The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Beyond energy storage: roles of glycogen metabolism in health and disease

Huafeng Zhang[1], Jingwei Ma[2], Ke Tang[3] and Bo Huang[3,4,5]

1 Department of Pathology, School of Basic Medicine, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

2 Department of Immunology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

3 Department of Biochemistry and Molecular Biology, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

4 Department of Immunology and National Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College, Beijing, China

5 Clinical Immunology Center, CAMS, Beijing, China

Keywords
G6P; gluconeogenesis; glycogen metabolism; PPP; UDPG

Correspondence
B. Huang, Department of Immunology and National Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College, Beijing 100005, China
Tel: +86 10 69156447
E-mail: tjhuangbo@hotmail.com

(Received 8 October 2020, revised 19 November 2020, accepted 25 November 2020)

doi:10.1111/febs.15648

Beyond storing and supplying energy in the liver and muscles, glycogen also plays critical roles in cell differentiation, signaling, redox regulation, and stemness under various physiological and pathophysiological conditions. Such versatile functions have been revealed by various forms of glycogen storage diseases. Here, we outline the source of carbon flux in glycogen metabolism and discuss how glycogen metabolism guides $CD8^+$ T-cell memory formation and maintenance. Likewise, we review how this affects macrophage polarization and inflammatory responses. Furthermore, we dissect how glycogen metabolism supports tumor development by promoting tumor-repopulating cell growth in hypoxic tumor microenvironments. This review highlights the essential role of the gluconeogenesis-glycogenesis-glycogenolysis-PPP metabolic chain in redox homeostasis, thus providing insights into potential therapeutic strategies against major chronic diseases including cancer.

Introduction

What is life like without sugar? For most healthy individuals, blood glucose levels are in the range from 72 to 99 $mg{\cdot}dL^{-1}$, which may increase to 140 $mg{\cdot}dL^{-1}$ 2 h after eating. The total amount of blood glucose is around 4 g for a healthy adult, which maintains the energy metabolism of tissues in the body [1]. The

Abbreviations
6PGD, 6-phosphogluconate dehydrogenase; 6PGDL, 6-phosphogluconolactone; AcAc, acetoacetate; AGL, amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase; BHB, β-hydroxybutyrate; ETC, electron transport chain; F6P, fructose 6-phosphate; FAO, fatty acid oxidation; Fbp1, fructose-bisphosphatase 1; G1P, glucose-1-phosphate; G3P, glyceraldehyde 3-phosphate; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; G6PD, G6P dehydrogenase; Glut1, glucose transporter 1; GSF, succinated glutathione; GSH, reduced glutathione; GSSG, oxidized glutathione; Gys1, glycogen synthase 1; NO, nitric oxide; OAA, oxaloacetate; Pck1, phosphoenolpyruvate carboxykinase 1; Pck2, mitochondrial phosphoenolpyruvate carboxykinase; PEP, phosphoenolpyruvate; Pgm1, phosphoglucomutase 1; PPP, pentose phosphate pathway; PYG, glycogen phosphorylase; R5P, ribose-5-P; RBCs, red blood cells; ROS, reactive oxygen species; S7P, sedoheptulose 7-phosphate; TCA, tricarboxylic acid; Teff, effector T; Tm, memory T; TRCs, tumor-repopulating cells; UDPG, UDP-glucose; UDP-GlcA, UDP-glucuronate; Ugp2, UDP-glucose pyrophosphorylase 2; α-KG, α-ketoglutaric acid.

majority of glucose (60%) in the human body is consumed by the brain daily via aerobic pathways, and the remainder is mainly utilized by erythrocytes, skeletal muscle, and the heart muscle [2,3]. After a meal, the liver removes excessive glucose from the blood and stores the sugar in the form of glycogen [4]. The liver has the highest glycogen content (~ 100 g) in the body with up to 10% of its weight serving as the body's glucose reservoir [5,6]. Glycogen stored in the liver is broken down to maintain a stable glucose concentration in the blood, thus insuring an adequate glucose supply for the brain and other tissues [7]. Unlike the liver, muscle has a much lower glycogen concentration (1–2% of the muscle mass), which is used for self-supply; however, the total amount of muscle glycogen in a 70 kg person can reach to 400 g, much higher than 100 g of the total quantity of liver glycogen [1,8]. This is attributed to the total mass of muscle, which is widely distributed in the body and is therefore much greater than that of the liver. The physiological function of muscle glycogen is to support the energy requirements for the muscle contraction. In line with this, glycogen content in muscle does not show significant decrease during fasting [9]. Throughout strenuous exercise or fasting, muscle or liver glycogen is broken down into glucose-1-phosphate (G1P), which is converted into glucose-6-phosphate (G6P) by phosphoglucomutase, followed by the flux of G6P into the glycolytic pathway for ATP generation [10]. Besides skeletal muscle and the liver, other tissues like the brain, kidneys, heart, and adipose tissue are also capable of storing glycogen [11]. The liver is the only tissue that can convert the stored glycogen into glucose and release the glucose into the extracellular space to maintain the homeostasis of glucose in the blood [12]. In addition, although the kidney can make glucose, it is a minor source compared with the liver. It is believed that most nonhepatic tissues, including the muscle and brain, release glucose from glycogen for their direct cellular energy needs rather than for the use by other tissues based on the lack of glucose-6-phosphatase (G6Pase) [13]. Collectively, glycogen is mostly found in skeletal muscle and the liver where energy is stored as a high-density branched polymer form of glucose. In this review, we will skip the conventional understanding of glycogen as a form of energy storage, which is an extensive subject itself, but turn attention to its emerging role beyond storing and supplying energy.

Does the source of G6P determine its role as a glycogen building block?

As the first step of glycogen synthesis, G6P is converted to G1P by phosphoglucomutase, a phosphotransferase that catalyzes the reversible transfer of phosphate between the 1 and 6 positions of α-D-glucose. G1P reacts with UTP under the catalyzation by UDP-glucose (UDPG) pyrophosphorylase, leading to the formation of UDPG, the direct glucose donor for the elongation of glycogen chains. UDPG is added to the nonreducing end of the glycogen by glycogen synthase [11], where G6P can function as an allosteric activator of this synthase [14,15]. Considering the pivotal role of G6P in glycogen synthesis, it is essential to clarify the source and flux of G6P during glycogen metabolism.

G6P, the central molecule in glycogen metabolism, can be supplied by three ways: glycolysis, gluconeogenesis, and glycogenolysis [16,17]. Cells use glycolysis to generate G6P by phosphorylation of glucose on the sixth carbon catalyzed by hexokinase or glucokinase (only in hepatocytes and pancreatic β-cells) [18]. After a meal, hepatocytes take up excessive glucose from the blood and convert the glucose immediately to G6P, which is subsequently stored as glycogen in the cells. During fasting, hepatocytes degrade glycogen and generate G6P in the cytosol, where G6P is translocated into and catalyzed to glucose by G6Pase in the endoplasmic reticulum (ER), thus releasing the glucose to the blood and maintaining the homeostasis. However, long-term fasting triggers the gluconeogenic pathway to generate G6P in hepatocytes. Glucogenic precursors such as glycerol, glycogenic amino acids (e.g., alanine), and lactate are first transformed to oxaloacetate (OAA) and then channeled to the gluconeogenic pathway to produce G6P [19,20]. Such fasting-derived hepatocytic G6P via gluconeogenesis aims to supply glucose for other organs rather than to become glycogen building block. Similar to fasting, exercise can also induce hepatocytic gluconeogenesis. In this case, skeletal muscle cells degrade glycogen into G6P, which fluxes into glycolysis causing an abundant lactate release. The released lactate in the blood is transported to the liver and the kidney where it can be converted to glucose via gluconeogenesis, which is then released into the blood, and accordingly utilized in the skeletal muscle as part of an internal cycle called the Cori cycle [21,22]. Thus, during fasting or exercise, gluconeogenesis may be activated in hepatocytes and results in the formation of G6P, which is then converted to glucose but not fluxed to glycogen. In contrast to this, G6P by gluconeogenesis in the fetal liver is fluxed to glycogen. The fetal liver contains each enzyme for glycogen synthesis and exhibits 24.6 $mg \cdot g^{-1}$ glycogen content between 121 and 130 days of gestation, and this glycogen content may even reach to 50 $mg \cdot g^{-1}$ at 40 weeks of gestation [23,24]. It is known that fetal hepatocytes

