Chapter II

Review of Literature

2. Review of Literature:

2.1. The Role of Immunometabolic Pathways in Immune Cell Function:

Metabolism is a complex network of interconnected biochemical reactions that involve the production and consumption of energy molecules (ATPs), as well as the biosynthesis of macromolecules from nutrients provided by the ambient microenvironment to sustain the life of a cell and its proper functionality [70, 71].

The metabolism of immune cells, known as "**Immunometabolism**", has been recognized as a central factor which influences differentiation, activation and responses of the immune cells. Immune cells have special and unique metabolic machinery that not only processes energy production but also shapes their phenotypes and regulates their functions [2, 72]. Upon activation, immune cells reprogram their metabolic pathways to produce metabolites that act as immune signaling molecules. These metabolic programs vary depending on the type/subtype and function of immune cells, and the stimulation ways (cytokine or antigen receptor) [73].

The **key metabolic pathways** that have been reported in the metabolism of immune cells are; **glycolysis**, **pentose phosphate pathway**, **TCA cycle** and **oxidative phosphorylation (OxPhos)**, **fatty acid oxidation**, **fatty acid synthesis** and **amino acid pathway**. These immunometabolic pathways work together as a complex network that shares fuel inputs and utilizes products from one pathway in others. The felicitous systematization of these pathways has critical roles in the regulation of cellular maintenance, proliferation and effector function, in addition to the apposite modulation of cellular signaling by generating energetic molecules and producing biosynthetic intermediates to fulfill these needs. Further, interrupting these metabolic pathways can alter immune cell fate leading to unfavorable immune modulation [1] (Figure 1).

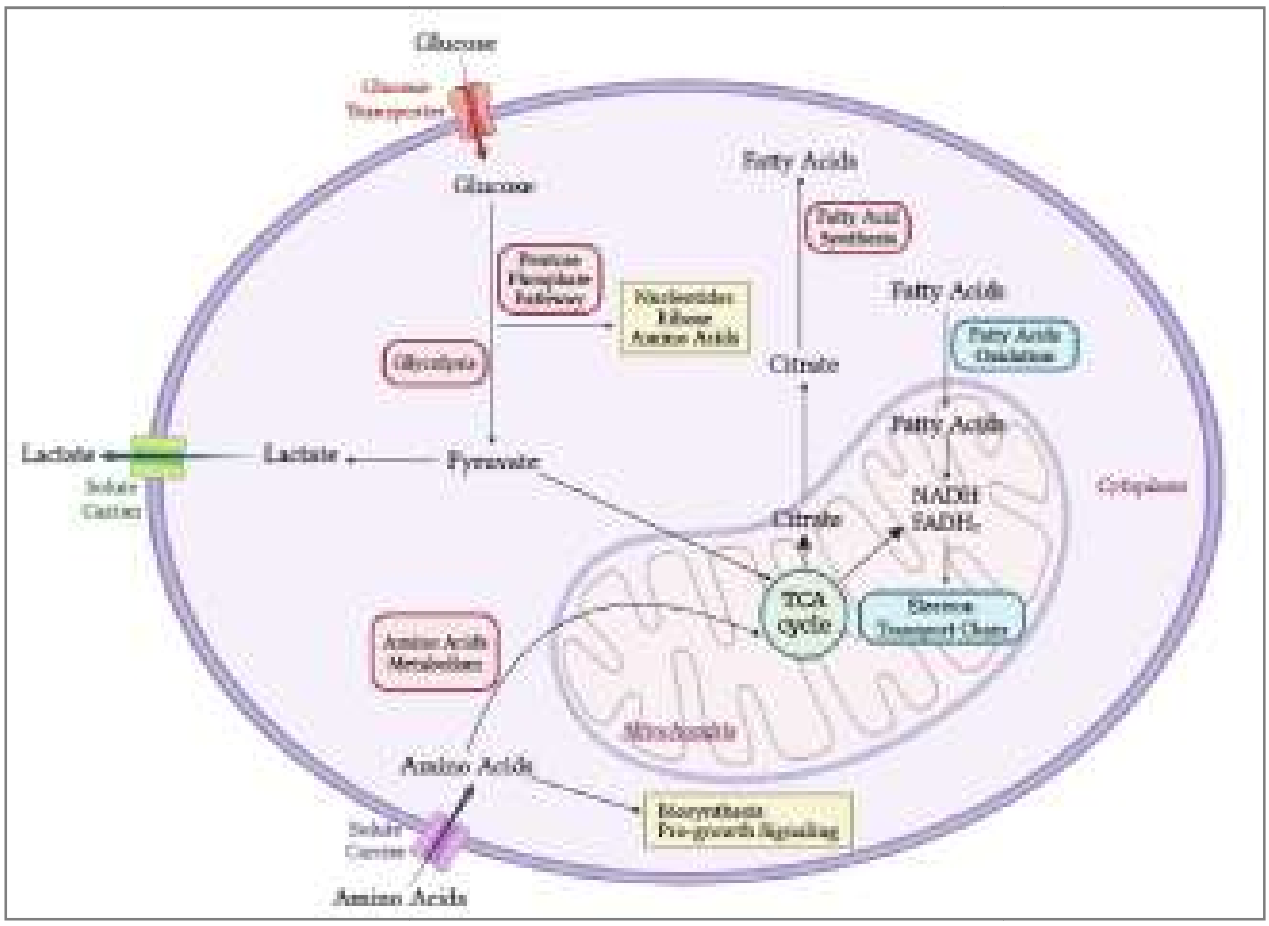


Figure (1): Scheme explains The Major Metabolic Pathways Characterized in Immune Cells. Adapted from ref. [1].

Glucose is taken up by immune cells through transporters like Glut1 and undergoes **glycolysis**, converting it into pyruvate. Pyruvate can either be converted into lactate and secreted or enter the **tricarboxylic acid (TCA) cycle**, where it generates NADH and $FADH_2$ as intermediates for the **electron transport chain (ETC)**, ultimately leading to ATP production. Glycolysis also supplies substrates for the **pentose phosphate pathway (PPP)**, which produces ribose for nucleotide synthesis and NADPH. NADPH is utilized in **fatty acid synthesis (FAS)**, which relies on citrate derived from the TCA cycle. Fatty acids can be oxidized, generating NADH and $FADH_2$, which fuel ATP production through the ETC. **Amino acids metabolism** contributes to the TCA cycle and is essential for cell growth and protein biosynthesis.

The following provides **a brief overview of the main metabolic pathways that have been reported in immune cells and their effects on immunological features;**

Glycolysis is a critical and dominant metabolic pathway for rapidly proliferating and effector immune cells, which converts glucose into both energy and biomolecules. The pathway starts with transporting glucose into the cytosol via transport receptors such as Glut1 and then is processed through a series of enzymatic reactions to generate pyruvate. Then, the pyruvate destination is depending on the presence of oxygen (O_2) (Figure 1). In the presence of O_2, pyruvate would be transported into the mitochondria to enter the TCA cycle while in the absence of O_2, pyruvate is processed in the cytoplasm to lactate which would be secreted from the cell, and this process is termed "anaerobic glycolysis" [74-76]. However, immune cells, including classically activated macrophages (M1; CAMs) [77], T cells [78], B cells [79], and NK cells [24, 29] can metabolize glucose to lactate in the presence of abundant oxygen in a process known as "aerobic glycolysis or Warburg effect" (Figure 2). Aerobic glycolysis was first characterized in cancer cells [80, 81] and later has been found as a distinct metabolic signature in immune cells upon activation to support their robust growth and proliferation [82, 83].

