism that dimer PKM2 promotes assembly of an active complex on the c-myc IRES, including hnRNP L, hnRNP K, PKM2, and possibly other molecules, to facilitate the c-myc IRES-dependent translation.

2.3.4 PKM2 dimer confers cancer cells glutamine addiction.

If dimer PKM2 mediates the effects of growth stimulation in activating glutaminolysis by promoting c-myc IRES-dependent translation, we expect that growth stimulations would upregulate c-myc and GLS-1 expression, and the regulatory effects would be PKM2 dependent. Indeed, c-myc and GLS-1 expression in SW480 cells were upregulated upon EGF and FGF stimulations (Fig. 4A). Upregulation of c-myc and GLS-1 by EGF were dependent on PKM2 (Fig. 4B). If c-myc IRES-dependent expression mediated the effects of PKM2 in regulation of GLS-1, we would expect that exogenous expression of c-myc would rescue the effects of PKM2 knockdown. Evidently, exogenous expression of c-myc in PKM2 knockdown cells restores the

GLS-1 expression (Fig. 4C) and glutamine consumption (Fig. 4D). Furthermore, growth stimulations are supposed to promote PKM2 and c-myc IRES-RNA interaction. We probed the PKM2 and c-myc IRES-RNA interaction by RIP in cells under EGF treatment. EGF treatment increased PKM2 and the c-myc IRES-RNA interaction (Fig. 4E). The pattern of changes of the PKM2-IRES interaction was consistent with the GLS-1 expression under EGF stimulation (Compare Figs. 4E to 4A). PKM2 dimer mediates effects of growth signals in regulating glutaminolysis. We would expect different effects of PKM2 in glutamine consumption in cells under growth stimulation vs non-growth stimulation. Indeed, knockdown of PKM2 exerted stronger effects on glutamine consumption under EGF treatment vs non-treatment (see Fig. 1B).

Upregulation of glutaminolysis commits cancer cells to glutamine addiction (Wise et al., 2008; Wise and Thompson, 2010). If dimer PKM2 upregulated glutaminolysis, it would be expected that an increase in PKM2 dimer would lead to an increase in the cells to glutamine addiction. Thus, we examined viability of SW480 cells with/without the TM mutant expression and PKM2 activator treatment under normal culture conditions or the culture condition with glutamine withdrawal. Expression of the TM mutant decreased viability of cells under glutamine withdraw from culture medium (Fig. 4F), while the PKM2 activator increased the viability of the cells under glutamine withdrawal (Fig. 4G). PKM2 dimer increases the cancer cell addiction to glutamine further supports the functional role of PKM2 in coordinating the regulation of metabolism of glucose and glutamine.

2.4 **Discussion**

Proliferation cancer cells limit carbon flow from glucose to the TCA cycle by reducing pyruvate kinase activity. An advantage of this change is pooling of glycolytic intermediates to meet the needs of high demand for biosynthesis in proliferation. Converting of the tetramer PKM2

to a dimer partially fulfills this regulation. However, change only in glycolysis is not sufficient to meet the metabolic needs for cancer progression and proliferation. It is well known that cancer cells switch to glutamine as an essential nutrient to support survival and growth (Eagle, 1955). It is not well understood how cancer cells coordinate the switch of the metabolism pathways. We demonstrate here that PKM2, a glycolytic enzyme, plays a role in regulating glutamine metabolism. PKM2 dimer, glycolytic inactive form, activates glutaminolysis upon growth stimulation. The reciprocal roles of PKM2 in regulation of glycolysis and glutaminolysis function well in coordinating the switch of the metabolism in cancer cells. In addition to providing the carbon inputs for the TCA cycle to act as a biosynthetic base and alternative resources for NADPH and lipids, glutamate, the immediate product of glutamine in glutaminolysis, provides reducing power by directly converting to GSH, which is the most abundant anti-oxidant in mammalian cells (Cairns et al., 2011; Gorrini et al., 2013; Lamonte et al., 2013). An important metabolism role of PKM2 in cancer cells is to help to cope with oxidative stress (Anastasiou et al., 2011). It is plausible that the upregulation of glutaminolysis by dimer PKM2 may help PKM2 to fulfill the role. Regulation of c-myc IRES-dependent translation by dimer PKM2 is intriguing. C-myc regulates PKM2 expression by controlling PKM2 pre-mRNA splicing (David et al., 2010). As a feedback, PKM2 regulates c-MYC gene transcription (Luo et al., 2011) (Yang et al., 2011, 2012). We showed here another feedback loop that dimer PKM2 regulates c-myc translation. Regulation of c-myc IRES-dependent translation by dimer PKM2 allows quick response of cancer cells to adoption of various stress conditions. It is well established that cancer cells employ very different, yet co-existing, pathways to cope both hypoxia condition and cell proliferation needs. It is not well understood how cancer cells coordinate or ‘‘fine-tune’’ the opposite signal pathways to allow them to proliferate under stressful hypoxia conditions (Gordan et al., 2007). Hypoxia/Hif1a and c-myc

control PKM2 expression (David et al., 2010; Luo et al., 2011); PKM2 plays a role in regulation of Hif1a activity (Luo et al., 2011; Palsson-McDermott et al., 2015). Furthermore, hypoxia and Hif1a along with c-myc also regulate metabolism adjustment and glutaminolysis in cancer cells (Goetzman and Prochownik, 2018). Thus, it is plausible that the functional role of PKM2 in coordinating glycolysis and glutaminolysis switch and in regulating c-myc IRES-dependent translation is a critical control point for cancer cells to ‘‘fine-tune’’ cell responses to hypoxia environments.

2.5 Limitations of the Study

Our data demonstrate the regulation of c-myc expression by dimer PKM2 via the IRES-dependent translation. Although our data suggest that PKM2 facilitates assembly of hnRNP L/K into c-Myc IRES complex, further study is needed to elucidate how assembly of hnRNP L/K into c-Myc IRES complex facilitated by PKM2 controls assembly of c-Myc IRES complex. Furthermore, whether dimer PKM2 is directly involved in the IRES-dependent c-myc translation, e.g. recruiting translation initiation factors to the IRES and/or modulating c-Myc IRES structure, remains to be elucidated. It is shown that c-myc regulates expression and cellular functions of PKM2, including metabolism function of PKM2. PKM2 may also play a role in growth-signaling-related regulation of C-MYC expression at the gene transcription level. The relationship among these different regulatory events and consequential cellular responses needs further investigations.

Figure 1. Dimer PKM2 regulates glutamine metabolism.

(A) – (C) and (D, left) Glutamine consumption in SW480 cells was measured by commercial kit. The glutamine consumption is presented as mmole per million cells per hour. In (A) – (C), PKM2 was knocked down (M2i) or cells were treated by non-targeting siRNA (NT) as control. Wild-type PKM1 (HA-M1)/PKM2 (HA-M2), the TM mutant (HA-TM), or empty vector (EV) was expressed in the PKM2 knockdown cells. In (B), the cells were serum starving overnight prior to the treatment (EGF+) or no treatment (EGF-) with EGF. In (D, left), the cells were treated with PKM2 activator ML265 (10 mM) or DMSO as control. Error bars represent mean ± S.E.M. (D, right) Pyruvate kinase activity of extracts from SW480 cells treated with 10 mM ML265 was measured by the pyruvate kinase activity kit. The pyruvate kinase activity is presented as relative to the extracts of DMSO treated cells (as 100) as reference. (E) – (G) Cellular levels of PKM2 (IB:PKM2), glutaminase 1 (IB:GLS), and c-myc (IB:c-myc) were measured by immunoblot analyses. PKM2 was knocked down in the cells (M2i). Wild type PKM1 (HA-M1)/PKM2 (HA-M2) or the TM mutant (HA-TM) was expressed in the PKM2 knockdown cells in (F) and (G). The cells were treated by PKM2 activator ML265 (ML265) in (G). Immunoblot of HA-tag (IB: HA) indicates the levels of exogenous expression of PKM1/PKM2 and the TM mutant. Cells were treated with non-target siRNA (NT) as controls in (E), (F), and(G). Numbers under the GLS-1 blot panel in F indicate the relative band intensities quantitated by ImagingJ. Immunoblot of GAPDH in (E) – (G) are a loading control.

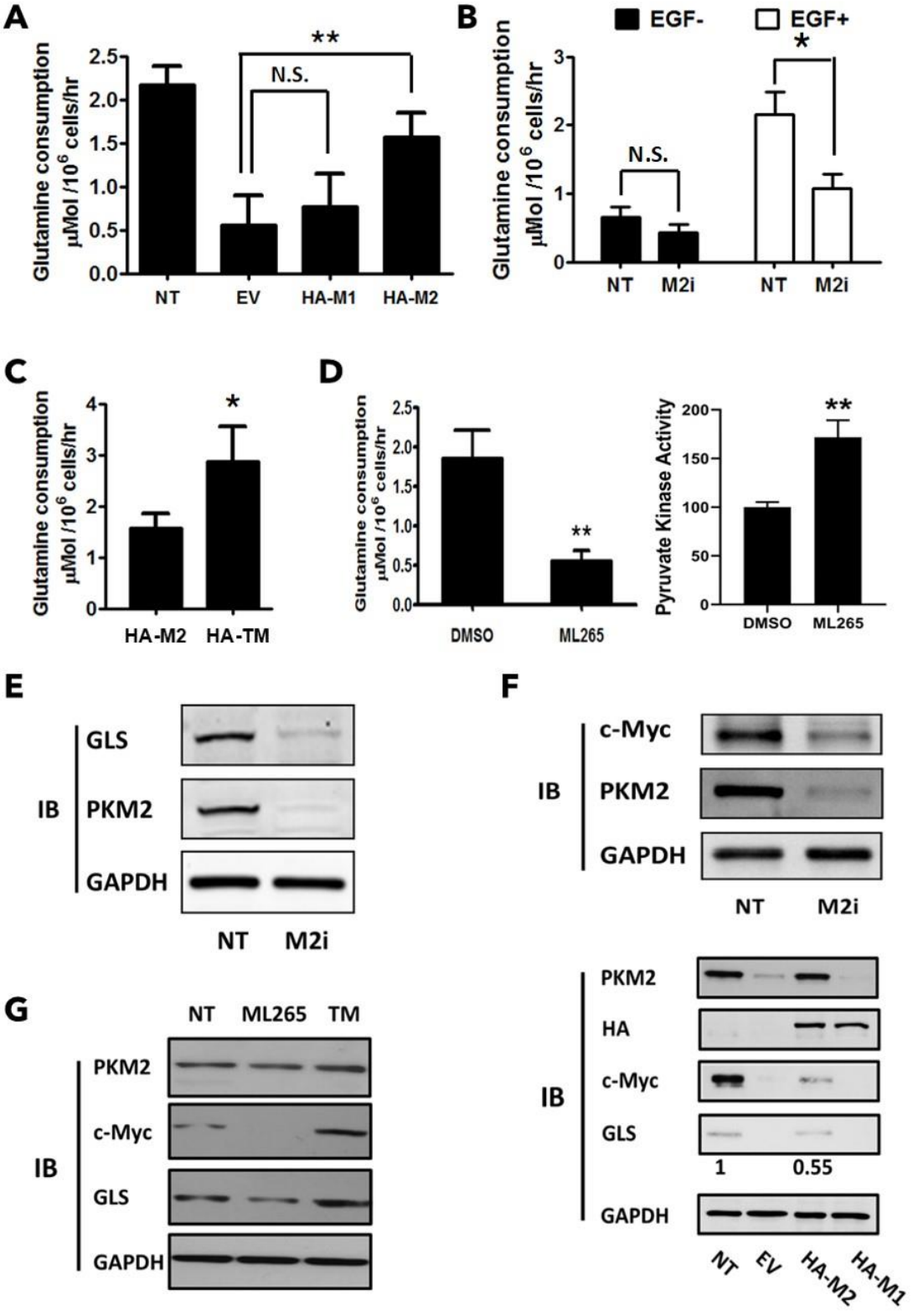


Figure 1 Dimer PKM2 regulates glutamine metabolism.

Figure 2. PKM2 regulates c-myc expression via translational control.

(A) Cellular levels of c-myc (IB:c-Myc) were measured by immunoblot analyses in SW480 cells that are treated by indicated concentrations of PKM2 activator ML265. (B) Cellular c-Myc premRNA and mRNA levels were probed by RT-PCR using primer pairs showing at the bottom of the panels. PKM2 was knocked down (M2i) or cells were treated with non-target siRNA (NT) as control. Immunoblot of PKM2 (IB:PKM2) indicates cellular levels of PKM2. (C) and (D) Cellular levels of c-myc (IB:c-myc) were measured by immunoblot. Cells were treated (+) or untreated (-) with EGF. Cells were also treated with the Cap analog m7G or CHX as translation inhibitors. In (D), PKM2 was knocked down, and the TM mutant (HA-TM) or empty vector (EV) was expressed in PKM2 knockdown cells. The numbers under each panel are quantitation of IB bands using ImagingJ. Immunoblot of GAPDH (IB:GAPDH) is a loading control for all immunoblot assays.

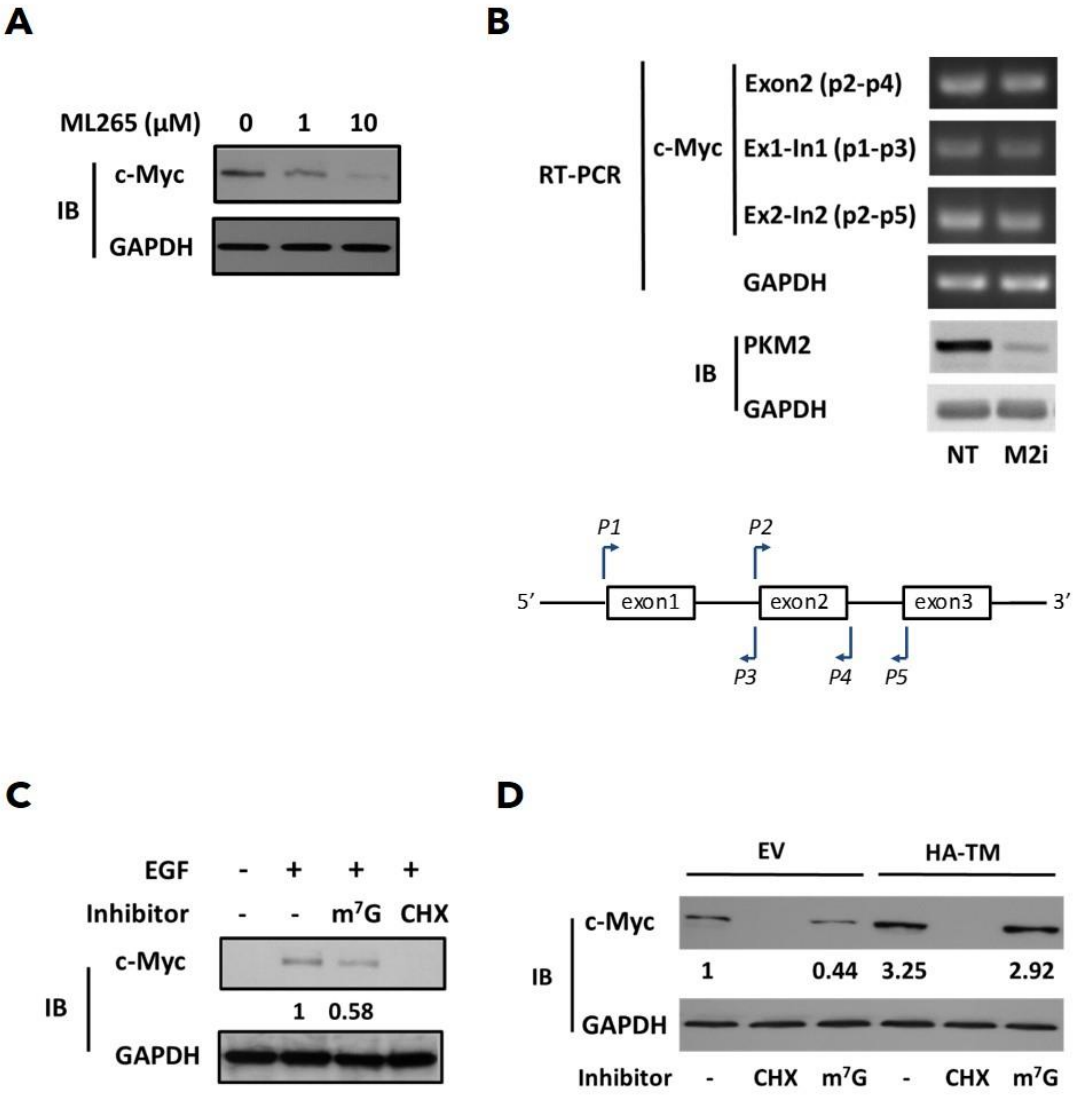
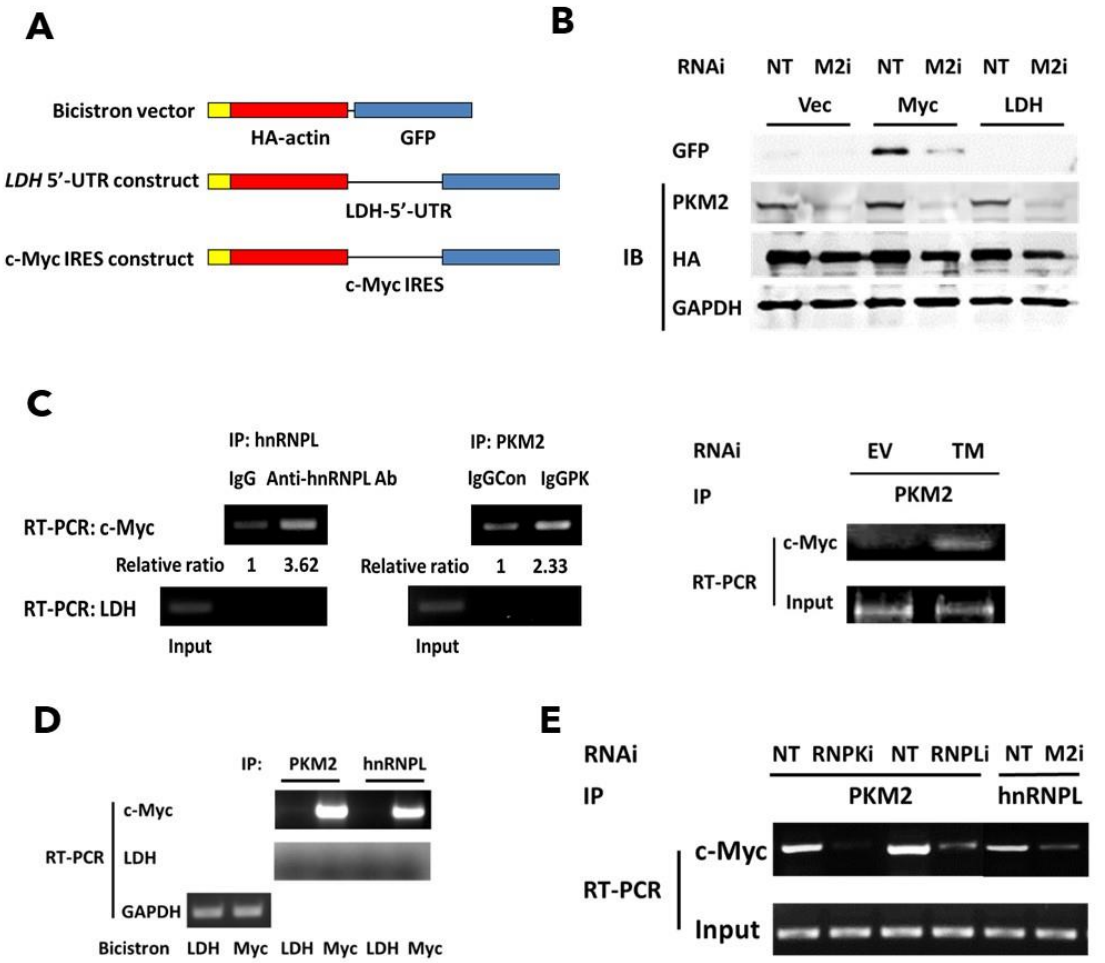


Figure 2 PKM2 regulates c-myc expression via translational control.

Figure 3. PKM2 Regulates c-myc Expression by IRES-Dependent Translation

(A) Diagram illustrates the construction of bicistronic vectors containing reading frames of HA-actin and GFP with 50-UTR of Idh or c-myc IRES insertions between two reading frames. (B) Cellular levels of GFP (IB:GFP) in the bicistronic vectors, Vec – no insertion, Myc – c-myc IRES as insertion, LDH, 50-UTR of ldh as insertion, transfected cells. PKM2 was knocked down (M2i) or cells were treated with non-target siRNA (NT) as control. Immunoblots of PKM2 (IB:PKM2) and HA-actin (IB:HA) indicate cellular levels of PKM2 and HA-tagged proteins. Immunoblot of GAPDH (IB:GAPDH) is a loading control. (C–E) RT-PCR analyses of RNA immunoprecipitation (RIP) of (C) cellular c-Myc mRNA using primer pair span c-myc IRES by antibodies against hnRNP L (left, IP:hnRNP L), PKM2 (middle, IP:PKM2), and PKM2 (right, IP: PKM2 for TM mutant). (D) mRNAs from exogenously expressed bicistronic vectors in the cells (MYC, c-myc IRES, or LDH, ldh-50-UTR) using primer pairs span c-myc IRES and GFP (c-Myc), 50UTR of ldh and GFP (LDH) and (E) cellular c-Myc IRES RNA by antibodies against hnRNP L (IP: hnRNP L) and PKM2 (IP: PKM2). Input in all RIP is a fraction of RT-PCR analyses of GAPDH mRNA of RNA extracts of the designated cells. In (C), IgG is a control antibody for anti-hnRNP L and IgGCon is a control for anti-PKM2 antibody IgGPK. Numbers under each top panel are relative band intensity quantified by ImagingJ. In (E), PKM2 (M2i), hnRNP L (RNPLi), or hnRNP K (RNPKi) were knocked down or cells were treated with non-target siRNA (NT) as control. (F) List of proteins that co-precipitated down with c-myc IRES analyzed by ms-MOLDI-tof/tof. The number of peptides and percent of amino acid sequence matching the corresponding genes is indicated.



F

Gene	Accession[a]	Protein Name	Coverage [b]	Spectra counts[c]
PKM	P14618	Pyruvate kinase PKM	42.75	29
HNRNPL	P14866	Heterogeneous nuclear ribonucleoprotein L	35.85	16
HNRNPK	P61978	Heterogeneous nuclear ribonucleoprotein K	26.83	15
HSPA9	P38646	Stress-70 protein	26.51	15
SYNCRIP	O60506	Heterogeneous nuclear ribonucleoprotein Q	22.95	13
PABPC1	P11940	Polyadenylate-binding protein 1	16.98	11
NCL	P19338	Nucleolin	14.65	10
KPNB1	Q14974	Importin subunit beta-1	12.10	9
RRP1B	Q14684	Ribosomal RNA processing protein1 homolog B	11.74	9

[a] Protein sequence identifier by Uniprot;

[b] percentage of protein sequence covered by identified peptides;

[c] total number of unique peptides matching protein sequence.

Figure 3 PKM2 Regulates c-myc Expression by IRES-Dependent Translation

Figure 4. PKM2 Mediates Cancer Cell Glutamine Addiction under Growth Stimulation

(A–C) Cellular levels of c-myc (IB:c-myc), GLS1 (IB:GLS), hnRNP L (IB:hnrnp L), and PKM2 (IB:PKM2) are analyzed by immunoblots (A left, B and C). The cells are under indicated treatments. In (A), the cells were treated with EGF (50 ng/mL) and FGF (20 ng/mL). (A left panel) Numbers under the GLS and c-Myc blots indicate the band intensities relative to band intensities of actin blots quantitated by ImagingJ. (A, Right panels) The relative changes of cellular levels of c-myc and GLS1 over time. Immunoblots of GAPDH and b-actin are loading controls. Immunoblot of HA-tag indicates cellular levels of exogenously expressed HA-c-myc. (D) Glutamine consumption in SW480 cells was measured by a commercial kit. The glutamine consumption is presented as mmole permillion cells per hour. The cells are under indicated RNAi treatments, or HA-c-myc is exogenously expressed in the indicated RNAi treatment cells. (E) RT-PCR analyses of RNA immunoprecipitation (RIP) of cellular c-Myc mRNA using PCR primer pair span c-Myc IRES by anti-PKM2 antibody (IP:PKM2). Input is a fraction of RT-PCR analyses ofGAPDH mRNA in the RNA extracts of the cells. The cells were treated by EGF at indicated time points. (F and G) Viability of SW480 cells with (with Gln) or without (without Gln) glutamine in medium was measured by cell counting (per view field). PKM2 wild-type (HA-PKM2) or TM mutant (HA-TM) was expressed in the cells in (F). Left panel in (F) is the cell viability measured at day 3 of culture. The cells were either treated with ML265 or DMSO as a control in (G). Error bars in D, F, and G represent mean G S.E.M.

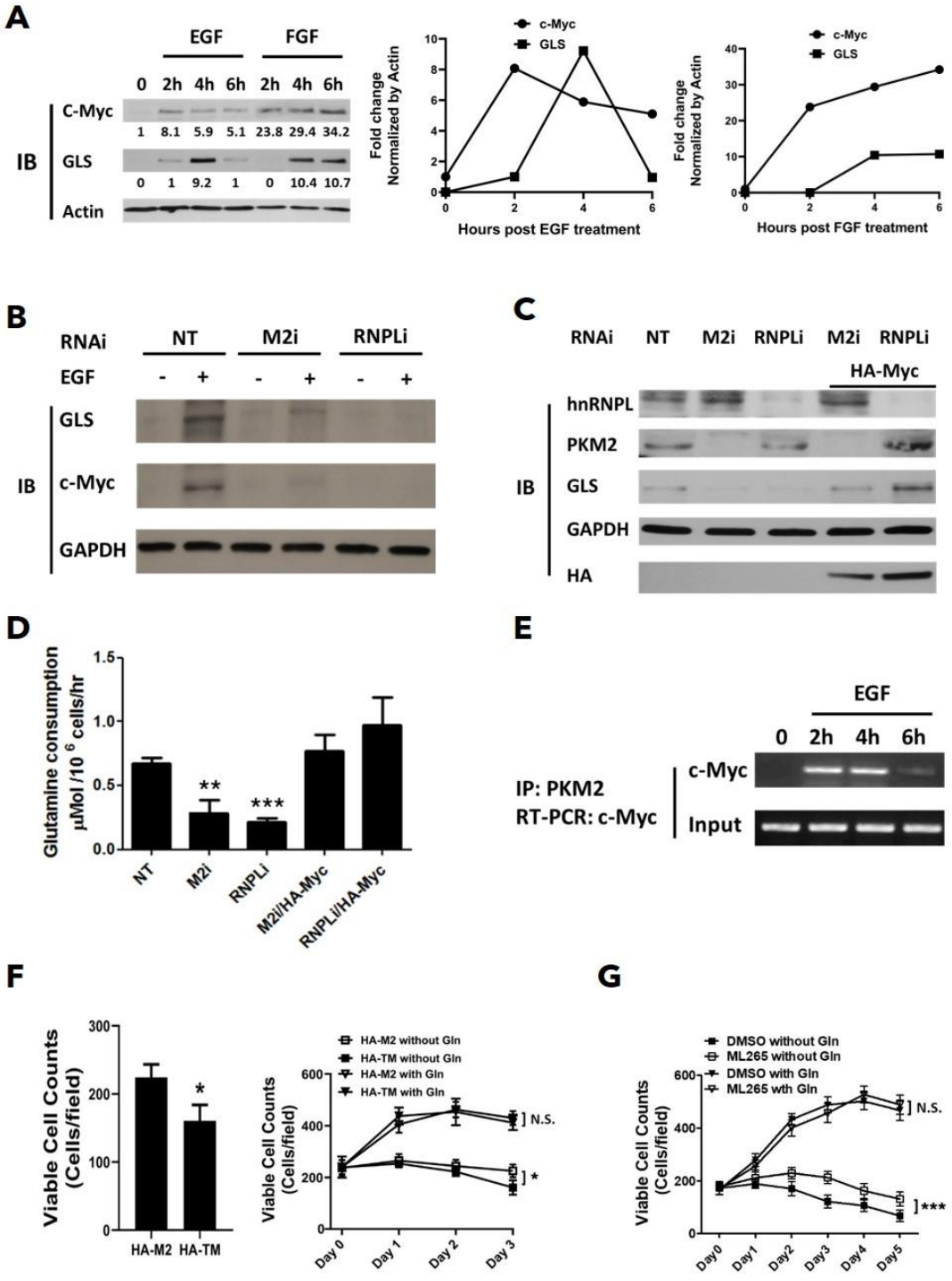


Figure 4 PKM2 Mediates Cancer Cell Glutamine Addiction under Growth Stimulation

Figure 5. Expression, pyruvate kinase activity, and dimer/tetramer status of PKM2 TM mutant. Related to Figure 1 and Figure 2 (A) Cellular levels of hnRNP L (IB:hnRNP L), hnRNP K (IB:hnRNP K), and PKM2 (IB:PKM2) were analyzed by immunoblot. The cells were treated with RNAi against hnRNP L (RNPLi), hnRNP K (RNPKi), PKM2 (M2i), and non-target RNAi (NT). Immunoblot of β-actin (IB:actin) is a control. (B) Proliferation of SW480 cells under 50 ng/ml EGF (+/-) treatment was analyzed by a proliferation kit, and is presented as fold change (proliferation) by comparing to the cells before treatment. Error bars represent mean ± S.E.M. (C) Chromatography profiles of TM mutant at concentrations of 20 uM. Elution volumes equivalent to tetramer, dimer, and aggregation are indicated by arrows. (D) Cellular levels of PKM2 (IB:PKM2), exogenously expressed HA-TM (IB:HA) were analyzed by immunoblot in the extracts of cells expressing HA-TM or empty vector (EV). Immunoblot of GAPDH (IB:GAPDH) is a loading control. (E) Pyruvate kinase activity of recombinant PKM2 (rPKM2) and TM mutant (rPKM2 TM) was measured by the pyruvate kinase activity kit. The pyruvate kinase activity is presented as relative to the rPKM2 (as 100) as reference.