use lactate, glycerol, and alanine to synthesize G6P via the gluconeogenic pathway and then use G6P as a substrate to synthesize glycogen [25,26]. In addition, after an intense exercise, lactate can also act as a substrate to resynthesize glycogen in skeletal muscle cells [10,27]. Previously, we demonstrated that $CD8^+$ memory T (Tm) cells have a considerable amount of glycogen storage and the gluconeogenesis-derived G6P is used to synthesize glycogen [28]. Collectively, although glycolysis-derived G6P is conventionally used as glycogen building block, gluconeogenesis-derived G6P can also act as the building block for glycogenesis in specific cell types under certain conditions.

UDPG can be a signal molecule beyond transferring glucose to glycogen

Glycogenesis is simply considered to be a metabolic event that stores energy, but it may not be simple when considering its crosstalk in cellular signaling. As aforementioned, UDPG is an intermediate metabolite of glycogenesis. Being a glycosyl donor, UDPG is catalyzed by glycogen synthase (UDPG-glycogen glucosyltransferase), thus transferring the glucose unit to glycogen. However, the UDPG can be shunted to other chemical reactions to generate other forms of nucleotide sugars such as UDP-glucuronate (UDP-GlcA), a central molecule in the regulation of proteoglycan and glycosaminoglycan [29,30]. UDP-GlcA can be converted to UDP-xylose in the ER and Golgi by UDP-xylose synthase, be used to synthesize hyaluronan at the plasma membrane, or result in hormone (e.g., dihydrotestosterone) glucuronidation in the ER [31].

In addition to converting to other nucleotide sugars, UDPG is an important signaling molecule. P2Y (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14) purinergic receptors, which belong to the superfamily of G protein-coupled receptors, are expressed in a wide variety of tissues [32]. Among them, P2Y14 is known to be recognized and activated by UDPG. Released UDPG can bind to the P2Y14 receptor in an autocrine or paracrine configuration, and the subsequent signals play important roles in a variety of cellular processes [33]. Extracellular UDPG has been identified and quantified with nanomolar sensitivity in various cell culture media and tissues [34], since UDPG in the ER/Golgi lumen can be released as cargo to the extracellular space via the constitutive secretory pathway [35,36]. Several studies have reported that an increased release of UDPG induces a pro-inflammatory response in immune cells with highly expressed P2Y14 receptor. The binding of UDPG to P2Y14 receptors results in RhoA activation, cytoskeleton rearrangements, and subsequent chemotaxis of neutrophils [37,38]. This chemoattraction of neutrophils by UDPG was recently demonstrated in cancer patients treated with drug-packaging microparticles [39]. Immature dendritic cells highly express P2Y14 and an increased release of UDPG may induce DC maturation through P2Y14-mediated calcium signaling in concert with other signals [40]. Also, mast cells express the P2Y14 receptor, which mediates UDPG-dependent degranulation and is therefore a potential novel therapeutic target for allergic conditions [41]. In addition to immune cells, the UDPG-P2Y14 signaling pathway can induce the secretion of IL-8 in airway epithelial cells [42]. Jokela *et al.* [43] found that UDPG released by keratinocytes stimulates epidermal inflammation and enhances hyaluronan synthesis by the phosphorylation of JAK and STAT3. In addition, extracellular UDPG and P2Y14 receptor have been associated with the commitment of mesenchymal stem cells to adipogenic and osteogenic differentiation [44] and to the chemotaxis of hematopoietic stem cells [45]. Collectively, UDPG can function as a very important signal molecule that regulates immunity, inflammation, and stem cell biology. Considering these functions, it is worthwhile to clarify whether there are certain cell types which do not synthesize glycogen but only use G6P to produce UDPG for signaling purposes.

Glycogenolysis-guided PPP regulates redox

The glycogenesis shunts G6P to glycogen for energy storage. The opposite reaction is the glycogenolysis, which breaks down glycogen back to G6P via two pathways. Cytosolic degradation of glycogen uses glycogen phosphorylase and the glycogen debranching enzyme. Lysosomal degradation relies on acid α-glucosidase to break down 5% of total muscle glycogen and 10% of total liver glycogen in lysosomes [11,13]. Glycogen phosphorylase is the rate-limiting enzyme for glycogenolysis, which catalyzes the release of G1P from the terminal α-1,4-glycosidic bond. Three tissue-specific isozymes are expressed in mice and humans, including a liver form (encoded by the *PYGL* gene on chromosome 14q22.1), muscle form (encoded by the *PYGM* gene on chromosome 11q13.1), and brain form (encoded by the *PYGB* gene on chromosome 20p11.21) [46,47]. The formed G1P is subsequently converted to G6P by phosphoglucomutase.

In the liver, glycogenolysis-derived G6P is hydrolyzed to glucose by G6Pase and the glucose is then

exported into bloodstream via the glucose transporter in order to maintain the homeostasis of blood glucose levels [7,48]. In nonhepatic tissues, including muscle and brain, G6P from glycogen mainly fluxes into glycolysis in order to satisfy the direct energy needs of the cells [8,49]. Despite the conventional notion that glycogenolysis-derived G6P fluxes to glycolysis for ATP production, an overlooked notion is that glycogenolysis-derived G6P may be directed to initiate the pentose phosphate pathway (PPP). In this metabolic process, the G6P is catalyzed to 6-phosphogluconolactone by G6P dehydrogenase (G6PD), concomitant with NADPH generation. Then, 6-phosphogluconolactone is converted to 6-phosphogluconate and the latter is used to produce ribulose-5-P, NADPH, and CO_2 by 6-phosphogluconate dehydrogenase (6PGD). Ribulose-5-P then initiates the nonoxidative phase of PPP by the direct conversion to ribose-5-P, which can be shunted toward nucleotide synthesis, or to the production of glyceraldehyde-3-P and fructose-6-P via a series of reactions, thus entering glycolysis. Although PPP-derived ribose-5-P is important for nucleotide metabolism, the oxidative phase-generated NADPH may be of paramount importance in nondividing cells. NADPH is a reducing agent required for the reduction of oxidized glutathione (GSSG) by supplying hydrogen to GSSG, thus insuring high levels of reduced glutathione (GSH) which protects cells from the toxicity of reactive oxygen species (ROS) [50,51]. Therefore, the PPP is extremely important for red blood cells (RBCs) [52]. RBCs are rich in heme iron and oxygen making these cells susceptible to oxidative damage; however, this can be neutralized by the highly efficient GSH antioxidative system [53,54]. RBCs are known to contain abundant glycogen [55], suggesting that glycogenolysis may be an active cellular process in RBCs. However, it remains to be addressed whether the resultant G6P is shunted to the PPP. Using the ^{13}C tracing method, we previously demonstrated that glycogenolysis-derived G6P is shunted to the PPP in both $CD8^+$ Tm cells and LPS-stimulated pro-inflammatory macrophages. Intriguingly, using the same ^{13}C tracing [28], Thwe *et al.* [56] found that activated dendritic cells shunted glycogenolysis-derived G6P to glycolysis. Thus, glycogenolysis-derived G6P faces a fluxing choice between glycolysis and PPP. Further investigations may determine what kind of situation or signal(s) persuades the cells to allow the shunt of glycogenolysis-derived G6P to PPP. In addition, given that the phosphorylation of glucose is the simplest way to generate G6P, why the cells use a more ineffective cycle of glycogenesis-glycogenolysis to produce G6P for PPP remains unexplained. Addressing these fundamental questions will undoubtedly provide deep insight into the regulation of redox by glycogen metabolism.

