Advances in Experimental Medicine and Biology 1011

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Immune Metabolism in Health and Tumor

Springer

Advances in Experimental Medicine and Biology

Volume 1011

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-94-024-1168-3 ISBN 978-94-024-1170-6 (eBook)
DOI 10.1007/978-94-024-1170-6

Library of Congress Control Number: 2017951122

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Science+Business Media B.V.
The registered company address is: Van Godewijckstraat 30, 3311 GX Dordrecht, The Netherlands

Contents

Chapter 1
Metabolism in Immune Cell Differentiation and Function

Nicole M. Chapman, Sharad Shrestha, and Hongbo Chi

Abstract The immune system is a central determinant of organismal health. Functional immune responses require quiescent immune cells to rapidly grow, proliferate, and acquire effector functions when they sense infectious agents or other insults. Specialized metabolic programs are critical regulators of immune responses, and alterations in immune metabolism can cause immunological disorders. There has thus been growing interest in understanding how metabolic processes control immune cell functions under normal and pathophysiological conditions. In this chapter, we summarize how metabolic programs are tuned and what the physiological consequences of metabolic reprogramming are as they relate to immune cell homeostasis, differentiation, and function.

Keywords Metabolism • T cells • Treg cells • NK cells • B cells • mTOR • AMPK

1.1 Introduction

The immune system is comprised of the innate and adaptive immune cells, which develop from bone marrow-derived progenitors cells. The response of the innate immune cells is more rapid than adaptive immune cells. Innate immune cells are activated by germ line-encoded receptors, including various pattern recognition receptors and cytokine receptors. The engagement of these and other receptors allows innate immune cells to engulf pathogens and other foreign antigens, to produce antimicrobial products, and to secrete cytokines and chemokines. Innate immune cells, especially dendritic cells (DCs), can serve as antigen-presenting cells (APCs), which process and present acquired antigens in the context of short peptides on major histocompatibility complex (MHC) molecules. Trafficking of these cells to secondary lymphoid tissues (e.g., spleen, lymph nodes) allows them to

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B. Li, F. Pan (eds.), *Immune Metabolism in Health and Tumor*, Advances in Experimental Medicine and Biology 1011, DOI 10.1007/978-94-024-1170-6_1

interact with adaptive immune cells. While the innate immune response is rapid, adaptive immunity takes days to weeks to form. T and B cells make up the adaptive immune system, and both of these cell populations express antigen receptors, called the T cell antigen receptor (TCR) and the B cell antigen receptor (BCR). The TCR must bind antigens expressed in the context of MHC molecules, whereas BCR engagement is MHC independent. These antigen receptors ensure that the adaptive immune response is selective and specific. Additionally, both T and B cells require co-stimulatory receptor engagement for maximum activation, and cytokines further shape their specific functions. The coordinated actions of the innate and adaptive immune responses promote pathogen clearance and the formation of long-term immunological memory, which allows for rapid immune responses following reinfection. However, dysregulated immune cell responses are connected to many diseases, ranging from tumorigenesis to autoimmunity. Therefore, it is of interest to understand how immune responses are regulated at the steady state and in various disease states.

Metabolism is the net result of both the breaking down (catabolic) and de novo synthesis (anabolic) of nutrients. In addition to providing the cell with energy, metabolic by-products from one pathway can tune other metabolic programs. Metabolites also serve as important regulators of gene transcription and protein translation, localization, activity, and expression. Metabolism plays a crucial role in shaping immune cell differentiation and function. In this chapter, we provide an overview of metabolic processes used by immune cells and how metabolic reprogramming is regulated at the molecular level (Figs. 1.1 and 1.2). We also summarize our current understanding of how metabolism shapes immune cell differentiation and function, with a particular emphasis being placed upon macrophages and DCs, natural killer (NK) cells, conventional T cells, and regulatory T (Treg) cells (Figs. 1.3, 1.4, and 1.5). We conclude with a brief discussion on how metabolism regulates B cell responses and future challenges facing the immunometabolism field.