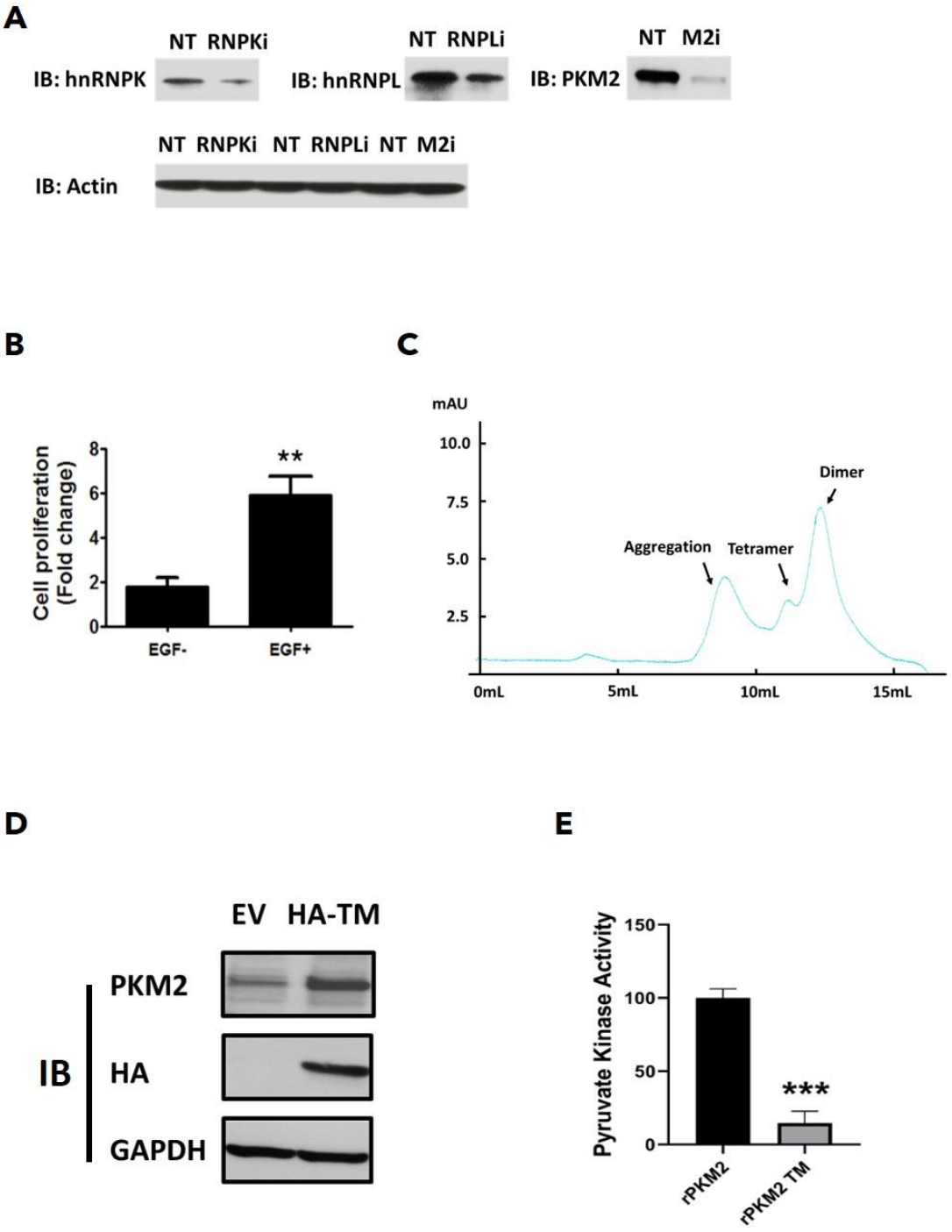


Figure 5 Expression, pyruvate kinase activity, and dimer/tetramer status of PKM2 TM mutant.

Figure 6. PKM2 regulates c-myc and GLS expression and glutamine consumption in breast cancer M4A4 cells.

Related to Figure 1. (A) and (B) Cellular levels of c-myc (IB:c-myc), GLS1 (IB:GLS), and PKM2 (IB:PKM2) were analyzed by immunoblot using indicated antibodies. Immunoblot of GAPDH (IB:GAPDH) is a loading control. (C) Glutamine consumption in M4A4 cells was measured by commercial kit. The glutamine consumption is presented as μmole per million cells per hour. Error bars represent mean ± S.E.M. In (A) & (C), the cells were serum starving overnight prior to the treatment (EGF+) or no treatment (EGF-) with EGF. In (B) & (C), PKM2 was knocked down (M2i) or cells were treated by non-targeting siRNA (NT) as control.

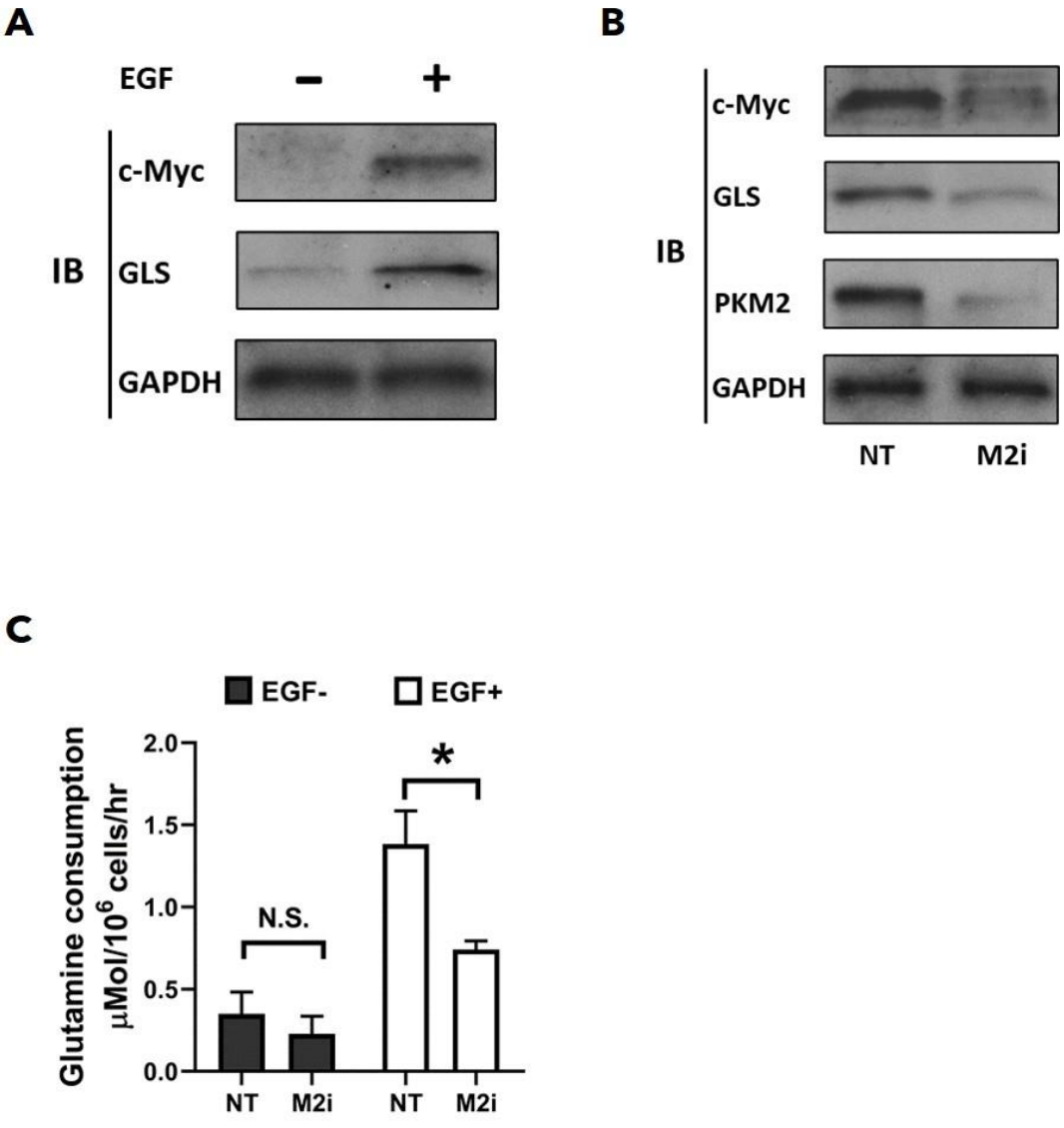


Figure 6 PKM2 regulates c-myc and GLS expression and glutamine consumption in breast cancer M4A4 cells.

Figure 7. PKM2 interacts with hnRNP L/K and c-Myc IRES. Related to Figure 3 &4. (A) Representative image of Coomassie blue staining of SDS-PAGE of co-immunoprecipitation of PKM2 with hnRNP L using anti-PKM2 antibody (IgGPK). IgGCon is IgG purified from prebleeding of the rabbit from which IgGPK was raised. PKM2, hnRNP L, and IgG heavy chain bands were indicated by the arrows. The hnRNP L and PKM2 bands were identified by ms-MALDItof/tof analyses. (B) and (C) Co-immunoprecipitation of PKM2 with hnRNP L using anti-PKM2 antibody (B, IP:PKM2) and anti-hnRNP L antibody (C, IP:hnRNPL). The extracts were treated by RNase A to remove RNA in the extracts in (B). IgG in (C) is mouse IgG as a control for antihnRNPL (IP:hnRNP L) of cellular mRNAs of c-Myc using primer pair span c-myc IRES (c-Myc) and LDH using primer pair span 5'-UTR of LDH mRNA (LDH). (bottom) RT-PCR analyses of cellular mRNA of c-myc, PKM2, GAPDH, and LDH. The cells were treated with RNAi against PKM2 (M2i) or non-target RNAi (NT). (E) Cellular levels of hnRNP L (IB:hnRNP L) and c-Myc (IB:c-Myc) were analyzed by immunoblots. The cells were treated by RNAi against hnRNP L (RNPLi) or non-target RNAi (NT). HA-tagger PKM2 TM mutant (HA-TM) was expressed in hnRNP L knockdown cells, indicatied by immunoblot of HA (IB:HA). Immunoblot of GAPDH in (B), (C), (D), and (E) is a loading control.

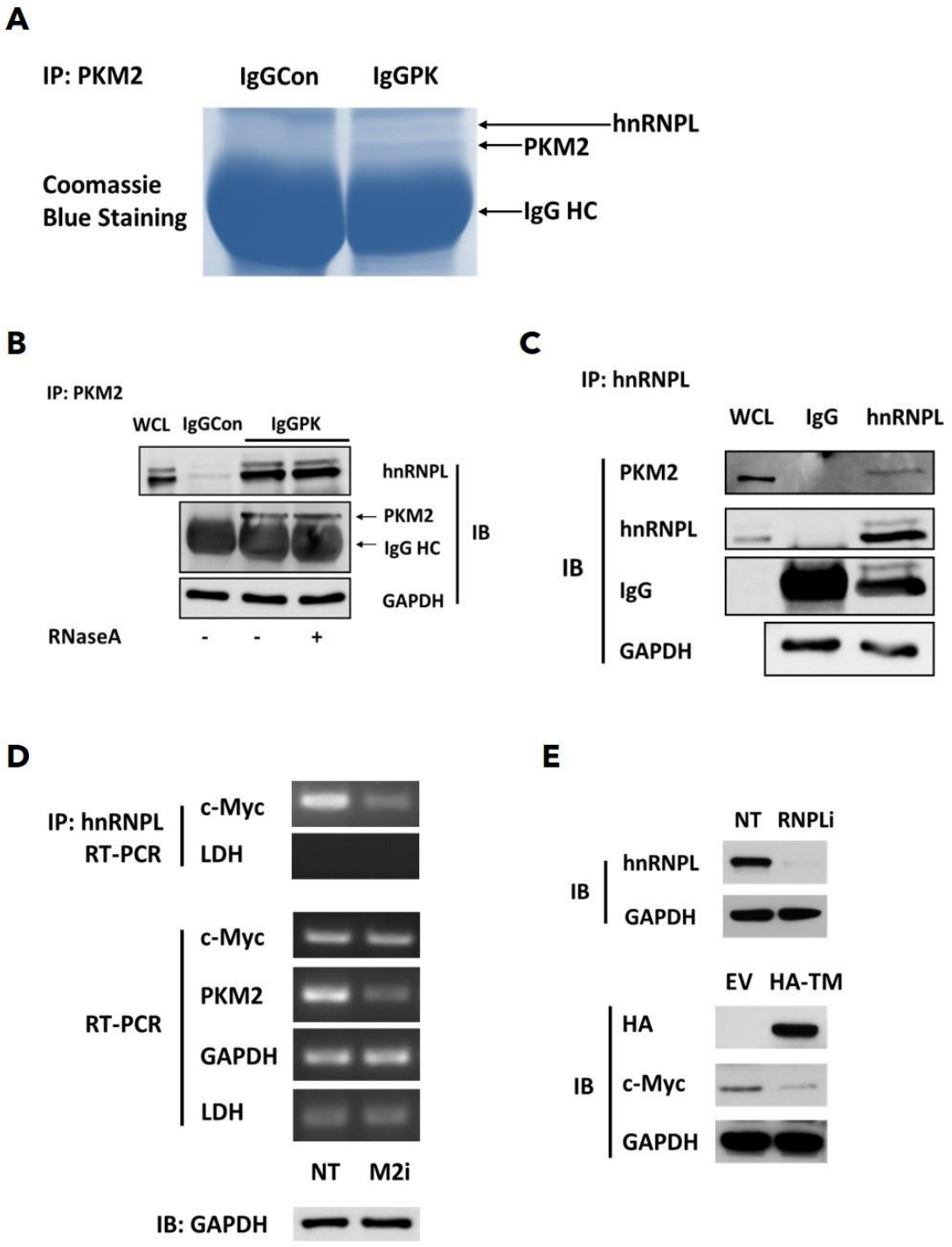


Figure 7 PKM2 interacts with hnRNP L/K and c-Myc IRES.

Figure 8. Schematic diagram of the proposed mechanisms of extracellular PKM2 promoting lung fibrosis progression. On binding to αvβ3 integrin on the surface of myofibroblasts, PKM2 activates FAK and PI3K, triggering (1) enhanced apoptosis resistance of myofibroblasts via activation of NF-κB and (2) upregulation of Arginase-1, generating proline for collagen synthesis. Both roles of PKM2 result in myofibroblast persistence and further fibrosis progression.

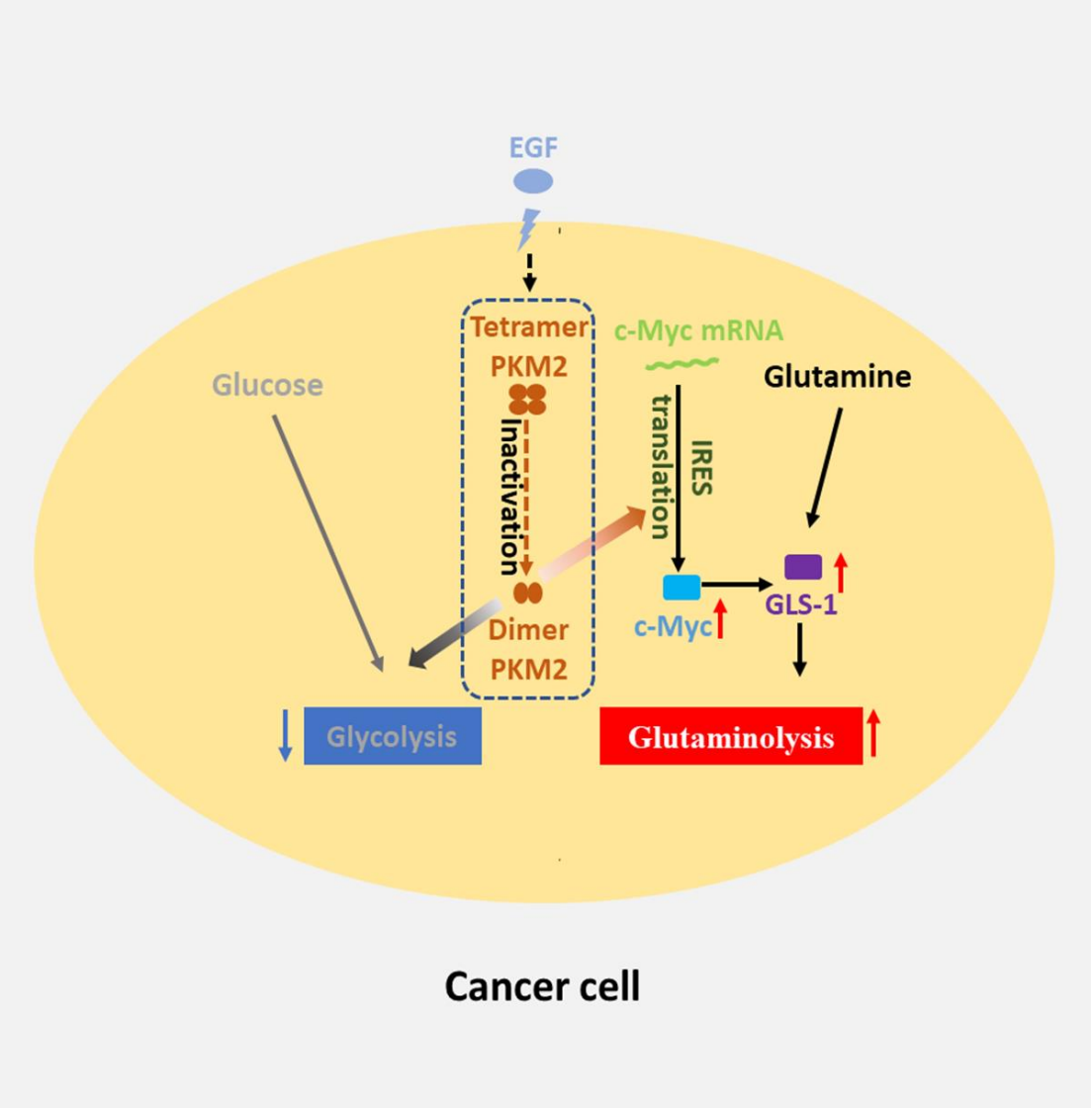


Figure 8 Schematic diagram of the proposed mechanisms of extracellular PKM2 promoting lung fibrosis progression.

3 EXTRACELLULAR PKM2 MODULATES CANCER IMMUNITY BY REGULATING MACROPHAGE POLARITY

3.1 Abstract

Cancer controls its immunity by educating its microenvironment, including regulation of polarization of cancer associated macrophages (CAMs). It is well documented that cancer cells release PKM2 to facilitate tumor progression. We report here that extracellular PKM2 (EcPKM2) modulates cancer immunity by facilitating M2 macrophage in tumors. EcPKM2 interacts with integrin αvβ3 on macrophage to activate the integrin-PI3K signal axis. Activation of integrin-PI3K by EcPKM2 suppresses PTEN expression, which subsequently upregulates arginase-1 (Arg-1) expression in macrophage. Our studies uncover a novel and important mechanism for modulation of cancer immunity. More importantly, an antibody disrupting the interaction between EcPKM2 and integrin αvβ3 is effective in converting M2 macrophages to M1 macrophages in tumors, suggesting a new therapeutic strategy and target for cancer immunotherapies. Combination of the PKM2 antibody with checkpoint blockades and chemotherapeutics provides synergistically enhanced treatment effects.

3.2 Introduction

Abundant infiltration of immune cells in tumors provides evidence of recognition of aberrations in cancer by the host immune system. A long puzzling question is “how do cancer cells avoid host immune destruction?” In fact, cancer turns host immune-responses into cancer promoting factors, meaning tumor infiltration immune cells often facilitate cancer progression and metastasis. Cancer cells educate immune cells in tumors through cross-talk between cancer cells and immune cells and the cells within the tumor microenvironment (J. Wang, Li, Cang, & Guo, 2019). A key component of tumor associated inflammation is CAMs. Macrophages in tumor come

from two sources, tissue-resident macrophage and circulating monocytes recruited to tumor site (). Macrophage is activated to different polarized status with two extreme phenotypes, classically activated M1 and alternatively activated M2. The functional roles of M1/M2 macrophages in modulating inflammation response are almost completely opposite with M1 as pro-inflammation while M2 anti-inflammation or pro-regeneration (Martinez & Gordon, 2014). Macrophage polarization is not completely fixed. M1/M2 are interconvertible dependent on the surrounding environment (Italiani & Boraschi, 2014). Macrophages are the most abundant immune cells in tumors. Levels of macrophages directly correlate with cancer progression (Hao et al., 2012; Nielsen & Schmid, 2017; Noy & Pollard, 2014). CAMs mostly adopt M1 at an early stage of cancer development. However, CAMs in late-stage cancer are predominately M2 subtype (). It is well documented that cancer cells

Pyruvate kinase is an enzyme that catalyzes the last reaction in glycolysis. There are four isoforms of pyruvate kinases, L/R and M1/M2, which are expressed in different tissue types or under different physiological conditions. PKM2 can form a homodimer or a homotetramer. The tetramer is active as pyruvate kinase (Elbers et al., 1991; Hacker et al., 1998), while the dimer is a protein kinase (Gao et al., 2013; X. Gao, H. Wang, J. J. Yang, X. Liu, & Z. R. Liu, 2012; W. Yang et al., 2012). Interestingly, a number of recent studies show that PKM2 is functionally involved in multiple cellular processes in different locations, including metabolism control, transcription regulation, and chromatin packaging (X. Gao et al., 2012; Luo et al., 2011; W. Yang et al., 2011). High serum levels of PKM2 have long been observed in patients of solid tumors () and with various inflammatory diseases (Hathurusinghe, Goonetilleke, & Siriwardena, 2007; Jeffery, Lewis, & Ayling, 2009; Staib, Hoffmann, & Schinkothe, 2006), suggesting a potential role of EcPKM2 in modulating inflammation responses in tumor and other inflammation associated diseases. Our

previous studies demonstrated that EcPKM2 facilitates tumor growth by promoting angiogenesis (L. Li, Y. Zhang, J. Qiao, J. J. Yang, & Z. R. Liu, 2014). During cutaneous wound repair, PKM2 is released by infiltration neutrophils at the wound site. The released PKM2 facilitates wound repair (Y. Zhang, L. Li, Y. Liu, & Z. R. Liu, 2016). In this report, we present evidence to show that EcPKM2 facilitates cancer progression by modulating tumor immunity. EcPKM2 promotes alternative activated macrophage (M2) in tumors. EcPKM2 promotes M2 macrophage by interacting with integrin $\alpha_v\beta_3$ on macrophage and consequently activating the integrin signaling, which leads to activating the integrin-PI3K signaling axis. Activation of integrin-PI3K results in suppression of PTEN and subsequent upregulation of arginase-1 (Arg-1) expression. Disruption of EcPKM2 and integrin $\alpha_v\beta_3$ interaction by a PKM2 antibody abolished the effects of EcPKM2 on promoting M2 macrophage and increasing M1 macrophage population in tumors, suggesting a new strategy and target in cancer immunotherapy. Combination of the PKM2 antibody with checkpoint blockades and chemotherapeutics provides synergistic cancer treatment effects.

3.3 Results

3.3.1 EcPKM2 in patient tumors and secretion of PKM2 by cultured cancer cells

Presence of high levels of PKM2 in blood circulation of cancer patients is well documented (Wong, De Melo, & Tang, 2013). The levels of circulating PKM2 correlated with tumor progression (Zahra, Dey, Ashish, Mishra, & Pandey, 2020a). We demonstrated previously that cancer cells release PKM2 to promote tumor angiogenesis (L. Li et al., 2014) and neutrophil release PKM2 to facilitate wound repair (Y. Zhang et al., 2016). ELISA analyses of culture medium of a panel of cancer cell lines supported that PKM2 is released from cultured cancer cells (Fig. 9A). To further confirm presence of EcPKM2 in tumor, we carried out immuno-histological analyses of tissue samples from cancer patients using an in-house developed rabbit monoclonal

antibody against PKM2, IgGPK (Y. Zhang et al., 2016). High levels of PKM2 were detected in cancer patient tissues. The EcPKM2 was apparent in the staining. As control, almost no PKM2 staining was observed in normal tissues (Fig. 9 B,C&D). Altogether, our results indicate that PKM2 is released to extracellular space of cancer patients, and PKM2 is secreted by cancer cells.

3.3.2 EcPKM2 interacts with integrin αvβ3 on macrophage and promotes M2 macrophage.

We previously observed that EcPKM2 interacts with integrin αvβ3 on myofibroblasts and activates the integrin signaling (unpublished data). It was noted that integrin αvβ3 is highly expressed in macrophages (Antonov et al., 2011). We probed integrin αvβ3 expression in cultured Raw264.7 cells and isolated bone marrow macrophages (BMM) by immunoblot. Evidently, the integrin was expressed in Raw264.7 and BMM cells (Fig. 10A). To test whether the integrin is expressed in cancer associated macrophage, immune cells, including macrophages, CD4+ and CD8+ T cells were isolated from the tumor from orthotopic model of murine breast cancer 4T1 cells. Expression of integrin αvβ3 in these isolated immune cells was analyzed by FACS. Clearly, macrophages expressed high levels of the integrin, CD8+ T cells expressed very low levels of the integrin, while CD4+ T cells did not (Fig. 10B). EcPKM2 interacts with integrin αvβ3 on angiogenic endothelial cells and myofibroblasts (unpublished data). We questioned whether EcPKM2 interacted with the integrin on macrophage. We first carried out cell attachment assay with Raw264.7 cells and culture plate coated with recombinant PKM2 (rPKM2) or PKM1 (rPKM1) expressed and purified from bacterial E. Coli. or BSA. Clearly, the cells attached to rPKM2 but not rPKM1 and BSA coated plates (Fig. 10C). To verify the interaction is integrin αvβ3 dependent, IgG or antibody against integrin αvβ3 LM609 were added into the attachment assays. LM609 blocked the Raw264.7 cells attaching to the rPKM2 coated plate, while IgG did not (Fig. 10D). To further confirm that rPKM2 interacts with tumor macrophage, macrophages

were isolated from 4T1 tumors that were treated with His-rPKM1 or His-rPKM2. The isolated macrophages were then sorted by his-tag. The FACS analyses suggested that rPKM2 interacted with macrophage in the tumor (Fig. 10E). We conclude that EcPKM2 interacted with macrophage in tumors.

Our next question is what the functional role(s) of the interaction between EcPKM2 and integrin αvβ3 on macrophage is. Careful examination of morphology of Raw264.7 cells that were treated by different agents, including rPKM2, rPKM1, LPS, or IL-4, revealed that the cells treated with rPKM2 resemble IL-4 treated cells in morphology (namely M2 polarized macrophage) (Fig. 11A). It is well documented that M2 macrophages secrete IL-10 (Martinez & Gordon, 2014), and the M2 macrophage express cell surface marker CD206 (Martinez & Gordon, 2014). Thus, we examined secretion of IL-10 in the culture medium of Raw264.7 that were treated with rPKM2/rPKM1 and LPS. The cells treated with rPKM1, or LPS did not secrete IL-10, while the rPKM2 treated cells secreted high levels of IL-10 (Fig. 11B). FACS analysis of Raw264.7 that treated with rPKM1/rPKM2, or IL-4 indicated that macrophages expressed high levels of CD206 upon IL-4 or rPKM2 treatment, while CD206 expression was almost not detectable in rPKM1 treated macrophages (Fig. 11C).

3.3.3 EcPKM2 increased M2 macrophages and decreased M1 macrophages in tumors

Cancer cells secret PKM2 and circulating PKM2 correlates with cancer progression. EcPKM2 promotes M2 macrophage *in vitro*. To test whether EcPKM2 promotes M2 macrophage *in vivo*, we employed the orthotopic 4T1 tumor model. The mice carrying 4T1 tumor were treated with rPKM2, rPKM1, or vehicle when tumor grew to an average size of 200 mm in diameter (Fig. 12A). At the end of experiments, tumor weights were examined. Clearly, rPKM2 increased tumor weight (Fig. 12B). FACS analyses of macrophages by F4/80 and CD206 sorting indicated that

rPKM2 treatment increased the percentage of M2 macrophage in 4T1 tumor compared to the rPKM1 group. FACS analyses indicated that rPKM2 reduced M1 macrophages in tumor (Fig. 12C). It is well documented that M2 macrophages induce CD4+ Treg cells in tumors. Thus, we analyzed CD4+ Treg cells in rPKM2 or rPKM1 treated 4T1 tumors. The Treg cells increased in rPKM2 treated 4T1 mice (Fig. 12D). Macrophage polarity largely affects cytokine profile in tumors, especially tumor associated M2 macrophage release high levels of IL-10, which plays a role in regulation of tumor immunity (Nielsen & Schmid, 2017; Sato et al., 2011). IL-10 increased in rPKM2 treated 4T1 mice (Fig. 12E). Examination of metastatic nodules in lung revealed that rPKM2 treatment increased both metastatic nodular number and size compared to the group treated with rPKM1 and vehicle (Fig. 12F). We asked whether rPKM2 treatment affected macrophage polarity in lung metastatic tumors. FACS analyses showed that rPKM2 increased M2 macrophages in lung metastatic tumors compared to rPKM1 (Fig. 12G). Similarly, rPKM2 treatment also increased Treg cells in lung metastatic tumor (Fig. 12H).

EcPKM2 promotes M2 macrophage by interacting with and activating integrin αvβ3. We reasoned that antibody disrupting PKM2 and integrin αvβ3 interaction would exert opposite effects in tumor. We used an in-house developed monoclonal anti-PKM2 antibody (PKM2 Ab) that disrupts PKM2 and integrin αvβ3 interaction (Yinwei Zhang et al., 2016). Tumor bearing mice were treated with PKM2 Ab or rabbit IgG as a control. PKM2 Ab treatment led to smaller tumors and less and smaller metastatic nodules in lung compared to the IgG treatment group (Fig. 13A&B). PKM2 Ab led to high M1 and low M2 macrophage in primary tumors (Fig.13D). FACS analyses of T-reg in the tumor also demonstrated that PKM2 Ab resulted in decrease in Treg (Fig. 13E). FACS analyses of M1/M2 macrophage and Treg in the lung metastatic tumors revealed that PKM2 Ab treatment decreased M2 macrophages and Treg in lung, while increased M1

macrophages (Fig. 13F&G). Histology analyses showed that PKM2 Ab decreased IL-10 in the lung (Fig. 13I). We conclude from our experiments that EcPKM2 promotes M2 macrophage, while antibody disrupts EcPKM2 and integrin αvβ3 interaction abrogates the effects of EcPKM2 on polarity of CAMs.

To test the commonality of the role of EcPKM2 in controlling CAM polarity in tumors, we analyzed the effects of rPKM2 with two additional tumor mouse models. Orthotopic xenograft of Bl16 cells, a murine melanoma cell line. Conditional mouse model of lung cancer is a genetic engineering mouse (GEM) lung cancer model. The GEM mice spontaneously develop lung cancer at age of 8 weeks due to specific expression of KrasG12D and Trp53R172H in the lung. B16 tumor bearing mice or 8-week-old GEM mice were treated with rPKM1/rPKM2 or vehicle. Analyses of macrophages in the tumors by FACS demonstrated that rPKM2 treatment increased M2 macrophages (Fig.14A&D). Conversely, PKM2 Ab treatment led to decrease in M2 macrophage (Fig. 14B, E&G) in tumors. Examination of macrophage in lung metastatic tumors of the treated B16 mice showed that rPKM2 increased M2 macrophages decreased M1 macrophages in lung metastatic tumors (Fig. 14C&E), while PKM2 Ab reduced M2 macrophages in B16 lung metastatic tumor (Fig. 14D&E). These results further support our conclusion that EcPKM2 promotes M2 macrophage in tumors.

3.3.4 EcPKM2 activates integrin-PI3K signaling axis and upregulates Arg-1 in macrophage

An open question was what is the cell surface receptor(s) that mediated the effects of EcPKM2 on macrophage. We previously showed that EcPKM2 interacted with integrin αvβ3 and activated the integrin signaling. Activated macrophage express high levels of integrin αvβ3, and EcPKM2 interacts the integrin (see Fig. 10A-E). Thus, a reasonable speculation would be that EcPKM2 activates the integrin signaling and downstream signal cascades. To test the speculation,

we first examined activation of integrin signaling. It is well established that activation of integrin signaling leads to activation of PI3K (Wegener & Campbell, 2008). Thus, we examined activation of PI3K in Raw264.7 upon rPKM2 treatments. PI3K activity assay demonstrated that PI3K was upregulated and activated in the cells (Fig. 15E). It is well established that PI3K – PTEN pathway regulates Arg-1 expression in macrophages and subsequently modulate macrophage polarity (Carracedo & Pandolfi, 2008; Chalhoub & Baker, 2009). We reasoned whether EcPKM2 regulated Arg-1 expression and subsequent macrophage polarity by the same pathway. We first probed Arg-1 levels and arginase activity in the rPKM2 treated Raw264.7 and BMM. Clearly, addition of rPKM2 but not rPKM1 into culture medium upregulates Arg-1 (Fig. 15A). The effects of rPKM2 were abrogated by addition of the antibody LM609 into culture medium (Fig. 15B) and integrin β3 knockdown in Raw264.7 cells (Fig. C&D), suggesting that upregulation of Arg-1 in macrophage by EcPKM2 were mediated by integrin αvβ3. To verify the role of EcPKM2 in regulating Arg-1 expression and promoting M2 polarity via suppression of PTEN expression, PTEN was exogenously expressed (Fig. 15F).