Glycogen metabolism regulates T-cell memory

T-cell immune response is of paramount importance in controlling viral infection and tumorigenesis [57–59]. T cells use their surface receptor TCR to interact with MHC class I-antigen peptide complex on the target cell surface, generating the signal 1 during T-cell activation [60]. Meanwhile, T cells use their costimulatory molecule CD28 to recognize the CD80/CD86 of target cells to form the signal 2 of T-cell activation. Activated T cells proliferate and differentiate into effector T (Teff) cells [60]. Following antigen clearance, more than 95% of the Teff cells enter apoptosis and the remaining Teff cells become memory T cells, which can survive months to years and launch a rapid and robust recall response upon the cognate antigen restimulation [61,62]. Despite numerous efforts to clarify T-cell memory formation and maintenance, the underlying molecular basis remains elusive. Metabolism is considered to play a key role in regulating T-cell memory. Studies have reported that mTOR, a metabolism-related signaling pathway, negatively regulates $CD8^+$ Tm cell differentiation [63]. Energy-regulating molecule AMPK and metformin, a drug which activates AMPK, have also been reported to regulate $CD8^+$ Tm cells [64–66]. Moreover, recent reports highlighted a critical role of fatty acid oxidation (FAO) in $CD8^+$ T-cell memory development [67,68]. $CD8^+$ Tm cells use cell-intrinsic lipolysis to generate free fatty acid and highly express carnitine palmitoyltransferase 1A, the FAO rate-limiting enzyme that mediates fatty acid translocation into mitochondria, thus supporting the metabolic programming necessary for $CD8^+$ T cell memory development [68]. Nevertheless, the mechanistic insight into how FAO is linked to T-cell memory development is still unexplained. Also, how mTOR inhibition and FAO synergistically regulate $CD8^+$ T-cell memory remains unclear. Our own studies may help explain the mechanism behind glycogen metabolism regulating T-cell memory.

It is a surprising observation that $CD8^+$ Tm cells store a large amount of glycogen [28]. The G6P molecule initiates the process of glycogen synthesis, which can be derived from glycolysis or gluconeogenesis. Despite the absence of G6Pase, phosphoenolpyruvate carboxykinase 1 (Pck1), and fructose-bisphosphatase 1 (Fbp1), the first two rate-limiting enzymes of gluconeogenesis, are upregulated in $CD8^+$ Tm cells, suggesting

that G6P might be derived from the gluconeogenic pathway. Tracing with ^{13}C carbon verified that $CD8^+$ Tm cells use gluconeogenesis-derived G6P to synthesize glycogen. Following glycogen synthesis, $CD8^+$ Tm cells further break down glycogen back into G6P, which may flux into glycolysis or PPP. The ^{13}C tracing experiments clearly demonstrate that $CD8^+$ Tm cells use glycogenolysis-derived G6P to trigger PPP, thus providing NADPH to reduce GSSG to GSH; and the increased GSH lowers ROS levels, which enhances the long-term survival of $CD8^+$ Tm cells [28]. Despite understanding the formation and maintenance of $CD8^+$ Tm cells, questions remain as to why and how $CD8^+$ Tm cells initiate glycogen metabolism.

Glycolysis and gluconeogenesis are two mechanisms which can provide G6P, the first substrate for the glycogenesis. Glucose-6-phosphatase is absent in $CD8^+$ Tm cells; however, Pck1 and Fbp1, the first two rate-limiting enzymes for gluconeogenesis, are upregulated. This implies that G6P might be provided via gluconeogenesis for glycogenesis, which is further verified by the ^{13}C tracing assay. The next key point is how $CD8^+$ Tm cells develop the gluconeogenic metabolic pathway. It is no doubt that abundant oxaloacetate fluxing to gluconeogenesis may result in the accumulation of acetyl-CoA in tricarboxylic acid cycle, leading to shunting acetyl-CoA toward ketogenesis. In fact, the formation of acetoacetate and β-hydroxybutyrate (BHB) is greatly increased in $CD8^+$ Tm cells [69]. In line with this, ketogenesis-related enzymes are upregulated in $CD8^+$ Tm cells. Although the ketogenesis-derived BHB can be used as an energy source for the tricarboxylic acid (TCA) cycle, the major role of BHB in $CD8^+$ Tm cells seems to exclude its function as an energy molecule. In addition to energy supply, BHB can also act as an epigenetic modifier by targeting histone β-hydroxybutyrylation. In $CD8^+$ Tm cells, BHB was found to modify H3K9 of *FoxO1* and *PGC-1α*, thus facilitating the expression of these two genes. Subsequently, FoxO1 and PGC-1α synergistically upregulate Pck1 expression, thus guiding the carbon flow along the gluconeogenic pathway toward glycogen [69]. These findings may explain why rapamycin or metformin favors $CD8^+$ T-cell memory formation. Rapamycin may inhibit AKT activity through its influence on mTORC2 assembly [70]. Metformin may also inhibit AKT activity by targeting insulin-like growth factor signaling. The inhibition of AKT relieves the subsequent phosphorylation of FoxO1, therefore allowing the translocation of FoxO1 into the nucleus to regulate Pck1 expression. Collectively, based on our findings and that of others, we propose an integrated metabolic chain in $CD8^+$ Tm cells as described in Fig. 1.

Glycogen metabolism is a key event for M1 macrophage polarization

Macrophages are fundamental innate immune cells, which can be polarized to a M1 phenotype against bacterial infections or to a M2 phenotype for tissue repair and wound healing [71,72]. It is known that classically activated M1 macrophages use glycolysis for energy supplement and alternatively activated M2 macrophages use oxidative metabolism to fuel their functions [73,74]. For M1 macrophages, in addition to glycolysis, recent studies also indicated the involvement of the TCA cycle in M1 polarization [75]. This is because the intermediate succinate can slow prolyl hydroxylase activity, thus allowing HIF1α to more efficiently exert its functions, including driving the macrophage M1 phenotype. Furthermore, during M1 polarization, carbon is also fluxed toward the PPP in addition to glycolysis. The PPP is important for the redox balance of M1 macrophages due to the NADPH generated during the oxidative phase [76]. However, the G6P source for the PPP in M1 macrophages is not clear. Our recent studies demonstrated that the G6P, which fluxes to PPP, probably is not directly derived from glycolysis but from glycogenolysis instead [77]. In LPS/IFN-γ-induced M1 macrophages, ^{13}C tracing showed glycogenolysis-derived G6P being channeled to the PPP. Abrogation of glycogenolysis by the inhibitor PZ0189 interferes with the PPP, ultimately leading to increased ROS levels and macrophage death. Meanwhile, the disruption of glycogenolysis or PPP can inhibit the M1 phenotype and reduce macrophage ability to clear bacteria, suggesting that the glycogenolysis-directed PPP plays a critical role in regulating the phenotype, function, and survival of M1 macrophages [77].