1.2 Mechanistic Regulation of Cellular Metabolism

1.2.1 Overview of Catabolic Metabolism

Immune cells utilize adenosine triphosphate (ATP) derived from diverse nutrients as an energy source to support their differentiation and specialized functions. Glucose is one major source of ATP for both resting and activated immune cells. After it has been taken up into the cell, glucose is converted into glucose-6-phosphate (G6P) by hexokinase (HK), of which there are four isoforms: HK1, HK2, HK3, and glucokinase (GCK; also called HK4). During glycolysis, several important intermediaries are produced, including 3-phosphoglycerate (3PG), phosphoenolpyruvate (PEP), and nicotinamide adenine dinucleotide (NADH) (Fig. 1.1). Glycolysis culminates in the production of two molecules of ATP and pyruvate, a metabolite that can be

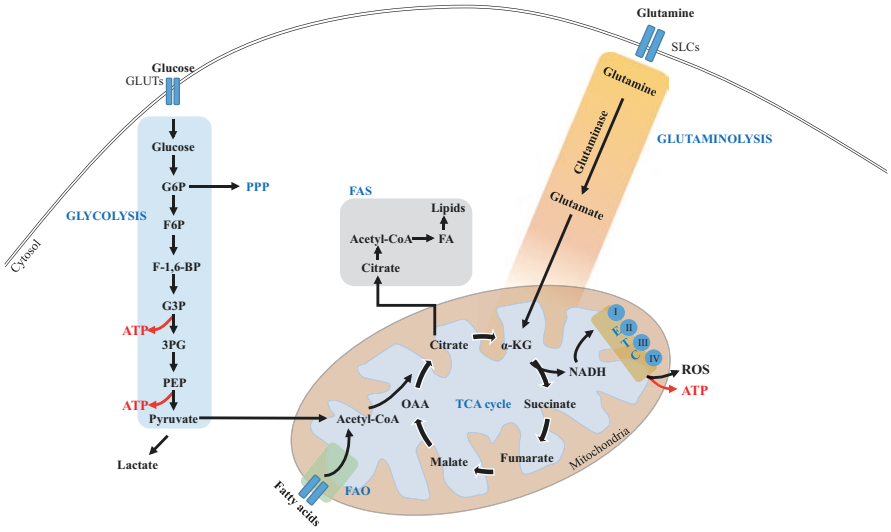

Fig. 1.1 Overview of cellular metabolism
The major metabolic pathways of immune cells are glycolysis, glutaminolysis, fatty acid synthesis (*FAS*), fatty acid oxidation (*FAO*), and oxidative phosphorylation (*OXPHOS*) fueled by the tricarboxylic acid (*TCA*) cycle. The pentose phosphate pathway (*PPP*) also controls select immune cell functions. Glycolysis, FAS, and glutaminolysis reactions occur in the cytosol, while the TCA and OXPHOS occur in the mitochondrial matrix. These pathways generate metabolites critical for multiple cellular functions and also produce cellular energy in the form of adenosine triphosphate (*ATP*). The reader is encouraged to visit Sect. 1.1 for a more detailed discussion of these pathways. *Abbreviations*: *G6P* glucose-6-phosphate, *F6P* fructose-6-phosphate, *F-1,6-BP* fructose-1, 6-bisphosphate, *G3P* glyceraldehyde-3-phosphate, *3-PG* 3-phosphoglycerate, *PEP* phosphoenolpyruvate, *FA* fatty acid, *ROS* reactive oxygen species, *SLCs* solute carrier family of amino acid transporters, *GLUTs* glucose transporters

further processed into lactate via lactate dehydrogenase (LDH). Glycolysis is favored under conditions where oxygen is limiting. However, seminal studies by Otto Warburg demonstrated that highly proliferative cells convert glucose into lactate in the presence of oxygen, a phenomenon termed the Warburg effect or aerobic glycolysis. Roles for aerobic glycolysis for energy production and for providing metabolic by-products that support immune cell functions have been recently uncovered, which we discuss throughout this chapter.