3.3.5 PKM2 Ab synergistically enhances the efficacy of checkpoint blockades.

Addition of checkpoint inhibitors to various chemotherapies represents an important advancement in the treatment of different type of cancers (D'Abreo & Adams, 2019; Vikas, Borcherding, & Zhang, 2018). It is well documented that macrophage plays a critical role in modulating cancer immunity by secreting pro- or anti-inflammatory soluble factors, such as cytokines/chemokines (Nielsen & Schmid, 2017; Noy & Pollard, 2014). The anti-PKM2 antibody PKM2 Ab that disrupts the interaction between EcPKM2 and integrin αvβ3 promotes M2 macrophage in tumors. It is thus intriguing to test anti-cancer effects of PKM2 Ab in combination with checkpoint blockade. B16 is a murine melanoma model that is well tested with checkpoint

blockade treatment. We therefore used this model to test the efficacy of a combination of PKM2 Ab + anti-PD-1 antibody (aPD-1). Tumor bearing mice were treated with PKM2 Ab, aPD-1, and PKM2 Ab + aPD-1. PKM2 Ab and aPD-1 alone provided marginal or modest benefits on the animal overall survival and tumor growth. The combination provided strong anti-cancer effects (Fig. 14I).

3.3.6 PKM2 Ab synergistically enhances efficacy of cancer chemotherapeutics.

It is now fully recognized that M2 CAMs strongly facilitates chemotherapy resistance and relapse after eradication of tumor by chemotherapy and/or radiation therapies. Antibody disrupts EcPKM2 and integrin αvβ3 interaction promotes CAMs M2 to M1 conversion. We reasoned that the PKM2 antibody may provide enhancement effects for cancer chemotherapies. To test this conjecture, we used the 4T1 murine breast cancer model. Tumor bearing mice were treated with PKM2 Ab and PKM2 Ab + a low dose of paclitaxel (PTX). PTX alone did not have a significant effect on the overall survival and tumor growth. PKM2 Ab provided modest benefits on the animal overall survival and tumor growth. However, the combination strongly prolonger the tumor bearing mice survival and inhibited tumor growth (Fig. 13J).

3.4 Discussion

Our study here revealed an unexpected role of EcPKM2 in facilitating tumor progression by inducing the macrophage into M2 type through activating integrin αvβ3. Along with our previous observations that EcPKM2 facilitates tumor growth (L. Li, Y. Zhang, J. Qiao, J. J. Yang, & Z.-R. Liu, 2014) and wound healing (Y. Zhang et al., 2016) by promoting angiogenesis, we propose a hypothesis that EcPKM2 may modulate cancer microenvironment by inducing macrophages into M2 type, which will enhance the immune suppressive environment. These

effects have been observed in different cancer types, implying that this could be a broad phenomenon.

We noticed that there are high levels of EcPKM2 in tumor tissues from patients with various cancer types, including breast cancer, lung cancer and melanoma; further *in vitro* analysis showed that different types of cancer cells secrete a high level of EcPKM2. These observations bring up a question, what is the function of EcPKM2 in cancer. By i.p. injection of rPKM2 in multiple mouse cancer types, we noticed that rPKM2 significantly promotes the tumor progression. Further investigations showed that M2 macrophage percentage is significantly increased in rPKM2-treated tumor mice and that EcPKM2 induces macrophage cells into M2 type. These results together indicate that EcPKM2 modulates cancer immunity by facilitating M2 macrophage polarization in tumors. Further investigations on the mechanism elucidated EcPKM2 interacts with integrin αvβ3 on macrophage to activate integrin-FAK-PI3K signal axis and activation of FAK-PI3K by EcPKM2 suppresses PTEN expression, which subsequently upregulates arginase-1 (Arg-1) expression in macrophage. Our studies uncover a novel and important mechanism for modulation of cancer immunity.

Extracellular PKM2 facilitates tumor progression *via* a novel mechanism of action via the activation of integrin αvβ3. It is intriguing that an antibody against PKM2 that disrupts the PKM2-integrin αvβ3 interaction is very effective in inhibiting tumor progression, indicating a potential anti-tumor strategy and target. Our study on the PKM2 antibody provides the proof of principle evidence for targeting extracellular PKM2 as a treatment of breast cancer, lung cancer and melanoma. Furthermore, combination of the PKM2 antibody with checkpoint blockades and chemotherapeutics provides synergistic treatment effects.

In fact, M2 macrophages are important in other diseases such as wound healing and organ fibrosis. In wound healing, M1 macrophage switch to M2 type is critical for the wound healing process; in the organ fibrosis diseases, M2 macrophage can promote fibrosis through many ways, including producing more pro-fibrotic factors TGF-β, releasing more collagen synthesis amino acids proline by upregulating arginase activity. It is very interesting to test whether extracellular PKM2 plays a similar role in progression of wound healing and organ fibrosis, and whether disrupting the PKM2-integrin αvβ3 interaction will interfere with the wound healing process or have treatment benefits for organ fibrosis. Another project of ours confirms that PKM2 also regulates M2 macrophages in bleomycin induced lung fibrosis (See appendix). We not only notice that EcPKM2 is highly expressed in lung fibrosis, but also find that the core cell type in fibrosis, myofibroblasts, secretes a high level of EcPKM2. Neutralizing EcPKM2 with PKM2Ab inhibits the lung fibrosis progression and the M2 type macrophages are significantly reduced.

Release of extracellular PKM2 and elevation in integrin αvβ3 expression are closely associated with a number of pathological conditions, particularly various inflammatory associated chronic diseases and cancers. It is conceivable that targeted disruption of PKM2 and αvβ3 interaction may be beneficial for treatment of these human diseases.

Figure 9. High level of EcPKM2 expressed in cancer.

(A) EcPKM2 level was measured with ELISA assay using the culture media individually collected from 4 different cell types, HEK, breast cancer cell line 4T1, melanoma cell line B16 and lung cancer cell line A549. (B) Representative images of immunohistochemistry analysis of PKM2 accumulation in normal human breast tissue and breast tissue from patients with breast cancer tissue. (C) Representative images of immunohistochemistry analysis of PKM2 accumulation in normal human skin tissue and skin tissue from patients with melanoma. (C) Representative images

of immunohistochemistry analysis of PKM2 accumulation in normal human lung tissue and lung tissue from patients with pulmonary fibrosis. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

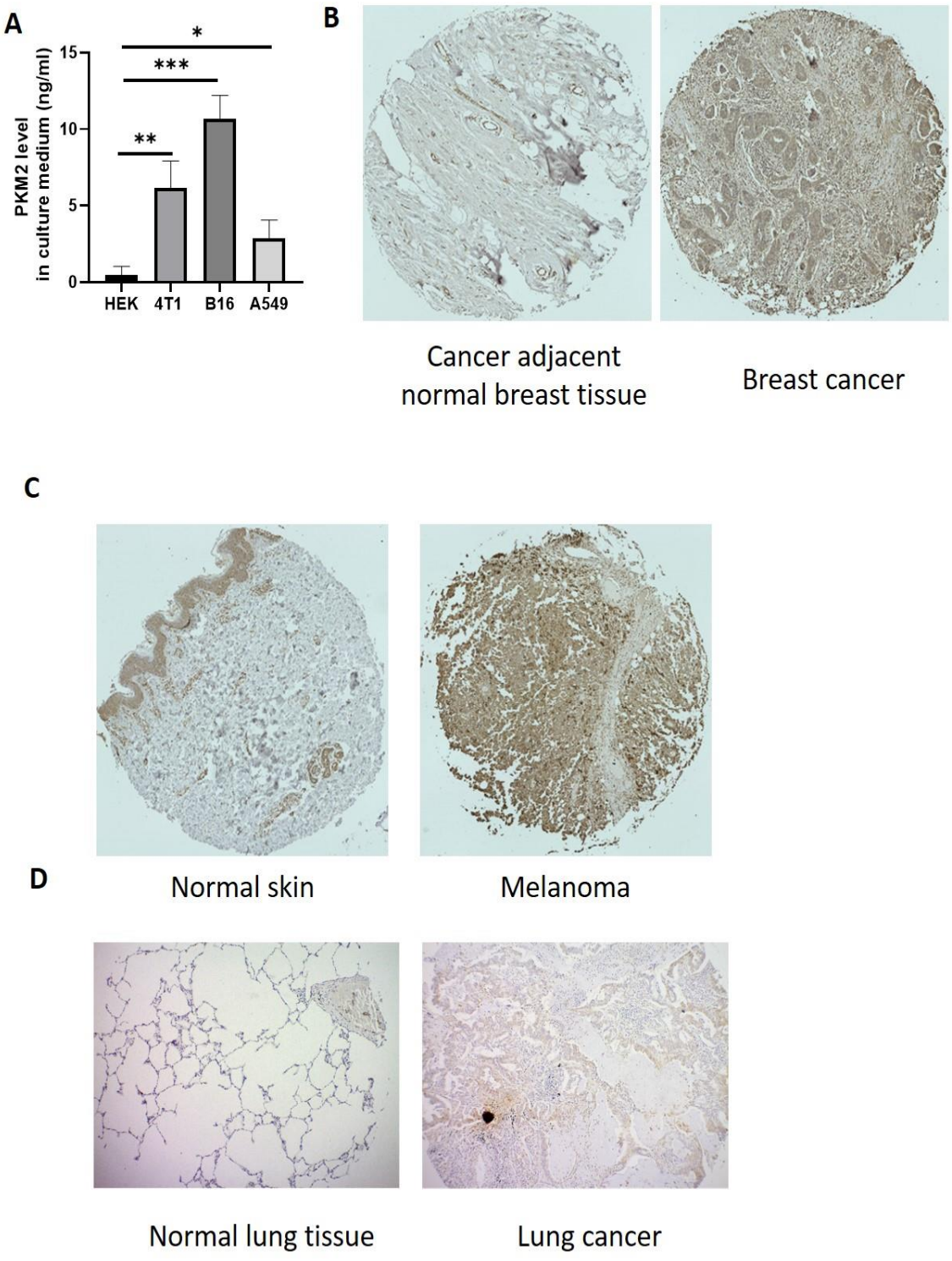


Figure 9 High level of EcPKM2 expressed in cancer.

Figure 10. Macrophage expresses αvβ3, EcPKM2 interacts with αvβ3 on macrophage.

(A) Representative western blots showing αv and β3 expression level in Raw 264.7 and bone marrow derived macrophages. GAPDH is used as a loading control. (B) Tumor tissues were collected from 4T1 breast cancer mice and subjected to the detection of αvβ3 level in macrophages, CD4 T cells and CD8 T cells with FACS analysis. (C and D) Representative microscope images of cell attachment assay. Raw 264.7 cells were seeded on plates pre-coated with BAS or rPKM1 or rPKM2 (C). Raw 264.7 cells were seeded on rPKM2-coated plates in the presence of the αvβ3 blocking antibody (LM609, 10 μg/ml) or IgG control (D), After incubation, unattached cells were washed away, and the remaining cells were counted in five randomly selected view field per images. Experiments were done in triplicate and data presented as means ± SD. rPKM1 serves as the isoform control for rPKM2. (E) Tumor tissues were collected from 4T1 breast cancer mice treated with rPKM1 or rPKM2 (rPKM1 and rPKM2 are his-tagged proteins) and macrophages were separated with FACS. Then his-tag positive macrophages were quantified in rPKM1 and rPKM2-treated groups. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

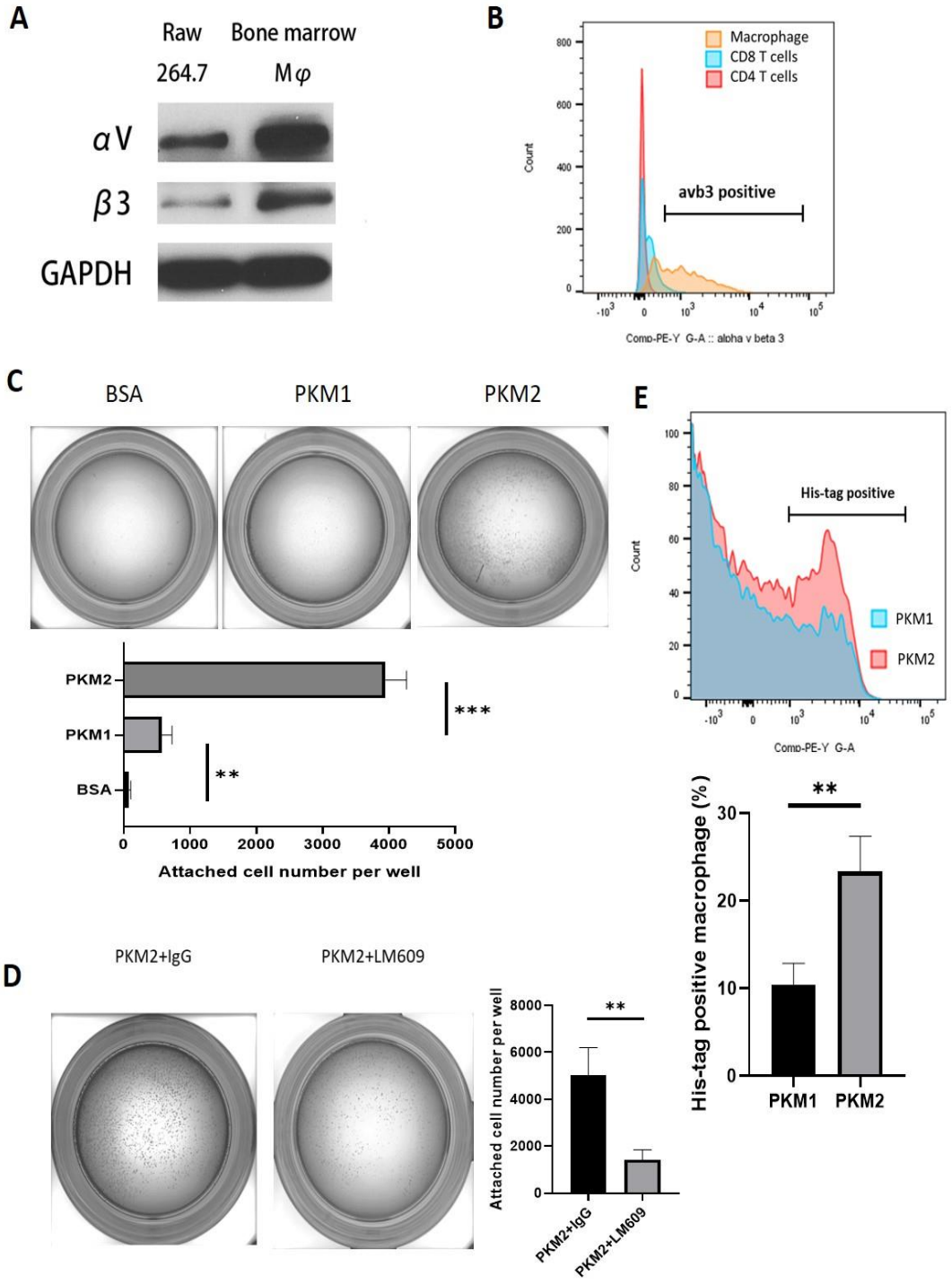


Figure 10 Macrophage expresses αvβ3, EcPKM2 interacts with αvβ3 on macrophage.

Figure 11. PKM2 can induce macrophage into M2 type.

(A) Raw 264.7 cells were treated with indicated agents and then stained by crystal violet. LPS is used as a typical inducer of M1 macrophages. IL4 is used as a typical inducer of M2 macrophages. M2 like cells were identified as spread filopodia shaped cells (S. P. C. Hsu et al., 2020). (B) The level of IL-10 cytokine was measured with ELISA assay using culture media of Raw264.7 treated with indicated agents. (D) Raw264.7 cells were treated with rPKM2 or IL4 and subjected to FACS analysis for $CD206^{+}$ macrophages. CD206 is a marker for M2 macrophages. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

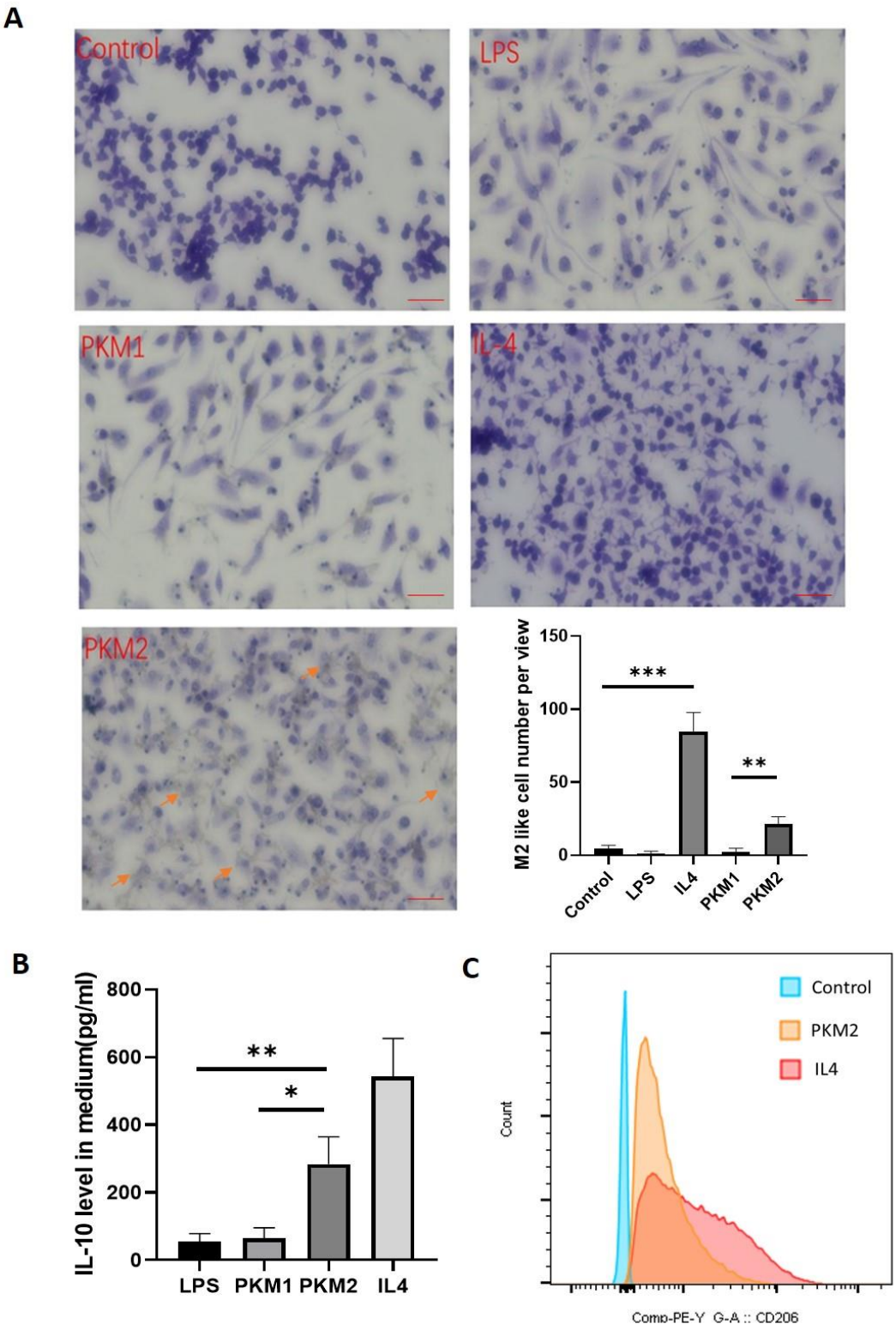


Figure 11 PKM2 can induce macrophage into M2 type.

Figure 12. PKM2 can induce macrophages into M2 type in the 4T1 mouse model.

(A) Experimental scheme of 4T1 breast cancer mouse model establishment and treatment with rPKM1/rPKM2. (B) Representative photographs of tumor tissues from mice treated with indicated agents. Tumor weights were measured at the endpoint. (C and D) Tumor tissues collected from mice treated with rPKM2 or rPKM2 were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206+$ positive M2 macrophages (C) and $CD4^{+}FoxP3^{+}$ T-reg cells (D). (E) Representative images of IL-10 immunohistochemistry staining in tumor tissues collected from 4T1 mice treated with rPKM1 or rPKM2. (F) Representative images of HE staining of lung tissues collected from 4T1 mice treated with rPKM1 or rPKM2. (G and H) Lung tissues collected from mice treated with rPKM1 or rPKM2 were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206^{+}$ M2 macrophages (F) and $CD4^{+}FoxP3^{+}$ T-reg cells (G). All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

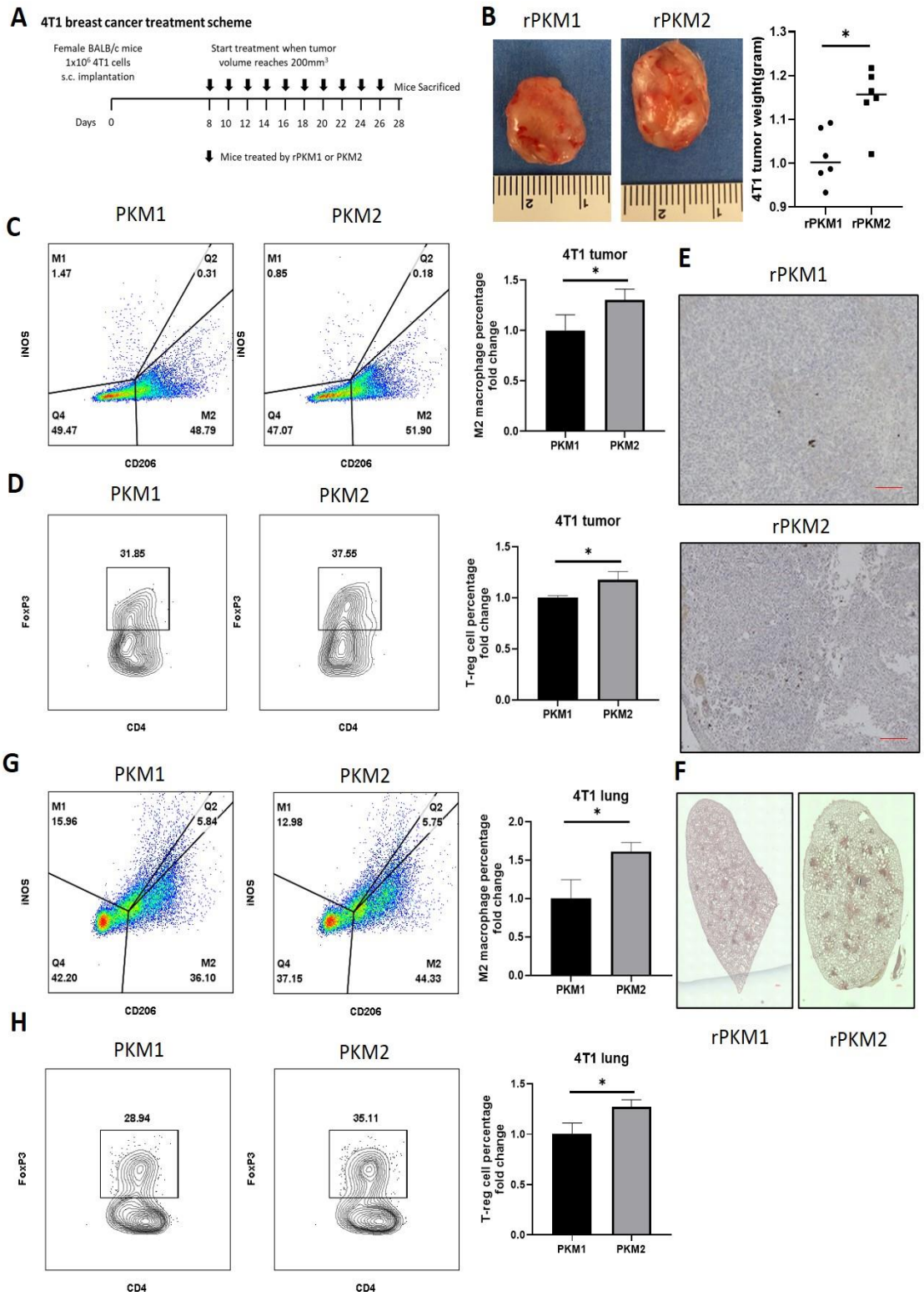


Figure 12 PKM2 can induce macrophages into M2 type in 4T1 mouse model.

Figure 13. PKM2 Ab can reduce M2 macrophage level in 4T1 mouse model.

(A) Representative photographs of tumor tissues from mice treated with IgG control or PKM2 antibody. (B) Tumor weights were measured at the endpoint. (C) Representative images of HE staining of lung tissues collected from 4T1 mice treated with IgG control or PKM2 antibody. (D and E) Tumor tissues collected from 4T1 mice treated with IgG control or PKM2 antibody were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206^{+}$ M2 macrophages (D) and $CD4^{+}FoxP3^{+}$ T-reg cells (E). (F and G) Lung tissues collected from 4T1 mice treated with IgG control or PKM2 antibody were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}$CD206+ positive M2 macrophages. Statistical analyses of D-G were displayed in (H). (I) Representative images of IL-10 immunohistochemistry staining in lung sections collected from 4T1 mice treated with IgG control or PKM2 antibody. (J) Survival rate of 4T1 mice treated with indicated agents. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

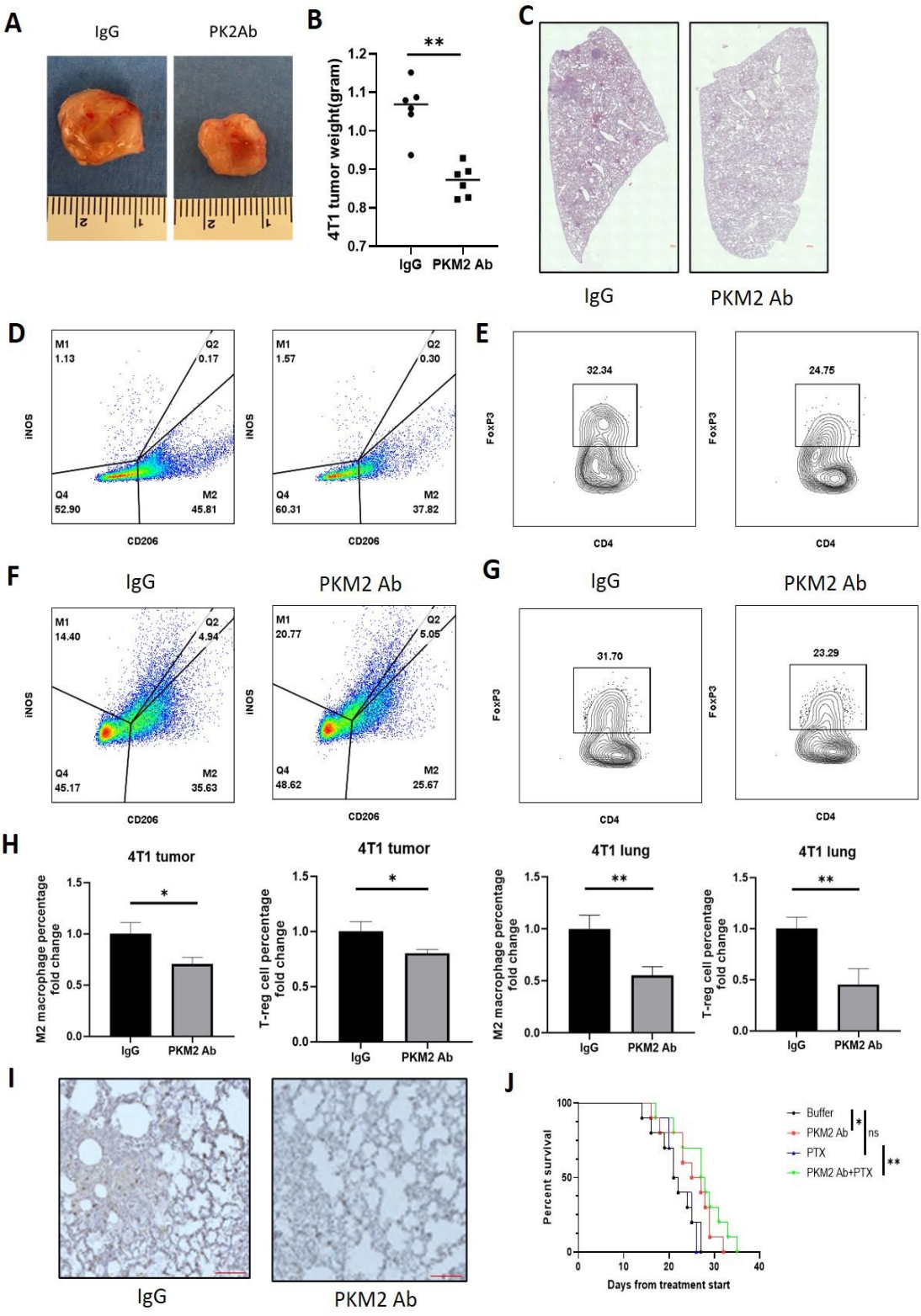


Figure 13 PKM2 Ab can reduce M2 macrophage level in 4T1 mouse model.

Figure 14. PKM2 and PKM2 Ab also work in mouse models of melanoma and lung cancer. (A and C) Tumor tissues (A) and lung tissues (C) collected from B16 melanoma mice treated with rPKM1 or rPKM2 were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206^{+}$ M2 macrophages. (B and D) Tumor tissues (B) and lung tissues (D) collected from B16 melanoma mice treated with IgG control or PKM2 antibody were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206^{+}$ M2 macrophages. Statistical analyses of A-D were displayed in (E). (F and H) Representative images of IL-10 immunohistochemistry staining in lung sections collected from B16 melanoma mice (F) and lung cancer mice (H) treated with IgG control or PKM2 antibody. (G) Lung tissues collected from lung cancer mice treated with IgG control or PKM2 antibody were dissociated and subjected to FACS analysis for $iNOS^{+}CD206^{-}$ M1 macrophages and $iNOS^{-}CD206^{+}$ M2 macrophages. (I) Survival rate of B16 mice treated with indicated agents. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

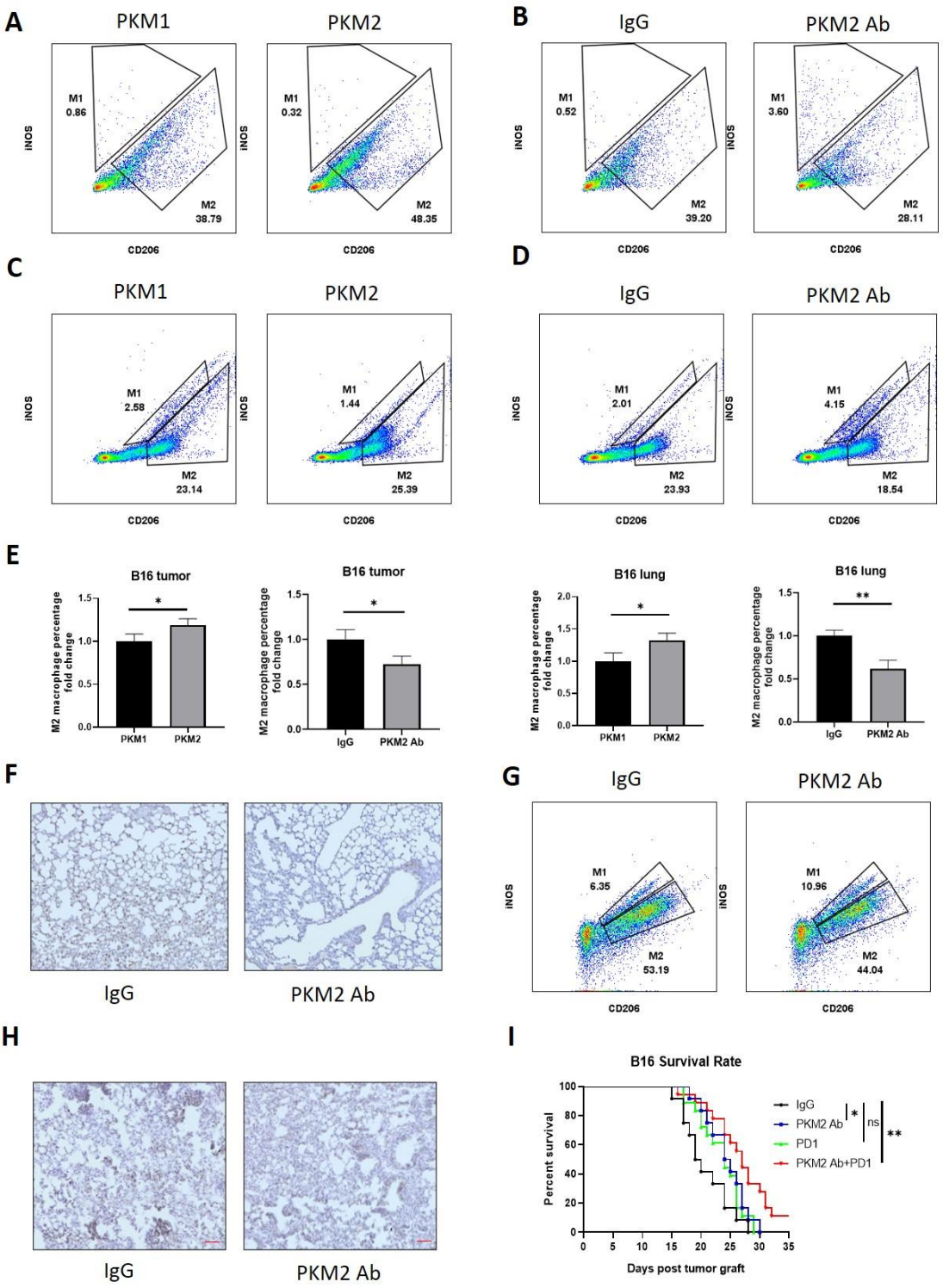


Figure 14 PKM2 and PKM2 Ab also work in mouse models of melanoma and lung cancer.