The innate and adaptive immune cells undergoing rapid activation in response to different stimulations (e.g. PRRs; Pattern recognition receptors, cytokine receptors or antigen receptors), enhance glycolysis to generate sufficient ATP and produce biosynthetic intermediates to carry out their particular effector functions, for instance; phagocytosis and inflammatory cytokine production in lipopolysaccharide (LPS)-activated macrophages [84], antigen presentation in dendritic cells (DCs) [85], production of cytokines in T cells (such as IL-17 in the case of Th17 cells) [86], activated effector CD8+ T cells [87], Th1 and Th2 cells [88] as well as antibodies in B cells [79] and IFNγ production by NK cells [24]. Glycolytic reprogramming involves upregulating glucose utilization and increasing glucose transporter expression (e.g, Glut-1) which is important in classically-activated macrophage [89], T cells [75] and NK cells [31] (Figure 2).

However, immune cells upregulate their glycolysis not only after activation to generate energy and enhance biosynthesis but also for adaptation and survival under metabolically restrictive conditions, like in the case of hypoxia. The hypoxic situation prevents efficient ATP synthesis through OxPhos then high rates of glycolysis can supply enough ATP to maintain energy homeostasis [90]. Although glycolysis is an inefficient way of generating ATP compared to the TCA cycle, it produces ATP rapidly and provides biosynthetic intermediates that fuel the

pentose phosphate pathway and fatty acid synthesis [1]. Moreover, glycolytic enzymes are important in providing products that are substrates for other metabolic pathways, such as pyruvate kinase, which regulates the funneling of earlier glycolytic intermediates into the pentose phosphate pathway or for use in serine synthesis. In addition, glycolytic enzymes have also a pivotal role in driving immunity response. For instance, the glycolytic enzyme GAPDH binds to *IFNγ* mRNA and prevents its translation. Once glycolysis conditions are met, GAPDH dissociates from *IFNγ* mRNA and enters the glycolytic pathway, allowing for the production of IFN-γ and achieving the effector function [91, 92].

The anabolic pathway that runs parallel glycolysis is the **pentose phosphate pathway (PPP),** also known as the hexose monophosphate shunt, which is an alternative pathway branching off from glycolysis which has roles in supporting cellular survival and proliferation, by generating ribose-5- phosphate (R5P) and NADPH (Figure 1). The R5P is an important precursor in the nucleotides and amino acids synthesis, while NADPH is an essential cofactor in fatty acid and lipid biosynthesis, and is required for maintaining a suitable cellular redox environment by the production of anti-oxidants, nitric oxide (NO) and reactive oxygen species (ROS) [93]. The regulation of PPP differs among immune cell subtypes. For instance, classically activated macrophages (M1), which are LPS and IFNγ-induced macrophages, enhance the flux of glucose intermediates through the PPP and allow for increased NADPH synthesis during respiratory bursts to both eliminate extracellular bacteria and synthesize antioxidants (e.g. glutathione and thioredoxin) which limit oxidative damage to the cell. By contrast, the PPP flux is limited in alternatively activated macrophages (M2) (IL4-induced macrophages), which perform tissue reparative functions and produce less ROS [94, 95]. NADPH derived from the PPP is also essential for the *de novo* fatty acid synthesis and cholesterol metabolism [96], which support phagocytosis [97] and the expansion of the endoplasmic reticulum and Golgi required for cytokine production upon response to oxidative stress in macrophages and DCs [98, 99].

The next pathway in the series of glucose metabolism is the **tricarboxylic acid cycle (TCA cycle)** (also known as the citric acid cycle or Krebs cycle) which plays a fundamental role in cellular energy production, and the generation of key metabolites necessary for cellular processes. The TCA cycle occurs in the mitochondrial matrix and serves as a central hub for various nutrient inputs that integrate different nutrient sources and contribute to ATP synthesis,

protein acetylation, and the synthesis of lipids essential for cellular membrane formation [76, 100]. The TCA cycle produces two crucial products; NADH and $FADH_2$. These molecules serve as electron carriers and provide reducing equivalents to the **electron transport chain (ETC)**, which supports efficient ATP generation through **oxidative phosphorylation (OxPhos)**. NADH and FADH2 donate electrons to the ETC, leading to the production of ATP [104]. The TCA cycle is fueled by glucose-derived pyruvate or fatty acids, in which they are converted into acetyl coenzyme A (acetyl-CoA) which then combines with oxaloacetate through aldol condensation to form citrate. Citrate, an important intermediate, can be further oxidized by the TCA cycle or hydrolyzed by ATP-citrate lyase (ACLY) to generate oxaloacetate and cytosolic acetyl-CoA, which contributes to protein acetylation and the synthesis of cholesterol and fatty acids needed for new membranes [76, 100]. Glutamate is another critical fuel for the TCA cycle through its direct conversion into α-ketoglutarate (αKG), an intermediate in the TCA cycle [76]. TCA cycle and OxPhos have a highly prominent metabolic role in ATP generation and are more often used by quiescent or non-proliferative cells whose primary requirements are energy and longevity such as memory CD8+T cells [100-102].

Alterations and rewiring in the TCA cycle lead to the accumulation of mitochondrial metabolites, other than ATP molecules, which in turn have a critical role in modulating the function of immune cells. These metabolites can play many pivotal roles as signaling molecules and affect important inflammatory pathways [72, 103]. For instance, **citrate** accumulation, caused by Isocitrate dehydrogenase 1 (IDH1) breakpoint in M1 macrophages and activated DCs, prevents glucose-derived pyruvate from continuing as citrate/isocitrate in the TCA cycle [99, 104]. Excess citrate has a role in inflammatory pathways in M1 macrophages through utilizing it in the fatty acids synthesis, which is substantial for membrane biogenesis to support antigen presentation [99], and have also a role in histone acetylation [104], and to generate key molecules for their effector functions that are nitric oxide (NO) and prostaglandins [105] and Itaconic acid which has antibacterial activity against many pathogenic species [106]. **Succinate** is another metabolite that can accumulate as a result of a broken TCA cycle in M1 macrophages, which can serve as an activation signal and promote IL-1β cytokine production in macrophages [48, 49, 107] (Figure 2). The **citrate–malate shuttle (CMS)**; is another breakpoint in the TCA cycle that has been found to play a crucial role in NK cells upon activation [29] (Figure 6). This shuttle involves the export of citrate from the mitochondria to the cytosol in exchange for malate,

facilitated by the mitochondrial citrate carrier (CIC). Once in the cytosol, citrate is enzymatically broken down by ATP-citrate lyase (ACLY) into acetyl-CoA and oxaloacetate. Oxaloacetate can then be converted to malate by malate dehydrogenase (MDH), allowing it to re-enter the mitochondria through CIC [29, 108].

The next highly efficient cellular catabolic process that plays a significant role in ATP generation is **Fatty acid oxidation (FAO)** where a single fatty acid molecule, such as palmitate, can yield over 100 molecules of ATP [109]. Within the mitochondria, fatty acids in the form of acyl-CoA undergo a β-oxidation process, resulting in the production of various products, including acetyl-CoA, NADH, and $FADH_2$. These products can be utilized in the TCA cycle and the electron transport chain to generate ATP. Acetyl-CoA, generated from FAO, serves as a substrate not only for ATP production but also for other important processes. It can be involved in de novo lipogenesis, which is the synthesis of fatty acids (Figure 1). Additionally, acetyl-CoA functions as a crucial cofactor for the acetylation of histones and non-histone proteins, which plays a role in regulating gene expression and cellular functions [110]. Several non-inflammatory immune cells, including M2 macrophages (activated with IL-4) [111], regulatory T cells [88, 112], and memory T cells [113], exhibit increased expression of genes involved in FAO, such as *Cpt1a*. This up-regulation of FAO-related genes is associated with enhanced cellular longevity and overall immune cell function and immune response efficiency in these cell types.