Pyruvate derived from aerobic glycolysis can also be converted to ATP energy via the tricarboxylic citric acid (TCA) cycle (also known as the citric acid cycle or the Krebs cycle) and mitochondrial oxidative phosphorylation (OXPHOS) (Fig. 1.1). To enter into the TCA cycle, pyruvate is transported into the mitochondria and oxidized to generate acetyl coenzyme A (acetyl-CoA). Citrate synthase then combines acetyl-CoA with oxaloacetate to generate citrate. During this multistep process, NADH and flavin adenine dinucleotide ($FADH_2$) are produced. These products are critical electron donors for the electron transport chain (ETC) that generates ATP via OXPHOS. The ETC is comprised of five protein complexes that shuttle electrons

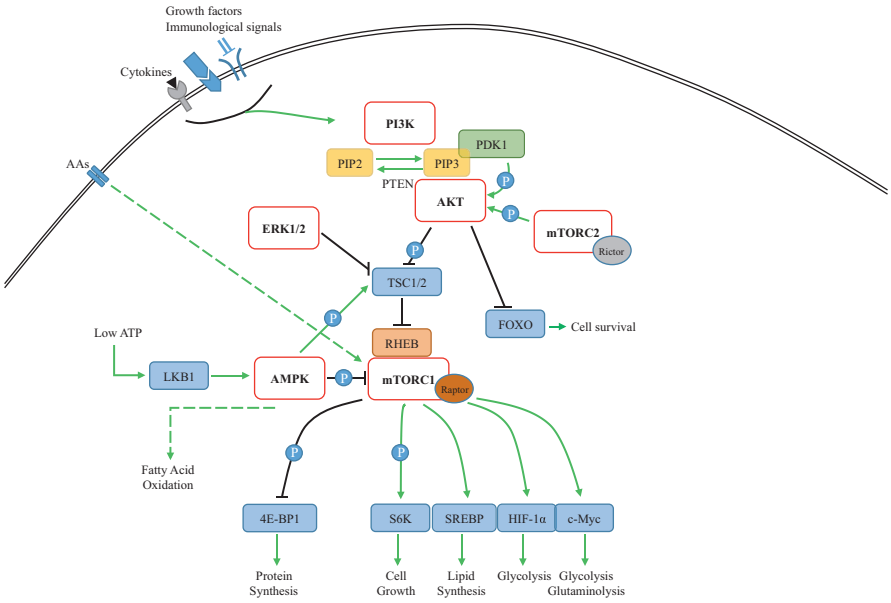

Fig. 1.2 Kinases and transcription factors cooperatively regulate immune cell metabolism
Immune cells receive immunological and environmental cues (growth factors, nutrients) that tune metabolic pathways. The alterations in cellular metabolism are shaped by intracellular serine/threonine kinases, including ERK1/2, AKT, mTORC1, mTORC2, LKB1, and AMPK. Additionally, the lipid kinase PI3K is a crucial regulator of immune responses, because it can modulate AKT, mTORC1, and mTORC2 activities, among many others. The mTORC1 pathway is a major determinant of metabolic fitness in immune cells. Upon its activation, mTORC1 phosphorylates 4E-BP1 and S6K to influence protein translation. Additionally, it induces expression of key metabolic enzymes, including c-MYC, HIF-1α, and SREBPs. The reader should refer to Sect. 1.2 for more details about how these pathways are tuned

through the inner mitochondrial membrane. NADH donates an electron to complex I; $FADH_2$ donates an electron to complex II and another electron to succinate to generate fumarate to feed back into the TCA cycle. Electrons transferred to complexes I and II are shuttled to complex III via coenzyme Q (also known as ubiquinone). Cytochrome c then transmits the electrons to complex IV. Electron movement across complexes I, III, and IV is coupled to proton pumping from the inner mitochondrial membrane matrix into the intermembrane space and thus creates a gradient that enables complex V (also known as ATP synthase) to produce ATP [417]. How OXPHOS controls immune responses is discussed below.