Figure 15. PKM2 induces macrophages to M2 type through αvβ3, PI3K and PTEN.

(A) Representative western blots showing expression of arginase 1 (Arg-1) after indicated treatments in Raw264.7 (upper panel) and bone marrow derived macrophages (lower panel). Actin serves as a loading control. (B) Raw264.7 were treated with rPKM1 or rPKM2 in the presence of the function-blocking anti-αvβ3 antibody (LM609) and analyzed for protein level of Arginase-1 using western blots. The rPKM1 serves as isoform control for rPKM2. Actin serves as a loading control. (C and D) The β3 was knocked down with siRNA in Raw264.7, followed by rPKM2 treatment and then analyzed for the protein level of Arginase-1 using western blots (C) and arginase activity with ELISA assay (D). Actin serves as a loading control for western blots. Scrambled siRNA was used as a control for β3 siRNA. (E) Raw264.7 were treated with rPKM1 or rPKM2 and assayed for PI3K activity. (F) PTEN was exogenously expressed using adenovirus ad-PTEN in Raw 264.7. The cells were then treated by rPKM2 and subjected to the immunoblot analysis of PTEN and ELISA assay of arginase activity. GAPDH serves as a loading control for western blot. All columns and error bars represent means ± SD. * $p<0.05$; ** $p<0.01$, *** $p<0.001$.

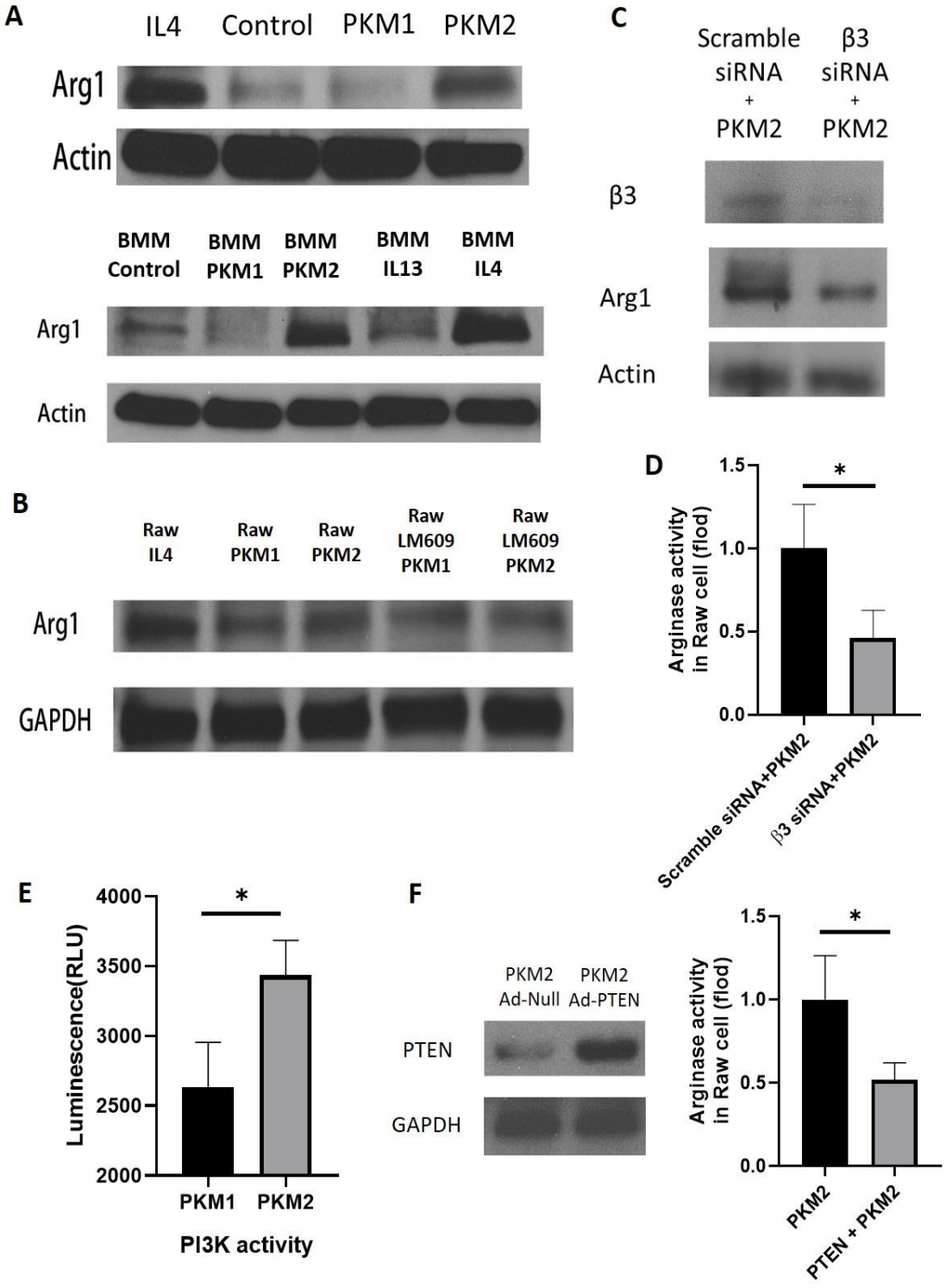


Figure 15 PKM2 induces macrophages to M2 type through αvβ3, PI3K and PTEN.

4 CONCLUSIONS AND DISCUSSIONS

Cancer cells reprogram their nutrition metabolism to meet their high bioenergetic and biosynthetic demands in support of rapid growth and continuous proliferation(Phan, Yeung, & Lee, 2014). Both glycolysis and glutaminolysis are altered in cancer cells(Akins, Nielson, & Le, 2018). Glycolysis is a universal pathway used by all living cells for energy production, which can occur in both aerobic and anaerobic states. However, about a century ago, Otto Warburg first discovered that the glycolysis rate is 200 times higher in cancer cells compared to healthy cells and cancer cells predominantly undergo anaerobic glycolysis regardless of whether oxygen is present(Akins et al., 2018; Warburg, 1924). This phenomenon is later termed the Warburg effect. After its discovery, scientists have come up with many theories for why cancer cells switch to inefficient anaerobic glycolysis. One widely accepted theory is that the Warburg effect allows cancer cells to maintain large pools of intermediates which provide building blocks for synthesis of nucleotides, fatty acids and amino acids, assisting the rapid proliferation and growth of cancer cells(Liberti & Locasale, 2016).

Warburg effect limits entry of pyruvate into TCA cycle in cancer cells. To compensate for the reduction in carbon coming from glycolysis, cancer cells switch to the glutaminolysis which utilizes glutamine to replenish the TCA cycle(Anderson, Mucka, Kern, & Feng, 2018). In addition, glutaminolysis also provides reducing power by converting glutamate to glutathione (GSH), the most abundant anti-oxidant in mammalian cells in handling oxidative stress(Anderson et al., 2018). Elevated glutaminolysis has been recognized as a critical hallmark of cancer(Cluntun, Lukey, Cerione, & Locasale, 2017). Not only that cancer patients have much lower concentrations of glutamine in their blood circulation compared to healthy people(Cluntun et al., 2017), but also, the reduction in glutamine consumption can restrain the cancer progression to some extent(Jiang,

Srivastava, & Zhang, 2019). Glutamine, the most abundant amino acid in blood circulation, is beneficial for cancer cells as it supplies more carbon and nitrogen for macromolecule biosynthesis and maintenance of redox balance(Alberghina & Gaglio, 2014; Kodama et al., 2020; Souba, 1993). By this means, with increased glutamine being converted via glutaminolysis, cancer cells display enhanced cell proliferation rate and reduced cell death(Matés, Pérez-Gómez, de Castro, Asenjo, & Márquez, 2002).

Glutaminolysis as well as glycolysis in cancer cells have been extensively studied, but rare attention has been put to how the cancer cells coordinate these two metabolic pathways. In a recent manuscript entitled "Pyruvate kinase M2 coordinates metabolism switch between glycolysis and glutaminolysis in cancer cells" (L. Li et al., 2020), we demonstrated that the pyruvate kinase M2 (PKM2), a critical enzyme in glycolysis, helps to switch the glutaminolysis pathway in cancer cells through IRES-dependent translation of c-Myc. This study uncovers an important mechanism how cancer cells coordinate glycolysis and glutaminolysis and also sheds light on the novel role of PKM2 as a regulator of glutaminolysis. Nevertheless, the unique way of PKM2 to regulate glutaminolysis in cancer cells also brings up some intriguing questions which are worthy of further investigations (Figure 1): Does PKM2 manipulate other proteins through IRES-dependent translation? Does PKM2 regulate glutaminolysis in other cell types besides cancer cells? What is the exact role of PKM2 in IRES-dependent translation of c-Myc?

4.1 The unique role of PKM2 in regulation of glutaminolysis

Pyruvate kinase is a key enzyme in glycolysis, which catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate at the last, yet a rate-limiting, step of glycolysis. Although there are several different isoforms of pyruvate kinase, cancer cells highly express M2 isoform

(Prakasam, Iqbal, Bamezai, & Mazurek, 2018). More interestingly, PKM2 in cancer cells is predominantly inactive form, dimeric PKM2(Zahra, Dey, Ashish, Mishra, & Pandey, 2020b). Compared to its active tetrameric PKM2, the low-activity-dimeric PKM2 has compromised affinity for its substrate PEP, leading to accumulation of glycolytic intermediates. Despite its glycolytic roles, PKM2 in cancer cells have additional non-glycolytic functions(M.-C. Hsu & Hung, 2018; Zahra et al., 2020b). For instance, PKM2 in cancer cells is identified to regulate HIF1α activity as a transcriptional activator(Azoitei et al., 2016); and PKM2 is also demonstrated to phosphorylate STAT3 using PEP as a phosphate donor in cancer cells(Xueliang Gao et al., 2012). These various non-canonical functions suggest that roles of PKM2 in cancer cells are complicated, and there might be other non-glycolytic functions which have not been elucidated yet. Here in our new study "Pyruvate kinase M2 coordinates metabolism switch between glycolysis and glutaminolysis in cancer cells", we revealed another novel non-glycolytic function of PKM2 in cancer cells.

In our recent study, we demonstrated the mechanism how PKM2 coordinates the glycolysis and glutaminolysis pathways. We showed for the first time a crucial role of inactive dimeric PKM2 in regulating IRES-dependent protein translation in cancer cells. Our investigations indicated PKM2 dimers, promoted by growth stimulations, elevate glutaminolysis by upregulating mitochondrial glutaminase I (GLS-1) via interacting with c-myc IRES-RNA and facilitating IRES-dependent translation of c-Myc. The traditional understanding of the role of PKM2 in cancer metabolism is that the inactive dimeric PKM2 support cancer cell growth by allowing the glycolytic intermediates to build-up and re-direct into anabolic pathways(Chi V Dang, 2012), an advance made in our latest manuscript is that inactive dimeric PKM2 can also modulate the augmentation of glutaminolysis, feeding synthesis materials to cancer cells.

4.2 Could dimeric PKM2 regulate IRES-dependent translation of other genes?

A number of proliferation and survival related genes are regulated at translational level (Ruggero, 2013). This is important to cancer cells since translational level regulation provides them with fast adjustment to stressful environments (B. Liu & Qian, 2014). Accumulating evidence shows that IRES-dependent translation is important for many cancer-related proteins(Grzmil & Hemmings, 2012; Xi et al., 2017; Y. Yang & Wang, 2019), suggesting that IRES-mediated translation is vital for cancer cells. Our work showed that PKM2 can regulate GLS-1 through IRES-dependent translation of c-Myc. Since c-Myc is a master regulator in cancer cells(Miller, Thomas, Islam, Muench, & Sedoris, 2012), PKM2 might regulate other c-Myc related pathways such as cell cycle, apoptosis and proliferation(C. V. Dang, 1999; Desbarats, Schneider, Müller, Bürgin, & Eilers, 1996; Hoffman & Liebermann, 2008; McMahon, 2014), which are worth further investigations.

Except c-Myc, many other key cancer-related proteins have been reported to be regulated by IRES-mediated translation, like VEGF, IGF1R, cyclin D1 and XIAP (Godet et al., 2019; Komar & Hatzoglou, 2011; Kucab & Dunn, 2003; Loges, Mazzone, Hohensinner, & Carmeliet, 2009; C. Vaklavas et al., 2015), Furthermore, IRES-mediated translation of these proteins have been found critical for many processes of cancer cells including metastasis and angiogenesis(Kucab & Dunn, 2003; Christos Vaklavas, Blume, & Grizzle, 2017; Walters & Thompson, 2016). Interestingly, dimeric PKM2 is found to promote cancer cell metastasis and angiogenesis(He et al., 2017; Liangwei Li et al., 2020). It is plausible that dimeric PKM2 might be able to regulate other cancer-related proteins through IRES-dependent translation. It is worthy to further test if PKM2 can regulate other targets through IRES-dependent translation in cancer cells, which may suggest a broader implication of dimeric PKM2 in reprogramming of cancer cells.

4.3 Could dimeric PKM2 regulate glutaminolysis in non-cancer cell types?

Besides tumor cells, metabolic reprogramming of glutaminolysis is observed in multiple cell types such as immune cells(Kim, 2018; Pearce & Pearce, 2013; S. Wu, Kuang, Ke, Pi, & Yang, 2021). The role of inactive dimeric PKM2 in regulation of glutaminolysis in non-cancer cell types merits further investigations.

Macrophages and neutrophils stimulated by LPS or bacteria utilize glutaminolysis to sustain ROS production by generating NADH(X. Zhang et al., 2020). Furthermore, macrophages and neutrophils upon microbial activation display an increased level of PKM2 expression(Palsson-McDermott et al., 2015; Yinwei Zhang et al., 2016) and PKM2 in LPS-activated macrophages are primarily dimers(Palsson-McDermott et al., 2015). Altogether, innate immune cells such as macrophages following microbial challenge might elevate dimeric PKM2 to rewire glutaminolysis through a similar mechanism as we observed in cancer cells. Noticeably, the role of dimeric PKM2 in macrophages might not be through the regulation of IRES-dependent translation of c-Myc as c-Myc can drive macrophages to alternative activated phenotype(Pello et al., 2012) which mainly have tetrameric PKM2(Palsson-McDermott et al., 2015). Nevertheless, it is still worthy to investigate whether dimeric PKM2 can regulate the glutaminolysis in innate immune cells and the underneath mechanisms if it can.

Besides the innate immune cells, it is well appreciated that dimeric PKM2 is essential for CD4 T cell activation(Angiari et al., 2020). Moreover, CD4 T cell activation also relies on glutaminolysis and c-Myc is indispensable in this process(Araujo, Khim, Mkhikian, Mortales, & Demetriou, 2017; Bandukwala et al., 2012; Wahl, Byersdorfer, Ferrara, Opipari, & Glick, 2012). So, it is possible

that dimeric PKM2 mediates glutaminolysis remolding in the process of T cell activation via regulating c-Myc by IRES-dependent translation.

In addition to immune cells, emerging evidence suggests glutaminolysis is required for the activation of myofibroblasts(Bernard et al., 2018; Gibb Andrew et al., 2019), whose persistence is a hallmark of fibrotic diseases of multiply tissues and organs(Bernard et al., 2018). Our lab has found that dimeric PKM2 expression is enriched in the activated myofibroblasts (unpublished data). Further investigations are needed to discover the role of dimeric PKM2 in gluaminolysis regulation in myofibroblasts.

4.4 What are the possible roles of PKM2 in IRES-dependent translation of c-Myc?

Our data have indicated that dimer PKM2 regulates c-Myc expression via the IRES-dependent translation. However, the detailed mechanism about how PKM2 regulates c-myc expression via IRES-dependent translation remains unclear. IRES-dependent translation involves multiple different proteins(Hellen & Sarnow, 2001; Y. Yang & Wang, 2019). Inferring from our observations, it is plausible that PKM2 may facilitate assembly of hnRNP L/K into c-myc IRES complex. However, further study is needed to elucidate how PKM2 regulates the assembly process of c-myc IRES complex. In addition, it is also unknown whether dimeric PKM2 is directly involved in the IRES-dependent c-Myc translation. Since the recruitment of translation initiation factors to IRES sequence and structure is critical for IRES-dependent translation(Fitzgerald & Semler, 2009; López de Quinto, Lafuente, & Martínez-Salas, 2001). It is reasonable to hypothesize that dimeric PKM2 may be directly involved in recruiting IRES associated translation initiation factors and/or modulating the IRES structure, which could be deciphered with further investigations.

4.5 What is implicated by EcPKM2 modulating macrophage polarity?

PKM2 has been identified as serving a variety of biological functions intracellularly. However, EcPKM2 has also been reported in many different kinds of diseases, like cancer. EcPKM2 has been reported related to angiogenesis and wound healing. But little is known about the function of EcPKM2 on immune cells. Here we observed that EcPKM2 can modulate macrophage polarity in different cancer types. EcPKM2 induces macrophages into M2 subtype through interacting with integrin on macrophages in cancer. M1 and M2 macrophages have very different functions in diseases. M1 subtype macrophages are generally considered "proinflammatory", which can clear bacteria; however, M2 subtype macrophages are generally considered "anti-inflammatory", that can release cytokines to facilitate tissue repair and growth.

In cancer, M2 type macrophages have been well known to help the tumor growth by providing anti-inflammatory effects. Along with our finding that EcPKM2, highly expressed in various cancers, can facilitate macrophages polarizing to M2 type; we proposed that it would be a novel treatment strategy by blocking the EcPKM2's effect on cancer associated macrophages. We expected to observe the decrease in M2 type macrophages in cancer and the reduction in tumor growth and progression. We used the PKM2 blocking antibody in mouse models of different cancers. As we expected, the PKM2 blocking antibody inhibited progression of these cancers in mice. Targeting EcPKM2 modulating macrophage polarity provides us a new strategy to treat diseases in which M2 macrophages play a critical role including cancer and certain organ fibrosis diseases.

What is more interesting is our finding of EcPKM2 modulating macrophage polarity might also provide us a new approach to resolve inflammation in a lot of inflammatory diseases. Inflammatory diseases rank among the most significant causes of death in the world, such as

chronic diseases. Chronic diseases are considered some of the greatest threats to human health, like diabetes and cardiovascular diseases. Since the EcPKM2 can induce macrophages into M2 type which can suppress the inflammation, administration of rPKM2 to the inflammatory diseases might help to resolve the inflammation and slow down the progression of these diseases or even cure them. Inflammatory diseases are complicated, specially, in many cases, there are different types of immune cells involved during different stages or time points. To use rPKM2 as an anti-inflammation treatment, we might need to further study the optimal time point and dosage based on the disease type.

5 METHODS AND MATERIALS

5.1 Reagents, Antibodies, and Cells

Table 1 Reagents, antibodies, and cell lines

Reagent or Resource	Source	Identifier
ML265	Cayman Chemical	13942
Ultravision Peroxidase block	Thermo Fisher Scientific	TA060H2O2Q
Ultravision Protein block	Thermo Fisher Scientific	TA060PBQ
Antibody diluent OP Quanto	Thermo Fisher Scientific	TA-125-ADQ
Betazoid DAB Chromogen kit	Biocare Medical	BDB2004L
Tris Base	Fisher Scientific	BP154-1
Tween-20	Sigma-Aldrich	P5927
Citrate buffer	Sigma-Aldrich	C9999-1000ML
Xylenes	Fisher Scientific	X5-4
Ethanol	Decon Lab	22032601
DirectPCR Lysis Reagent (Mouse Tail)	Viagen Biotech	102-T
PCR Master Mix	Thermo Scientific	F548S
100bp DNA Ladder	Thermo Scientific	SM1143
Molecular Biology Agarose	Bio-Rad	1613101
PKM2 siRNA	Thermo Fisher Scientific	s10575
hnRNP L siRNA	Thermo Fisher Scientific	s6741
hnRNP K siRNA	Thermo Fisher Scientific	s6739
Antibodies		
GLS1	Proteintech	19958-1-AP
c-myc	Thermo Fisher Scientific	13-2500
HA-tag	Abcam	ab9110
GFP	Abcam	ab290
hnPNP L	Abcam	ab6106

GAPDH	Santa Cruz Biotechnology	sc-32233
hnRNP K	Abcam	ab52600
β-actin	Yurogen	MA5-18035
CD16/CD32	Thermo Fisher Scientific	16-0161-82
F4/80	BioLegend	123111
CD45	BioLegend	103108
iNOS	Thermo Fisher Scientific	12-5920-80
CD206	Thermo Fisher Scientific	48-2061-82
CD3e	Thermo Fisher Scientific	17-0031-82
CD4	BioLegend	100447
FoxP3	BioLegend	320008
CD25	Thermo Fisher Scientific	48-0251-82
Chemicals, Peptides, and Recombinant Proteins		
Recombinant murine EGF		236-EG
Recombinant murine FGF		233-FB
Commercial Assay kits		
Picro sirus red staining kit	IHC world	IW-3012
Masson's trichrome kit	IHC world	IW-3006
Human IGF1 ELISA kit	R&D systems	DG100
Novaultra Oil red O stain kit	IHC world	IW-3008
Annexin V-FITC Apoptosis Staining Kit	abcam	ab14085
BrdU Cell Proliferation Assay	Millipore	2752
Glutamine Colorimetric Assay Kit	Biovision	K556-100
Endotoxin Removal Spin Columns	Thermo Fisher Scientific	88274
Endotoxin Assay Kit	GenScript	L00350
Experimental Models: Cell Lines		
SW480	ATCC	CCL-228
Raw 264.7	ATCC	TIB-71

5.2 Primers

Table 2 primer sequences

Name	sequence
c-Myc P1	5’-TCCAGCGAGAGGCAGAGGGAGCGA-3’
c-Myc P2	5’-TCTGCGACCCGGACGACGAGACCT-3’
c-Myc P3	5’-GGCAAGTGGACTTCGGTGCTTACC-3’
c-Myc P4	5’-TGGAGGTGGAGCAGACGCTGTGGC-3’
c-Myc P5	5’-TTGACAGGCCTGGGCGGGCTTCG-3’
GLS1 F	5’-GATGGGCAACAGTGTTAAG-3’
GLS1 R	5’-CTCTCCCAGACTTTCCATTC-3’
c-Myc F	5’-TGAGGAGACACCGCCCAC-3’
c-Myc R	5’-CATCGATTTCTTCCTCATCTTC-3’
Actin F	5’-GAGCAAGAGAGGCATCCTC-3’
Actin R	5’-GCACAGCCTGGATAGCAACG-3’
Actin-clone F	5’-CAGAAGCTTGATGATGATATCGCCGC-3’
Actin-clone R	5’- CAGGGTACCCTACTAGAAGCATTTGCGGTGG -3’
c-Myc-5UTR F	5’-CAGGGTACCAATTCCAGCGAGAGGCAGAGG-3’
c-Myc-5UTR R	5’- GTAGGATCCGCGTCGCGGGAGGCTGCTG -3’
LDH-5UTR F	5’- CAGGGTACCTTAGTCTGATTTCCGCCCACC -3’
LDH-5UTR R	5’- GCAGGATCCGTGTCACTACAGCTTCTTTAATGT -3’

5.3 Cell culture

SW480 cells were maintained in L15 medium supplemented with 2mM Glutamine and 10% Fetal Bovine Serum (FBS) in 37°C with no CO2. SW480 were treated with or without EGF (50 ng/ml) and FGF (20 ng/ml) respectively for 2 h, 4 h, and 6 h in serum free medium. Cell lysates from indicted time points were subjected for SDS-PAGE and western blot analysis regarding to the levels of c-Myc and GLS1.

5.4 Construction of PKM2 and mutant expression, bicistronic, and HA-myc expression vectors

PKM2 expression vector is the same as previous studies (PMID: 22306293). PKM2 triple mutant (TM) was constructed based on PKM2 gene with three mutations on the following amino acid residues: R399E, K422A, N523A. C-Myc was cloned into pHM6 vector. For bicistronic vectors, β-actin gene with HA tag was inserted into pHM6 vector by HindIII and KpnI; Lactate dehydrogenase (LDH) gene 5'-UTR or c-Myc gene 5'-UTR was inserted by KpnI and BamHI.

5.5 Glutamine consumption measurement

Intracellular glutamine consumption was measured by hydrolyzing glutamine to glutamate to produce colored signal at OD450 nm by assay kit from Biovision. Briefly, cell lysates were deproteinized by 10K Spin Column by centrifuging at 10,000 X g for 20min at 4°C. Glutamate concentration [Glu] of the deproteinized samples was firstly determined. Glutamine was then converted to glutamate and total converted glutamate concentration [Glut] was measured. Glutamine concentration was calculated by the equation of [Gln]=[Glut]-[Glu]. SW480 cells were starved with glucose and glutamine overnight. Fresh culture media containing 2mM D-Glutamine were applied to the cells on the second day for 2 hours and cells were lyzed at the indicated time points. Glutamine consumption was described by glutamine concentration changes Δ[Gln] per 106 cells over time.

5.6 Biotinylation of c-Myc 5'-UTR

5'-UTR of c-Myc was biotinylated following the MEGAshortscript Kit manual (ThermoFisher). Firstly, T7 RNA polymerase promoter sequence TAATACGACTCACTATAGGG was incorporated into the upstream of c-Myc 5'-UTR by PCR. Briefly, 5µl pfu DNA polymerase buffer, 5 µl of primer mix, 1 µl DNA polymerase, 1 µl of dNTP

mix and 5ng of DNA template was prepared in DNase-free water to final volume 50 µl in a 500 µl PCR tube. PCR products were mixed thoroughly, and Eppendorf Thermocycler was set up with 40 cycles: 30sec at 95°C, 30sec at 60°C and 30sec at 72°C. For in vitro transcription, 2 µl of reaction buffer, 2 µl of 75mM ATP, 2 µl of 75mM CTP, 2 µl of 75mM GTP, 1.6 µl of 75mM UTP, 0.4 µl of 75mM Biotin-14-CTP, 2 µl MEGAshortscript T7 enzyme and 200ng of DNA template are mixed in water to a final volume 20 µl in a PCR tube. The reaction was incubated at 37°C for 4 hours in Thermocycler. The DNA template was removed by DNase. Biotinylated C-myc IRES was purified by Ambion NucAway Spin column.

5.7 Western blot

After the treatment, the culture medium was removed from the plates or dishes and cells were washed three times with ice-cold PBS buffer. Then the cells were scraped off with a rubber scraper and centrifuged at 1000 rpm, 4 ℃ for 10 min. The superannuants were discarded, and the cell pellets were resuspended with RIPA buffer premixed with protease inhibitors cocktail and phosphatase inhibitors. The mixtures were placed on a rotator and incubated at 4 ℃ for 30 min, after which, the lysates were centrifuged at 14000 rpm, 4 ℃ for 10 min. The supernatants were transferred to new tubes and stored at -20 /-80℃ freezer. The protein concentrations were determined using the Quick Start Bradford 1×Dye Reagent (Bio-Rad, Cat. # 5000205) and a Biophotometer. For western blot analysis, an equal amount of protein sample was taken out and mixed with 5× loading buffer (175 mM Tris-HCl pH 7.0, 5 mM EDTA, 10% SDS, 20% sucrose, 0.01% bromophenol blue, and 28.8 mM β-mercaptoethanol) and heated at 95℃ for 10 min. The samples and protein ladder were loaded in SDS-PAGE gel and ran at constant voltage 80 V first at the stacking stage, and then at 80 V at the separating stage.

After the SDS-PAGE, the proteins were transferred to a nitrocellulose membrane at a constant current of 250 mA for 2 h at 4℃. The membrane was blocked in the blocking solution, 5% BSA prepared in TBST (20mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) at room temperature for 1 h. After blocking, the membrane was incubated with the primary antibody diluted in the blocking solution on a flat shaker at 4 ℃ overnight, followed by three times washing with TBST buffer, 10 min each time. Then the membrane was incubated with respective secondary antibody diluted in blocking solution on a flat shaker at 4 ℃ overnight, followed by three times washing with TBST buffer, 10 min each time. The specific bands were detected by autoradiography using chemiluminescence reagents.

5.8 RNA-pulldown assay and PKM2 immunoprecipitation

SW480 cells cultured in the media containing EGF or buffer for 4 hours and cells were disrupted by polysome extraction buffer and the cell lysates were collected after centrifugation at highest speed for 10min. The cell lysates were incubated with biotinylated c-Myc IRES in assay buffer (20mM Tris-HCl pH 8.0, 1mM EDTA, 200mM NaCl and 1% triton X-100 containing protease inhibitor and RNA inhibitor) at 4°C for 4 hours. Then c-Myc IRES was pulled down by streptavidin-conjugated dynabeads and dynabeads were washed three times with assay buffer without RNA inhibitor. Pull-down samples were treated with RNase and dynabeads were removed. The samples were then subjected to incubation with in-house polyclonal anti-PKM2 antibody conjugated Protein A agarose beads 4°C for overnight. Protein A agarose beads were washed three times with assay buffer and eluted by 45µl of 0.1M Glycine-HCl pH 2.5. The elution was neutralized by addition of 5 µl of 1M Tris-HCl pH 8.5

5.9 Identification of c-MYC IRES interacting proteins by Mass Spectrometry

RNA-pulldown and IP samples proceeded to Trypsin digestion. Briefly, samples were concentrated by Vacuum concentrator and incubated in with 8M Urea and 5mM DTT at 37°C for 1 hour. 15mM iodoacetamide was applied to samples for 30min in the dark at room temperature. The samples were trppsinized by Trypsin Gold from Promega in trypsin digestion buffer pH 8.0 at 37°C for overnight. The digested peptides were concentrated and desalted by C18 ZipTip pipette tips from Millipore. All HPLC-MS/MS experiments were performed on a Orbitrap Elite mass spectrometer equipped with NanoLC Ultimate 3000 high-performance liquid chromatography system.