In addition to the need of energy production, immune cells also require anabolic processes to support their cellular growth, differentiation, and proliferation. **Fatty acid synthesis (FAS)** is one such process that involves the production of lipids from precursors derived from other cell-intrinsic metabolic pathways within the cell, including glycolysis, TCA cycle, and pentose phosphate pathway (Figure 1). FAS relies on the activity of specific transcription factors and enzymes, such as sterol regulatory element binding protein (SREBP), fatty acid synthase (FASN), and acetyl CoA carboxylase (ACC). These factors and enzymes convert metabolic intermediates, such as citrate derived from TCA cycle or glycerol derived from glycolysis, into triacylglycerols and phospholipids, which are essential components of cellular structures [111, 113, 115]. FAS supports immune cells in carrying out their functions effectively. For instance, it has been found that FAS is necessary for differentiation and inflammatory responses of M1 macrophages [106, 101, 114]. The up-regulation of FAS is also found to be crucial for the

activation of DCs mediated by Toll-like receptors (TLRs) and their stimulation of CD8 + T cell responses [116]. Furthermore, the synthesis of fatty acids and sterols is vital for the efficient proliferation and functionality of CD8+ T cells and B cells following their activation through antigen receptors [117, 118].

The last immunometabolic pathway is **Amino Acid Metabolism** which has a critical role in immune cells not only as primary units for protein synthesis but also in contributing to other metabolic pathways in fueling them for ATP generation and supporting the nucleotide biosynthesis and redox balance. Beyond protein synthesis and increasing the biomass, amino acids metabolism plays an important role in supporting various effector functions and responses of immune cells [119] (Figure 1). Illustratively, **Glutamine** has an important role in the cytotoxic and antimicrobial functions of macrophages and adequate supplies of glutamine are also required for IL-1β secretion by M1 [LPS+IFNγ] macrophages in response to LPS stimulation. Glutamine is also required in promoting M2 macrophage polarization in response to IL-4 stimulation, but not in the case of the development of LPS-stimulated M1 macrophages [120]. Increased glutamine metabolism is required for regulating the responses of T cells [121, 122] and B cells [123] upon activation by antigen receptor stimulation. In T cells, but not in B cells, glutamine metabolism might have a role in controlling reactive oxygen species (ROS) levels resulting in hypoxic stress [123].

In the context of NK cells, research has focused on the role of glutamine and amino acid transporters, while further investigation is needed to understand the involvement of other amino acids in NK cell functions. Glutamine serves as a significant energy source for metabolically active cells by contributing to the TCA cycle through a process called “Glutaminolysis”, which fuels OxPhos. However, while glutamine does enter the TCA cycle via glutaminolysis, this glutamine-dependent TCA cycle is not essential for maintaining elevated levels of OxPhos in activated NK cells. Additionally, researchers revealed a crucial role of glutamine transported by SLC7A5, in activated NK cells through the regulation of cMyc expression, which acts as a vital metabolic regulator in controlling NK cell growth and effector responses [124] (Figure 6). Further, IL-12 stimulated NK cells have been found to increase the expression of CD25, high affinity IL2-receptor, and up-regulate amino acid transporters including SLC7A5, SLC1A5, and SLC3A2, upon stimulation with IL-2 [124, 32]. Blocking the transporters, SLC1A5 and

SLC3A2, has been shown to decrease IFN-γ production and degranulation that are mediated by NKG2D activating receptor [32]. In addition, stimulated NK cells with IL-18 have also been shown to promote the expression of SLC7A5/SLC3A2 and facilitate NK cell proliferation through the mTORC1 pathway [143].

Arginine, an essential amino acid, plays a crucial role as a precursor for various potent immunomodulatory metabolites, including nitric oxide (NO) and polyamines. l-arginine serves as a substrate for four main enzymes: NO synthases (NOSs), arginases (ARGs), glycine aminidotransferase, and l-arginine decarboxylase [125]. The metabolic pathway involving arginine in macrophages has significant effects on their function. Macrophages utilize arginine in two distinct metabolic pathways, the nitric oxide synthesis pathway and the arginase pathway, which define classically (M1) and alternatively (M2) activated macrophage subsets, respectively [126]. M1 macrophages direct arginine towards the nitric oxide synthesis pathway mediated by inducible nitric oxide synthase (iNOS), enabling them to perform their inflammatory functions against bacteria and tumor cells [127]. On the other hand, arginine used through the arginase pathway in M2 macrophages, that needed in the series of events for wound healing and tissue repair. In this pathway, arginase-1 converts arginine into L-ornithine which is further broken down to produce polyamines and L-proline. These compounds support macrophage growth and division, and also to provide essential building blocks for collagen production [127, 128]. Notably, arginase activity has been found to be positively correlated with disease severity in conditions such as visceral leishmaniasis and HIV infection [129]. Activated T cells increase arginine metabolism to enhance T cell survival [130] and to promote the proliferation of human T cells [131] as well as to regulate the expression of components of the T cell receptor [132]. The availability of L-arginine modulates the phenotypic and functional properties of NK cells, influencing their cytotoxicity and proliferation [133].

Tryptophan is an essential amino acid that plays a key role in modulating the immune response and its availability is an important factor in controlling protein biosynthesis and other important metabolic functions [134, 135]. The immune system utilizes tryptophan catabolism as a means to restrict the growth of pathogens and malignant cells by depriving them of a necessary substrate for anabolic growth. Consequently, tryptophan depletion has gained interest as an antipathogenic or antitumor response in numerous studies [135, 136]. Considerable research on the

immunoregulatory role of tryptophan metabolism in the immune system has focused on the role of the enzyme "Indoleamine-2, 3-dioxygenase (IDO)". IDO is expressed in various tissues and catalyzes the initial and rate-limiting step in tryptophan breakdown within the kynurenine pathway [136]. It was shown in a study of the role of tryptophan metabolism in the immune system that tryptophan is required for T cell proliferation in vitro [137], and driving IDO expression and tryptophan catabolism in antigen-presenting cells dampen T cell stimulation [138]. IFN-γ, a proinflammatory cytokine, induces tryptophan catabolism by promoting the expression of IDO during an immune response [136]. Moreover, metabolites generated from tryptophan catabolism, such as kynurenine, may play important roles in modulating immune cell function through the activation of the aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor [139]. Enhanced tryptophan metabolism resulting from increased IDO expression has been observed in both tumor cells and tumor-associated stromal cells, contributing to the propensity of tumor cells to evade antitumor immunity in many types of tumors [140]. Studies have also demonstrated that IDO can influence the function of NK cells. Illustratively, l-kynurenine, a catabolite of tryptophan produced by IDO activity has been found to hinder the cytokine-mediated up-regulation of the expression and function of specific triggering receptors responsible for the induction of NK-cell-mediated killing. Specifically, l-kynurenine has an impact on restricting NKp46 and NKG2D receptors, while it does not affect other surface receptors such as NKp30 or CD16. Consequently, NK cells treated with l-kynurenine exhibit impaired ability to eliminate target cells recognized via NKp46 and NKG2D, but they retain the ability to kill targets such as DCs that are primarily recognized via the NKp30 receptor. The inhibitory effect of l-kynurenine, which occurs at both the transcriptional and the protein levels, can be reverted, as NK cells were found to regain their functional competence after washing [141]. IDO was found to have the ability to inhibit the proliferation of CD4+T and CD8+T lymphocytes and NK cells, while the proliferation of B lymphocytes was not affected [142].

Methionine is an essential amino acid that serves multiple functions in cells, including protein synthesis and providing methyl groups for various biochemical processes. Once inside the cells, methionine regulates various cellular functions, particularly within the nucleus. Methionine is converted to S-adenosylmethionine (SAM) through the methionine cycle, which is important for processes such as folate metabolism, redox balance, polyamine synthesis, and methylation

reactions [144] (Figure 5). Recent studies have also shown that methionine contributes to DNA and RNA methylation, and promotes T-cell differentiation and proliferation. The uptake of methionine by T cells is mediated by the methionine transporter SLC7A5, which becomes upregulated upon T cell activation [145]. This transporter is considered the limiting factor for methyl group generation during T cell activation. However, tumor cells can also import methionine through transporters like SLC43A2, allowing them to outcompete T cells for limited methionine availability [146].