Reactive oxygen species (ROS) are produced during the process of mitochondrial OXPHOS. Superoxide is generated when electrons are not efficiently passed along the ETC and transferred to oxygen. Superoxide generated from complex I or complex II is released into the mitochondrial matrix, while superoxide generated from complex III can be present in either the matrix or intermembrane space. Superoxide dismutase (SOD) localized in either the matrix (SOD2) or intermembrane

space (SOD1) can convert superoxide to hydrogen peroxide, which readily crosses the inner and outer mitochondrial membranes and can participate in antimicrobial responses or peroxisome metabolism. Alternatively, superoxide found in the intermembrane space can be exported into the cytosol via a voltage-dependent anion exchange channel [417]. Metabolic and cellular stresses that accompany active immune responses increase superoxide production, the biological consequences of which are discussed below.

Pyruvate is also generated from fatty acids via the process of fatty acid β-oxidation (FAO). This catabolic process occurs within the mitochondria. To begin FAO, free fatty acids are modified by fatty acyl-CoA synthetase (FACS; also called fatty acyl-CoA synthase) to form a fatty acyl-CoA. This product is not permeable to the mitochondrial membrane, so carnitine palmitoyltransferase 1 (CPT1) adds a carnitine moiety onto the fatty acyl-CoA to generate fatty acylcarnitine. The carnitine translocase (CAT) allows the fatty acylcarnitine to enter into the inner mitochondrial membrane via carnitine exchange. Within the inner mitochondrial membrane, CPT2 converts the fatty acylcarnitine back into fatty acyl-CoA. FAO is complete when the fatty acyl-CoA is converted into acetyl-CoA, which enters into the TCA cycle. NADH and $FADH_2$ are also produced during this process to feed the ETC-OXPHOS pathway and generate ATP (Fig. 1.1).

Dietary proteins can also be used as an energy source. Proteins are first hydrolyzed into individual amino acids, which can feed into various parts of the TCA cycle to fuel ATP production. Alanine, glycine, threonine, cysteine, serine, and tryptophan can be converted directly into pyruvate. Additionally, asparagine and aspartate can be used to produce oxaloacetate, which is converted into PEP by the mitochondrial enzyme phosphoenolpyruvate carboxykinase 2 (PCK2). PEP can then be subsequently converted back into pyruvate to generate acetyl-CoA. Catabolism of additional amino acids, including arginine, glutamine, and glutamate, also produces TCA cycle intermediates. The breakdown of glutamine via glutaminolysis is crucial for immune cell biology. During this process, glutamine is converted into α-ketoglutarate (α-KG) via a two-step process requiring the activities of glutaminase (GLS), which converts glutamine to glutamate, and glutamate dehydrogenase (GDH), which converts glutamate to α-KG [128, 404]. This TCA cycle intermediate can then fuel OXPHOS for cellular energy. Because glutaminolysis can produce TCA cycle intermediates, it can also promote anabolic processes by serving as a carbon or nitrogen donor to support cell growth and proliferation. We discuss how amino acids contribute to biosynthesis in more detail below.

1.2.2 Overview of Anabolic Metabolism

Anabolic processes are also important for immune cell fitness. Aside from entering into glycolysis, G6P can also be shuttled into the cytosolic pentose phosphate pathway (PPP) after its conversion into 6-phosphogluconolactone by G6P dehydrogenase (G6PD). NAD^+ and NADPH are two by-products of the PPP. NAD^+ is an

important electron acceptor for catabolic processes driving OXPHOS, while NADPH is utilized in reducing reactions important for the production of pentose sugars, nuclear hormone receptor ligands, and fatty acids. Derivatives from the PPP can also integrate back into glycolysis to feed ATP production and are also important for generating nucleotides. We will discuss how the PPP influences immune cell functions in this chapter. The glycolytic by-products 3PG and pyruvate are important for serine, cysteine, glycine, and alanine biosynthesis. Moreover, oxaloacetate and α-KG generated via the TCA cycle are important for aspartate, asparagine, proline, and arginine synthesis. Thus, glycolysis and TCA cycle intermediates support cell growth and functions through both catabolic and anabolic processes.