The breakdown of glycogen implicates the existence of glycogen synthesis in M1 macrophages. Several lines of evidence demonstrate that a highly efficient glycogenesis exists in M1 macrophages: (a) PAS staining displays red color; (b) abundant glycogen is detected by the colorimetric assay in M1 macrophages; (c) glycogen particles are observed under a transmission electron microscope; and (d) ^{13}C tracing shows G6P flux to glycogen. Identification of the glycogenesis-glycogenolysis-PPP metabolic chain provides deep insights into how glucose metabolism is involved in macrophage polarization toward the M1 phenotype. This, however, raises a question about the futile use of G6P. The distinctive compartmentalization of the involved enzymes may provide a rational explanation,

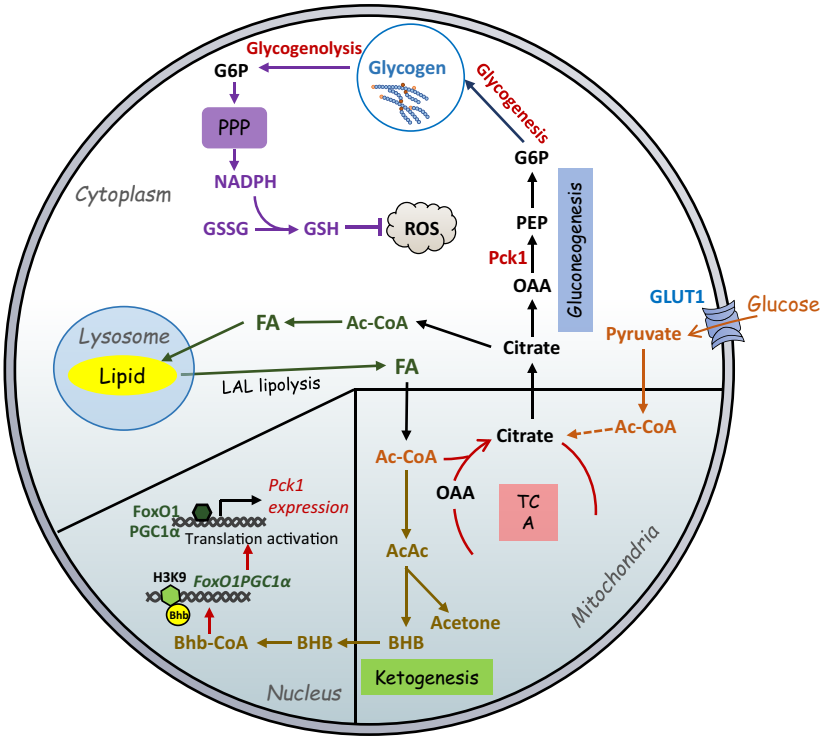

Fig. 1. Glycogen metabolic chain in CD8$^+$ Tm cells. CD8$^+$ Tm cells use extracellular glucose for their *de novo* fatty acid synthesis and then use cell-intrinsic lysosomal acid lipase (LAL)-mediated lipolysis of lipid to fuel fatty acid oxidation (FAO). Mitochondrial citrate enters the cytoplasm where it decomposes into acetyl-CoA and oxaloacetate. Acetyl-CoA is used for fatty acid biosynthesis. CD8$^+$ Tm cells markedly upregulate Pck1 to drive gluconeogenesis and allow G6P to flux into glycogen synthesis. G6P from glycogenolysis is channeled to PPP that generates abundant NADPH, insuring high levels of GSH in CD8$^+$ Tm cells. On the other hand, insufficient OAA in mitochondria results in the diversion of acetyl-CoA to the ketogenic pathway. Ketogenesis-generated BHB epigenetically modifies FoxO1 and PGC-1α, leading to FoxO1 and PGC-1α-dependent Pck1 upregulation.

but a more reasonable answer is that the glycogen metabolic intermediate, UDPG/P2Y14, can activate an important signaling pathway, as previously mentioned. In this case, the UDPG-P2Y14 signaling regulates the inflammatory response of M1 macrophages by regulating the expression and activity of STAT1. STAT1 is a key transcription factor that regulates a panel of pro-inflammatory factors such as TNF-α, IL-12, and nitric oxide (NO) [77]. Transcription factors such as retinoic acid receptor RARβ, ZNF-148, and IRF-1 are known to regulate the expression of STAT1 [78–81]. Notably, the UDPG-P2Y14 signaling results in the upregulation of RARβ, which directly binds to the *STAT1* promoter, thus promoting STAT1 expression in M1 macrophages. Besides upregulating STAT1 expression, this UDPG-P2Y14 signaling also induces STAT1 phosphorylation by downregulating tyrosine phosphatase TC45, thus activating STAT1 and subsequent driving macrophage M1 polarization [77]. The elucidation of this mechanism reveals glycogen metabolism as a prospective intervention target against inflammatory diseases.

Glycogen metabolism benefits hypoxic tumor-repopulating cells

Hypoxia is a physiological and physiopathological phenomenon, which commonly appears in malignant tissues due to the disorganized tumor vascular network, leading to intercapillary distance beyond normal oxygen diffusion [82]. Hypoxia causes a lack of O_2 as the electron recipient in the electron transport chain (ETC) and thus slows down the electron transfer along the ETC [83,84]. Despite obstructing energy generation, hypoxia is commonly associated with increased metastasis, a worse prognosis in cancer patients and is believed to be an important driving force for cancer progression [85,86]. The underlying mechanistic understanding involves in glucose metabolism. Slowing down the ETC due to hypoxia results in NADH and FADH2 accumulation, thus hindering the TCA cycle [87,88]. As a feedback regulation, glycolysis is known to be strengthened in hypoxia. One molecular basis lies in the upregulation of HIF-1α, which regulates various glycolysis enzymes. Notably, HIF-1α also regulates glycogenesis enzymes including glycogen synthase [89,90]. Indeed, the existence of glycogen in various hypoxic tumor cell lines has been reported [91,92]. Studies had demonstrated that blocking glycogen breakdown with a small molecular inhibitor can induce apoptosis in pancreatic tumor cells [93]. Also, blocking glycogen degradation can induce glioblastoma cell senescence via the decrease of PPP-derived NADPH [94]. Although it is clear that glycogen metabolism promotes hypoxic tumor growth [90,91], dissecting the

intrinsic mechanisms utilizing glycogen metabolism to reprogram hypoxic tumors is necessary.

Tumor-repopulating cells (TRCs) are a very small population of tumor cells with a characteristic of mechanical softness, which can self-renew and are highly tumorigenic [95–98]. Studies have reported that hypoxia can promote tumorigenic cell development and confer the cells a stem-like phenotype [99]. Notably, hypoxia can induce differentiated tumor cell growth retardation or even apoptosis but can also promote TRC growth. This alternate consequence is due to the extremely elevated ROS levels in differentiated tumor cells but a merely moderately high ROS level in TRCs under hypoxic conditions. Hypoxia-driven HIF-1α downregulates mitochondrial phosphoenolpyruvate carboxykinase (Pck2), resulting in the hindrance of fumarate carbon flow. On the other hand, hypoxia promotes TRCs to use glutamine as a supplemental carbon source. These two conditions lead to fumarate accumulation. Fumarate is an unsaturated electrophilic metabolite. Thus, the excessive fumarate can covalently modify cysteine residues of glutathione and generate succinated glutathione (GSF) [100]. GSF is then catalyzed by glutathione reductase in the consumption of NADPH. Such fumarate-GSF forms a futile cycle but impedes GSH from clearing free radicals, thus increasing ROS levels. However, the question remains as to why hypoxic TRCs only moderately increase ROS but hypoxic differentiated tumor cells strikingly increase it. In our ongoing studies, we found that TRCs upregulate gluconeogenesis rate-limiting enzymes Pck1 and Fbp1, which can drive the carbon flow from gluconeogenesis to glycogenesis. Following this, glycogenolysis-derived G6P further fluxes to the PPP and the generated NADPH can therefore lower ROS levels in hypoxic TRCs. However, the gluconeogenic activity is very low in hypoxic differentiated tumor cells, which cannot form the glycogen-PPP metabolic pathway. Thus, a gluconeogenesis-glycogenesis-glycogenolysis-PPP metabolic chain seems to be initiated by TRCs in response to hypoxia, leading to hypoxic TRC growth.