5.10 Mass spectrometry data quantitative analysis

Mass spectrometry raw data files were analyzed by MaxQuant with Thermo Foundation 2.0. Data was analyzed according to previous study PMID19651621. Brief, relative protein abundance was estimated by the number of MS/MS spectral counts representing each identified protein. The quantitation of the spectral counts must meet three requirements: 1. Proteins with at least two unique peptides in three independent experiments were considered as positive partners; 2. Proteins must be identified in two independent experiments; 3. The spectral count for a given protein in the EGF treated group should be at least two folds higher than the one in the buffer treated group; 4 The p value of the spectral counts for a given protein between EGF and buffer group should be <0.05.

5.11 Animal model and treatment.

All animal experiments were performed according to the guidelines of Georgia State University and were approved by IACUC. 6-week old male C57BL/6J mice were administrated 25mg/kg bleomycin twice a week for 5 weeks and treated with appropriate agents for the indicated

time period. rPKM1 and rPKM2 are recombinant proteins expressed in bacteria. IgG is normal rabbit IgG used as control. PKM2 Ab are in-house developed rabbit antibody against human PKM2. At the end of the treatments, animals were sacrificed, and organs were harvested and processed for embedding and sectioning or immediately collected for FACS analyses. Statistical analyses were done in comparison to the control group.

4T1 cells (0.5-1× 10^6) or B16-F10 (0.2× 10^6) were suspended in PBS and implanted in 100μl volume of 1:1 mixture of cell suspension and growth factor-reduced Matrigel (BD Biosciences) into the mammary glands of BALB/c female mice or C57BL/6J mice (aged 6-10 weeks) dorsal site subcutaneously, respectively. The tumor volumes were monitored by measuring two principal perpendicular diameters of the tumor with calipers and calculating the tumor volume using the formula: $V = 0.5 \times W^2 \times L$. W=width (mm), L=length (mm). When the tumor reached indicated volume, the mice were treated with appropriate agents for the indicated time period. rPKM1 and rPKM2 are recombinant proteins expressed in bacteria. IgG is normal rabbit IgG used as control. PKM2 Ab is in-house developed rabbit antibody against human PKM2. At the end of the treatments, animals were sacrificed, and organs were harvested and processed for embedding and sectioning or immediately collected for FACS analyses. Statistical analyses were done in comparison to the control group. To determine the effects of application of different agents on lung metastatic tumor frequency in vivo, the visible tumor number was counted immediately after lungs were harvested. Lung tissue blocks were sectioned into 6-μm sections and stained by H&E. The number of metastases was scored using 5 sections per mouse.

5.12 Flow cytometry.

Freshly harvested lung or tumor tissues were minced and dissociated using collagenase D (Sigma 11088858001, 0.5mg/ml) and DNase I for 20 min at 37 °C on a shaker. Cell suspensions

were subsequently passed through 40um cell strainer to get single cell suspension. Non-specific Fc-mediated interactions were blocked by incubating cells with CD16/CD31 antibodies.

Cells were labeled with fluorophore-conjugated antibodies. At last step, Cells were resuspended in FACS staining buffer and acquired using LSRFortessa flow cytometer (BD Biosciences). Data were analyzed by FlowJo software (Tree Star).

5.13 Hematoxylin and eosin (H&E) staining

The paraffin slides were baked at 60 ℃ for 2 hours, and rehydrated by immersing into the following solution in order (xylene, 100% ethanol, 90% ethanol, 70% ethanol, 50% ethanol, 30% ethanol). Next, the slides were incubated with Mayer's Hematoxylin for 10 min and washed under tap water for 10 min. The slides were immersed in Eosin for 30 sec and washed under tap water. The slides were immersed into the previous solutions in reverse order for dehydration, after which the slides were mounted with mounting medium and covered by coverslips.

5.14 Immunohistochemistry staining.

Tissues were fixed in 10% formalin and embedded in paraffin blocks then cut into 6 μm sections.

The tissue sections were baked at 60 ℃ for 2 hours and then dewaxed and rehydrated by immersion in the following solutions in order (three times xylene, twice 100% ethanol, 90% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, and distilled water). Antigen retrieval was performed by incubating tissue sections in 10 mM Sodium Citrate buffer, pH 6.0 in a pressure cooker for 10 min. After the sections were cooled down at the room temperature, they were washed in PBS for 5 min. The endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min, followed by washing with PBS for 5 min. The sections were then blocked with the blocking buffer, 5% BSA in PBST (0.1% Tween-20 in PBS) and then incubated with the primary antibody diluted

in the blocking buffer at 4 ℃ overnight. The sections were washed with PBST three times, 5 min each time. The sections were then incubated with respective HRP polymer at room temperature for 30 min, followed by the same washing step as above. The DAB substrate chromogen solution was prepared freshly by mixing 1 ml of DAB diluent with 1 drop of DAB concentrate using ImmPACT DAB Peroxidase (HRP) Substrate (Vector Laboratories, Cat. # SK 4150). DAB substrate was added onto the slides and the color development was monitored carefully under a microscope. Hematoxylin was used for counterstain. The sections were dehydrated by immersion in the same set of solutions in reverse order and then mounted with mounting medium.

IHC staining results were quantified using FRIDA (FRamework for Image Dataset Analysis) software. All images for analysis were saved in the same folder and a mask was selected by picking positive staining regions in different images. Once the mask was defined, the software calculates the positive area for all images.

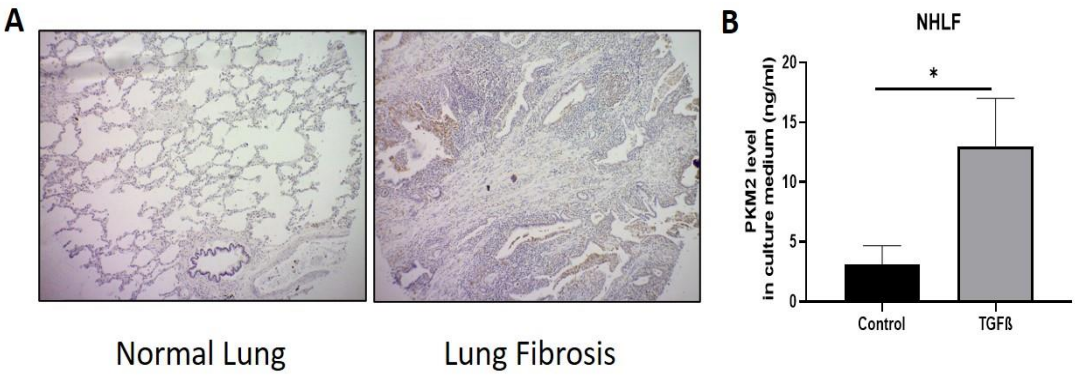
5.15 Statistical calculations

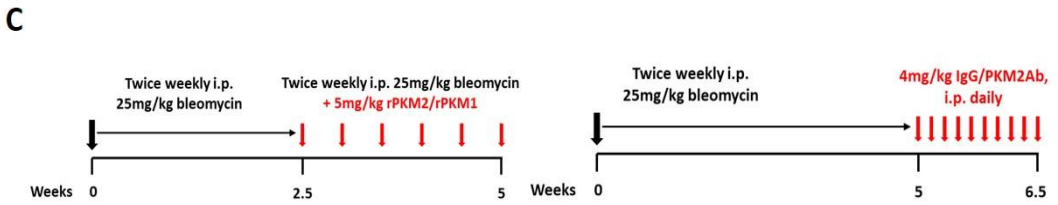
Statistical analyses were carried out using the GraphPad Prism 6.0 software. All experiments were carried out in 5 times minimum. Statistical significance was assayed by either Student's t-test and/or one-way ANOVA for multiple comparisons followed by post-hoc Tukey's test. Box plots show range, median and quartiles. In all figures, *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$, **** $P < 0.0001$; n.s. denotes not significant. All data are presented as mean ± s.e.m. or as box plots.

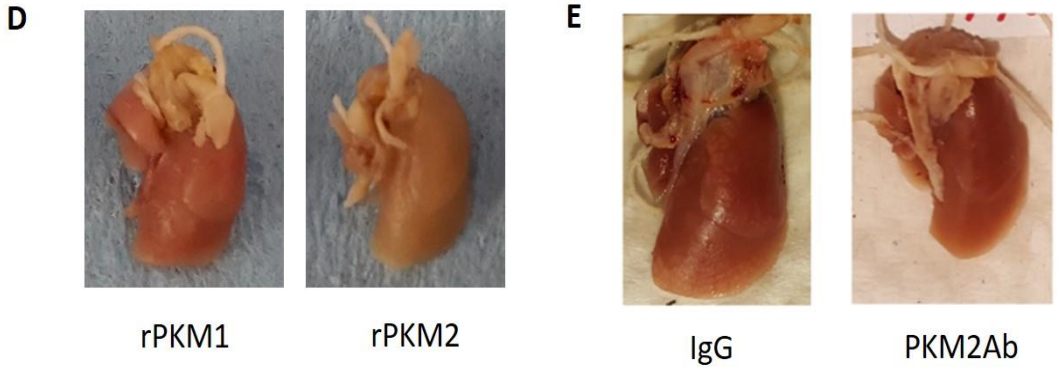
Appendix: EcPKM2 regulates lung fibrosis development in mice by modulating M2 macrophage polarization.

Appendix 1. EcPKM2 is involved in lung fibrosis progression.

(A) Representative images of immunohistochemistry analysis of PKM2 accumulation in normal human lung tissues and lung tissues from patients with pulmonary fibrosis. (B) PKM2 levels in the culture medium of normal human lung fibroblasts (NHLF) measured by ELISA. The cells were treated with or without TGF-β1 (10 ng/ml). All columns and error bars represent means ± SD. * $p<0.05$. (C) Experimental scheme of bleomycin (BLM)-induced lung fibrosis model establishment and treatment with rPKM1/rPKM2 (left panel) or IgG/PKM2 antibody (right panel). (D and E) Representative photographs of lungs from mice treated with indicated agents 1-day post-fixation with 10% formalin.

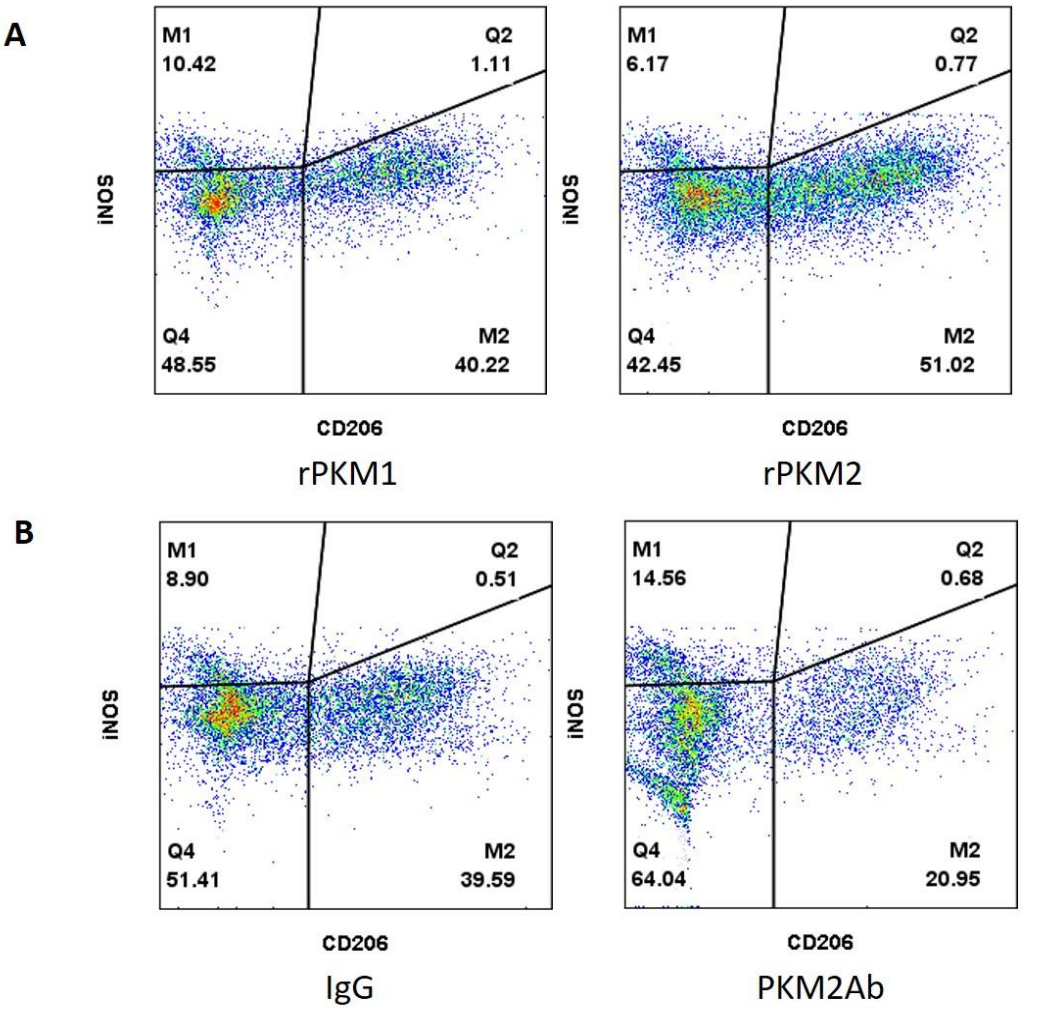

A
Normal Lung
Lung Fibrosis
B
NHLF
PKM2 level in culture medium (ng/ml)
20
15
10
5
0
*
Control
TGFß

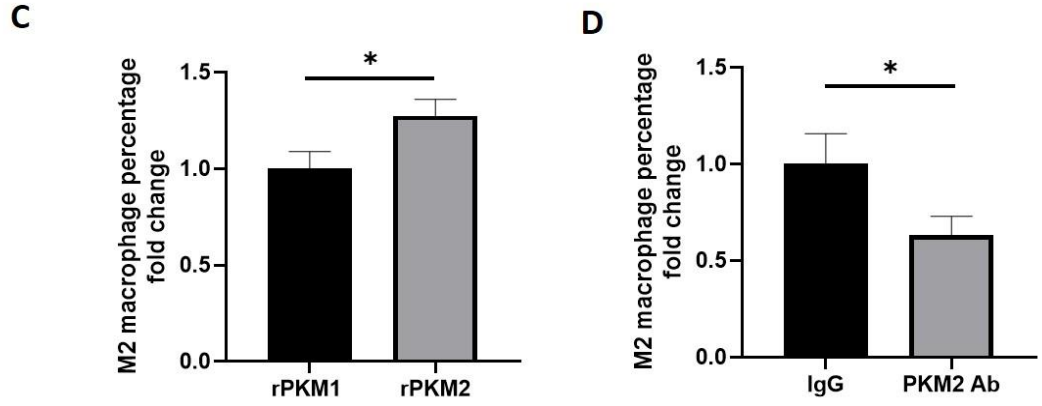

C
Twice weekly i.p. 25mg/kg bleomycin
Twice weekly i.p. 25mg/kg bleomycin + 5mg/kg rPKM2/rPKM1
Weeks 0 2.5 5
Twice weekly i.p. 25mg/kg bleomycin
4mg/kg IgG/PKM2Ab, i.p. daily
Weeks 0 5 6.5


D
rPKM1
rPKM2
E
IgG
PKM2Ab

Appendix 2. EcPKM2 regulates the development of bleomycin-induced lung fibrosis in mice by modulating M2 macrophage polarization.

(A and B) Lung tissues collected from bleomycin-induced lung fibrosis mice treated with rPKM1/rPKM2 (A) or IgG/PKM2 antibody (B) were dissociated and subjected to FACS analysis for $CD206^+$ M2 macrophages. Statistical analysis of (A) and (B) was displayed in (C) and (D), respectively. All columns and error bars represent means ± SD. * $p<0.05$.


A
M1
10.42
Q2
1.11
Q4
48.55
M2
40.22
iNOS
CD206
rPKM1
M1
6.17
Q2
0.77
Q4
42.45
M2
51.02
iNOS
CD206
rPKM2
B
M1
8.90
Q2
0.51
Q4
51.41
M2
39.59
iNOS
CD206
IgG
M1
14.56
Q2
0.68
Q4
64.04
M2
20.95
iNOS
CD206
PKM2Ab


C
M2 macrophage percentage fold change
1.5
1.0
0.5
0.0
*
rPKM1
rPKM2
D
M2 macrophage percentage fold change
1.5
1.0
0.5
0.0
*
IgG
PKM2 Ab

REFERENCES

Akins, N. S., Nielson, T. C., & Le, H. V. (2018). Inhibition of Glycolysis and Glutaminolysis: An Emerging Drug Discovery Approach to Combat Cancer. Current topics in medicinal chemistry, 18(6), 494-504. doi:10.2174/1568026618666180523111351

Alberghina, L., & Gaglio, D. (2014). Redox control of glutamine utilization in cancer. Cell death & disease, 5(12), e1561-e1561. doi:10.1038/cddis.2014.513

Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Frontiers in Pharmacology, 8(561). doi:10.3389/fphar.2017.00561

Anastasiou, D. et al. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science 334, 1278-1283 (2011).

Anastasiou, D. et al. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. Nat Chem Biol (2012).

Anderson, N. M., Mucka, P., Kern, J. G., & Feng, H. (2018). The emerging role and targetability of the TCA cycle in cancer metabolism. Protein & cell, 9(2), 216-237. doi:10.1007/s13238-017-0451-1

Angiari, S., Runtsch, M. C., Sutton, C. E., Palsson-McDermott, E. M., Kelly, B., Rana, N., . . . O'Neill, L. A. J. (2020). Pharmacological Activation of Pyruvate Kinase M2 Inhibits CD4+ T Cell Pathogenicity and Suppresses Autoimmunity. Cell metabolism, 31(2), 391-405.e398. doi:https://doi.org/10.1016/j.cmet.2019.10.015

Antonov, A. S., Antonova, G. N., Munn, D. H., Mivechi, N., Lucas, R., Catravas, J. D., & Verin, A. D. (2011). αVβ3 integrin regulates macrophage inflammatory responses via PI3 kinase/Akt-

dependent NF-κB activation. Journal of cellular physiology, 226(2), 469-476. doi:10.1002/jcp.22356

Appelbaum, F. R., Gundacker, H., Head, D. R., Slovak, M. L., Willman, C. L., Godwin, J. E., . . . Petersdorf, S. H. (2006). Age and acute myeloid leukemia. Blood, 107(9), 3481-3485. doi:10.1182/blood-2005-09-3724

Araujo, L., Khim, P., Mkhikian, H., Mortales, C.-L., & Demetriou, M. (2017). Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation. eLife, 6, e21330. doi:10.7554/eLife.21330

Azoitei, N., Becher, A., Steinestel, K., Rouhi, A., Diepold, K., Genze, F., . . . Seufferlein, T. (2016). PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. Molecular cancer, 15(1), 3. doi:10.1186/s12943-015-0490-2

Bade, B. C., & Dela Cruz, C. S. (2020). Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med, 41(1), 1-24. doi:10.1016/j.ccm.2019.10.001

Bailar, J. C., & Gornik, H. L. (1997). Cancer undefeated. New England Journal of Medicine, 336(22), 1569-1574.

Bandukwala, H. S., Gagnon, J., Togher, S., Greenbaum, J. A., Lamperti, E. D., Parr, N. J., . . . Rao, A. (2012). Selective inhibition of CD4+ T-cell cytokine production and autoimmunity by BET protein and c-Myc inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 109(36), 14532-14537. doi:10.1073/pnas.1212264109

Bashir, B., & Wilson, M. A. (2019). Novel Immunotherapy Combinations. Current Oncology Reports, 21(11), 96. doi:10.1007/s11912-019-0851-x

Bernard, K., Logsdon, N. J., Benavides, G. A., Sanders, Y., Zhang, J., Darley-Usmar, V. M., & Thannickal, V. J. (2018). Glutaminolysis is required for transforming growth factor-β1-induced

myofibroblast differentiation and activation. The Journal of biological chemistry, 293(4), 1218-1228. doi:10.1074/jbc.RA117.000444

Bonilla, F. A., & Oettgen, H. C. (2010). Adaptive immunity. Journal of Allergy and Clinical Immunology, 125(2, Supplement 2), S33-S40. doi:https://doi.org/10.1016/j.jaci.2009.09.017

Boros, L. G., Cascante, M., & Lee, W. N. (2002). Metabolic profiling of cell growth and death in cancer: applications in drug discovery. Drug Discov Today, 7(6), 364-372. doi:10.1016/s1359-6446(02)02179-7

Brabletz, T., Hlubek, F., Spaderna, S., Schmalhofer, O., Hiendlmeyer, E., Jung, A., & Kirchner, T. (2005). Invasion and Metastasis in Colorectal Cancer: Epithelial-Mesenchymal Transition, Mesenchymal-Epithelial Transition, Stem Cells and β-Catenin. Cells Tissues Organs, 179(1-2), 56-65. doi:10.1159/000084509

Carracedo, A., & Pandolfi, P. P. (2008). The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene, 27(41), 5527-5541. doi:10.1038/onc.2008.247

Chabner, B. A., & Roberts, T. G. (2005). Chemotherapy and the war on cancer. Nature Reviews Cancer, 5(1), 65-72. doi:10.1038/nrc1529

Chalhoub, N., & Baker, S. J. (2009). PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol, 4, 127-150. doi:10.1146/annurev.pathol.4.110807.092311

Chaneton, B., & Gottlieb, E. (2012). Rocking cell metabolism: revised functions of the key glycolytic regulator PKM2 in cancer. Trends in biochemical sciences, 37(8), 309-316.

Chen, M., Zhang, J., & Manley, J. L. (2010). Turning on a fuel switch of cancer: hnRNP proteins regulate alternative splicing of pyruvate kinase mRNA. Cancer research, 70(22), 8977-8980.

Chen, X., & Song, E. (2019). Turning foes to friends: targeting cancer-associated fibroblasts. Nat Rev Drug Discov, 18(2), 99-115. doi:10.1038/s41573-018-0004-1

Cluntun, A. A., Lukey, M. J., Cerione, R. A., & Locasale, J. W. (2017). Glutamine Metabolism in Cancer: Understanding the Heterogeneity. Trends in cancer, 3(3), 169-180. doi:10.1016/j.trecan.2017.01.005

Couzin-Frankel, J. (2013). Cancer Immunotherapy. Science (New York, N.Y.), 342(6165), 1432. doi:10.1126/science.342.6165.1432

Cairns, R.A., Harris, I.S. & Mak, T.W. Regulation of cancer cell metabolism. Nature reviews. Cancer 11, 85-95 (2011).

Chappell, S.A. et al. A mutation in the c-myc-IRES leads to enhanced internal ribosome entry in multiple myeloma: a novel mechanism of oncogene de-regulation. Oncogene 19, 4437-4440 (2000).

Chen, J.Q. & Russo, J. Dysregulation of glucose transport, glycolysis, TCA cycle and glutaminolysis by oncogenes and tumor suppressors in cancer cells. Biochimica et biophysica acta 1826, 370-384 (2012).

Dang, C.V. Glutaminolysis: supplying carbon or nitrogen or both for cancer cells? Cell cycle 9, 3884-3886 (2010).

David, C.J., Chen, M., Assanah, M., Canoll, P. & Manley, J.L. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature 463, 364-368 (2010).

DeBerardinis, R.J. & Chandel, N.S. Fundamentals of cancer metabolism. Science advances 2, e1600200 (2016).

DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G. & Thompson, C.B. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell metabolism 7, 11-20 (2008).

DeBerardinis, R.J. et al. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proceedings of the National Academy of Sciences of the United States of America 104, 19345-19350 (2007).

D'Abreo, N., & Adams, S. (2019). Immune-checkpoint inhibition for metastatic triple-negative breast cancer: safety first? Nat Rev Clin Oncol, 16(7), 399-400. doi:10.1038/s41571-019-0216-2

Dang, C. V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. Molecular and cellular biology, 19(1), 1-11. doi:10.1128/mcb.19.1.1

Dang, C. V. (2012). Links between metabolism and cancer. Genes & development, 26(9), 877-890.

Darvin, P., Toor, S. M., Sasidharan Nair, V., & Elkord, E. (2018). Immune checkpoint inhibitors: recent progress and potential biomarkers. Experimental & Molecular Medicine, 50(12), 1-11. doi:10.1038/s12276-018-0191-1

Dayton, T. L., Jacks, T., & Vander Heiden, M. G. (2016). PKM 2, cancer metabolism, and the road ahead. EMBO reports, 17(12), 1721-1730.

de Haes, J. C. J. M., & van Knippenberg, F. C. E. (1985). The quality of life of cancer patients: A review of the literature. Social Science & Medicine, 20(8), 809-817. doi:https://doi.org/10.1016/0277-9536(85)90335-1

De Laurentiis, M., Cianniello, D., Caputo, R., Stanzione, B., Arpino, G., Cinieri, S., . . . De Placido, S. (2010). Treatment of triple negative breast cancer (TNBC): current options and future perspectives. Cancer Treatment Reviews, 36, S80-S86. doi:https://doi.org/10.1016/S0305-7372(10)70025-6

Desbarats, L., Schneider, A., Müller, D., Bürgin, A., & Eilers, M. (1996). Myc: a single gene controls both proliferation and apoptosis in mammalian cells. Experientia, 52(12), 1123-1129. doi:10.1007/BF01952111

Dong, G., Mao, Q., Xia, W., Xu, Y., Wang, J., Xu, L., & Jiang, F. (2016). PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncology letters, 11(3), 1980-1986. doi:10.3892/ol.2016.4168

Dudley, M. E., & Rosenberg, S. A. (2003). Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nature Reviews Cancer, 3(9), 666-675. doi:10.1038/nrc1167

Eagle, H. The specific amino acid requirements of a human carcinoma cell (Stain HeLa) in tissue culture. The Journal of experimental medicine 102, 37-48 (1955).

Elbers, J. R., van Unnik, J. A., Rijksen, G., van Oirschot, B. A., Roholl, P. J., Oosting, J., & Staal, G. E. (1991). Pyruvate kinase activity and isozyme composition in normal fibrous tissue and fibroblastic proliferations. Cancer, 67(10), 2552-2559. doi:10.1002/1097-0142(19910515)67:10<2552::aid-cncr2820671027>3.0.co;2-k

Eliceiri, B. P., & Cheresh, D. A. (2000). Role of alpha v integrins during angiogenesis. Cancer J, 6 Suppl 3, S245-249.

Fass, L. (2008). Imaging and cancer: A review. Molecular Oncology, 2(2), 115-152. doi:https://doi.org/10.1016/j.molonc.2008.04.001

Ferguson, E.C. & Rathmell, J.C. New roles for pyruvate kinase M2: working out the Warburg effect. Trends Biochem Sci 33, 359-362 (2008).

Fitzgerald, K. D., & Semler, B. L. (2009). Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1789(9), 518-528. doi:https://doi.org/10.1016/j.bbagrm.2009.07.004

Fothergill-Gilmore, L. A., & Michels, P. A. (1993). Evolution of glycolysis. Progress in biophysics and molecular biology, 59(2), 105-235.

Franco, O. E., Shaw, A. K., Strand, D. W., & Hayward, S. W. (2010). Cancer associated fibroblasts in cancer pathogenesis. Seminars in Cell & Developmental Biology, 21(1), 33-39. doi:https://doi.org/10.1016/j.semcdb.2009.10.010

Gao, X. et al. Reciprocal regulation of protein kinase and pyruvate kinase activities of pyruvate kinase m2 by growth signals. The Journal of biological chemistry 288, 15971-15979 (2013).

Gao, X., Wang, H., Yang, J.J., Liu, X. & Liu, Z.R. Pyruvate kinase m2 regulates gene transcription by acting as a protein kinase. Molecular cell 45, 598-609 (2012).

Gao, P. et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 458, 762-765 (2009).

Goetzman, E.S. & Prochownik, E.V. The Role for Myc in Coordinating Glycolysis, Oxidative Phosphorylation, Glutaminolysis, and Fatty Acid Metabolism in Normal and Neoplastic Tissues. Frontiers in endocrinology 9, 129 (2018).

Gorrini, C., Harris, I.S. & Mak, T.W. Modulation of oxidative stress as an anticancer strategy. Nature reviews. Drug discovery 12, 931-947 (2013).

Gao, X., Wang, H., Yang, J. J., Chen, J., Jie, J., Li, L., . . . Liu, Z. R. (2013). Reciprocal regulation of protein kinase and pyruvate kinase activities of pyruvate kinase M2 by growth signals. The Journal of biological chemistry, 288(22), 15971-15979. doi:10.1074/jbc.M112.448753

Gao, X., Wang, H., Yang, J. J., Liu, X., & Liu, Z.-R. (2012). Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Molecular cell, 45(5), 598-609. doi:10.1016/j.molcel.2012.01.001

10.1016/j.molcel.2012.01.001

Gibb Andrew, A., Lombardi Alyssa, A., Murray Emma, K., Tan, Y., Lorkiewicz Pawel, K., Huynh Anh, T., . . . Elrod John, W. (2019). Abstract 517: Glutaminolysis is Required to Initiate Myofibroblast Differentiation and Persistence During Stress. Circulation Research, 125(Suppl_1), A517-A517. doi:10.1161/res.125.suppl_1.517

Godet, A.-C., David, F., Hantelys, F., Tatin, F., Lacazette, E., Garmy-Susini, B., & Prats, A.-C. (2019). IRES Trans-Acting Factors, Key Actors of the Stress Response. International journal of molecular sciences, 20(4), 924. doi:10.3390/ijms20040924

Goodman, S. L., & Picard, M. (2012). Integrins as therapeutic targets. Trends in Pharmacological Sciences, 33(7), 405-412. doi:https://doi.org/10.1016/j.tips.2012.04.002

Gordon, S. (2007). The macrophage: Past, present and future. European Journal of Immunology, 37(S1), S9-S17. doi:https://doi.org/10.1002/eji.200737638

Gordan, J.D., Thompson, C.B. & Simon, M.C. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer cell 12, 108-113 (2007).

Grzmil, M., & Hemmings, B. A. (2012). Translation Regulation as a Therapeutic Target in Cancer. Cancer research, 72(16), 3891. doi:10.1158/0008-5472.CAN-12-0026

Gui, D. Y., Lewis, C. A., & Vander Heiden, M. G. (2013). Allosteric regulation of PKM2 allows cellular adaptation to different physiological states. Science signaling, 6(263), pe7-pe7.

Gupta, V., & Bamezai, R. N. (2010). Human pyruvate kinase M2: a multifunctional protein. Protein science, 19(11), 2031-2044.

Hacker, H. J., Steinberg, P., & Bannasch, P. (1998). Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. Carcinogenesis, 19(1), 99-107. doi:10.1093/carcin/19.1.99

Hao, N.-B., Lü, M.-H., Fan, Y.-H., Cao, Y.-L., Zhang, Z.-R., & Yang, S.-M. (2012). Macrophages in Tumor Microenvironments and the Progression of Tumors. Clinical and Developmental Immunology, 2012, 948098. doi:10.1155/2012/948098

Harding, H. P., Zhang, Y., & Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature, 397(6716), 271-274.

Harris, J. R., Lippman, M. E., Veronesi, U., & Willett, W. (1992). Breast Cancer. New England Journal of Medicine, 327(5), 319-328. doi:10.1056/nejm199207303270505

Hathurusinghe, H. R., Goonetilleke, K. S., & Siriwardena, A. K. (2007). Current status of tumor M2 pyruvate kinase (tumor M2-PK) as a biomarker of gastrointestinal malignancy. Ann Surg Oncol, 14(10), 2714-2720. doi:10.1245/s10434-007-9481-x

He, X., Du, S., Lei, T., Li, X., Liu, Y., Wang, H., . . . Wang, Y. (2017). PKM2 in carcinogenesis and oncotherapy. Oncotarget, 8(66), 110656-110670. doi:10.18632/oncotarget.22529

Hellen, C. U., & Sarnow, P. (2001). Internal ribosome entry sites in eukaryotic mRNA molecules. Genes & development, 15(13), 1593-1612. doi:10.1101/gad.891101

Hensley, C.T., Wasti, A.T. & DeBerardinis, R.J. Glutamine and cancer: cell biology, physiology, and clinical opportunities. The Journal of clinical investigation 123, 3678-3684 (2013).