Fatty acid and cholesterol synthesis are crucial anabolic processes supporting cellular functions. To initiate fatty acid synthesis (FAS) in the cytosol, acetyl-CoA is shuttled out of the mitochondria by citrate and subsequently converted into malonyl-CoA via acetyl-CoA carboxylase (ACC) in a manner that requires biotin (also called vitamin B_7). Malonyl-CoA is then reduced by fatty acid synthase (FASN) and its cofactor NADPH to generate palmitate, a 16-carbon fatty acid. Among several functions, palmitate serves as the backbone for other long-chain fatty acids and phospholipids and can also modify proteins, a process termed palmitoylation. Additionally, accumulation of palmitate perturbs FAS via the feedback inhibition of ACC. Acetyl-CoA can also serve as a substrate for cholesterol biosynthesis. The sequential activities of thiolase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase (HMGCS), and HMG-CoA reductase (HMGCR) convert acetyl-CoA into mevalonate, the metabolic precursor required for cholesterol synthesis. Cholesterol biosynthesis is important for maintaining the plasma membrane and for producing sterol hormones, vitamin D, and various oxysterols [e.g., 25-hydroxycholesterol (25-HC)]. Mevalonate-derived isoprenoids produced during cholesterol biogenesis also serve important cellular functions. For instance, isoprenoids modify proteins in a process termed prenylation [85]. Statins are effective inhibitors of HMGCR function and thus block cholesterol and isoprenoids-dependent functions. How FAS and cholesterol biosynthesis control immune cell fate is discussed throughout this chapter.

Serine metabolism is also crucial for anabolic processes supporting nucleotide and lipid biosynthesis. Glucose-derived 3PG serves as a precursor for serine biosynthesis, whose rate-limiting step is catalyzed by phosphoglycerate dehydrogenase (PHGDH). In the presence of glutamine-derived glutamate, 3-phosphohydroxypyruvate is catalyzed into 3-phosphoserine via the enzymatic activity of phosphoserine aminotransferase (PSAT). This reaction also generates α-KG that can be used for other cellular purposes. Dephosphorylation of 3-phosphoserine generates serine, which is important for one-carbon metabolism [306]. One-carbon metabolism is a process whereby a carbon unit derived from serine or glycine is cycled through the folate and methionine cycles to fuel biosynthetic pathways. This pathway aids in lipid, nucleotide, and protein biosynthesis and the generation of products important for redox reactions (i.e., those that neutralize ROS species) and methylation reactions (e.g., for epigenetic modifications like DNA methylation) [232, 306]. Dietary folate (also called vitamin B_9) is essential for

one-carbon metabolism. After entering the cell, folate is reduced to tetrahydrofolate (THF). Then, a carbon unit from serine or glycine is donated to THF via the enzymatic activity of serine hydroxymethyl transferase (SHMT), forming methylene-THF (me-THF) and glycine. In addition to folate, vitamins B_2, B_6, and B_{12} serve as important cofactors for these reactions. How serine and one-carbon metabolism regulate cancer and immune cell biology is under active investigation [232, 306].