Open questions and concluding remarks

It has been more than 160 years since the discovery of liver glycogen by Claude Bernard [101]. Now, a basic understanding of glycogen storing and supplying energy has been established. However, despite numerous research achievements, there is still much to learn about glycogen and its metabolism. Oxidative phosphorylation is the root of energy generation. Electrons are transferred along the ETC and the released energy pumps proton from the matrix to the intermembrane space to form a proton gradient. The membrane-spanning enzyme ATP synthase catalyzes the addition of a phosphate to ADP by capturing energy released from protons across the inner membrane into the matrix. Such generated energy creates the molecule ATP, which has become the spring of cellular life. However, the imperfection lies in that electrons may leak from the ETC and react with molecular O_2 to generate a superoxide anion, the prototype of ROS. Thus, the cellular respiration by glycolysis, the TCA cycle, and oxidative phosphorylation generates energy to create a life but it also produces ROS which can ultimately destroy the life. In contrast, the carbon flow along the gluconeogenesis-glycogenesis-glycogenolysis-PPP and back to glycolysis not only generates ATP but also mobilizes the machinery to quench ROS. From a biochemical view, the glycogen-PPP-glycolysis metabolic mode has a greater advantage in maintaining cellular homeostasis and survival. In this sense, we propose that glycogen metabolism may play a critical role in maintaining a healthy state of cellular life rather than just in storing and supplying energy. We also propose that the whole metabolic chain of the gluconeogenesis-glycogenesis-glycogenolysis-PPP may provide useful targets for drug intervention against major chronic diseases (Fig. 2). Regardless, some fundamental questions must be addressed.

For instance, why do cells use gluconeogenesis-derived G6P rather than glycolysis-derived G6P to initiate glycogenesis? Similarly, why do cells use glycogenolysis-derived G6P rather than gluconeogenesis or glycolysis-derived G6P to initiate PPP? The spatial distribution of glycogen is highly heterogeneous in cells. The distinctive compartmentalization of glycogen and the involved enzymes might be a reason. The existence of carbohydrate metabolic compartments in the cytoplasm has been demonstrated before [102–104]. These compartments are considered the product of F-actin remodeling by forming spherical structures with a diameter 0.5–1 μm, which can contain glycogen metabolic enzymes GYS and PYG [105,106]. In addition, mechanobiology is necessary to address how glycogen is transported by cytoskeletal movement.

Another example is about the regulation of glycogenesis and glycogenolysis. Glycogen synthesis and degradation are two opposite processes. The active forms of the key glycogen metabolic enzymes glycogen synthase and glycogen phosphorylase are also opposite with dephosphorylation and phosphorylation, respectively. Signals such as insulin, glucagon, and epinephrine also have opposite roles in regulating glycogen

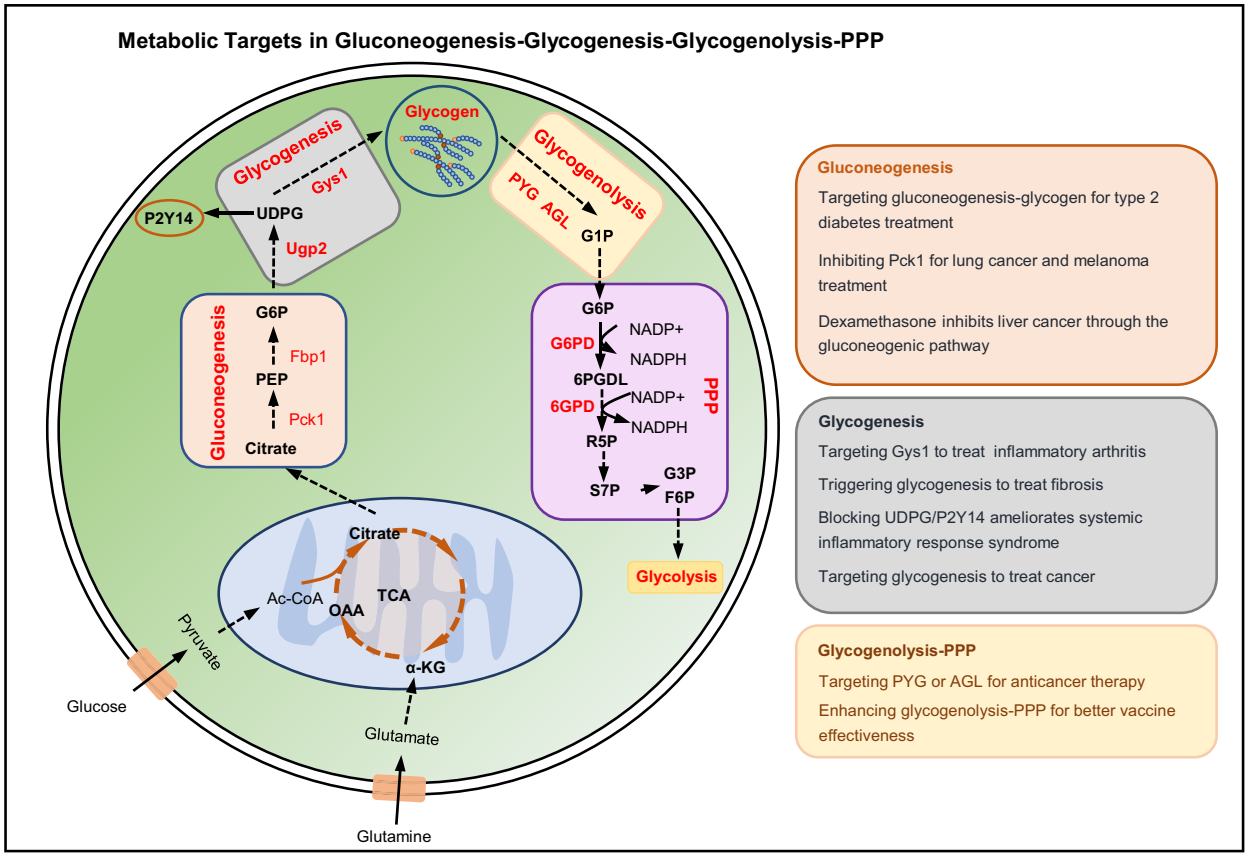

Fig. 2. Potential metabolic targets in the chain of gluconeogenesis-glycogenesis-glycogenolysis-PPP for drug intervention against major chronic diseases.

synthesis and degradation. How can tumor cells, macrophages, and T cells mobilize glycogen synthesis and degradation simultaneously?

All in all, over the last decade glycogen metabolism has become an avidly investigated topic in the metabolic field. From a conventional view, the function of glycogen seems clear with muscle glycogen generating ATP for energy demand and hepatic glycogen releasing glucose for other tissues. However, the significance of glycogen may be far beyond storing and supplying energy. The roles of glycogen in redox homeostasis, energy sensing, metabolic integration, cell differentiation, and inflammation regulation are far more unclear and worthy of investigation in the future.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant numbers 81788101, 81701544), CAMS Initiative for Innovative Medicine (2016-I2M-1-007), and Program for HUST Academic Frontier Youth Team (2018QYTD01).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

BH developed the concepts of reviewing this topic. HZ, JM, KT, and BH wrote the manuscript. BH provided critical review and supervised the project.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1111/febs.15648.
[Correction added on 04 February 2021, after first online publication: URL for peer review history has been corrected.]