Methionine restriction can affect histone methylation patterns and gene expression involved in Th17 cell proliferation and cytokine production. Methionine-deficient diets have been shown to reduce the expansion of pathogenic Th17 cells and mitigate T cell-mediated inflammation [147, 148]. In addition, the mice on the methionine restriction diet exhibited a decrease in the cytotoxicity of NK cells in the spleen compared to the mice on the basic diet [149].

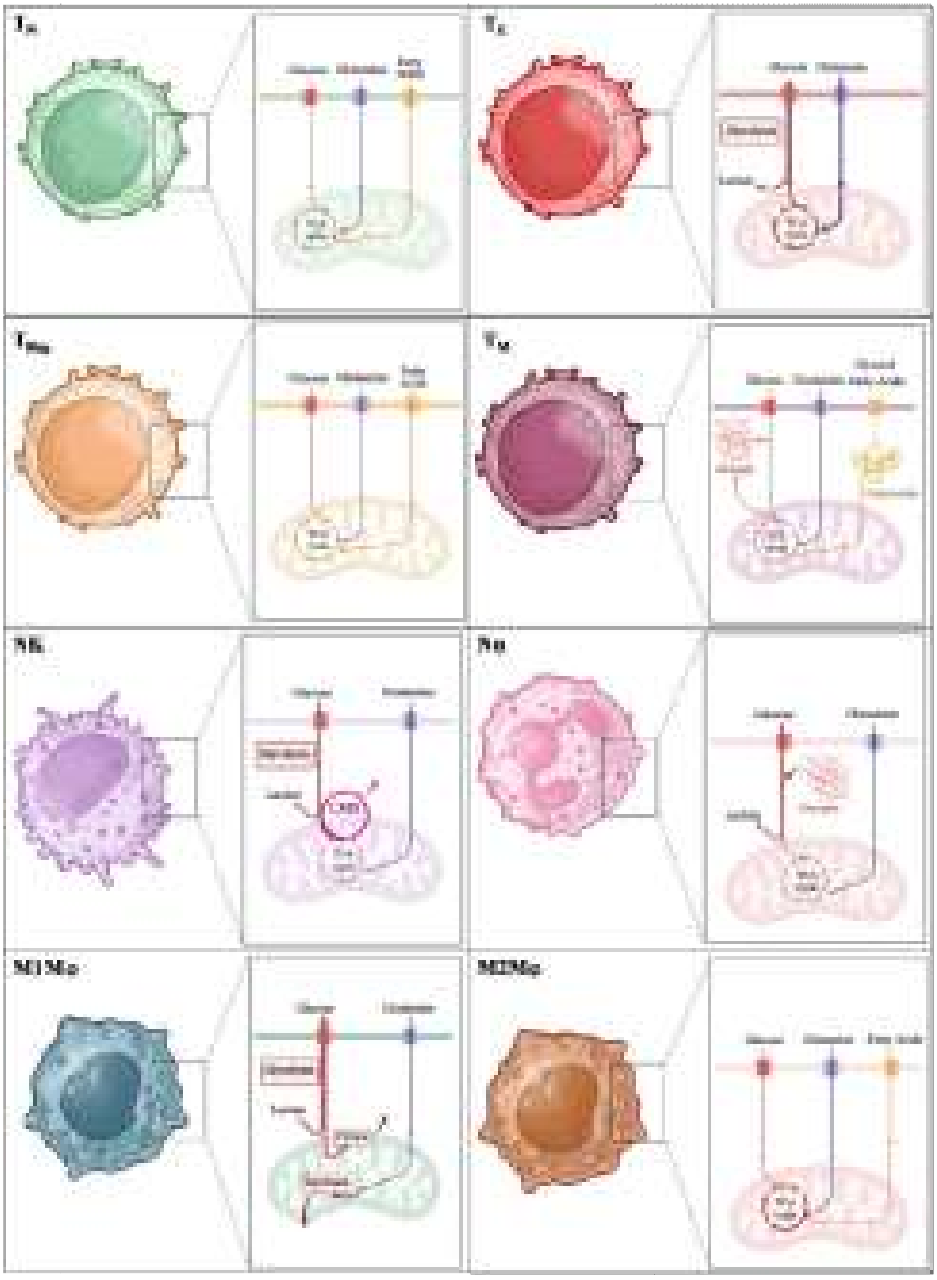
In the context of tumors, competition for methionine can lead to reduced intracellular levels of methionine and SAM in CD8+ T cells. This, in turn, affects histone methylation and gene expression related to immune function, attenuating the anti-tumor capabilities of T cells [146]. Elevated SAM levels have been found to reprogram the chromatin accessibility of CD8+ T cells, impairing their anti-tumor function [150]. Further, the transporter SLC43A2 also plays a critical role in the survival of regulatory T cells (Tregs). Decreased expression of SLC43A2 reduces methionine uptake, leading to increased apoptosis of T-regs [151]. In macrophages, methionine has been reported to have several effects on their immune response. It reduces the activation of the mitogen-activated protein kinase (MAPK) signaling pathway and decreases the production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interferon-beta (IFN-β) following treatment with lipopolysaccharide (LPS). Additionally, methionine enhances the levels of intracellular S-adenosylmethionine (SAM), a molecule involved in various cellular processes including DNA methylation. Similar anti-inflammatory effects were observed with SAM treatment, supporting the role of methionine in modulating the immune response. Furthermore, methionine treatment resulted in increased global DNA methylation in macrophages. The anti-inflammatory effects of methionine were partially reversed when macrophages were treated with 5-aza-2'-deoxycytidine, an inhibitor of DNA

methyltransferase, suggesting that DNA methylation is involved in the mechanism by which methionine exerts its anti-inflammatory effects [152].

Serine is considered a non-essential amino acid; however, plays important roles in various metabolic processes, including protein and glutathione synthesis. It also acts as a crucial donor of one-carbon units for the folate cycle, which is involved in nucleotide synthesis, methylation reactions, and the generation of NADPH [153, 154] (Figure 5). In activated T cells, the rate of serine synthesis increases following antigenic stimulation, which provides intracellular glycine and one-carbon metabolites necessary to support T cell proliferation [155]. Another study discovered that during rapid T cell proliferation, naïve T cells induce the reprogramming of mitochondrial biogenesis and remodeling. Among the various pathways induced, one-carbon metabolism, which is fueled by serine, was found to be the most highly upregulated pathway. Disrupting the key enzyme involved in mitochondrial serine metabolism, called serine hydroxymethyltransferase (SHMT2), was found to inhibit T cell expansion [156].

Serine in the TME has been found to activate mTOR, which in turn inhibits the expression of FOXP3, a key transcription factor involved in the function of regulatory T cells (Tregs). This inhibition of FOXP3 expression hinders the immunosuppressive capacity of Tregs. However, the conversion of serine to glutathione, facilitated by the enzyme glutamate cysteine ligase (Gclc), has been shown to preserve the immunosuppressive function of Tregs. When Gclc is absent, leading to a depletion of glutathione, it results in autoimmunity and enhanced anti-tumor immune responses [157].

Figure (2): Distinctive Metabolic Features of Immune Cell Subtypes.



Adapted from [159].