1.2.3 Nutrient Transporters Regulate Catabolic Metabolism

To initiate catabolic or anabolic processes, nutrients must be made available to the cells. Extracellular nutrients are delivered to cells via nutrient transporters. Glucose transporters (GLUTs) mediate glucose uptake into many cell types. GLUT1 is the preferential transporter expressed by immune cells, but other GLUTs can play redundant or compensatory in the absence of GLUT1. Glucose diffusion via GLUTs is a passive process, so the expression of GLUTs in the plasma membrane is tightly controlled to regulate glucose import. In activated T cells, for example, strong TCR or suboptimal TCR signals combined with CD28 co-stimulatory signals upregulate GLUT1 expression [121, 173]. Then, GLUT1 translocates from the cytosol to the membrane via mechanisms requiring CD28 co-stimulation and phosphatidylinositol-3-kinase (PI3K)-AKT activation [418]. The physiological consequences of altering glucose transport into immune cells are discussed in this chapter.

Extracellular fatty acids must enter into the cytoplasm for transport into the mitochondria for FAO. Free fatty acids can readily diffuse across the plasma membrane into the cytosol. However, most fatty acids require transport facilitated by protein surface receptors. Fatty acid transport proteins (FATPs) are transmembrane proteins involved in fatty acid uptake, but these proteins do not appear to be expressed at high levels in immune cells [99]. Instead, fatty acid translocase (FAT or CD36) facilitates long-chain fatty acid and oxidized fatty acid transport across the plasma membrane [354]. Additionally, G protein-coupled receptors can recognize fatty acids of different lengths and promote their transport into immune cells. GPR40 [also known as free fatty acid receptor 1 (FFA1)] and GPR120 bind long-chain fatty acids; GPR84 recognizes medium-chain fatty acids; and short-chain fatty acids (SCFAs) are ligands for GPR43 (also known as FFA2) and GPR41 (also known as FFA3) [87]. The roles of these transporters as they relate to immunity are discussed in later sections of this chapter.

Essential amino acids cannot be synthesized via intrinsic metabolic programs and must therefore be obtained from dietary sources. There are nine essential amino acids: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Under conditions of cellular stress, glutamine and other amino acid levels become limiting, so cells also utilize extracellular sources of these amino acids for energy and biosynthetic processes. The system L amino acid transporters allow neutral amino acids to enter into immune cells. LAT1 (also known as Slc7a5) is an anti-porter that transports leucine into the cell and glutamine out of the

cell and is a crucial regulator of immune cell responses as discussed below [312]. ASCT2 (also known as Slc1a5) transports glutamine into cells and also has reported roles in immune responses [265]. Despite the importance of extracellular amino acids, the breakdown of amino acids by various enzymes, including cytosolic branched-chain aminotransferase (BCATc), indoleamine 2,3-dioxygenase (IDO), and arginase (Arg), also plays crucial roles in controlling metabolic programs and immune cell fates (Fig. 1.2).

1.2.4 Intracellular Kinases Regulate Metabolic Programs

Four major kinase pathways cooperate to control metabolic reprogramming in immune cells: PI3K, mechanistic target of rapamycin (mTOR), AMP-activated protein kinase (AMPK), and mitogen-activated protein kinases [MAPKs; including the extracellular-related kinases (ERKs), the p38 kinases, and the c-Jun N-terminal kinases (JNKs)]. Below, we review how these pathways are coupled to metabolic reprogramming (Fig. 1.2).

1.2.4.1 PI3K

Phospholipids are important second messengers that control protein localization, expression, and functions. The PI3K pathway is a major regulator of phospholipid turnover. The class I PI3Ks proteins are a heterodimer containing a regulatory subunit (p85α, p85β, and p55) and a catalytic subunit (p110α, p110β, p110δ, or p110γ). To promote PI3K activity, the Src homology 2 (SH2) domain of the PI3K regulatory subunit binds membrane-associated proteins containing a pYXXM motif, where pY is a phosphotyrosine, M is a methionine, and X is any amino acid. Alternatively, PI3K activity is triggered downstream of the membrane-anchored RAS GTPases (HRAS, NRAS, and KRAS), which directly bind to the RAS-binding domain (RBD) within the catalytic p110 subunit [61]. The p85 regulatory subunit also associates with GAB, which can bind growth factor receptor bound 2 (Grb2) and be indirectly recruited to membrane proteins containing pYXN motifs [61]. After binding the plasma membrane, the catalytic PI3K subunit converts phosphatidylinositol-(4,5)-bisphosphate (PIP_2) into phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3). Among other functions, PIP_3 allows proteins containing pleckstrin homology (PH) domains to bind the plasma membrane, which modulates their enzymatic activity. Phosphatase and tensin homolog (PTEN) and SH2 domain-containing inositol 5′-phosphatase (SHIP) antagonize PI3K signaling by converting PIP_3 to PIP_2 and PI-(3,4)-P_2, respectively. These phospholipids and their by-products are also important signaling molecules for immune cells [338, 408].