References

1 Wasserman DH (2009) Four grams of glucose. *Am J Physiol Endocrinol Metab* **296**, E11–E21.

2 Caravas J & Wildman DE (2014) A genetic perspective on glucose consumption in the cerebral cortex during human development. *Diabetes Obes Metab* **16**, 21–25.
3 Berg JM, Tymoczko JL & Stryer L (2002) Section 30.2 Each Organ Has a Unique Metabolic Profile Biochemistry. 5th edn. W H Freeman, New York.
4 Hers HG (1976) The control of glycogen metabolism in the liver. *Annu Rev Biochem* **45**, 167–189.
5 den Otter W & van Boxtel AB (1971) Relation between the glycogen content of the liver and liver weight, and its meaning for enzymology. *Experientia* **27**, 1271–1272.
6 Bonjorn VM, Latour MG, Bélanger P & Lavoie J-M (2002) Influence of prior exercise and liver glycogen content on the sensitivity of the liver to glucagon. *J Appl Physiol (1985)* **92**, 188–194.
7 Gonzalez JT, Fuchs CJ, Betts JA & van Loon LJ (2016) Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am J Physiol Endocrinol Metab* **311**, E543–E553.
8 Knuiman P, Hopman MT & Mensink M (2015) Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. *Nutr Metab (Lond)* **12**, 59.
9 Vendelbo MH, Clasen BF, Treebak JT, Moller L, Krusenstjerna-Hafstrom T, Madsen M, Nielsen TS, Stodkilde-Jorgensen H, Pedersen SB, Jorgensen JO *et al.* (2012) Insulin resistance after a 72-h fast is associated with impaired AS160 phosphorylation and accumulation of lipid and glycogen in human skeletal muscle. *Am J Physiol Endocrinol Metab* **302**, E190–E200.
10 Hargreaves M & Spriet LL (2020) Skeletal muscle energy metabolism during exercise. *Nat Metab* **2**, 817–828.
11 Adeva-Andany MM, Gonzalez-Lucan M, Donapetry-Garcia C, Fernandez-Fernandez C & Ameneiros-Rodriguez E (2016) Glycogen metabolism in humans. *BBA Clin* **5**, 85–100.
12 McGarry JD, Kuwajima M, Newgard CB, Foster DW & Katz J (1987) From dietary glucose to liver glycogen: the full circle round. *Annu Rev Nutr* **7**, 51–73.
13 Prats C, Graham TE & Shearer J (2018) The dynamic life of the glycogen granule. *J Biol Chem* **293**, 7089–7098.
14 Bouskila M, Hunter RW, Ibrahim AF, Delattre L, Peggie M, van Diepen JA, Voshol PJ, Jensen J & Sakamoto K (2010) Allosteric regulation of glycogen synthase controls glycogen synthesis in muscle. *Cell Metab* **12**, 456–466.
15 Palm DC, Rohwer JM & Hofmeyr JH (2013) Regulation of glycogen synthase from mammalian skeletal muscle – a unifying view of allosteric and covalent regulation. *FEBS J* **280**, 2–27.
16 Agius L, Centelles J & Cascante M (2002) Multiple glucose 6-phosphate pools or channelling of flux in diverse pathways? *Biochem Soc Trans* **30**, 38–43.
17 Gomis RR, Favre C, Garcia-Rocha M, Fernandez-Novell JM, Ferrer JC & Guinovart JJ (2003) Glucose 6-phosphate produced by gluconeogenesis and by glucokinase is equally effective in activating hepatic glycogen synthase. *J Biol Chem* **278**, 9740–9746.
18 Matschinsky FM & Wilson DF (2019) The central role of glucokinase in glucose homeostasis: a perspective 50 years after demonstrating the presence of the enzyme in Islets of Langerhans. *Front Physiol* **10**, 148.
19 Sen S & Das C (2018) Managing the sugar factory: a new feather in the cap for nuclear factor Y. *J Biol Chem* **293**, 7905–7906.
20 Zhang X, Yang S, Chen J & Su Z (2019) Unraveling the regulation of hepatic gluconeogenesis. *Front Endocrinol* **9**, 802.
21 Cori CF & Cori GT (1946) Carbohydrate metabolism. *Annu Rev Biochem* **15**, 193–218.
22 Brooks GA (2018) The science and translation of lactate shuttle theory. *Cell Metab* **27**, 757–785.
23 Greengard O (1977) Enzymic differentiation of human liver: comparison with the rat model. *Pediatr Res* **11**, 669–676.
24 Capkova A & Jirasek JE (1968) Glycogen reserves in organs of human foetuses in the first half of pregnancy. *Biol Neonat* **13**, 129–142.
25 Levitsky LL, Paton JB & Fisher DE (1988) Precursors to glycogen in ovine fetuses. *Am J Physiol* **255**, E743–E747.
26 Prior RL (1980) Glucose and lactate metabolism in vivo in ovine fetus. *Am J Physiol* **239**, E208–E214.
27 Stevenson RW, Mitchell DR, Hendrick GK, Rainey R, Cherrington AD & Frizzell RT (1987) Lactate as substrate for glycogen resynthesis after exercise. *J Appl Physiol (1985)* **62**, 2237–2240.
28 Ma R, Ji T, Zhang H, Dong W, Chen X, Xu P, Chen D, Liang X, Yin X, Liu Y *et al.* (2018) A Pck1-directed glycogen metabolic program regulates formation and maintenance of memory CD8(+) T cells. *Nat Cell Biol* **20**, 21–27.
29 Bar-Peled M, Griffith CL & Doering TL (2001) Functional cloning and characterization of a UDP-glucuronic acid decarboxylase: the pathogenic fungus *Cryptococcus neoformans* elucidates UDP-xylose synthesis. *Proc Natl Acad Sci USA* **98**, 12003–12008.
30 Strominger JL (1964) Nucleotide intermediates in the biosynthesis of heteropolymeric polysaccharides. *Biophys J* **4**, L139–L153.
31 Zimmer BM, Barycki JJ & Simpson MA (2020) Integration of sugar metabolism and proteoglycan synthesis by UDP-glucose dehydrogenase. *J Histochem Cytochem*. https://doi.org/10.1369/0022155420947500

32 Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA *et al.* (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* **58**, 281–341.

33 Abbracchio MP, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Miras-Portugal MT, King BF, Gachet C, Jacobson KA, Weisman GA *et al.* (2003) Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. *Trends Pharmacol Sci* **24**, 52–55.

34 Lazarowski ER, Shea DA, Boucher RC & Harden TK (2003) Release of cellular UDP-glucose as a potential extracellular signaling molecule. *Mol Pharmacol* **63**, 1190–1197.

35 Lazarowski ER & Harden TK (2015) UDP-sugars as extracellular signaling molecules: cellular and physiologic consequences of P2Y14 receptor activation. *Mol Pharmacol* **88**, 151–160.

36 Sesma JI, Esther CJ, Kreda SM, Jones L, O'Neal W, Nishihara S, Nicholas RA & Lazarowski ER (2009) Endoplasmic reticulum/golgi nucleotide sugar transporters contribute to the cellular release of UDP-sugar signaling molecules. *J Biol Chem* **284**, 12572–12583.

37 Sesma JI, Kreda SM, Steinckwich-Besancon N, Dang H, Garcia-Mata R, Harden TK & Lazarowski ER (2012) The UDP-sugar-sensing P2Y(14) receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils. *Am J Physiol Cell Physiol* **303**, C490–C498.

38 Sesma JI, Weitzer CD, Livraghi-Butrico A, Dang H, Donaldson S, Alexis NE, Jacobson KA, Harden TK & Lazarowski ER (2016) UDP-glucose promotes neutrophil recruitment in the lung. *Purinergic Signal* **12**, 627–635.