Immune cell subtypes exhibit diverse metabolic configurations, with variations in the utilization of glucose, glutamine, fatty acids, and different metabolic pathways such as Glycolysis, OxPhos, and TCA cycle. These metabolic differences contribute to the functional characteristics and responses of immune cells. **Naïve T cells (T_N)** have low metabolic rates and rely on glucose and glutamine as fuel sources. **Effector T cells (T_E)** have increased levels of both aerobic glycolysis and OxPhos fueled by glucose and glutamine. **Memory T cells (T_M)** store fuel in the form of glycogen and triacylglycerides, utilizing glucose and fatty acid uptake. They primarily rely on OxPhos rather than glycolysis. TM cells exhibit metabolic flexibility, engaging in various opposing metabolic pathways. **Regulatory T cells (T_{Reg})** import fatty acids for biosynthesis and energy generation through fatty acid oxidation. In other immune cells, **Natural Killer (NK) cells** primarily utilize glucose for aerobic glycolysis and OxPhos via the citrate-malate shuttle (CMS). **M1 macrophages (M1Mφ)** break the tricarboxylic acid (TCA) cycle, metabolizing glucose to lactate and citrate, while glutamine is metabolized to succinate. **M2 macrophages (M2Mφ)** maintain an intact TCA cycle and rely on oxidative metabolism fueled by fatty acids, glutamine, and glucose. **Neutrophils (Nφ)** primarily rely on glycolysis and have minimal OxPhos, utilizing both glucose uptake and internal glycogen stores.

2.2. The Interplay between Signaling Pathways and Metabolic Pathways in Immune Cells:

Immune cells in their resting state exhibit minimal metabolic activity that is essential for maintaining basic cellular homeostasis. However, when immune cells are stimulated, their energy and biosynthesis requirements increase substantially. This activated state necessitates metabolic reprogramming, enabling immune cells to meet their energy needs and generate cytokines crucial for an effective immune response. Consequently, the effective functioning of immune cells relies on their capacity to swiftly and efficiently adjust their metabolism in response to various stimuli. Metabolic reprogramming in response to immune cell activation involves a precise network of signaling pathways that regulate the transition between anabolic and catabolic processes [158-161]. Different immune cell subsets exhibit distinct metabolic strategies and signaling pathways upon activation, leading to variations in their metabolic profiles and specific demands for nutrients [159, 161] (Figure 2).

Innate immune cells, such as macrophages and dendritic cells play a crucial role in the initial response to pathogens. They employ various signaling pathways, including toll-like receptors (TLRs) and cytokine receptors, for coordinating their metabolic responses to different stimuli [161, 162]. Among these pathways, the **mammalian target of rapamycin (mTOR) pathway** is particularly important in regulating the metabolic response of innate and adaptive immune cells. Activation of the mTOR pathway in macrophages and NK cells promotes glycolysis and the production of pro-inflammatory cytokines [163] (Figure 3).

On the other hand, adaptive immune cells like T cells and B cells require metabolic reprogramming to meet the energy demands during activation and proliferation. These cells rely on specific signaling pathways to transition from a quiescent to an activated state [164]. The **PI3K-AKT-mTOR pathway** is a key player in the metabolic response of T cells. It facilitates glucose and amino acid uptake, as well as ATP production and biomass generation for cell growth [165, 166]. Additionally, the **AMPK pathway** is critical in T cell metabolism, particularly under conditions of low energy availability or stress. AMPK promotes catabolic pathways such as fatty acid oxidation and autophagy to generate ATP and maintain cellular homeostasis [165-168] (Figure 3).

2.2.1. The mTOR pathway:

mTOR is a serine/threonine kinase that forms two distinct complexes in functions: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [169-174]. **mTORC1** is relatively sensitive to rapamycin, while **mTORC2** is relatively resistant to it [170, 175]. Both complexes share common components, including the mTOR kinase, mLST8 (a scaffolding protein), DEPTOR (an mTOR regulatory subunit), and the Tti1/Tel2 complex, which is important for their assembly and stability. However, each complex also has unique subunits that contribute to substrate specificity, subcellular localization, and complex-specific regulation. **Raptor** and **Rictor** are the defining components of mTORC1 and mTORC2, respectively [173, 174] (Figure 3). mTORC1 is characterized by its association with Raptor [176], a scaffolding protein that plays a role in mTORC1 assembly, stability, substrate specificity, and regulation. It also involves PRAS40, a factor that inhibits mTORC1 activity until relieved by growth factor receptor signaling. The structure of mTORC1 forms a lozenge-shaped dimer, with the kinase domains close at the center of the structure and Raptor and mLST8 binding on the periphery. These complexes regulate cell growth, proliferation, and metabolism and play crucial roles in immune cells.

mTORC1 activation involves the canonical PI3K-PDK1-AKT-TSC1/2-mTORC1 pathway. While less is known about the upstream activator of mTORC2, PtdIns (3,4,5) P3, generated by PI3K, has been identified as a critical factor [177]. mTORC2 is defined as phosphorylates AKT at Serine473, enhancing its kinase activity [178] (Figure 3).

The mTOR pathway is also involved in regulating NK cell metabolism and function. It promotes NK cell proliferation and enhances their ability to produce cytokines and exert cytotoxicity (Figure 6). Inhibition of the mTOR pathway can impair NK cell function and reduce their anti-tumor activity [24, 26, 143].

Both mTORC1 and mTORC2 are necessary for NK cell activity, but they operate through different mechanisms. While mTORC1 is involved in early NK cell development, mTORC2 may function during terminal maturation, and their functions may be complementary rather than redundant [179, 180]. Specifically, mTORC1 sustains mTORC2 activity through IL-15 stimulation mediated by CD122 [180]. Furthermore, researchers found that mouse NK cell

education is associated with increased basal activity in the mTOR/AKT pathway, which corresponds to the number of educating receptors. This heightened activity relies on the SHP-1 phosphatase, an enzyme necessary for maintaining optimal NK cell reactivity, and it is crucial for the improved response of educated NK cells. The mTOR/AKT pathway upon stimulation enhances signaling by activating NK cell receptors. Inhibiting mTOR pharmacologically results in a proportional decrease in NK cell reactivity. Conversely, acute cytokine stimulation restores the reactivity of less responsive NK cells by activating mTOR. These findings demonstrate that mTOR functions as a molecular regulator of NK cell reactivity, modulated by educating receptors [181].

In summary, immune cells employ different signaling pathways to coordinate their metabolic responses and maintain cellular balance. The mTOR pathway is crucial in innate immune cells, while the PI3K-AKT-mTOR and AMPK pathways are essential for metabolic reprogramming in activated T cells. Nutrient availability plays a significant role in shaping the fate and functionality of immune cells, and these signaling pathways interact with immunometabolism to regulate immune cell biology and function (Figure 3).