Hitosugi, T. et al. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal 2, ra73 (2009).

Henderson, N. C., Arnold, T. D., Katamura, Y., Giacomini, M. M., Rodriguez, J. D., McCarty, J. H., . . . Sheppard, D. (2013). Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med, 19(12), 1617-1624. doi:10.1038/nm.3282

Henderson, N. C., & Sheppard, D. (2013). Integrin-mediated regulation of TGFbeta in fibrosis. Biochim Biophys Acta, 1832(7), 891-896. doi:10.1016/j.bbadis.2012.10.005

Hinds, P. W., & Weinberg, R. A. (1994). Tumor suppressor genes. Curr Opin Genet Dev, 4(1), 135-141. doi:10.1016/0959-437x(94)90102-3

Hoffman, B., & Liebermann, D. A. (2008). Apoptotic signaling by c-MYC. Oncogene, 27(50), 6462-6472. doi:10.1038/onc.2008.312

Hood , J. D., Frausto , R., Kiosses , W. B., Schwartz , M. A., & Cheresh , D. A. (2003). Differential αv integrin–mediated Ras-ERK signaling during two pathways of angiogenesis. Journal of Cell Biology, 162(5), 933-943. doi:10.1083/jcb.200304105

Horton, M. A. (1997). The αvβ3 integrin "vitronectin receptor". The International Journal of Biochemistry & Cell Biology, 29(5), 721-725. doi:https://doi.org/10.1016/S1357-2725(96)00155-0

Hsu, M.-C., & Hung, W.-C. (2018). Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. Molecular cancer, 17(1), 35. doi:10.1186/s12943-018-0791-3

Israelsen, William J., Dayton, Talya L., Davidson, Shawn M., Fiske, Brian P., Hosios, Aaron M., Bellinger, G., . . . Vander Heiden, Matthew G. (2013). PKM2 Isoform-Specific Deletion Reveals a Differential Requirement for Pyruvate Kinase in Tumor Cells. Cell, 155(2), 397-409. doi:10.1016/j.cell.2013.09.025

Israelsen, W. J., & Vander Heiden, M. G. (2015). Pyruvate kinase: function, regulation and role in cancer. Paper presented at the Seminars in Cell & Developmental Biology.

Italiani, P., & Boraschi, D. (2014). From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. Frontiers in immunology, 5, 514-514. doi:10.3389/fimmu.2014.00514

Jeffery, J., Lewis, S. J., & Ayling, R. M. (2009). Fecal dimeric M2-pyruvate kinase (tumor M2-PK) in the differential diagnosis of functional and organic bowel disorders. Inflamm Bowel Dis, 15(11), 1630-1634. doi:10.1002/ibd.20946

Jiang, J., Srivastava, S., & Zhang, J. (2019). Starve Cancer Cells of Glutamine: Break the Spell or Make a Hungry Monster? Cancers, 11(6), 804. doi:10.3390/cancers11060804

Jin, L., Alesi, G. N., & Kang, S. (2016). Glutaminolysis as a target for cancer therapy. Oncogene, 35(28), 3619-3625. doi:10.1038/onc.2015.447

Kennedy, R., & Celis, E. (2008). Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunological Reviews, 222(1), 129-144. doi:https://doi.org/10.1111/j.1600-065X.2008.00616.x

Kim, J. (2018). Regulation of Immune Cell Functions by Metabolic Reprogramming. Journal of immunology research, 2018, 8605471-8605471. doi:10.1155/2018/8605471

Kim, J.H. et al. Heterogeneous nuclear ribonucleoprotein C modulates translation of c-myc mRNA in a cell cycle phase-dependent manner. Molecular and cellular biology 23, 708-720 (2003).

Kodama, M., Oshikawa, K., Shimizu, H., Yoshioka, S., Takahashi, M., Izumi, Y., . . . Nakayama, K. I. (2020). A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer. Nature Communications, 11(1), 1320. doi:10.1038/s41467-020-15136-9

Komar, A. A., & Hatzoglou, M. (2011). Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. Cell cycle (Georgetown, Tex.), 10(2), 229-240. doi:10.4161/cc.10.2.14472

Kucab, J., & Dunn, S. (2003). Role of IGF-1R in mediating breast cancer invasion and metastasis. Breast disease, 17, 41-47.

Kumar, C. C. (1998). Signaling by integrin receptors. Oncogene, 17(11), 1365-1373. doi:10.1038/sj.onc.1202172

Lamonte, G. et al. Acidosis induces reprogramming of cellular metabolism to mitigate oxidative stress. Cancer & metabolism 1, 23 (2013).

Le, A. et al. Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in B cells. Cell metabolism 15, 110-121 (2012).

Lee, S.-Y., Obata, Y., Yoshida, M., Stockert, E., Williamson, B., Jungbluth, A. A., . . . Scanlan, M. J. (2003). Immunomic analysis of human sarcoma. Proceedings of the National Academy of Sciences, 100(5), 2651. doi:10.1073/pnas.0437972100

Levine, A. J., & Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science (New York, N.Y.), 330(6009), 1340-1344. doi:10.1126/science.1193494

Li, L., Peng, G., Liu, X., Zhang, Y., Han, H., & Liu, Z.-R. (2020). Pyruvate Kinase M2 Coordinates Metabolism Switch between Glycolysis and Glutaminolysis in Cancer Cells. iScience, 23(11), 101684. doi:https://doi.org/10.1016/j.isci.2020.101684

Li, L., Peng, G., Liu, X., Zhang, Y., Han, H., & Liu, Z. R. (2020). Pyruvate Kinase M2 Coordinates Metabolism Switch between Glycolysis and Glutaminolysis in Cancer Cells. iScience, 23(11), 101684. doi:10.1016/j.isci.2020.101684

Li, L., Zhang, Y., Qiao, J., Yang, J. J., & Liu, Z.-R. (2014). Pyruvate kinase M2 in blood circulation facilitates tumor growth by promoting angiogenesis. The Journal of biological chemistry, 289(37), 25812-25821. doi:10.1074/jbc.M114.576934

Li, X. B., Gu, J. D., & Zhou, Q. H. (2015). Review of aerobic glycolysis and its key enzymes - new targets for lung cancer therapy. Thorac Cancer, 6(1), 17-24. doi:10.1111/1759-7714.12148

Li, Y.-h., Li, X.-f., Liu, J.-t., Wang, H., Fan, L.-l., Li, J., & Sun, G.-p. (2018). PKM2, a potential target for regulating cancer. Gene, 668, 48-53.

Li, Z., Yang, P., & Li, Z. (2014). The multifaceted regulation and functions of PKM2 in tumor progression. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1846(2), 285-296.

Li, Z. Z., Wang, F., Liu, S., Li, H., & Wang, Y. (2020). Ablation of PKM2 ameliorated ER stress‐induced apoptosis and associated inflammation response in IL‐1β‐treated chondrocytes via blocking Rspo2‐mediated Wnt/β‐catenin signaling. Journal of cellular biochemistry, 121(10), 4204-4213.

Liang, J., Cao, R., Wang, X., Zhang, Y., Wang, P., Gao, H., . . . Yang, W. (2017). Mitochondrial PKM2 regulates oxidative stress-induced apoptosis by stabilizing Bcl2. Cell Res, 27(3), 329-351. doi:10.1038/cr.2016.159

Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? Trends in biochemical sciences, 41(3), 211-218. doi:10.1016/j.tibs.2015.12.001

Liu, B., & Qian, S.-B. (2014). Translational reprogramming in cellular stress response. Wiley interdisciplinary reviews. RNA, 5(3), 301-315. doi:10.1002/wrna.1212

Liu, Z., Wang, F., & Chen, X. (2008). Integrin αvβ3-targeted cancer therapy. Drug Development Research, 69(6), 329-339. doi:https://doi.org/10.1002/ddr.20265

Loges, S., Mazzone, M., Hohensinner, P., & Carmeliet, P. (2009). Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. Cancer Cell, 15(3), 167-170. doi:10.1016/j.ccr.2009.02.007

López de Quinto, S., Lafuente, E., & Martínez-Salas, E. (2001). IRES interaction with translation initiation factors: functional characterization of novel RNA contacts with eIF3, eIF4B, and eIF4GII. RNA (New York, N.Y.), 7(9), 1213-1226. doi:10.1017/s1355838201010433

Lowe, S. W., & Lin, A. W. (2000). Apoptosis in cancer. Carcinogenesis, 21(3), 485-495. doi:10.1093/carcin/21.3.485

Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annual review of cell and developmental biology, 27, 441-464.

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., . . . Semenza, G. L. (2011). Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell, 145(5), 732-744. doi:10.1016/j.cell.2011.03.054

Mazurek, S., Boschek, C.B., Hugo, F. & Eigenbrodt, E. Pyruvate kinase type M2 and its role in tumor growth and spreading. Seminars in cancer biology 15, 300-308 (2005).

Mazurek, S. Pyruvate kinase type M2: a key regulator within the tumour metabolome and a tool for metabolic profiling of tumours. Ernst Schering Found Symp Proc, 99-124 (2007).

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime reports, 6, 13-13. doi:10.12703/P6-13

Matés, J. M., Pérez-Gómez, C., de Castro, I. N., Asenjo, M., & Márquez, J. (2002). Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. The International Journal of Biochemistry & Cell Biology, 34(5), 439-458. doi:https://doi.org/10.1016/S1357-2725(01)00143-1

Mattevi, A., Bolognesi, M., & Valentini, G. (1996). The allosteric regulation of pyruvate kinase. FEBS letters, 389(1), 15-19.

Mazurek, S., Boschek, C. B., Hugo, F., & Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol, 15(4), 300-308. doi:10.1016/j.semcancer.2005.04.009

McKeehan, W. L. (1982). Glycolysis, glutaminolysis and cell proliferation. Cell biology international reports, 6(7), 635-650.

McMahon, S. B. (2014). MYC and the control of apoptosis. Cold Spring Harbor perspectives in medicine, 4(7), a014407-a014407. doi:10.1101/cshperspect.a014407

Medzhitov, R., & Janeway Jr, C. A. (1998). Innate immune recognition and control of adaptive immune responses. Seminars in immunology, 10(5), 351-353. doi:https://doi.org/10.1006/smim.1998.0136

Méndez-Lucas, A., Li, X., Hu, J., Che, L., Song, X., Jia, J., . . . Tschaharganeh, D. F. (2017). Glucose catabolism in liver tumors induced by c-MYC can be sustained by various PKM1/PKM2 ratios and pyruvate kinase activities. Cancer research, 77(16), 4355-4364.

Miller, D. M., Thomas, S. D., Islam, A., Muench, D., & Sedoris, K. (2012). c-Myc and cancer metabolism. Clinical cancer research : an official journal of the American Association for Cancer Research, 18(20), 5546-5553. doi:10.1158/1078-0432.CCR-12-0977

Morgan, H. P., O'Reilly, F. J., Wear, M. A., O'Neill, J. R., Fothergill-Gilmore, L. A., Hupp, T., & Walkinshaw, M. D. (2013). M2 pyruvate kinase provides a mechanism for nutrient sensing and regulation of cell proliferation. Proceedings of the National Academy of Sciences, 110(15), 5881-5886.

Munger, J. S., Huang, X., Kawakatsu, H., Griffiths, M. J., Dalton, S. L., Wu, J., . . . Sheppard, D. (1999). The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for regulating pulmonary inflammation and fibrosis. Cell, 96(3), 319-328. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10025398

Nakamura, K., & Smyth, M. J. (2020). Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. Cellular & Molecular Immunology, 17(1), 1-12. doi:10.1038/s41423-019-0306-1

Nielsen, S. R., & Schmid, M. C. (2017). Macrophages as Key Drivers of Cancer Progression and Metastasis. Mediators of Inflammation, 2017, 9624760. doi:10.1155/2017/9624760

Noy, R., & Pollard, J. W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity, 41(1), 49-61. doi:10.1016/j.immuni.2014.06.010

Orphanides, G., & Reinberg, D. (2002). A unified theory of gene expression. Cell, 108(4), 439-451.

Palsson-McDermott, E.M. et al. Pyruvate Kinase M2 Regulates Hif-1alpha Activity and IL-1beta Induction and Is a Critical Determinant of the Warburg Effect in LPS-Activated Macrophages. Cell metabolism 21, 347 (2015).

Pan, T. et al. Elevated expression of glutaminase confers glucose utilization via glutaminolysis in prostate cancer. Biochemical and biophysical research communications 456, 452-458 (2015).

Palsson-McDermott, E. M., Curtis, A. M., Goel, G., Lauterbach, M. A. R., Sheedy, F. J., Gleeson, L. E., . . . O'Neill, L. A. J. (2015). Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the warburg effect in LPS-activated macrophages. Cell metabolism, 21(1), 65-80. doi:10.1016/j.cmet.2014.12.005

Paulin, F.E. et al. Aberrant translational control of the c-myc gene in multiple myeloma. Oncogene 13, 505-513 (1996).

Pearce, E. L., & Pearce, E. J. (2013). Metabolic pathways in immune cell activation and quiescence. Immunity, 38(4), 633-643. doi:10.1016/j.immuni.2013.04.005

Pello, O. M., De Pizzol, M., Mirolo, M., Soucek, L., Zammataro, L., Amabile, A., . . . Locati, M. (2012). Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. Blood, 119(2), 411-421. doi:10.1182/blood-2011-02-339911

Phan, L. M., Yeung, S.-C. J., & Lee, M.-H. (2014). Cancer metabolic reprogramming: importance, main features, and potentials for precise targeted anti-cancer therapies. Cancer biology & medicine, 11(1), 1-19. doi:10.7497/j.issn.2095-3941.2014.01.001

Poh, A. R., & Ernst, M. (2018). Targeting Macrophages in Cancer: From Bench to Bedside. Frontiers in Oncology, 8(49). doi:10.3389/fonc.2018.00049

Prakasam, G., Iqbal, M. A., Bamezai, R. N. K., & Mazurek, S. (2018). Posttranslational Modifications of Pyruvate Kinase M2: Tweaks that Benefit Cancer. Frontiers in Oncology, 8, 22-22. doi:10.3389/fonc.2018.00022

Rajkumar, S. V., Gertz, M. A., Kyle, R. A., & Greipp, P. R. (2002). Current Therapy for Multiple Myeloma. Mayo Clinic Proceedings, 77(8), 813-822. doi:https://doi.org/10.4065/77.8.813

Rastrelli, M., Tropea, S., Rossi, C. R., & Alaibac, M. (2014). Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. In Vivo, 28(6), 1005-1011.

Reed, N. I., Jo, H., Chen, C., Tsujino, K., Arnold, T. D., DeGrado, W. F., & Sheppard, D. (2015). The alphavbeta1 integrin plays a critical in vivo role in tissue fibrosis. Sci Transl Med, 7(288), 288ra279. doi:10.1126/scitranslmed.aaa5094

Richter, J. D., & Sonenberg, N. (2005). Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature, 433(7025), 477-480.

Rőszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators of Inflammation, 2015, 816460. doi:10.1155/2015/816460

Ruggero, D. (2013). Translational control in cancer etiology. Cold Spring Harbor perspectives in biology, 5(2), a012336. doi:10.1101/cshperspect.a012336

Sato, T., Terai, M., Tamura, Y., Alexeev, V., Mastrangelo, M. J., & Selvan, S. R. (2011). Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. Immunologic Research, 51(2), 170-182. doi:10.1007/s12026-011-8262-6

Sawyers, C. (2004). Targeted cancer therapy. Nature, 432(7015), 294-297. doi:10.1038/nature03095

Scott, A. M., Allison, J. P., & Wolchok, J. D. (2012). Monoclonal antibodies in cancer therapy. Cancer Immunity Archive, 12(1), 14. Retrieved from http://cancerimmunolres.aacrjournals.org/content/12/1/14.abstract

Souba, W. W. (1993). Glutamine and cancer. Annals of surgery, 218(6), 715-728. doi:10.1097/00000658-199312000-00004

Staib, P., Hoffmann, M., & Schinkothe, T. (2006). Plasma levels of tumor M2-pyruvate kinase should not be used as a tumor marker for hematological malignancies and solid tumors. Clin Chem Lab Med, 44(1), 28-31. doi:10.1515/CCLM.2006.006

Subkhankulova, T., Mitchell, S.A. & Willis, A.E. Internal ribosome entry segment-mediated initiation of c-Myc protein synthesis following genotoxic stress. The Biochemical journal 359, 183-192 (2001).

Svitkin, Y. V., Ovchinnikov, L., Dreyfuss, G., & Sonenberg, N. (1996). General RNA binding proteins render translation cap dependent. The EMBO journal, 15(24), 7147-7155.

Thoma, C., Bergamini, G., Galy, B., Hundsdoerfer, P. & Hentze, M.W. Enhancement of IRES-mediated translation of the c-myc and BiP mRNAs by the poly(A) tail is independent of intact eIF4G and PABP. Molecular cell 15, 925-935 (2004).

Vaklavas, C., Blume, S. W., & Grizzle, W. E. (2017). Translational Dysregulation in Cancer: Molecular Insights and Potential Clinical Applications in Biomarker Development. Frontiers in Oncology, 7(158). doi:10.3389/fonc.2017.00158

Vaklavas, C., Meng, Z., Choi, H., Grizzle, W. E., Zinn, K. R., & Blume, S. W. (2015). Small molecule inhibitors of IRES-mediated translation. Cancer Biol Ther, 16(10), 1471-1485. doi:10.1080/15384047.2015.1071729

Vaupel, P., Schmidberger, H., & Mayer, A. (2019). The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. Int J Radiat Biol, 95(7), 912-919. doi:10.1080/09553002.2019.1589653

Vikas, P., Borcherding, N., & Zhang, W. (2018). The clinical promise of immunotherapy in triple-negative breast cancer. Cancer Manag Res, 10, 6823-6833. doi:10.2147/CMAR.S185176

Wahl, D. R., Byersdorfer, C. A., Ferrara, J. L. M., Opipari, A. W., Jr., & Glick, G. D. (2012). Distinct metabolic programs in activated T cells: opportunities for selective immunomodulation. Immunological Reviews, 249(1), 104-115. doi:10.1111/j.1600-065X.2012.01148.x

Walters, B., & Thompson, S. R. (2016). Cap-Independent Translational Control of Carcinogenesis. Frontiers in Oncology, 6, 128-128. doi:10.3389/fonc.2016.00128

Wan, L., Xia, T., Du, Y., Liu, J., Xie, Y., Zhang, Y., . . . Shi, C. (2019). Exosomes from activated hepatic stellate cells contain GLUT1 and PKM2: a role for exosomes in metabolic switch of liver nonparenchymal cells. The FASEB Journal, 33(7), 8530-8542.

Wang, J., Li, D., Cang, H., & Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. Cancer medicine, 8(10), 4709-4721. doi:10.1002/cam4.2327

Wang, P., Sun, C., Zhu, T., & Xu, Y. (2015). Structural insight into mechanisms for dynamic regulation of PKM2. Protein & cell, 6(4), 275-287.

Warburg, O. (1924). Über den Stoffwechsel der Carcinomzelle. Naturwissenschaften, 12(50), 1131-1137. doi:10.1007/BF01504608

Wegener, K. L., & Campbell, I. D. (2008). Transmembrane and cytoplasmic domains in integrin activation and protein-protein interactions (review). Mol Membr Biol, 25(5), 376-387. doi:10.1080/09687680802269886

Wise, D.R. & Thompson, C.B. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci 35, 427-433 (2010).

Wise, D.R. et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proceedings of the National Academy of Sciences of the United States of America 105, 18782-18787 (2008).

Wong, N., De Melo, J. & Tang, D. PKM2, a Central Point of Regulation in Cancer Metabolism. International journal of cell biology 2013, 242513 (2013).

Wong, N., Ojo, D., Yan, J., & Tang, D. (2015). PKM2 contributes to cancer metabolism. Cancer letters, 356(2), 184-191.

Wu, S., Kuang, H., Ke, J., Pi, M., & Yang, D.-H. (2021). Metabolic Reprogramming Induces Immune Cell Dysfunction in the Tumor Microenvironment of Multiple Myeloma. Frontiers in Oncology, 10(3061). doi:10.3389/fonc.2020.591342

Wu, X., Cai, J., Zuo, Z., & Li, J. (2019). Collagen facilitates the colorectal cancer stemness and metastasis through an integrin/PI3K/AKT/Snail signaling pathway. Biomedicine & Pharmacotherapy, 114, 108708. doi:https://doi.org/10.1016/j.biopha.2019.108708

Wybran, J., & Fudenberg, H. H. (1973). Thymus-Derived Rosette-Forming Cells in Various Human Disease States: Cancer, Lymphoma, Bacterial and Viral Infections, and Other Diseases. The Journal of Clinical Investigation, 52(5), 1026-1032. doi:10.1172/JCI107267

Xi, S., Zhao, M., Wang, S., Ma, L., Wang, S., Cong, X., . . . Huang, Y. (2017). IRES-Mediated Protein Translation Overcomes Suppression by the p14ARF Tumor Suppressor Protein. Journal of Cancer, 8(6), 1082-1088. doi:10.7150/jca.17457

Yang, L., Venneti, S. & Nagrath, D. Glutaminolysis: A Hallmark of Cancer Metabolism. Annual review of biomedical engineering 19, 163-194 (2017).

Yang, W., Xia, Y., Hawke, D., Li, X., Liang, J., Xing, D., . . . Lu, Z. (2012). PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell, 150(4), 685-696. doi:10.1016/j.cell.2012.07.018

Yang, W., Xia, Y., Hawke, D., Li, X., Liang, J., Xing, D., . . . Lu, Z. (2012). PKM2 Phosphorylates Histone H3 and Promotes Gene Transcription and Tumorigenesis. Cell, 150(4), 685-696. doi:10.1016/j.cell.2012.07.018

Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W., . . . Lu, Z. (2011). Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature, 480(7375), 118-122. doi:nature10598 [pii]10.1038/nature10598

Yang, Y., & Wang, Z. (2019). IRES-mediated cap-independent translation, a path leading to hidden proteome. Journal of Molecular Cell Biology, 11(10), 911-919. doi:10.1093/jmcb/mjz091

Zahra, K., Dey, T., Ashish, Mishra, S. P., & Pandey, U. (2020a). Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. Frontiers in Oncology, 10, 159-159. doi:10.3389/fonc.2020.00159

Zahra, K., Dey, T., Ashish, Mishra, S. P., & Pandey, U. (2020b). Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. Frontiers in Oncology, 10, 159. Retrieved from https://www.frontiersin.org/article/10.3389/fonc.2020.00159

Zhang, J., Pavlova, N. N., & Thompson, C. B. (2017). Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. The EMBO journal, 36(10), 1302-1315.

Zhang, X., Zink, F., Hezel, F., Vogt, J., Wachter, U., Wepler, M., . . . Hartmann, C. (2020). Metabolic substrate utilization in stress-induced immune cells. Intensive Care Medicine Experimental, 8(1), 28. doi:10.1186/s40635-020-00316-0

Zhang, Y., Li, L., Liu, Y., & Liu, Z.-R. (2016). PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society, 24(2), 328-336. doi:10.1111/wrr.12411

Zhang, Y., Li, L., Liu, Y., & Liu, Z. R. (2016). PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound Repair Regen, 24(2), 328-336. doi:10.1111/wrr.12411

Zheng, B., & Clemmons, D. R. (1998). Blocking ligand occupancy of the αVβ3 integrin inhibits insulin-like growth factor I signaling in vascular smooth muscle cells. Proceedings of the National Academy of Sciences, 95(19), 11217. doi:10.1073/pnas.95.19.11217

Akins, N. S., Nielson, T. C., & Le, H. V. (2018). Inhibition of Glycolysis and Glutaminolysis: An Emerging Drug Discovery Approach to Combat Cancer. Current topics in medicinal chemistry, 18(6), 494-504. doi:10.2174/1568026618666180523111351

Alberg, A. J., Brock, M. V., Ford, J. G., Samet, J. M., & Spivack, S. D. (2013). Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest

Physicians evidence-based clinical practice guidelines. Chest, 143(5 Suppl), e1S-e29S. doi:10.1378/chest.12-2345

Alberghina, L., & Gaglio, D. (2014). Redox control of glutamine utilization in cancer. Cell death & disease, 5(12), e1561-e1561. doi:10.1038/cddis.2014.513

Anastasiou, D., Yu, Y., Israelsen, W. J., Jiang, J.-K., Boxer, M. B., Hong, B. S., . . . Vander Heiden, M. G. (2012). Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. Nature chemical biology, 8(10), 839-847. doi:10.1038/nchembio.1060

Anderson, N. M., Mucka, P., Kern, J. G., & Feng, H. (2018). The emerging role and targetability of the TCA cycle in cancer metabolism. Protein & cell, 9(2), 216-237. doi:10.1007/s13238-017-0451-1

Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers in immunology, 5, 491-491. doi:10.3389/fimmu.2014.00491

Asomaning, K., Miller, D. P., Liu, G., Wain, J. C., Lynch, T. J., Su, L., & Christiani, D. C. (2008). Second hand smoke, age of exposure and lung cancer risk. Lung cancer (Amsterdam, Netherlands), 61(1), 13-20. doi:10.1016/j.lungcan.2007.11.013

Azoitei, N., Becher, A., Steinestel, K., Rouhi, A., Diepold, K., Genze, F., . . . Seufferlein, T. (2016). PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. Molecular cancer, 15(1), 3. doi:10.1186/s12943-015-0490-2

Balkwill, F. R., Capasso, M., & Hagemann, T. (2012). The tumor microenvironment at a glance. Journal of Cell Science, 125(23), 5591. doi:10.1242/jcs.116392

Bingle, L., Brown, N. J., & Lewis, C. E. (2002). The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol, 196(3), 254-265. doi:10.1002/path.1027

Boodhoo, N., Kamble, N., Sharif, S., & Behboudi, S. (2020). Glutaminolysis and Glycolysis Are Essential for Optimal Replication of Marek's Disease Virus. Journal of virology, 94(4), e01680-01619. doi:10.1128/JVI.01680-19

Boros, L. G., Cascante, M., & Lee, W. N. (2002). Metabolic profiling of cell growth and death in cancer: applications in drug discovery. Drug Discov Today, 7(6), 364-372. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11893545

Brittan, M., Hunt, T., Jeffery, R., Poulsom, R., Forbes, S., Hodivala-Dilke, K., . . . Wright, N. (2002). Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut, 50(6), 752-757.

Bronte, V., Serafini, P., Mazzoni, A., Segal, D. M., & Zanovello, P. (2003). L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends in immunology, 24(6), 301-305.

Buschow, S. I., van Balkom, B. W. M., Aalberts, M., Heck, A. J. R., Wauben, M., & Stoorvogel, W. (2010). MHC class II-associated proteins in B-cell exosomes and potential functional implications for exosome biogenesis. Immunology & Cell Biology, 88(8), 851-856. doi:https://doi.org/10.1038/icb.2010.64

Campbell, D. J., & Koch, M. A. (2011). Treg cells: patrolling a dangerous neighborhood. Nat Med, 17(8), 929-930. doi:10.1038/nm.2433

Carmeliet, P., & Jain, R. K. (2011). Molecular mechanisms and clinical applications of angiogenesis. Nature, 473(7347), 298-307. doi:10.1038/nature10144

Chen, J., Zhang, S., Wu, J., Wu, S., Xu, G., & Wei, D. (2019). Essential Role of Nonessential Amino Acid Glutamine in Atherosclerotic Cardiovascular Disease. DNA and Cell Biology, 39(1), 8-15. doi:10.1089/dna.2019.5034

Clower, C. V., Chatterjee, D., Wang, Z., Cantley, L. C., Vander Heiden, M. G., & Krainer, A. R. (2010). The alternative splicing repressors hnRNP A1/A2 and PTB influence pyruvate kinase isoform expression and cell metabolism. Proceedings of the National Academy of Sciences, 107(5), 1894. doi:10.1073/pnas.0914845107

Cluntun, A. A., Lukey, M. J., Cerione, R. A., & Locasale, J. W. (2017). Glutamine Metabolism in Cancer: Understanding the Heterogeneity. Trends in cancer, 3(3), 169-180. doi:10.1016/j.trecan.2017.01.005

Cruzat, V., Macedo Rogero, M., Noel Keane, K., Curi, R., & Newsholme, P. (2018). Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. Nutrients, 10(11), 1564. doi:10.3390/nu10111564

Damsky, W. E., Rosenbaum, L. E., & Bosenberg, M. (2010). Decoding melanoma metastasis. Cancers, 3(1), 126-163. doi:10.3390/cancers3010126

Dang, C. V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. Molecular and cellular biology, 19(1), 1-11. doi:10.1128/mcb.19.1.1

Dang, C. V. (2012). MYC on the path to cancer. Cell, 149(1), 22-35. doi:10.1016/j.cell.2012.03.003

David, C. J., Chen, M., Assanah, M., Canoll, P., & Manley, J. L. (2010). HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature, 463(7279), 364-368. doi:10.1038/nature08697

Dayton, T. L., Gocheva, V., Miller, K. M., Israelsen, W. J., Bhutkar, A., Clish, C. B., . . . Vander Heiden, M. G. (2016). Germline loss of PKM2 promotes metabolic distress and hepatocellular carcinoma. Genes & development, 30(9), 1020-1033. doi:10.1101/gad.278549.116

De Larco, J. E., Wuertz, B. R., & Furcht, L. T. (2004). The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. Clinical Cancer Research, 10(15), 4895-4900.

DeBerardinis, R. J., & Chandel, N. S. (2016). Fundamentals of cancer metabolism. Science Advances, 2(5), e1600200. doi:10.1126/sciadv.1600200

Desmoulière, A., Guyot, C., & Gabbiani, G. (2004). The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. Int J Dev Biol, 48(5-6), 509-517. doi:10.1387/ijdb.041802ad

Dominguez-Sola, D., & Gautier, J. (2014). MYC and the control of DNA replication. Cold Spring Harbor perspectives in medicine, 4(6), a014423. doi:10.1101/cshperspect.a014423

Dong, G., Mao, Q., Xia, W., Xu, Y., Wang, J., Xu, L., & Jiang, F. (2016). PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncology letters, 11(3), 1980-1986. doi:10.3892/ol.2016.4168

Donovan, J., & Slingerland, J. (2000). Transforming growth factor-beta and breast cancer: Cell cycle arrest by transforming growth factor-beta and its disruption in cancer. Breast cancer research : BCR, 2(2), 116-124. doi:10.1186/bcr43

Dougan, M., & Dranoff, G. (2009). Immune therapy for cancer. Annu Rev Immunol, 27, 83-117. doi:10.1146/annurev.immunol.021908.132544

Elbers, J. R., van Unnik, J. A., Rijksen, G., van Oirschot, B. A., Roholl, P. J., Oosting, J., & Staal, G. E. (1991). Pyruvate kinase activity and isozyme composition in normal fibrous tissue and fibroblastic proliferations. Cancer, 67(10), 2552-2559. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=2015555

Erez, N., Truitt, M., Olson, P., Arron, S. T., & Hanahan, D. (2010). Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kappaB-Dependent Manner. Cancer Cell, 17(2), 135-147. doi:10.1016/j.ccr.2009.12.041

Fisher, D. T., Appenheimer, M. M., & Evans, S. S. (2014). The two faces of IL-6 in the tumor microenvironment. Seminars in immunology, 26(1), 38-47. doi:10.1016/j.smim.2014.01.008

Fridlender, Z. G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., . . . Albelda, S. M. (2009). Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell, 16(3), 183-194. doi:10.1016/j.ccr.2009.06.017

Fridman, W. H., Pagès, F., Sautès-Fridman, C., & Galon, J. (2012). The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer, 12(4), 298-306. doi:10.1038/nrc3245

Friman, T., Gustafsson, R., Stuhr, L. B., Chidiac, J., Heldin, N.-E., Reed, R. K., . . . Rubin, K. (2012). Increased fibrosis and interstitial fluid pressure in two different types of syngeneic murine carcinoma grown in integrin β3-subunit deficient mice. PLoS One, 7(3), e34082.