39 Gao Y, Zhang H, Zhou N, Xu P, Wang J, Gao Y, Jin X, Liang X, Lv J, Zhang Y *et al.* (2020) Methotrexate-loaded tumour-cell-derived microvesicles can relieve biliary obstruction in patients with extrahepatic cholangiocarcinoma. *Nat Biomed Eng* **4**, 743–753.

40 Skelton L, Cooper M, Murphy M & Platt A (2003) Human immature monocyte-derived dendritic cells express the G protein-coupled receptor GPR105 (KIAA0001, P2Y14) and increase intracellular calcium in response to its agonist, uridine diphosphoglucose. *J Immunol* **171**, 1941–1949.

41 Gao ZG, Ding Y & Jacobson KA (2010) UDP-glucose acting at P2Y14 receptors is a mediator of mast cell degranulation. *Biochem Pharmacol* **79**, 873–879.

42 Muller T, Bayer H, Myrtek D, Ferrari D, Sorichter S, Ziegenhagen MW, Zissel G, Virchow JC, Luttmann W, Norgauer J *et al.* (2005) The P2Y14 receptor of airway epithelial cells-coupling to intracellular $Ca2^{+}$ and IL-8 secretion. *Am J Resp Cell Mol* **33**, 601–609.

43 Jokela TA, Karna R, Makkonen KM, Laitinen JT, Tammi RH & Tammi MI (2014) Extracellular UDP-glucose activates P2Y14 receptor and induces signal transducer and activator of transcription 3 (STAT3) Tyr705 phosphorylation and binding to hyaluronan synthase 2 (HAS2) promoter, stimulating hyaluronan synthesis of keratinocytes. *J Biol Chem* **289**, 18569–18581.

44 Zippel N, Limbach CA, Ratajski N, Urban C, Luparello C, Pansky A, Kassack MU & Tobiasch E (2012) Purinergic receptors influence the differentiation of human mesenchymal stem cells. *Stem Cells Dev* **21**, 884–900.

45 Lee BC, Cheng T, Adams GB, Attar EC, Miura N, Lee SB, Saito Y, Olszak I, Dombkowski D, Olson DP *et al.* (2003) P2Y-like receptor, GPR105 (P2Y14), identifies and mediates chemotaxis of bone-marrow hematopoietic stem cells. *Genes Dev* **17**, 1592–1604.

46 Zhu Y, Fan Z, Wang R, Xie R, Guo H, Zhang M, Guo B, Sun T, Zhang H, Zhuo L *et al.* (2020) Single-cell analysis for glycogen localization and metabolism in cultured astrocytes. *Cell Mol Neurobiol* **40**, 801–812.

47 Li C & Hu Z (2020) Is liver glycogen fragility a possible drug target for diabetes? *FASEB J* **34**, 3–15.

48 Roach PJ, Depaoli-Roach AA, Hurley TD & Tagliabracci VS (2012) Glycogen and its metabolism: some new developments and old themes. *Biochem J* **441**, 763–787.

49 Rich LR, Harris W & Brown AM (2019) The role of brain glycogen in supporting physiological function. *Front Neurosci* **13**, 1176.

50 Lewis CA, Parker SJ, Fiske BP, McCloskey D, Gui DY, Green CR, Vokes NI, Feist AM, Vander HM & Metallo CM (2014) Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells. *Mol Cell* **55**, 253–263.

51 Riganti C, Gazzano E, Polimeni M, Aldieri E & Ghigo D (2012) The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. *Free Radic Biol Med* **53**, 421–436.

52 Metere A, Iorio E, Scorza G, Camerini S, Casella M, Crescenzi M, Minetti M & Pietraforte D (2014) Carbon monoxide signaling in human red blood cells: evidence for pentose phosphate pathway activation and protein deglutathionylation. *Antioxid Redox Signal* **20**, 403–416.

53 Mohanty JG, Nagababu E & Rifkind JM (2014) Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. *Front Physiol* **5**, 84.

54 Moses SW, Bashan N & Gutman A (1972) Glycogen metabolism in the normal red blood cell. *Blood* **40**, 836–843.

55 Moses SW, Bashan N, Gutman A & Ockerman PA (1974) Glycogen metabolism in glycogen-rich erythrocytes. *Blood* **44**, 275–284.
56 Thwe PM, Pelgrom LR, Cooper R, Beauchamp S, Reisz JA, D'Alessandro A, Everts B & Amiel E (2017) Cell-intrinsic glycogen metabolism supports early glycolytic reprogramming required for dendritic cell immune responses. *Cell Metab* **26**, 558–567.
57 Harty JT, Tvinnereim AR & White DW (2000) CD8+ T cell effector mechanisms in resistance to infection. *Annu Rev Immunol* **18**, 275–308.
58 St PM & Ohashi PS (2020) The roles of CD8(+) T cell subsets in antitumor immunity. *Trends Cell Biol* **30**, 695–704.
59 Waldman AD, Fritz JM & Lenardo MJ (2020) A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* **20**, 1–18.
60 Smith-Garvin JE, Koretzky GA & Jordan MS (2009) T cell activation. *Annu Rev Immunol* **27**, 591–619.
61 Mueller SN, Gebhardt T, Carbone FR & Heath WR (2013) Memory T cell subsets, migration patterns, and tissue residence. *Annu Rev Immunol* **31**, 137–161.
62 Farber DL, Yudanin NA & Restifo NP (2014) Human memory T cells: generation, compartmentalization and homeostasis. *Nat Rev Immunol* **14**, 24–35.
63 Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, Larsen CP & Ahmed R (2009) mTOR regulates memory CD8 T-cell differentiation. *Nature* **460**, 108–112.
64 Araki K & Ahmed R (2013) AMPK: a metabolic switch for CD8+ T-cell memory. *Eur J Immunol* **43**, 878–881.
65 Rolf J, Zarrouk M, Finlay DK, Foretz M, Viollet B & Cantrell DA (2013) AMPKalpha1: a glucose sensor that controls CD8 T-cell memory. *Eur J Immunol* **43**, 889–896.
66 Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG & Choi Y (2009) Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* **460**, 103–107.
67 Raud B, Roy DG, Divakaruni AS, Tarasenko TN, Franke R, Ma EH, Samborska B, Hsieh WY, Wong AH, Stuve P *et al.* (2018) Etomoxir actions on regulatory and memory T cells are independent of Cpt1a-mediated fatty acid oxidation. *Cell Metab* **28**, 504–515.
68 O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, Qiu J, Smith AM, Lam WY, DiPlato LM *et al.* (2014) Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* **41**, 75–88.
69 Zhang H, Tang K, Ma J, Zhou L, Liu J, Zeng L, Zhu L, Xu P, Chen J, Wei K *et al.* (2020) Ketogenesis-generated beta-hydroxybutyrate is an epigenetic regulator of CD8(+) T-cell memory development. *Nat Cell Biol* **22**, 18–25.
70 Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL & Sabatini DM (2006) Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell* **22**, 159–168.
71 Varol C, Mildner A & Jung S (2015) Macrophages: development and tissue specialization. *Annu Rev Immunol* **33**, 643–675.
72 Mosser DM, Hamidzadeh K & Goncalves R (2020) Macrophages and the maintenance of homeostasis. *Cell Mol Immunol* **15**, 1–9.
73 Van den Bossche J, O'Neill LA & Menon D (2017) Macrophage immunometabolism: where are we (going)? *Trends Immunol* **38**, 395–406.
74 Viola A, Munari F, Sanchez-Rodriguez R, Scolaro T & Castegna A (2019) The metabolic signature of macrophage responses. *Front Immunol* **10**, 1462.
75 Palmieri EM, Gonzalez-Cotto M, Baseler WA, Davies LC, Ghesquiere B, Maio N, Rice CM, Rouault TA, Cassel T, Higashi RM *et al.* (2020) Nitric oxide orchestrates metabolic rewiring in M1 macrophages by targeting aconitase 2 and pyruvate dehydrogenase. *Nat Commun* **11**, 698.
76 Nagy C & Haschemi A (2015) Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and the pentose phosphate pathway. *Front Immunol* **6**, 164.
77 Ma J, Wei K, Liu J, Tang K, Zhang H, Zhu L, Chen J, Li F, Xu P, Chen J *et al.* (2020) Glycogen metabolism regulates macrophage-mediated acute inflammatory responses. *Nat Commun* **11**, 1769.
78 Kolla V, Weihua X & Kalvakolanu DV (1997) Modulation of interferon action by retinoids. Induction of murine STAT1 gene expression by retinoic acid. *J Biol Chem* **272**, 9742–9748.
79 Bai L & Merchant JL (2003) Transcription factor ZBP-89 is required for STAT1 constitutive expression. *Nucleic Acids Res* **31**, 7264–7270.
80 Shang Y, Baumrucker CR & Green MH (1999) The induction and activation of STAT1 by all-trans-retinoic acid are mediated by RAR beta signaling pathways in breast cancer cells. *Oncogene* **18**, 6725–6732.
81 Wong LH, Sim H, Chatterjee-Kishore M, Hatzinisiriou I, Devenish RJ, Stark G & Ralph SJ (2002) Isolation and characterization of a human STAT1 gene regulatory element. Inducibility by interferon (IFN) types I and II and role of IFN regulatory factor-1. *J Biol Chem* **277**, 19408–19417.
82 Wilson WR & Hay MP (2011) Targeting hypoxia in cancer therapy. *Nat Rev Cancer* **11**, 393–410.
83 Kung-Chun Chiu D, Pui-Wah Tse A, Law CT, Ming-Jing XuI, Lee D, Chen M, Kit-Ho Lai R, Wai-Hin