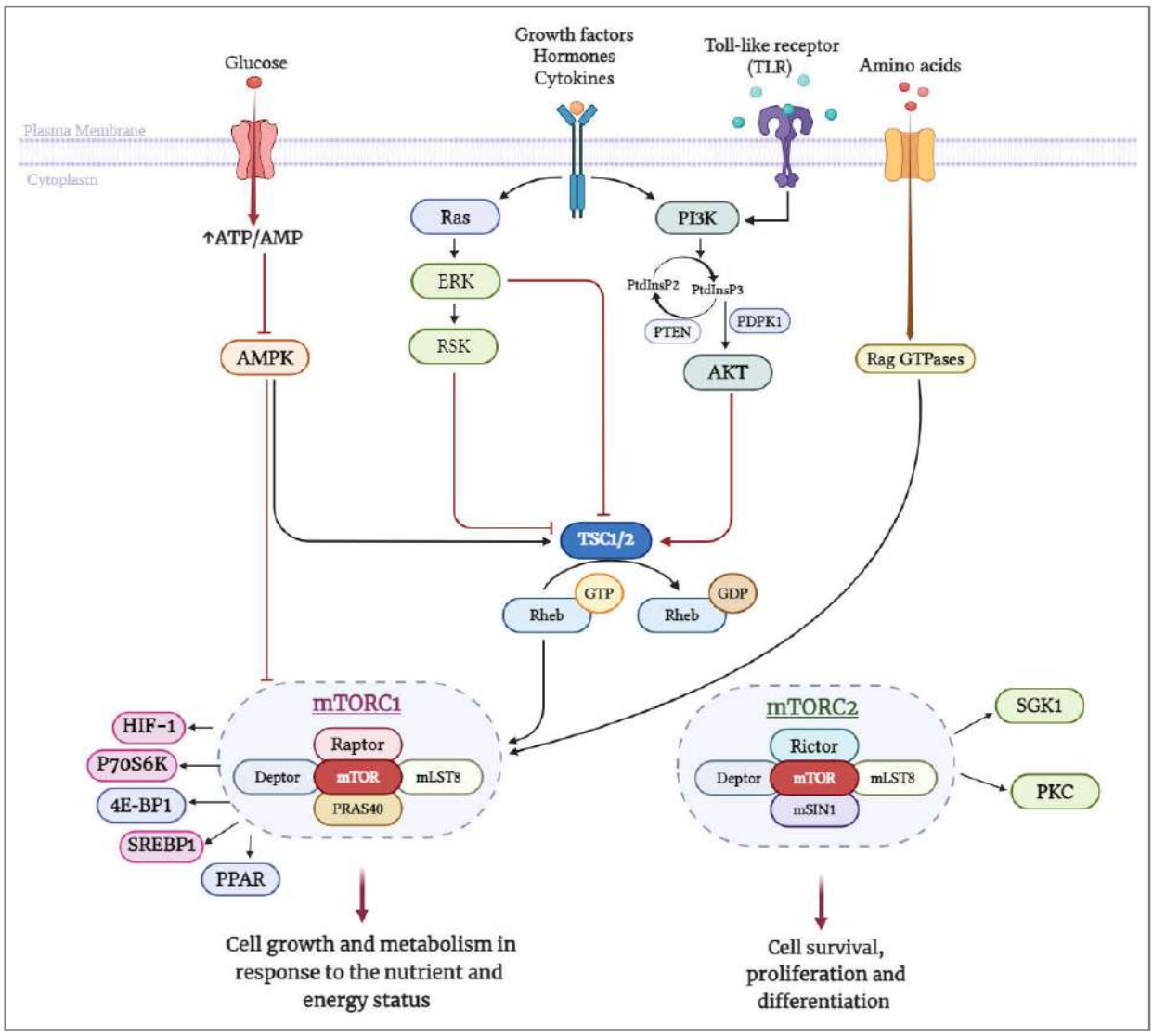


Figure (3): mTOR signaling pathway in innate immune cells. Adapted from [163]

The mTOR pathway is a central signaling pathway that integrates different signals and coordinates cellular processes such as metabolism, proliferation, and immune responses. The figure illustrates that mTOR activation can occur through multiple upstream signals, including growth factors, cytokines, Toll-like receptor (TLR) ligands and nutrient availability. Once activated, mTOR regulates the activity of downstream effectors such as S6K1 (ribosomal protein S6 kinase 1) and 4EBP1 (eukaryotic translation initiation factor 4E-binding protein 1), which control protein synthesis and cell growth.

2.3. Metabolism in Tumor Microenvironment (TME):

Tumor microenvironment (TME), also known as the tumor stroma, is a complex and diverse medium surrounding tumor cells. TME includes various cells interacting with each other, paracrine signaling molecules released in the vicinity, and extracellular matrix (ECM) as well as newly formed blood vessels and a slew of secreted factors generated by tumor and non-tumor cells [182, 185]. The genesis of cell diversity within the TME of solid tumor is derived from the surrounding host tissues and could be hematopoietic or mesenchymal. The hematopoietic cells in TME comprise subsets of tumor-infiltrating immune cells from both the innate and adaptive immune systems, such as T cells, neutrophils, B cells, NK cells, macrophages, and DCs, while The mesenchymal component of the TME consists of fibroblasts, adipocytes, endothelial cells, and pericytes (also known as Rouget cells) [183-185].

The interaction between cells in the TME influences oxygen availability, thus affecting cellular metabolism and anti-tumor activity of infiltrated immune cells [38, 186]. The various heterogeneous tumor-infiltrated immune cell types work in a coordinated manner targeting tumor antigens. Their differentiation, proliferation and effector functionality are regulated by multiple signals influenced by the metabolic activity within the TME [38, 43, 187].

Solid tumors create a hostile microenvironment characterized by a consistent decrease in oxygen levels and a lack of nutrients delivered through the bloodstream. Consequently, hypoxia, acidosis, nutrients deprivation and metabolites accumulation are critical characteristics of the TME [185].

Cancer cells and immune cells have similar metabolic requirements, making the TME as a battleground where they compete for nutritional and energy resources, and strive to adapt and survive amidst the continuously changing microenvironment [37, 38, 43, 186]

2.3.1. Cancer Cell Metabolism Shaping the TME:

Cancer cells exhibit uncontrolled growth and metabolic changes that enable their survival, rapid proliferation, evasion of immune surveillance, and metastatic dissemination. These cancerous behaviors arise as a consequence of multiple genetic mutations and epigenetic modifications which drive metabolic reprogramming to support biosynthesis, and energy generation. The accelerated and aberrant growth of tumor cells leads to increased consumption of oxygen and nutrients, as well as the induction of angiogenesis in the TME, leading to develop a pro-tumorigenic milieu that favors the survival of tumor cells. Thus, the TME undergoes a gradual depletion of oxygen and metabolic precursors from the periphery towards the center of the solid tumor [185].

Metabolic reprogramming in cancer cells is mainly represented by elevated glycolysis which converts glucose to lactate even in the presence of sufficient O_2 supply which is known as aerobic glycolysis (Warburg phenotype). Therefore, reduced blood perfusion, hypoxic stress, and preference for the use of glycolysis rather than TCA cycle and OxPhos by the cancer cells for their energy needs, result in increased lactic acid production then decreasing the extracellular pH and creating acidosis in the TME. Acute acidosis suppresses cancer cell proliferation and increases apoptosis, however, chronic acidosis acts as a selective pressure leading to multiple genomic mutations that benefit cancer cell growth and adaptation within the new milieu. This remodeling of the TME fosters tumor growth, proliferation, and metastasis, enhancing invasiveness and providing protection against immune interventions [40, 41, 188] (Figure 4).

Multiple mutations in the genes encode metabolic enzymes in the TCA cycle that have been found in many types of cancer which lead to aberrant accumulation of metabolites in the TME, termed oncometabolites. Oncometabolites have been emerged as novel hallmarks in cancer cells which play crucial roles in neoplastic transformation, cancer metabolism, tumor aggressiveness, and resistance to therapy [189-192]. The abnormal buildup of oncometabolites has serious repercussions on cellular processes downstream, resulting in substantial alterations to metabolic regulation within cancer cells and further modifying the characteristics of the TME. Among these oncometabolites, **succinate** is a TCA cycle metabolite that has been associated with cancer initiation and progression [193] (Figure 4).

Emerging research has revealed succinate involvement in various cellular processes and its potential impact on cancer development and progression. **Succinate accumulation** in the mitochondrial matrix and extracellular spaces can be a result of various causes including; inhibition or dysfunction of succinate dehydrogenase (SDH), and a hypoxic environment. This accumulation, in turn, can disrupt cellular processes, including epigenetic regulation and cellular signaling pathways. For instance, succinate has been implicated in the regulation of hypoxia-inducible factor (HIF) signaling. Succinate accumulation can stabilize HIF-α subunits, leading to the activation of HIF-dependent pathways involved in angiogenesis, cellular metabolism, and tumor growth. However, it is important to note that the role of succinate in cancer is complex and context-dependent. Its effects can vary depending on the specific tumor type, genetic alterations, and microenvironmental conditions [46, 193, 194]. Moreover, succinate has been linked to inflammation and immune responses within the TME and is considered a key metabolic factor in cancer–immune cycle. It can modulate the production of pro-inflammatory cytokines and influence immune cell function, potentially impacting tumor progression and the anti-tumor immune response [45-50, 52, 53].