AGC kinases are important regulators of cell proliferation, cell growth, cytoskeletal rearrangements, survival, and cellular metabolism. The activation of these kinases requires phosphorylation of both a residue in the activation segment of the

kinase domain and a hydrophobic motif residue. PI3K-dependent mechanisms activate 3-phosphoinositide-dependent kinase 1 (PDK1). PDK1 phosphorylates other AGC kinases, including AKT, ribosomal S6 kinase (S6K, also called $p70^{S6K}$), serum- and glucocorticoid-regulated kinase (SGK), and protein kinase C (PKC). PDK1 is constitutively active, because it *trans*-autophosphorylates its own activation loop residue. PDK1 is expressed throughout the cell and is made assessable to its substrates via multiple mechanisms. The PH domain of PDK1 binds PIP_3 and, to a lesser extent, PIP_2, which helps localize PDK1 in proximity with its substrate AKT (isoforms AKT1, AKT2, and AKT3). Further, PDK1 binds soluble inositol phosphates in the cytosol. This association, coupled with substrate interactions mediated by its PIF (PDK1-interacting fragment) pocket, promotes the PDK1-dependent phosphorylation of proteins like S6K and SGK. PDK1-dependent functions are linked to metabolic reprogramming and immune cell functions as discussed below.

Like PDK1, AKT is recruited to the plasma membrane via PH domain-PIP_3 interactions. Binding of AKT to PIP_3 promotes a conformational change in AKT, which enables the PDK1-dependent phosphorylation of AKT threonine 308. The phosphorylation of AKT serine 473 by mTOR complex 2 (mTORC2) promotes maximal AKT activity by allowing the PDK1-PIF pocket to bind AKT S473, driving the phosphorylation of AKT T308. Of note, the activity of mTORC2 is also regulated by PIP_3 [127], although the receptor-specific activation mechanisms of mTORC2 activation remain unclear. This model for AKT activation is, however, context specific, as excess PIP_3 levels can drive AKT activation when mTORC2 activity is inhibited [166]. Glycogen synthase kinase 3 (GSK3), tuberous sclerosis 2 (TSC2), and forkhead box O (FOXO) proteins are substrates of AKT. Further, AKT activity influences glucose metabolism, in part by regulating the expression of GLUT1 and mTORC1 activity as discussed more below.

S6K and SGK do not have PH domains, but the phosphorylation of their activation loop and hydrophobic motif residues is linked to PI3K activity. The S6Ks are phosphorylated on their hydrophobic motif residues by mTOR complex 1 (mTORC1). Phosphorylation of the hydrophobic domain residue of S6K allows the PIF domain of PDK1 to bind S6K and phosphorylate its activation segment residue. Similarly, the mTORC2-dependent phosphorylation of the hydrophobic motif residue of SGK promotes PDK1 binding to and subsequent activation of SGK. The S6K signaling network influences mRNA processing, translation initiation and elongation, protein folding, and cell growth. The mTORC1-S6K axis also influences cellular metabolism by modulating the expression of sterol regulation element-binding proteins (SREBPs). SGK can influence cellular metabolism by phosphorylating FOXO transcription factors [43]. We discuss how mTORC1, mTORC2, SREBPs, and FOXOs govern metabolic processes below.