Gabrilovich, D. I., Ostrand-Rosenberg, S., & Bronte, V. (2012). Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol, 12(4), 253-268. doi:10.1038/nri3175

Gao, X., Wang, H., Yang, J. J., Chen, J., Jie, J., Li, L., . . . Liu, Z. R. (2013). Reciprocal regulation of protein kinase and pyruvate kinase activities of pyruvate kinase m2 by growth signals. J Biol Chem, 288(22), 15971-15979. doi:10.1074/jbc.M112.448753

Gao, X., Wang, H., Yang, J. J., Liu, X., & Liu, Z.-R. (2012). Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Molecular cell, 45(5), 598-609. doi:10.1016/j.molcel.2012.01.001

Gao, X., Wang, H., Yang, J. J., Liu, X., & Liu, Z. R. (2012). Pyruvate kinase m2 regulates gene transcription by acting as a protein kinase. Mol Cell, 45(5), 598-609. doi:S1097-2765(12)00003-2 [pii]

10.1016/j.molcel.2012.01.001

Gordon, S., & Plüddemann, A. (2017). Tissue macrophages: heterogeneity and functions. BMC Biology, 15(1), 53. doi:10.1186/s12915-017-0392-4

Granot, Z., Henke, E., Comen, E. A., King, T. A., Norton, L., & Benezra, R. (2011). Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell, 20(3), 300-314. doi:10.1016/j.ccr.2011.08.012

Gupta, M., Han, J. J., Stenson, M., Maurer, M., Wellik, L., Hu, G., . . . Witzig, T. E. (2012). Elevated serum IL-10 levels in diffuse large B-cell lymphoma: a mechanism of aberrant JAK2 activation. Blood, 119(12), 2844-2853. doi:10.1182/blood-2011-10-388538

Hacker, H. J., Steinberg, P., & Bannasch, P. (1998). Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. Carcinogenesis, 19(1), 99-107. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9472700

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. Cell, 100(1), 57-70. doi:10.1016/s0092-8674(00)81683-9

Hellen, C. U., & Sarnow, P. (2001). Internal ribosome entry sites in eukaryotic mRNA molecules. Genes & development, 15(13), 1593-1612. doi:10.1101/gad.891101

Henke, E., Nandigama, R., & Ergün, S. (2020). Extracellular Matrix in the Tumor Microenvironment and Its Impact on Cancer Therapy. Frontiers in Molecular Biosciences, 6, 160. Retrieved from https://www.frontiersin.org/article/10.3389/fmolb.2019.00160

Hirayama, D., Iida, T., & Nakase, H. (2017). The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis. International journal of molecular sciences, 19(1), 92. doi:10.3390/ijms19010092

Horton, M. A. (1997). The αvβ3 integrin "vitronectin receptor". The International Journal of Biochemistry & Cell Biology, 29(5), 721-725.

Hosios, A. M., Fiske, B. P., Gui, D. Y., & Vander Heiden, M. G. (2015). Lack of Evidence for PKM2 Protein Kinase Activity. Molecular cell, 59(5), 850-857. doi:10.1016/j.molcel.2015.07.013

Hsieh, C.-S., Lee, H.-M., & Lio, C.-W. J. (2012). Selection of regulatory T cells in the thymus. Nature Reviews Immunology, 12(3), 157-167.

Hsu, M.-C., Hung, W.-C., Yamaguchi, H., Lim, S.-O., Liao, H.-W., Tsai, C.-H., & Hung, M.-C. (2016). Extracellular PKM2 induces cancer proliferation by activating the EGFR signaling pathway. American journal of cancer research, 6(3), 628-638. Retrieved from https://pubmed.ncbi.nlm.nih.gov/27152240

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4851842/

Huang, B., Pan, P.-Y., Li, Q., Sato, A. I., Levy, D. E., Bromberg, J., . . . Chen, S.-H. (2006). Gr-1+ CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer research, 66(2), 1123-1131.

Iqbal, M. A., Siddiqui, F. A., Gupta, V., Chattopadhyay, S., Gopinath, P., Kumar, B., . . . Bamezai, R. N. K. (2013). Insulin enhances metabolic capacities of cancer cells by dual regulation of

glycolytic enzyme pyruvate kinase M2. Molecular cancer, 12, 72-72. doi:10.1186/1476-4598-12-72

Israelsen, William J., Dayton, Talya L., Davidson, Shawn M., Fiske, Brian P., Hosios, Aaron M., Bellinger, G., . . . Vander Heiden, Matthew G. (2013). PKM2 Isoform-Specific Deletion Reveals a Differential Requirement for Pyruvate Kinase in Tumor Cells. Cell, 155(2), 397-409. doi:10.1016/j.cell.2013.09.025

Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical reviews in immunology, 32(1), 23-63. doi:10.1615/critrevimmunol.v32.i1.30

Jain, R. K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science (New York, N.Y.), 307(5706), 58-62.

Jiang, J., Srivastava, S., & Zhang, J. (2019). Starve Cancer Cells of Glutamine: Break the Spell or Make a Hungry Monster? Cancers, 11(6), 804. doi:10.3390/cancers11060804

Jin, L., Alesi, G. N., & Kang, S. (2016). Glutaminolysis as a target for cancer therapy. Oncogene, 35(28), 3619-3625. doi:10.1038/onc.2015.447

Jing, C., Qu, X., Li, Z., Wu, C., Zhao, M., Wang, Y., . . . Zhou, X. (2017). EGFRwt/vIII-PKM2-β-catenin cascade affects proliferation and chemo-sensitivity in head and neck squamous cell carcinoma. American journal of cancer research, 7(12), 2491-2502. Retrieved from https://pubmed.ncbi.nlm.nih.gov/29312802

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5752689/

Johnson, D. E., O'Keefe, R. A., & Grandis, J. R. (2018). Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nature reviews. Clinical oncology, 15(4), 234-248. doi:10.1038/nrclinonc.2018.8

Keller, Kirstie E., Doctor, Zainab M., Dwyer, Zachary W., & Lee, Y.-S. (2014). SAICAR Induces Protein Kinase Activity of PKM2 that Is Necessary for Sustained Proliferative Signaling of Cancer Cells. Molecular cell, 53(5), 700-709. doi:https://doi.org/10.1016/j.molcel.2014.02.015

Kim, H., Kim, S. H., Hwang, D., An, J., Chung, H. S., Yang, E. G., & Kim, S. Y. (2020). Extracellular pyruvate kinase M2 facilitates cell migration by upregulating claudin-1 expression in colon cancer cells. Biochem Cell Biol, 98(2), 219-226. doi:10.1139/bcb-2019-0139

Kim, M. H., & Kim, H. (2013). Oncogenes and tumor suppressors regulate glutamine metabolism in cancer cells. Journal of cancer prevention, 18(3), 221-226. doi:10.15430/jcp.2013.18.3.221

Kim, S. C., Tonkonogy, S. L., Albright, C. A., Tsang, J., Balish, E. J., Braun, J., . . . Sartor, R. B. (2005). Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology, 128(4), 891-906. doi:10.1053/j.gastro.2005.02.009

Kodama, M., Oshikawa, K., Shimizu, H., Yoshioka, S., Takahashi, M., Izumi, Y., . . . Nakayama, K. I. (2020). A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer. Nature Communications, 11(1), 1320. doi:10.1038/s41467-020-15136-9

Komar, A. A., & Hatzoglou, M. (2011). Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. Cell cycle (Georgetown, Tex.), 10(2), 229-240. doi:10.4161/cc.10.2.14472

Kraman, M., Bambrough, P. J., Arnold, J. N., Roberts, E. W., Magiera, L., Jones, J. O., . . . Fearon, D. T. (2010). Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein–α. Science (New York, N.Y.), 330(6005), 827-830.

Kumar, C. C. (1998). Signaling by integrin receptors. Oncogene, 17(11), 1365-1373.

Kumari, S., Badana, A. K., G, M. M., G, S., & Malla, R. (2018). Reactive Oxygen Species: A Key Constituent in Cancer Survival. Biomarker insights, 13, 1177271918755391-1177271918755391. doi:10.1177/1177271918755391

Kusmartsev, S., Nagaraj, S., & Gabrilovich, D. I. (2005). Tumor-associated CD8+ T cell tolerance induced by bone marrow-derived immature myeloid cells. The journal of immunology, 175(7), 4583-4592.

Landskron, G., De la Fuente, M., Thuwajit, P., Thuwajit, C., & Hermoso, M. A. (2014). Chronic Inflammation and Cytokines in the Tumor Microenvironment. Journal of Immunology Research, 2014, 149185. doi:10.1155/2014/149185

Li, B., & Wang, J. H. (2011). Fibroblasts and myofibroblasts in wound healing: force generation and measurement. J Tissue Viability, 20(4), 108-120. doi:10.1016/j.jtv.2009.11.004

Li, L., Zhang, Y., Qiao, J., Yang, J. J., & Liu, Z.-R. (2014). Pyruvate kinase M2 in blood circulation facilitates tumor growth by promoting angiogenesis. The Journal of biological chemistry, 289(37), 25812-25821. doi:10.1074/jbc.M114.576934

Liang, J., Cao, R., Wang, X., Zhang, Y., Wang, P., Gao, H., . . . Yang, W. (2017). Mitochondrial PKM2 regulates oxidative stress-induced apoptosis by stabilizing Bcl2. Cell Res, 27(3), 329-351. doi:10.1038/cr.2016.159

Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? Trends in biochemical sciences, 41(3), 211-218. doi:10.1016/j.tibs.2015.12.001

Lin, E. Y., Li, J.-F., Gnatovskiy, L., Deng, Y., Zhu, L., Grzesik, D. A., . . . Pollard, J. W. (2006). Macrophages regulate the angiogenic switch in a mouse model of breast cancer. Cancer research, 66(23), 11238-11246.

Long, L., Chen, M., Yuan, Y., Ming, A. L., Guo, W., Wu, K., & Chen, H. (2020). High expression of PKM2 synergizes with PD-L1 in tumor cells and immune cells to predict worse survival in human lung adenocarcinoma. Journal of Cancer, 11(15), 4442-4452. doi:10.7150/jca.42610

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., . . . Semenza, G. L. (2011). Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell, 145(5), 732-744. doi:10.1016/j.cell.2011.03.054

Luo, W., & Semenza, G. L. (2012). Emerging roles of PKM2 in cell metabolism and cancer progression. Trends in endocrinology and metabolism: TEM, 23(11), 560-566. doi:10.1016/j.tem.2012.06.010

Márquez, J., Alonso, F. J., Matés, J. M., Segura, J. A., Martín-Rufián, M., & Campos-Sandoval, J. A. (2017). Glutamine Addiction In Gliomas. Neurochem Res, 42(6), 1735-1746. doi:10.1007/s11064-017-2212-1

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime reports, 6, 13-13. doi:10.12703/P6-13

Masisi, B. K., El Ansari, R., Alfarsi, L., Rakha, E. A., Green, A. R., & Craze, M. L. (2020). The role of glutaminase in cancer. Histopathology, 76(4), 498-508. doi:https://doi.org/10.1111/his.14014

Matés, J. M., Pérez-Gómez, C., de Castro, I. N., Asenjo, M., & Márquez, J. (2002). Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. The International Journal of Biochemistry & Cell Biology, 34(5), 439-458. doi:https://doi.org/10.1016/S1357-2725(01)00143-1

Matsuda, S., Adachi, J., Ihara, M., Tanuma, N., Shima, H., Kakizuka, A., . . . Matsuda, T. (2016). Nuclear pyruvate kinase M2 complex serves as a transcriptional coactivator of arylhydrocarbon receptor. Nucleic acids research, 44(2), 636-647. doi:10.1093/nar/gkv967

Mazurek, S., Boschek, C. B., Hugo, F., & Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol, 15(4), 300-308. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15908230

Mazurek, S., Drexler, H. C., Troppmair, J., Eigenbrodt, E., & Rapp, U. R. (2007). Regulation of pyruvate kinase type M2 by A-Raf: a possible glycolytic stop or go mechanism. Anticancer Res, 27(6b), 3963-3971.

Miller, D. M., Thomas, S. D., Islam, A., Muench, D., & Sedoris, K. (2012). c-Myc and cancer metabolism. Clinical cancer research : an official journal of the American Association for Cancer Research, 18(20), 5546-5553. doi:10.1158/1078-0432.CCR-12-0977

Morfouace, M., Lalier, L., Oliver, L., Cheray, M., Pecqueur, C., Cartron, P. F., & Vallette, F. M. (2014). Control of glioma cell death and differentiation by PKM2-Oct4 interaction. Cell death & disease, 5(1), e1036-e1036. doi:10.1038/cddis.2013.561

Murphy, M. J., Wilson, A., & Trumpp, A. (2005). More than just proliferation: Myc function in stem cells. Trends in Cell Biology, 15(3), 128-137. doi:10.1016/j.tcb.2005.01.008

Murray, P. J. (2005). The primary mechanism of the IL-10-regulated antiinflammatory response is to selectively inhibit transcription. Proceedings of the National Academy of Sciences of the United States of America, 102(24), 8686. doi:10.1073/pnas.0500419102

Newsholme, P., Lima, M. M. R., Procopio, J., Pithon-Curi, T. C., Doi, S. Q., Bazotte, R. B., & Curi, R. (2003). Glutamine and glutamate as vital metabolites. Brazilian Journal of Medical and

Biological Research, 36, 153-163. Retrieved from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-879X2003000200002&nrm=iso

Noy, R., & Pollard, J. W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity, 41(1), 49-61. doi:10.1016/j.immuni.2014.06.010

Ojalvo, L. S., Whittaker, C. A., Condeelis, J. S., & Pollard, J. W. (2010). Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. The journal of immunology, 184(2), 702-712.

Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., . . . Weinberg, R. A. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell, 121(3), 335-348. doi:10.1016/j.cell.2005.02.034

Palsson-McDermott, E. M., Dyck, L., Zasłona, Z., Menon, D., McGettrick, A. F., Mills, K. H. G., & O'Neill, L. A. (2017). Pyruvate Kinase M2 Is Required for the Expression of the Immune Checkpoint PD-L1 in Immune Cells and Tumors. Frontiers in immunology, 8, 1300-1300. doi:10.3389/fimmu.2017.01300

Panieri, E., & Santoro, M. M. (2016). ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell death & disease, 7(6), e2253-e2253. doi:10.1038/cddis.2016.105

Patsenker, E., Popov, Y., Stickel, F., Schneider, V., Ledermann, M., Sägesser, H., . . . Schuppan, D. (2009). Pharmacological inhibition of integrin alphavbeta3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. Hepatology (Baltimore, Md.), 50(5), 1501-1511. doi:10.1002/hep.23144

Pavlova, N. N., & Thompson, C. B. (2016). The Emerging Hallmarks of Cancer Metabolism. Cell metabolism, 23(1), 27-47. doi:10.1016/j.cmet.2015.12.006

Pekarek, L. A., Starr, B. A., Toledano, A. Y., & Schreiber, H. (1995). Inhibition of tumor growth by elimination of granulocytes. The Journal of experimental medicine, 181(1), 435-440.

Phan, L. M., Yeung, S.-C. J., & Lee, M.-H. (2014). Cancer metabolic reprogramming: importance, main features, and potentials for precise targeted anti-cancer therapies. Cancer biology & medicine, 11(1), 1-19. doi:10.7497/j.issn.2095-3941.2014.01.001

Poltavets, V., Kochetkova, M., Pitson, S. M., & Samuel, M. S. (2018). The Role of the Extracellular Matrix and Its Molecular and Cellular Regulators in Cancer Cell Plasticity. Frontiers in Oncology, 8, 431-431. doi:10.3389/fonc.2018.00431

Poole, C. J., & van Riggelen, J. (2017). MYC-Master Regulator of the Cancer Epigenome and Transcriptome. Genes, 8(5), 142. doi:10.3390/genes8050142

Prakasam, G., Iqbal, M. A., Bamezai, R. N. K., & Mazurek, S. (2018). Posttranslational Modifications of Pyruvate Kinase M2: Tweaks that Benefit Cancer. Frontiers in Oncology, 8, 22-22. doi:10.3389/fonc.2018.00022

Qian, B. Z., & Pollard, J. W. (2010). Macrophage diversity enhances tumor progression and metastasis. Cell, 141(1), 39-51. doi:10.1016/j.cell.2010.03.014

Radisky, D. C., Kenny, P. A., & Bissell, M. J. (2007). Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? J Cell Biochem, 101(4), 830-839. doi:10.1002/jcb.21186

Rajala, R. V. S., Rajala, A., Kooker, C., Wang, Y., & Anderson, R. E. (2016). The Warburg Effect Mediator Pyruvate Kinase M2 Expression and Regulation in the Retina. Scientific Reports, 6(1), 37727. doi:10.1038/srep37727

Reynolds, L. E., Wyder, L., Lively, J. C., Taverna, D., Robinson, S. D., Huang, X., . . . Hodivala-Dilke, K. M. (2002). Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins. Nat Med, 8(1), 27-34. doi:10.1038/nm0102-27

Robinson, S. D., Reynolds, L. E., Wyder, L., Hicklin, D. J., & Hodivala-Dilke, K. M. (2004). Beta3-integrin regulates vascular endothelial growth factor-A-dependent permeability. Arterioscler Thromb Vasc Biol, 24(11), 2108-2114. doi:10.1161/01.ATV.0000143857.27408.de

Rosenberg, S. A., Restifo, N. P., Yang, J. C., Morgan, R. A., & Dudley, M. E. (2008). Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nature reviews. Cancer, 8(4), 299-308. doi:10.1038/nrc2355

Rőszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators of Inflammation, 2015, 816460. doi:10.1155/2015/816460

Saha, S. K., Islam, S. M. R., Abdullah-Al-Wadud, M., Islam, S., Ali, F., & Park, K. S. (2019). Multiomics Analysis Reveals that GLS and GLS2 Differentially Modulate the Clinical Outcomes of Cancer. Journal of clinical medicine, 8(3), 355. doi:10.3390/jcm8030355

Sato, T., Terai, M., Tamura, Y., Alexeev, V., Mastrangelo, M. J., & Selvan, S. R. (2011). Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. Immunologic Research, 51(2), 170-182. doi:10.1007/s12026-011-8262-6

Schiller, H. B., & Fassler, R. (2013). Mechanosensitivity and compositional dynamics of cell-matrix adhesions. EMBO Rep, 14(6), 509-519. doi:10.1038/embor.2013.49

Semenza, G. L. (2013). HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. The Journal of Clinical Investigation, 123(9), 3664-3671. doi:10.1172/JCI67230

Shojaei, F., Singh, M., Thompson, J. D., & Ferrara, N. (2008). Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. Proceedings of the National Academy of Sciences, 105(7), 2640-2645.

Sica, A., & Bronte, V. (2007). Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest, 117(5), 1155-1166. doi:10.1172/jci31422

Silvera, D., Formenti, S. C., & Schneider, R. J. (2010). Translational control in cancer. Nature Reviews Cancer, 10(4), 254-266. doi:10.1038/nrc2824

Sinha, P., Clements, V. K., Bunt, S. K., Albelda, S. M., & Ostrand-Rosenberg, S. (2007). Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. The journal of immunology, 179(2), 977-983.

Sonenberg, N., & Hinnebusch, A. G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell, 136(4), 731-745. doi:10.1016/j.cell.2009.01.042

Song, Z., Wei, B., Lu, C., Li, P., & Chen, L. (2017). Glutaminase sustains cell survival via the regulation of glycolysis and glutaminolysis in colorectal cancer. Oncol Lett, 14(3), 3117-3123. doi:10.3892/ol.2017.6538

Souba, W. W. (1993). Glutamine and cancer. Annals of surgery, 218(6), 715-728. doi:10.1097/00000658-199312000-00004

Spaeth, E. L., Dembinski, J. L., Sasser, A. K., Watson, K., Klopp, A., Hall, B., . . . Marini, F. (2009). Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. PLoS One, 4(4), e4992. doi:10.1371/journal.pone.0004992

Sriram, A., Bohlen, J., & Teleman, A. A. (2018). Translation acrobatics: how cancer cells exploit alternate modes of translational initiation. EMBO reports, 19(10), e45947. doi:https://doi.org/10.15252/embr.201845947

Stutchbury, B., Atherton, P., Tsang, R., Wang, D.-Y., & Ballestrem, C. (2017). Distinct focal adhesion protein modules control different aspects of mechanotransduction. Journal of cell science, 130(9), 1612-1624. doi:10.1242/jcs.195362

Sugimoto, H., Mundel, T. M., Kieran, M. W., & Kalluri, R. (2006). Identification of fibroblast heterogeneity in the tumor microenvironment. Cancer Biol Ther, 5(12), 1640-1646. doi:10.4161/cbt.5.12.3354

Sun, Z., Guo, S. S., & Fässler, R. (2016). Integrin-mediated mechanotransduction. The Journal of cell biology, 215(4), 445-456. doi:10.1083/jcb.201609037

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, n/a(n/a). doi:https://doi.org/10.3322/caac.21660

Takada, Y., Ye, X., & Simon, S. (2007). The integrins. Genome Biology, 8(5), 215. doi:10.1186/gb-2007-8-5-215

Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C., & Brown, R. A. (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol, 3(5), 349-363. doi:10.1038/nrm809

Vander Heiden, M. G., Cantley, L. C., & Thompson, C. B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science (New York, N.Y.), 324(5930), 1029-1033. doi:10.1126/science.1160809

Wang, C., Zhang, S., Liu, J., Tian, Y., Ma, B., Xu, S., . . . Luo, Y. (2020). Secreted Pyruvate Kinase M2 Promotes Lung Cancer Metastasis through Activating the Integrin Beta1/FAK

Signaling Pathway. Cell Reports, 30(6), 1780-1797.e1786. doi:https://doi.org/10.1016/j.celrep.2020.01.037

Wang, Z., Liu, F., Fan, N., Zhou, C., Li, D., Macvicar, T., . . . Zhao, Y. (2020). Targeting Glutaminolysis: New Perspectives to Understand Cancer Development and Novel Strategies for Potential Target Therapies. Frontiers in Oncology, 10(2321). doi:10.3389/fonc.2020.589508

Warburg, O. (1924). Über den Stoffwechsel der Carcinomzelle. Naturwissenschaften, 12(50), 1131-1137. doi:10.1007/BF01504608

Wei, S. C., Fattet, L., Tsai, J. H., Guo, Y., Pai, V. H., Majeski, H. E., . . . Yang, J. (2015). Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway. Nature cell biology, 17(5), 678-688. doi:10.1038/ncb3157

Weis, S. M., & Cheresh, D. A. (2011). αV integrins in angiogenesis and cancer. Cold Spring Harbor perspectives in medicine, 1(1), a006478-a006478. doi:10.1101/cshperspect.a006478

Wendt, M. K., Allington, T. M., & Schiemann, W. P. (2009). Mechanisms of the epithelial-mesenchymal transition by TGF-beta. Future oncology (London, England), 5(8), 1145-1168. doi:10.2217/fon.09.90

Weng, S., Zemany, L., Standley, K. N., Novack, D. V., La Regina, M., Bernal-Mizrachi, C., . . . Semenkovich, C. F. (2003). Beta3 integrin deficiency promotes atherosclerosis and pulmonary inflammation in high-fat-fed, hyperlipidemic mice. Proc Natl Acad Sci U S A, 100(11), 6730-6735. doi:10.1073/pnas.1137612100

Whiteside, T. L. (2008). The tumor microenvironment and its role in promoting tumor growth. Oncogene, 27(45), 5904-5912. doi:10.1038/onc.2008.271

Williams, A. L., Khadka, V., Tang, M., Avelar, A., Schunke, K. J., Menor, M., & Shohet, R. V. (2018). HIF1 mediates a switch in pyruvate kinase isoforms after myocardial infarction. Physiol Genomics, 50(7), 479-494. doi:10.1152/physiolgenomics.00130.2017

Willis, B. C., duBois, R. M., & Borok, Z. (2006). Epithelial origin of myofibroblasts during fibrosis in the lung. Proc Am Thorac Soc, 3(4), 377-382. doi:10.1513/pats.200601-004TK

Winkler, J., Abisoye-Ogunniyan, A., Metcalf, K. J., & Werb, Z. (2020). Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nature Communications, 11(1), 5120. doi:10.1038/s41467-020-18794-x

Wong, N., De Melo, J., & Tang, D. (2013). PKM2, a Central Point of Regulation in Cancer Metabolism. International Journal of Cell Biology, 2013, 242513. doi:10.1155/2013/242513

Wozniak, M. A., Modzelewska, K., Kwong, L., & Keely, P. J. (2004). Focal adhesion regulation of cell behavior. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1692(2), 103-119. doi:https://doi.org/10.1016/j.bbamcr.2004.04.007

Yang, L., Pang, Y., & Moses, H. L. (2010). TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. Trends in immunology, 31(6), 220-227. doi:10.1016/j.it.2010.04.002

Yang, L., Venneti, S., & Nagrath, D. (2017). Glutaminolysis: A Hallmark of Cancer Metabolism. Annual Review of Biomedical Engineering, 19(1), 163-194. doi:10.1146/annurev-bioeng-071516-044546

Yang, P., Li, Z., Wang, Y., Zhang, L., Wu, H., & Li, Z. (2015). Secreted pyruvate kinase M2 facilitates cell migration via PI3K/Akt and Wnt/β-catenin pathway in colon cancer cells. Biochem Biophys Res Commun, 459(2), 327-332. doi:10.1016/j.bbrc.2015.02.112

Yang, W., & Lu, Z. (2015). Pyruvate kinase M2 at a glance. Journal of Cell Science, 128(9), 1655. doi:10.1242/jcs.166629

Yang, W., Xia, Y., Cao, Y., Zheng, Y., Bu, W., Zhang, L., . . . Lu, Z. (2012). EGFR-induced and PKCε monoubiquitylation-dependent NF-κB activation upregulates PKM2 expression and promotes tumorigenesis. Molecular cell, 48(5), 771-784. doi:10.1016/j.molcel.2012.09.028

Yang, W., Xia, Y., Hawke, D., Li, X., Liang, J., Xing, D., . . . Lu, Z. (2012). PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell, 150(4), 685-696. doi:10.1016/j.cell.2012.07.018

Yang, W., Zheng, Y., Xia, Y., Ji, H., Chen, X., Guo, F., . . . Lu, Z. (2012). ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. Nature cell biology, 14(12), 1295-1304. doi:10.1038/ncb2629

Yin, L., Duan, J.-J., Bian, X.-W., & Yu, S.-c. (2020). Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Research, 22(1), 61. doi:10.1186/s13058-020-01296-5

Youn, J. I., & Gabrilovich, D. I. (2010). The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. Eur J Immunol, 40(11), 2969-2975. doi:10.1002/eji.201040895

Zahra, K., Dey, T., Ashish, Mishra, S. P., & Pandey, U. (2020). Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. Frontiers in Oncology, 10, 159-159. doi:10.3389/fonc.2020.00159

Zhang, Y., Li, L., Liu, Y., & Liu, Z.-R. (2016). PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound repair and regeneration :

official publication of the Wound Healing Society [and] the European Tissue Repair Society, 24(2), 328-336. doi:10.1111/wrr.12411

Zhang, Z., Deng, X., Liu, Y., Liu, Y., Sun, L., & Chen, F. (2019). PKM2, function and expression and regulation. Cell & Bioscience, 9(1), 52. doi:10.1186/s13578-019-0317-8

Zumsteg, A., & Christofori, G. (2009). Corrupt policemen: inflammatory cells promote tumor angiogenesis. Curr Opin Oncol, 21(1), 60-70. doi:10.1097/CCO.0b013e32831bed7e

Akins, N. S., Nielson, T. C., & Le, H. V. (2018). Inhibition of Glycolysis and Glutaminolysis: An Emerging Drug Discovery Approach to Combat Cancer. Current topics in medicinal chemistry, 18(6), 494-504. doi:10.2174/1568026618666180523111351

Alberghina, L., & Gaglio, D. (2014). Redox control of glutamine utilization in cancer. Cell death & disease, 5(12), e1561-e1561. doi:10.1038/cddis.2014.513

Alsaab, H. O., Sau, S., Alzhrani, R., Tatiparti, K., Bhise, K., Kashaw, S. K., & Iyer, A. K. (2017). PD-1 and PD-L1 Checkpoint Signaling Inhibition for Cancer Immunotherapy: Mechanism, Combinations, and Clinical Outcome. Frontiers in Pharmacology, 8(561). doi:10.3389/fphar.2017.00561

Anderson, N. M., Mucka, P., Kern, J. G., & Feng, H. (2018). The emerging role and targetability of the TCA cycle in cancer metabolism. Protein & cell, 9(2), 216-237. doi:10.1007/s13238-017-0451-1

Angiari, S., Runtsch, M. C., Sutton, C. E., Palsson-McDermott, E. M., Kelly, B., Rana, N., . . . O'Neill, L. A. J. (2020). Pharmacological Activation of Pyruvate Kinase M2 Inhibits CD4+ T Cell

Pathogenicity and Suppresses Autoimmunity. Cell metabolism, 31(2), 391-405.e398. doi:https://doi.org/10.1016/j.cmet.2019.10.015

Antonov, A. S., Antonova, G. N., Munn, D. H., Mivechi, N., Lucas, R., Catravas, J. D., & Verin, A. D. (2011). αVβ3 integrin regulates macrophage inflammatory responses via PI3 kinase/Akt-dependent NF-κB activation. Journal of cellular physiology, 226(2), 469-476. doi:10.1002/jcp.22356

Appelbaum, F. R., Gundacker, H., Head, D. R., Slovak, M. L., Willman, C. L., Godwin, J. E., . . . Petersdorf, S. H. (2006). Age and acute myeloid leukemia. Blood, 107(9), 3481-3485. doi:10.1182/blood-2005-09-3724

Araujo, L., Khim, P., Mkhikian, H., Mortales, C.-L., & Demetriou, M. (2017). Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation. eLife, 6, e21330. doi:10.7554/eLife.21330

Azoitei, N., Becher, A., Steinestel, K., Rouhi, A., Diepold, K., Genze, F., . . . Seufferlein, T. (2016). PKM2 promotes tumor angiogenesis by regulating HIF-1α through NF-κB activation. Molecular cancer, 15(1), 3. doi:10.1186/s12943-015-0490-2

Bade, B. C., & Dela Cruz, C. S. (2020). Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med, 41(1), 1-24. doi:10.1016/j.ccm.2019.10.001

Bailar, J. C., & Gornik, H. L. (1997). Cancer undefeated. New England Journal of Medicine, 336(22), 1569-1574.

Bandukwala, H. S., Gagnon, J., Togher, S., Greenbaum, J. A., Lamperti, E. D., Parr, N. J., . . . Rao, A. (2012). Selective inhibition of CD4+ T-cell cytokine production and autoimmunity by BET protein and c-Myc inhibitors. Proceedings of the National Academy of Sciences of the United States of America, 109(36), 14532-14537. doi:10.1073/pnas.1212264109

Bashir, B., & Wilson, M. A. (2019). Novel Immunotherapy Combinations. Current Oncology Reports, 21(11), 96. doi:10.1007/s11912-019-0851-x

Bernard, K., Logsdon, N. J., Benavides, G. A., Sanders, Y., Zhang, J., Darley-Usmar, V. M., & Thannickal, V. J. (2018). Glutaminolysis is required for transforming growth factor-β1-induced myofibroblast differentiation and activation. The Journal of biological chemistry, 293(4), 1218-1228. doi:10.1074/jbc.RA117.000444

Bonilla, F. A., & Oettgen, H. C. (2010). Adaptive immunity. Journal of Allergy and Clinical Immunology, 125(2, Supplement 2), S33-S40. doi:https://doi.org/10.1016/j.jaci.2009.09.017

Boros, L. G., Cascante, M., & Lee, W. N. (2002). Metabolic profiling of cell growth and death in cancer: applications in drug discovery. Drug Discov Today, 7(6), 364-372. doi:10.1016/s1359-6446(02)02179-7

Brabletz, T., Hlubek, F., Spaderna, S., Schmalhofer, O., Hiendlmeyer, E., Jung, A., & Kirchner, T. (2005). Invasion and Metastasis in Colorectal Cancer: Epithelial-Mesenchymal Transition, Mesenchymal-Epithelial Transition, Stem Cells and β-Catenin. Cells Tissues Organs, 179(1-2), 56-65. doi:10.1159/000084509

Carracedo, A., & Pandolfi, P. P. (2008). The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene, 27(41), 5527-5541. doi:10.1038/onc.2008.247

Chabner, B. A., & Roberts, T. G. (2005). Chemotherapy and the war on cancer. Nature Reviews Cancer, 5(1), 65-72. doi:10.1038/nrc1529

Chalhoub, N., & Baker, S. J. (2009). PTEN and the PI3-kinase pathway in cancer. Annu Rev Pathol, 4, 127-150. doi:10.1146/annurev.pathol.4.110807.092311

Chaneton, B., & Gottlieb, E. (2012). Rocking cell metabolism: revised functions of the key glycolytic regulator PKM2 in cancer. Trends in biochemical sciences, 37(8), 309-316.