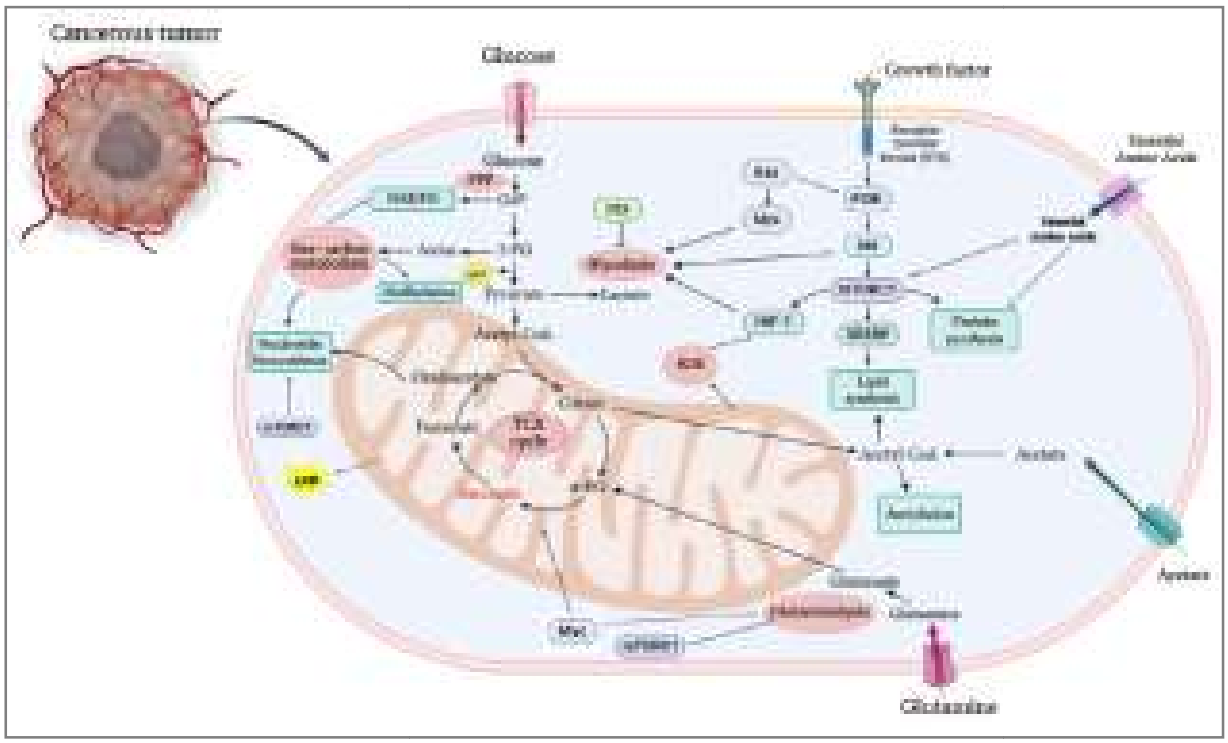


Figure (4): The Metabolic Signaling Pathway in Cancer Cells. Adapted from [321]

Metabolic alterations in cancer cells are highly heterogeneous and can vary depending on the tumor type, stage, and microenvironment. Cancer cells exhibit metabolic plasticity, allowing them to adapt to changing nutrient availability and environmental conditions. **The key features of cancer metabolism**, demonstrate the metabolic adaptations that support the unique energy and biosynthetic requirements of cancer cells: **1.** Cancer cells exhibit a metabolic shift towards increased glycolysis, even in the presence of oxygen. This phenomenon, known as the Warburg effect, allows cancer cells to produce energy (ATP) and generate metabolic intermediates for biosynthesis. **2.** Cancer cells upregulate glucose transporters, such as Glut-1, to facilitate increased glucose uptake from the extracellular environment. This provides a constant supply of glucose for glycolysis. Cancer cells preferentially metabolize glucose through Aerobic glycolysis, producing lactate. This metabolic adaptation helps generate ATP quickly and supports the increased biosynthetic demands of cancer cells. **3.** Cancer cells exhibit changes in mitochondrial metabolism. While some tumors show reduced mitochondrial activity, others maintain active mitochondrial function to support various metabolic pathways, including OxPhos and TCA cycle. **4.** Cancer cells divert glycolytic intermediates, such as glucose-6-phosphate (G6P), into biosynthetic pathways to produce nucleotides, lipids, and amino acids necessary for cell proliferation and growth. **5.** Cancer cells also support their growth and biosynthesis by metabolizing glutamine in glutaminolysis which provides carbon and nitrogen for the synthesis of macromolecules. **6.** Cancer cells exhibit dysregulated lipid metabolism, including increased *de novo* lipogenesis and enhanced uptake of fatty acids. These processes provide building blocks for membrane synthesis and energy storage. **7.** Cancer cells exhibit altered regulation of ROS. While increased ROS levels can induce DNA damage and senescence, cancer cells develop mechanisms to counteract excessive ROS, allowing them to survive and proliferate. **8.** Cancer cells rewire amino acid metabolism to meet their increased demands for protein synthesis, energy production, and redox balance. Amino acids, such as glutamine, serine, and glycine, play critical roles in cancer cell metabolism.

Besides, metabolic reprogramming in cancer cells also depends on the **Folate-mediated one-carbon metabolism (FOCM)** that provides the precursors and intermediates to support the high demand for macromolecules synthesis (amino acids, nucleotides, fatty acids) and epigenetic modifications that are needed for cancer cells rapid growth and proliferation [153, 154, 195-198]. FOCM encompasses a set of interconnected metabolic pathways that involve the transfer of one-carbon units, typically in the form of methyl groups, for various biosynthetic reactions and cellular processes (Figure 5). It contributes to the synthesis of nucleotides for DNA and RNA production, amino acids and protein synthesis, the generation of S-adenosylmethionine (SAM) for methylation reactions involved in epigenetic regulation, the production of critical energetic metabolites such as ATP, as well as the maintenance of redox balance. Therefore, this metabolism acts as a central hub that integrates nutrient availability and metabolic demands to ensure proper cellular physiology. Its function as an integrator of nutrient status highlights its crucial role in maintaining cellular homeostasis and adapting to changing environmental conditions [63, 199] (Figure 5).

Nutrients such as folate, choline, methionine, and vitamins B_2, B_6 and B_{12} play essential roles in providing the necessary one-carbon units and cofactors for these metabolic pathways. The availability and balance of these nutrients influence the activity and efficiency of **one-carbon metabolism** (Figures 4 and 5). The activity of FOCM is firmly regulated and responds to changing nutrient availability and cellular demands. When nutrient levels are sufficient, FOCM can support cellular growth, proliferation, and the synthesis of biomolecules. Conversely, under nutrient-limiting conditions, it can be downregulated to conserve resources and prioritize essential cellular functions [195-200]. One of the consequences of altered metabolic pathways in cancer is the disruption of one-carbon metabolism. The abnormal activity of glycolysis and the Warburg effect not only lead to increased glucose consumption and lactate production but also affect the availability and utilization of key metabolites, including folate [201, 208] (Figure 5).

In tumors, the demand for nucleotides, DNA and RNA synthesis, and methylation reactions is high due to the rapidly dividing cancer cells. **Folate**, as a critical component of FOCM, plays a pivotal role in these cellular processes. However, the dysregulation of folate metabolism in cancer cells can have profound effects on DNA synthesis, methylation patterns, and nucleotide availability [197, 199-202].

The altered activity of enzymes involved in folate metabolism, such as dihydrofolate reductase (DHFR), thymidylate synthase (TS), and methylenetetrahydrofolate reductase (MTHFR), can lead to imbalances in the production of key metabolites required for DNA replication and methylation reactions. These imbalances can result in genomic instability, aberrant DNA methylation patterns, and impaired nucleotide synthesis, all of which are associated with cancer development and progression [203,204]. Moreover, disrupted folate metabolism can have implications beyond cancer cells themselves. The TME, characterized by hypoxia and acidosis, can further impact folate metabolism. Hypoxia, resulting from inadequate oxygen supply, can influence the activity of folate transporters (such as RFC) and enzymes involved in folate metabolism and alter the availability of folate derivatives, affecting DNA synthesis and methylation reactions in both cancer and environing cells [205, 206].