Yuen V, Wing-Sum Cheu J, Wai-Hung Ho D *et al.* (2019) Hypoxia regulates the mitochondrial activity of hepatocellular carcinoma cells through HIF/HEY1/PINK1 pathway. *Cell Death Dis* **10**, 934.
84 Fuhrmann DC & Brüne B (2017) Mitochondrial composition and function under the control of hypoxia. *Redox Biol* **12**, 208–215.
85 Gilkes DM, Semenza GL & Wirtz D (2014) Hypoxia and the extracellular matrix: drivers of tumour metastasis. *Nat Rev Cancer* **14**, 430–439.
86 Muz B, de la Puente P, Azab F & Azab AK (2015) The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia (Auckl)* **3**, 83–92.
87 Wheaton WW & Chandel NS (2011) Hypoxia. 2. Hypoxia regulates cellular metabolism. *Am J Physiol Cell Physiol* **300**, C385–C393.
88 Martinez-Reyes I & Chandel NS (2020) Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun* **11**, 102.
89 Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J & Ratcliffe PJ (2009) Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. *J Biol Chem* **284**, 16767–16775.
90 Pelletier J, Bellot G, Gounon P, Lacas-Gervais S, Pouyssegur J & Mazure NM (2012) Glycogen synthesis is induced in hypoxia by the hypoxia-inducible factor and promotes cancer cell survival. *Front Oncol* **2**, 18.
91 Pescador N, Villar D, Cifuentes D, Garcia-Rocha M, Ortiz-Barahona A, Vazquez S, Ordonez A, Cuevas Y, Saez-Morales D, Garcia-Bermejo ML *et al.* (2010) Hypoxia promotes glycogen accumulation through hypoxia inducible factor (HIF)-mediated induction of glycogen synthase 1. *PLoS One* **5**, e9644.
92 Shen GM, Zhang FL, Liu XL & Zhang JW (2010) Hypoxia-inducible factor 1-mediated regulation of PPP1R3C promotes glycogen accumulation in human MCF-7 cells under hypoxia. *FEBS Lett* **584**, 4366–4372.
93 Lee WN, Guo P, Lim S, Bassilian S, Lee ST, Boren J, Cascante M, Go VL & Boros LG (2004) Metabolic sensitivity of pancreatic tumour cell apoptosis to glycogen phosphorylase inhibitor treatment. *Br J Cancer* **91**, 2094–2100.
94 Favaro E, Bensaad K, Chong MG, Tennant DA, Ferguson DJ, Snell C, Steers G, Turley H, Li JL, Gunther UL *et al.* (2012) Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells. *Cell Metab* **16**, 751–764.
95 Liu J, Tan Y, Zhang H, Zhang Y, Xu P, Chen J, Poh YC, Tang K, Wang N & Huang B (2012) Soft fibrin gels promote selection and growth of tumorigenic cells. *Nat Mater* **11**, 734–741.
96 Liu Y, Liang X, Yin X, Lv J, Tang K, Ma J, Ji T, Zhang H, Dong W, Jin X *et al.* (2017) Blockade of IDO-kynurenine-AhR metabolic circuitry abrogates IFN-gamma-induced immunologic dormancy of tumor-repopulating cells. *Nat Commun* **8**, 15207.
97 Liu Y, Lv J, Liu J, Liang X, Jin X, Xie J, Zhang L, Chen D, Fiskesund R, Tang K *et al.* (2018) STAT3/p53 pathway activation disrupts IFN-beta-induced dormancy in tumor-repopulating cells. *J Clin Invest* **128**, 1057–1073.
98 Liu Y, Lv J, Liang X, Yin X, Zhang L, Chen D, Jin X, Fiskesund R, Tang K, Ma J *et al.* (2018) Fibrin stiffness mediates dormancy of tumor-repopulating cells via a Cdc42-driven Tet2 epigenetic program. *Cancer Res* **78**, 3926–3937.
99 Tang K, Yu Y, Zhu L, Xu P, Chen J, Ma J, Zhang H, Fang H, Sun W, Zhou L *et al.* (2019) Hypoxia-reprogrammed tricarboxylic acid cycle promotes the growth of human breast tumorigenic cells. *Oncogene* **38**, 6970–6984.
100 Sullivan LB, Martinez-Garcia E, Nguyen H, Mullen AR, Dufour E, Sudarshan S, Licht JD, Deberardinis RJ & Chandel NS (2013) The proto-oncometabolite fumarate binds glutathione to amplify ROS-dependent signaling. *Mol Cell* **51**, 236–248.
101 Young FG (1957) Claude Bernard and the discovery of glycogen; a century of retrospect. *Br Med J* **1**, 1431–1437.
102 Lynch RM & Paul RJ (1983) Compartmentation of glycolytic and glycogenolytic metabolism in vascular smooth muscle. *Science* **222**, 1344–1346.
103 de Mas IM, Selivanov VA, Marin S, Roca J, Oresic M, Agius L & Cascante M (2011) Compartmentation of glycogen metabolism revealed from ^{13}C isotopologue distributions. *BMC Syst Biol* **5**, 175.
104 O'Connell JD, Zhao A, Ellington AD & Marcotte EM (2012) Dynamic reorganization of metabolic enzymes into intracellular bodies. *Annu Rev Cell Dev Biol* **28**, 89–111.
105 Prats C, Cadefau JA, Cusso R, Qvortrup K, Nielsen JN, Wojtaszewski JF, Hardie DG, Stewart G, Hansen BF & Ploug T (2005) Phosphorylation-dependent translocation of glycogen synthase to a novel structure during glycogen resynthesis. *J Biol Chem* **280**, 23165–23172.
106 Prats C, Helge JW, Nordby P, Qvortrup K, Ploug T, Dela F & Wojtaszewski JF (2009) Dual regulation of muscle glycogen synthase during exercise by activation and compartmentalization. *J Biol Chem* **284**, 15692–15700.