Chen, M., Zhang, J., & Manley, J. L. (2010). Turning on a fuel switch of cancer: hnRNP proteins regulate alternative splicing of pyruvate kinase mRNA. Cancer research, 70(22), 8977-8980.

Chen, X., & Song, E. (2019). Turning foes to friends: targeting cancer-associated fibroblasts. Nat Rev Drug Discov, 18(2), 99-115. doi:10.1038/s41573-018-0004-1

Cluntun, A. A., Lukey, M. J., Cerione, R. A., & Locasale, J. W. (2017). Glutamine Metabolism in Cancer: Understanding the Heterogeneity. Trends in cancer, 3(3), 169-180. doi:10.1016/j.trecan.2017.01.005

Couzin-Frankel, J. (2013). Cancer Immunotherapy. Science (New York, N.Y.), 342(6165), 1432. doi:10.1126/science.342.6165.1432

D'Abreo, N., & Adams, S. (2019). Immune-checkpoint inhibition for metastatic triple-negative breast cancer: safety first? Nat Rev Clin Oncol, 16(7), 399-400. doi:10.1038/s41571-019-0216-2

Dang, C. V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. Molecular and cellular biology, 19(1), 1-11. doi:10.1128/mcb.19.1.1

Dang, C. V. (2012). Links between metabolism and cancer. Genes & development, 26(9), 877-890.

Darvin, P., Toor, S. M., Sasidharan Nair, V., & Elkord, E. (2018). Immune checkpoint inhibitors: recent progress and potential biomarkers. Experimental & Molecular Medicine, 50(12), 1-11. doi:10.1038/s12276-018-0191-1

Dayton, T. L., Jacks, T., & Vander Heiden, M. G. (2016). PKM 2, cancer metabolism, and the road ahead. EMBO reports, 17(12), 1721-1730.

de Haes, J. C. J. M., & van Knippenberg, F. C. E. (1985). The quality of life of cancer patients: A review of the literature. Social Science & Medicine, 20(8), 809-817. doi:https://doi.org/10.1016/0277-9536(85)90335-1

De Laurentiis, M., Cianniello, D., Caputo, R., Stanzione, B., Arpino, G., Cinieri, S., . . . De Placido, S. (2010). Treatment of triple negative breast cancer (TNBC): current options and future perspectives. Cancer Treatment Reviews, 36, S80-S86. doi:https://doi.org/10.1016/S0305-7372(10)70025-6

Desbarats, L., Schneider, A., Müller, D., Bürgin, A., & Eilers, M. (1996). Myc: a single gene controls both proliferation and apoptosis in mammalian cells. Experientia, 52(12), 1123-1129. doi:10.1007/BF01952111

Dong, G., Mao, Q., Xia, W., Xu, Y., Wang, J., Xu, L., & Jiang, F. (2016). PKM2 and cancer: The function of PKM2 beyond glycolysis. Oncology letters, 11(3), 1980-1986. doi:10.3892/ol.2016.4168

Dudley, M. E., & Rosenberg, S. A. (2003). Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nature Reviews Cancer, 3(9), 666-675. doi:10.1038/nrc1167

Elbers, J. R., van Unnik, J. A., Rijksen, G., van Oirschot, B. A., Roholl, P. J., Oosting, J., & Staal, G. E. (1991). Pyruvate kinase activity and isozyme composition in normal fibrous tissue and fibroblastic proliferations. Cancer, 67(10), 2552-2559. doi:10.1002/1097-0142(19910515)67:10<2552::aid-cncr2820671027>3.0.co;2-k

Eliceiri, B. P., & Cheresh, D. A. (2000). Role of alpha v integrins during angiogenesis. Cancer J, 6 Suppl 3, S245-249.

Fass, L. (2008). Imaging and cancer: A review. Molecular Oncology, 2(2), 115-152. doi:https://doi.org/10.1016/j.molonc.2008.04.001

Fitzgerald, K. D., & Semler, B. L. (2009). Bridging IRES elements in mRNAs to the eukaryotic translation apparatus. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1789(9), 518-528. doi:https://doi.org/10.1016/j.bbagrm.2009.07.004

Fothergill-Gilmore, L. A., & Michels, P. A. (1993). Evolution of glycolysis. Progress in biophysics and molecular biology, 59(2), 105-235.

Franco, O. E., Shaw, A. K., Strand, D. W., & Hayward, S. W. (2010). Cancer associated fibroblasts in cancer pathogenesis. Seminars in Cell & Developmental Biology, 21(1), 33-39. doi:https://doi.org/10.1016/j.semcdb.2009.10.010

Gao, X., Wang, H., Yang, J. J., Chen, J., Jie, J., Li, L., . . . Liu, Z. R. (2013). Reciprocal regulation of protein kinase and pyruvate kinase activities of pyruvate kinase M2 by growth signals. The Journal of biological chemistry, 288(22), 15971-15979. doi:10.1074/jbc.M112.448753

Gao, X., Wang, H., Yang, J. J., Liu, X., & Liu, Z.-R. (2012). Pyruvate kinase M2 regulates gene transcription by acting as a protein kinase. Molecular cell, 45(5), 598-609. doi:10.1016/j.molcel.2012.01.001

Gao, X., Wang, H., Yang, J. J., Liu, X., & Liu, Z. R. (2012). Pyruvate kinase m2 regulates gene transcription by acting as a protein kinase. Mol Cell, 45(5), 598-609. doi:S1097-2765(12)00003-2 [pii]

10.1016/j.molcel.2012.01.001

Gibb Andrew, A., Lombardi Alyssa, A., Murray Emma, K., Tan, Y., Lorkiewicz Pawel, K., Huynh Anh, T., . . . Elrod John, W. (2019). Abstract 517: Glutaminolysis is Required to Initiate Myofibroblast Differentiation and Persistence During Stress. Circulation Research, 125(Suppl_1), A517-A517. doi:10.1161/res.125.suppl_1.517

Godet, A.-C., David, F., Hantelys, F., Tatin, F., Lacazette, E., Garmy-Susini, B., & Prats, A.-C. (2019). IRES Trans-Acting Factors, Key Actors of the Stress Response. International journal of molecular sciences, 20(4), 924. doi:10.3390/ijms20040924

Goodman, S. L., & Picard, M. (2012). Integrins as therapeutic targets. Trends in Pharmacological Sciences, 33(7), 405-412. doi:https://doi.org/10.1016/j.tips.2012.04.002

Gordon, S. (2007). The macrophage: Past, present and future. European Journal of Immunology, 37(S1), S9-S17. doi:https://doi.org/10.1002/eji.200737638

Grzmil, M., & Hemmings, B. A. (2012). Translation Regulation as a Therapeutic Target in Cancer. Cancer research, 72(16), 3891. doi:10.1158/0008-5472.CAN-12-0026

Gui, D. Y., Lewis, C. A., & Vander Heiden, M. G. (2013). Allosteric regulation of PKM2 allows cellular adaptation to different physiological states. Science signaling, 6(263), pe7-pe7.

Gupta, V., & Bamezai, R. N. (2010). Human pyruvate kinase M2: a multifunctional protein. Protein science, 19(11), 2031-2044.

Hacker, H. J., Steinberg, P., & Bannasch, P. (1998). Pyruvate kinase isoenzyme shift from L-type to M2-type is a late event in hepatocarcinogenesis induced in rats by a choline-deficient/DL-ethionine-supplemented diet. Carcinogenesis, 19(1), 99-107. doi:10.1093/carcin/19.1.99

Hao, N.-B., Lü, M.-H., Fan, Y.-H., Cao, Y.-L., Zhang, Z.-R., & Yang, S.-M. (2012). Macrophages in Tumor Microenvironments and the Progression of Tumors. Clinical and Developmental Immunology, 2012, 948098. doi:10.1155/2012/948098

Harding, H. P., Zhang, Y., & Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature, 397(6716), 271-274.

Harris, J. R., Lippman, M. E., Veronesi, U., & Willett, W. (1992). Breast Cancer. New England Journal of Medicine, 327(5), 319-328. doi:10.1056/nejm199207303270505

Hathurusinghe, H. R., Goonetilleke, K. S., & Siriwardena, A. K. (2007). Current status of tumor M2 pyruvate kinase (tumor M2-PK) as a biomarker of gastrointestinal malignancy. Ann Surg Oncol, 14(10), 2714-2720. doi:10.1245/s10434-007-9481-x

He, X., Du, S., Lei, T., Li, X., Liu, Y., Wang, H., . . . Wang, Y. (2017). PKM2 in carcinogenesis and oncotherapy. Oncotarget, 8(66), 110656-110670. doi:10.18632/oncotarget.22529

Hellen, C. U., & Sarnow, P. (2001). Internal ribosome entry sites in eukaryotic mRNA molecules. Genes & development, 15(13), 1593-1612. doi:10.1101/gad.891101

Henderson, N. C., Arnold, T. D., Katamura, Y., Giacomini, M. M., Rodriguez, J. D., McCarty, J. H., . . . Sheppard, D. (2013). Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med, 19(12), 1617-1624. doi:10.1038/nm.3282

Henderson, N. C., & Sheppard, D. (2013). Integrin-mediated regulation of TGFbeta in fibrosis. Biochim Biophys Acta, 1832(7), 891-896. doi:10.1016/j.bbadis.2012.10.005

Hinds, P. W., & Weinberg, R. A. (1994). Tumor suppressor genes. Curr Opin Genet Dev, 4(1), 135-141. doi:10.1016/0959-437x(94)90102-3

Hoffman, B., & Liebermann, D. A. (2008). Apoptotic signaling by c-MYC. Oncogene, 27(50), 6462-6472. doi:10.1038/onc.2008.312

Hood , J. D., Frausto , R., Kiosses , W. B., Schwartz , M. A., & Cheresh , D. A. (2003). Differential αv integrin–mediated Ras-ERK signaling during two pathways of angiogenesis. Journal of Cell Biology, 162(5), 933-943. doi:10.1083/jcb.200304105

Horton, M. A. (1997). The αvβ3 integrin “vitronectin receptor”. The International Journal of Biochemistry & Cell Biology, 29(5), 721-725. doi:https://doi.org/10.1016/S1357-2725(96)00155-0

Hsu, M.-C., & Hung, W.-C. (2018). Pyruvate kinase M2 fuels multiple aspects of cancer cells: from cellular metabolism, transcriptional regulation to extracellular signaling. Molecular cancer, 17(1), 35. doi:10.1186/s12943-018-0791-3

Israelsen, William J., Dayton, Talya L., Davidson, Shawn M., Fiske, Brian P., Hosios, Aaron M., Bellinger, G., . . . Vander Heiden, Matthew G. (2013). PKM2 Isoform-Specific Deletion Reveals a Differential Requirement for Pyruvate Kinase in Tumor Cells. Cell, 155(2), 397-409. doi:10.1016/j.cell.2013.09.025

Israelsen, W. J., & Vander Heiden, M. G. (2015). Pyruvate kinase: function, regulation and role in cancer. Paper presented at the Seminars in Cell & Developmental Biology.

Italiani, P., & Boraschi, D. (2014). From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. Frontiers in immunology, 5, 514-514. doi:10.3389/fimmu.2014.00514

Jeffery, J., Lewis, S. J., & Ayling, R. M. (2009). Fecal dimeric M2-pyruvate kinase (tumor M2-PK) in the differential diagnosis of functional and organic bowel disorders. Inflamm Bowel Dis, 15(11), 1630-1634. doi:10.1002/ibd.20946

Jiang, J., Srivastava, S., & Zhang, J. (2019). Starve Cancer Cells of Glutamine: Break the Spell or Make a Hungry Monster? Cancers, 11(6), 804. doi:10.3390/cancers11060804

Jin, L., Alesi, G. N., & Kang, S. (2016). Glutaminolysis as a target for cancer therapy. Oncogene, 35(28), 3619-3625. doi:10.1038/onc.2015.447

Kennedy, R., & Celis, E. (2008). Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunological Reviews, 222(1), 129-144. doi:https://doi.org/10.1111/j.1600-065X.2008.00616.x

Kim, J. (2018). Regulation of Immune Cell Functions by Metabolic Reprogramming. Journal of immunology research, 2018, 8605471-8605471. doi:10.1155/2018/8605471

Kodama, M., Oshikawa, K., Shimizu, H., Yoshioka, S., Takahashi, M., Izumi, Y., . . . Nakayama, K. I. (2020). A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer. Nature Communications, 11(1), 1320. doi:10.1038/s41467-020-15136-9

Komar, A. A., & Hatzoglou, M. (2011). Cellular IRES-mediated translation: the war of ITAFs in pathophysiological states. Cell cycle (Georgetown, Tex.), 10(2), 229-240. doi:10.4161/cc.10.2.14472

Kucab, J., & Dunn, S. (2003). Role of IGF-1R in mediating breast cancer invasion and metastasis. Breast disease, 17, 41-47.

Kumar, C. C. (1998). Signaling by integrin receptors. Oncogene, 17(11), 1365-1373. doi:10.1038/sj.onc.1202172

Lee, S.-Y., Obata, Y., Yoshida, M., Stockert, E., Williamson, B., Jungbluth, A. A., . . . Scanlan, M. J. (2003). Immunomic analysis of human sarcoma. Proceedings of the National Academy of Sciences, 100(5), 2651. doi:10.1073/pnas.0437972100

Levine, A. J., & Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. Science (New York, N.Y.), 330(6009), 1340-1344. doi:10.1126/science.1193494

Li, L., Peng, G., Liu, X., Zhang, Y., Han, H., & Liu, Z.-R. (2020). Pyruvate Kinase M2 Coordinates Metabolism Switch between Glycolysis and Glutaminolysis in Cancer Cells. iScience, 23(11), 101684. doi:https://doi.org/10.1016/j.isci.2020.101684

Li, L., Peng, G., Liu, X., Zhang, Y., Han, H., & Liu, Z. R. (2020). Pyruvate Kinase M2 Coordinates Metabolism Switch between Glycolysis and Glutaminolysis in Cancer Cells. iScience, 23(11), 101684. doi:10.1016/j.isci.2020.101684

Li, L., Zhang, Y., Qiao, J., Yang, J. J., & Liu, Z.-R. (2014). Pyruvate kinase M2 in blood circulation facilitates tumor growth by promoting angiogenesis. The Journal of biological chemistry, 289(37), 25812-25821. doi:10.1074/jbc.M114.576934

Li, L., Zhang, Y., Qiao, J., Yang, J. J., & Liu, Z. R. (2014). Pyruvate kinase M2 in blood circulation facilitates tumor growth by promoting angiogenesis. J Biol Chem, 289(37), 25812-25821. doi:10.1074/jbc.M114.576934

Li, X. B., Gu, J. D., & Zhou, Q. H. (2015). Review of aerobic glycolysis and its key enzymes - new targets for lung cancer therapy. Thorac Cancer, 6(1), 17-24. doi:10.1111/1759-7714.12148

Li, Y.-h., Li, X.-f., Liu, J.-t., Wang, H., Fan, L.-l., Li, J., & Sun, G.-p. (2018). PKM2, a potential target for regulating cancer. Gene, 668, 48-53.

Li, Z., Yang, P., & Li, Z. (2014). The multifaceted regulation and functions of PKM2 in tumor progression. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1846(2), 285-296.

Li, Z. Z., Wang, F., Liu, S., Li, H., & Wang, Y. (2020). Ablation of PKM2 ameliorated ER stress‐induced apoptosis and associated inflammation response in IL‐1β‐treated chondrocytes via blocking Rspo2‐mediated Wnt/β‐catenin signaling. Journal of cellular biochemistry, 121(10), 4204-4213.

Liang, J., Cao, R., Wang, X., Zhang, Y., Wang, P., Gao, H., . . . Yang, W. (2017). Mitochondrial PKM2 regulates oxidative stress-induced apoptosis by stabilizing Bcl2. Cell Res, 27(3), 329-351. doi:10.1038/cr.2016.159

Liberti, M. V., & Locasale, J. W. (2016). The Warburg Effect: How Does it Benefit Cancer Cells? Trends in biochemical sciences, 41(3), 211-218. doi:10.1016/j.tibs.2015.12.001

Liu, B., & Qian, S.-B. (2014). Translational reprogramming in cellular stress response. Wiley interdisciplinary reviews. RNA, 5(3), 301-315. doi:10.1002/wrna.1212

Liu, Z., Wang, F., & Chen, X. (2008). Integrin αvβ3-targeted cancer therapy. Drug Development Research, 69(6), 329-339. doi:https://doi.org/10.1002/ddr.20265

Loges, S., Mazzone, M., Hohensinner, P., & Carmeliet, P. (2009). Silencing or fueling metastasis with VEGF inhibitors: antiangiogenesis revisited. Cancer Cell, 15(3), 167-170. doi:10.1016/j.ccr.2009.02.007

López de Quinto, S., Lafuente, E., & Martínez-Salas, E. (2001). IRES interaction with translation initiation factors: functional characterization of novel RNA contacts with eIF3, eIF4B, and eIF4GII. RNA (New York, N.Y.), 7(9), 1213-1226. doi:10.1017/s1355838201010433

Lowe, S. W., & Lin, A. W. (2000). Apoptosis in cancer. Carcinogenesis, 21(3), 485-495. doi:10.1093/carcin/21.3.485

Lunt, S. Y., & Vander Heiden, M. G. (2011). Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annual review of cell and developmental biology, 27, 441-464.

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., . . . Semenza, G. L. (2011). Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell, 145(5), 732-744. doi:10.1016/j.cell.2011.03.054

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000prime reports, 6, 13-13. doi:10.12703/P6-13

Matés, J. M., Pérez-Gómez, C., de Castro, I. N., Asenjo, M., & Márquez, J. (2002). Glutamine and its relationship with intracellular redox status, oxidative stress and cell proliferation/death. The International Journal of Biochemistry & Cell Biology, 34(5), 439-458. doi:https://doi.org/10.1016/S1357-2725(01)00143-1

Mattevi, A., Bolognesi, M., & Valentini, G. (1996). The allosteric regulation of pyruvate kinase. FEBS letters, 389(1), 15-19.

Mazurek, S., Boschek, C. B., Hugo, F., & Eigenbrodt, E. (2005). Pyruvate kinase type M2 and its role in tumor growth and spreading. Semin Cancer Biol, 15(4), 300-308. doi:10.1016/j.semcancer.2005.04.009

McKeehan, W. L. (1982). Glycolysis, glutaminolysis and cell proliferation. Cell biology international reports, 6(7), 635-650.

McMahon, S. B. (2014). MYC and the control of apoptosis. Cold Spring Harbor perspectives in medicine, 4(7), a014407-a014407. doi:10.1101/cshperspect.a014407

Medzhitov, R., & Janeway Jr, C. A. (1998). Innate immune recognition and control of adaptive immune responses. Seminars in immunology, 10(5), 351-353. doi:https://doi.org/10.1006/smim.1998.0136

Méndez-Lucas, A., Li, X., Hu, J., Che, L., Song, X., Jia, J., . . . Tschaharganeh, D. F. (2017). Glucose catabolism in liver tumors induced by c-MYC can be sustained by various PKM1/PKM2 ratios and pyruvate kinase activities. Cancer research, 77(16), 4355-4364.

Miller, D. M., Thomas, S. D., Islam, A., Muench, D., & Sedoris, K. (2012). c-Myc and cancer metabolism. Clinical cancer research : an official journal of the American Association for Cancer Research, 18(20), 5546-5553. doi:10.1158/1078-0432.CCR-12-0977

Morgan, H. P., O'Reilly, F. J., Wear, M. A., O'Neill, J. R., Fothergill-Gilmore, L. A., Hupp, T., & Walkinshaw, M. D. (2013). M2 pyruvate kinase provides a mechanism for nutrient sensing and regulation of cell proliferation. Proceedings of the National Academy of Sciences, 110(15), 5881-5886.

Munger, J. S., Huang, X., Kawakatsu, H., Griffiths, M. J., Dalton, S. L., Wu, J., . . . Sheppard, D. (1999). The integrin alpha v beta 6 binds and activates latent TGF beta 1: a mechanism for

regulating pulmonary inflammation and fibrosis. Cell, 96(3), 319-328. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10025398

Nakamura, K., & Smyth, M. J. (2020). Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. Cellular & Molecular Immunology, 17(1), 1-12. doi:10.1038/s41423-019-0306-1

Nielsen, S. R., & Schmid, M. C. (2017). Macrophages as Key Drivers of Cancer Progression and Metastasis. Mediators of Inflammation, 2017, 9624760. doi:10.1155/2017/9624760

Noy, R., & Pollard, J. W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity, 41(1), 49-61. doi:10.1016/j.immuni.2014.06.010

Orphanides, G., & Reinberg, D. (2002). A unified theory of gene expression. Cell, 108(4), 439-451.

Palsson-McDermott, E. M., Curtis, A. M., Goel, G., Lauterbach, M. A. R., Sheedy, F. J., Gleeson, L. E., . . . O'Neill, L. A. J. (2015). Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the warburg effect in LPS-activated macrophages. Cell metabolism, 21(1), 65-80. doi:10.1016/j.cmet.2014.12.005

Pearce, E. L., & Pearce, E. J. (2013). Metabolic pathways in immune cell activation and quiescence. Immunity, 38(4), 633-643. doi:10.1016/j.immuni.2013.04.005

Pello, O. M., De Pizzol, M., Mirolo, M., Soucek, L., Zammataro, L., Amabile, A., . . . Locati, M. (2012). Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. Blood, 119(2), 411-421. doi:10.1182/blood-2011-02-339911

Phan, L. M., Yeung, S.-C. J., & Lee, M.-H. (2014). Cancer metabolic reprogramming: importance, main features, and potentials for precise targeted anti-cancer therapies. Cancer biology & medicine, 11(1), 1-19. doi:10.7497/j.issn.2095-3941.2014.01.001

Poh, A. R., & Ernst, M. (2018). Targeting Macrophages in Cancer: From Bench to Bedside. Frontiers in Oncology, 8(49). doi:10.3389/fonc.2018.00049

Prakasam, G., Iqbal, M. A., Bamezai, R. N. K., & Mazurek, S. (2018). Posttranslational Modifications of Pyruvate Kinase M2: Tweaks that Benefit Cancer. Frontiers in Oncology, 8, 22-22. doi:10.3389/fonc.2018.00022

Rajkumar, S. V., Gertz, M. A., Kyle, R. A., & Greipp, P. R. (2002). Current Therapy for Multiple Myeloma. Mayo Clinic Proceedings, 77(8), 813-822. doi:https://doi.org/10.4065/77.8.813

Rastrelli, M., Tropea, S., Rossi, C. R., & Alaibac, M. (2014). Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. In Vivo, 28(6), 1005-1011.

Reed, N. I., Jo, H., Chen, C., Tsujino, K., Arnold, T. D., DeGrado, W. F., & Sheppard, D. (2015). The alphavbeta1 integrin plays a critical in vivo role in tissue fibrosis. Sci Transl Med, 7(288), 288ra279. doi:10.1126/scitranslmed.aaa5094

Richter, J. D., & Sonenberg, N. (2005). Regulation of cap-dependent translation by eIF4E inhibitory proteins. Nature, 433(7025), 477-480.

Rőszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators of Inflammation, 2015, 816460. doi:10.1155/2015/816460

Ruggero, D. (2013). Translational control in cancer etiology. Cold Spring Harbor perspectives in biology, 5(2), a012336. doi:10.1101/cshperspect.a012336

Sato, T., Terai, M., Tamura, Y., Alexeev, V., Mastrangelo, M. J., & Selvan, S. R. (2011). Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. Immunologic Research, 51(2), 170-182. doi:10.1007/s12026-011-8262-6

Sawyers, C. (2004). Targeted cancer therapy. Nature, 432(7015), 294-297. doi:10.1038/nature03095

Scott, A. M., Allison, J. P., & Wolchok, J. D. (2012). Monoclonal antibodies in cancer therapy. Cancer Immunity Archive, 12(1), 14. Retrieved from http://cancerimmunolres.aacrjournals.org/content/12/1/14.abstract

Souba, W. W. (1993). Glutamine and cancer. Annals of surgery, 218(6), 715-728. doi:10.1097/00000658-199312000-00004

Staib, P., Hoffmann, M., & Schinkothe, T. (2006). Plasma levels of tumor M2-pyruvate kinase should not be used as a tumor marker for hematological malignancies and solid tumors. Clin Chem Lab Med, 44(1), 28-31. doi:10.1515/CCLM.2006.006

Svitkin, Y. V., Ovchinnikov, L., Dreyfuss, G., & Sonenberg, N. (1996). General RNA binding proteins render translation cap dependent. The EMBO journal, 15(24), 7147-7155.

Vaklavas, C., Blume, S. W., & Grizzle, W. E. (2017). Translational Dysregulation in Cancer: Molecular Insights and Potential Clinical Applications in Biomarker Development. Frontiers in Oncology, 7(158). doi:10.3389/fonc.2017.00158

Vaklavas, C., Meng, Z., Choi, H., Grizzle, W. E., Zinn, K. R., & Blume, S. W. (2015). Small molecule inhibitors of IRES-mediated translation. Cancer Biol Ther, 16(10), 1471-1485. doi:10.1080/15384047.2015.1071729

Vaupel, P., Schmidberger, H., & Mayer, A. (2019). The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. Int J Radiat Biol, 95(7), 912-919. doi:10.1080/09553002.2019.1589653

Vikas, P., Borcherding, N., & Zhang, W. (2018). The clinical promise of immunotherapy in triple-negative breast cancer. Cancer Manag Res, 10, 6823-6833. doi:10.2147/CMAR.S185176

Wahl, D. R., Byersdorfer, C. A., Ferrara, J. L. M., Opipari, A. W., Jr., & Glick, G. D. (2012). Distinct metabolic programs in activated T cells: opportunities for selective immunomodulation. Immunological Reviews, 249(1), 104-115. doi:10.1111/j.1600-065X.2012.01148.x

Walters, B., & Thompson, S. R. (2016). Cap-Independent Translational Control of Carcinogenesis. Frontiers in Oncology, 6, 128-128. doi:10.3389/fonc.2016.00128

Wan, L., Xia, T., Du, Y., Liu, J., Xie, Y., Zhang, Y., . . . Shi, C. (2019). Exosomes from activated hepatic stellate cells contain GLUT1 and PKM2: a role for exosomes in metabolic switch of liver nonparenchymal cells. The FASEB Journal, 33(7), 8530-8542.

Wang, J., Li, D., Cang, H., & Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. Cancer medicine, 8(10), 4709-4721. doi:10.1002/cam4.2327

Wang, P., Sun, C., Zhu, T., & Xu, Y. (2015). Structural insight into mechanisms for dynamic regulation of PKM2. Protein & cell, 6(4), 275-287.

Warburg, O. (1924). Über den Stoffwechsel der Carcinomzelle. Naturwissenschaften, 12(50), 1131-1137. doi:10.1007/BF01504608

Wegener, K. L., & Campbell, I. D. (2008). Transmembrane and cytoplasmic domains in integrin activation and protein-protein interactions (review). Mol Membr Biol, 25(5), 376-387. doi:10.1080/09687680802269886

Wong, N., De Melo, J., & Tang, D. (2013). PKM2, a Central Point of Regulation in Cancer Metabolism. International Journal of Cell Biology, 2013, 242513. doi:10.1155/2013/242513

Wong, N., Ojo, D., Yan, J., & Tang, D. (2015). PKM2 contributes to cancer metabolism. Cancer letters, 356(2), 184-191.

Wu, S., Kuang, H., Ke, J., Pi, M., & Yang, D.-H. (2021). Metabolic Reprogramming Induces Immune Cell Dysfunction in the Tumor Microenvironment of Multiple Myeloma. Frontiers in Oncology, 10(3061). doi:10.3389/fonc.2020.591342

Wu, X., Cai, J., Zuo, Z., & Li, J. (2019). Collagen facilitates the colorectal cancer stemness and metastasis through an integrin/PI3K/AKT/Snail signaling pathway. Biomedicine & Pharmacotherapy, 114, 108708. doi:https://doi.org/10.1016/j.biopha.2019.108708

Wybran, J., & Fudenberg, H. H. (1973). Thymus-Derived Rosette-Forming Cells in Various Human Disease States: Cancer, Lymphoma, Bacterial and Viral Infections, and Other Diseases. The Journal of Clinical Investigation, 52(5), 1026-1032. doi:10.1172/JCI107267

Xi, S., Zhao, M., Wang, S., Ma, L., Wang, S., Cong, X., . . . Huang, Y. (2017). IRES-Mediated Protein Translation Overcomes Suppression by the p14ARF Tumor Suppressor Protein. Journal of Cancer, 8(6), 1082-1088. doi:10.7150/jca.17457

Yang, W., Xia, Y., Hawke, D., Li, X., Liang, J., Xing, D., . . . Lu, Z. (2012). PKM2 Phosphorylates Histone H3 and Promotes Gene Transcription and Tumorigenesis. Cell, 150(4), 685-696. doi:10.1016/j.cell.2012.07.018

Yang, W., Xia, Y., Hawke, D., Li, X., Liang, J., Xing, D., . . . Lu, Z. (2012). PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell, 150(4), 685-696. doi:10.1016/j.cell.2012.07.018

Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W., . . . Lu, Z. (2011). Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature, 480(7375), 118-122. doi:nature10598 [pii]10.1038/nature10598

Yang, Y., & Wang, Z. (2019). IRES-mediated cap-independent translation, a path leading to hidden proteome. Journal of Molecular Cell Biology, 11(10), 911-919. doi:10.1093/jmcb/mjz091

Zahra, K., Dey, T., Ashish, Mishra, S. P., & Pandey, U. (2020a). Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. Frontiers in Oncology, 10, 159. Retrieved from https://www.frontiersin.org/article/10.3389/fonc.2020.00159

Zahra, K., Dey, T., Ashish, Mishra, S. P., & Pandey, U. (2020b). Pyruvate Kinase M2 and Cancer: The Role of PKM2 in Promoting Tumorigenesis. Frontiers in Oncology, 10, 159-159. doi:10.3389/fonc.2020.00159

Zhang, J., Pavlova, N. N., & Thompson, C. B. (2017). Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. The EMBO journal, 36(10), 1302-1315.

Zhang, X., Zink, F., Hezel, F., Vogt, J., Wachter, U., Wepler, M., . . . Hartmann, C. (2020). Metabolic substrate utilization in stress-induced immune cells. Intensive Care Medicine Experimental, 8(1), 28. doi:10.1186/s40635-020-00316-0

Zhang, Y., Li, L., Liu, Y., & Liu, Z. R. (2016). PKM2 released by neutrophils at wound site facilitates early wound healing by promoting angiogenesis. Wound Repair Regen, 24(2), 328-336. doi:10.1111/wrr.12411

Zheng, B., & Clemmons, D. R. (1998). Blocking ligand occupancy of the αVβ3 integrin inhibits insulin-like growth factor I signaling in vascular smooth muscle cells. Proceedings of the National Academy of Sciences, 95(19), 11217. doi:10.1073/pnas.95.19.11217