Furthermore, acidosis in the TME can affect the uptake and utilization of folate by cancer cells and immune cells. The acidic pH can interfere with the transporters responsible for folate uptake such as RFC and PCFT, leading to altered folate availability and metabolism [223, 226].

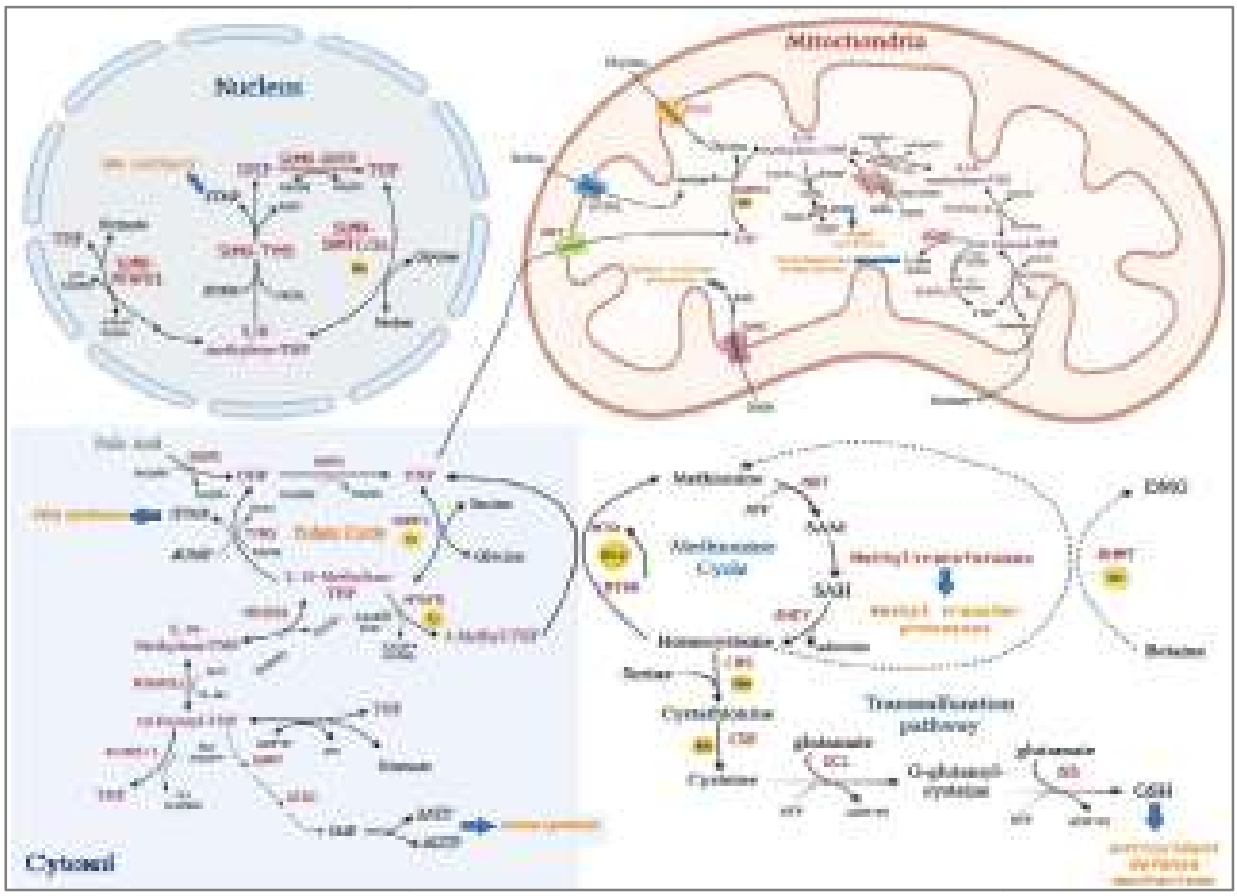


Figure (5): Folate-Mediated One-Carbon Metabolism (FOCM). Adapted from [322]

Folate-mediated one-carbon metabolism (FOCM) is a complex set of biochemical reactions that involve the utilization of folate and its derivatives to transfer one-carbon units for various cellular processes. The key steps involved in FOCM: Cellular uptake of dietary folate or folic acid (FA) is mediated by three distinct transporters; reduced folate carrier (RFC), folate receptors (FRs) and proton-coupled folate transporters (PCFT). Folate or FA is converted to dihydrofolate (DHF) and then converted to tetrahydrofolate (THF) by dihydrofolate reductase (DHFR) reaction. This step is essential for recycling THF and maintaining a steady supply for further one-carbon transfer reactions.THF serves as a coenzyme and carrier of one-carbon units. It can accept or donate one-carbon groups in various forms, such as methyl (CH3), formyl (CHO), or methylene (CH2) groups. One-carbon units carried by THF can participate in various other metabolic pathways, including the synthesis of certain amino acids, purines, and methylation reactions involved in the regulation of gene expression. One-carbon units carried by THF, usually in the form of 5-methyl-THF, are utilized in methylation reactions. For example, 5-methyl-THF can donate its methyl group to homocysteine, converting it back to methionine. Methionine is an essential amino acid and serves as a precursor for protein synthesis. 10-formyltetrahydrofolate (10-FTHF), another derivative of THF, plays a crucial role in the synthesis of purines and thymidylate, which are essential components of DNA and RNA. Glycine, another amino acid derived from THF, can be converted to serine. Serine is involved in numerous cellular processes and serves as a precursor for the synthesis of other important molecules. Glycine can also enter the glycine cleavage system, where it is converted to CO_2, NH_3, and a one-carbon unit in the form of N^5, N^10-methylenetetrahydrofolate (5,10-methylene-THF).

Folate is the generic term that refers to an essential group of water-soluble B_9 vitamin derivatives.

Naturally, folate is found in leafy green vegetables, yeast, and legumes as well as in some animal sources (e.g. liver and kidney) and dairy products [315]. The synthetic more stable form of folate, folic acid (FA), is a fully oxidized derivative (pteroylmono-glutamic acid) used in dietary supplements and food fortification. Folate found in food is polyglutamates, while FA is monoglutamate [315]. Consumed food folates and FA differ from each other in their bioavailability. FA in daily supplements has a higher absorption rate across small intestines than folates in natural and fortified food [316]. While food folates are hydrolyzed to the monoglutamate form in the intestines, before being absorbed by active transport across the intestinal mucosa, FA is rapidly absorbed by a passive diffusion mechanism [315]. Then, the monoglutamate form is reduced to tetrahydrofolate (THF) and converted to methyl or formyl forms. In humans, the main circulating folate form is 5- methyl-THF [317]. However, unaltered FA can also be found in the blood, known as unmetabolized folic acid (UMFA) [66]. Reduced Folates play a crucial role in regulating various intracellular metabolic processes, including; producing energy, epigenetic controlling and genome stability, biosynthesis and repairing of nucleic acids (DNA, RNA), proteins and phospholipid biosynthesis that are needed by proliferating cells.

Folate cycle (also known as; one-carbon (C1) metabolism) begins when dietary folate or FA is converted to an intermediate metabolite, dihydrofolate (DHF) then to THF by dihydrofolate reductase (DHFR). THF is the reactant in the folate cycle and is utilized as a source of intermediates in other metabolic pathways related to C1-metabolism [318, 319]. C1 metabolism provides many metabolic intermediates essential for other catabolism and biosynthesis pathways. For instance; 5-methylenetetrahydrofolate (5-MTHF), a methyl donor, is needed for remethylation of homocysteine to methionine. Methionine is an essential amino acid required for protein synthesis as well as for cellular methylation reactions through its conversion to S-adenosylmethionine (SAM). SAM is a cofactor required for many methyl transfer reactions including DNA and chromatin methylation that regulate gene expression. 10-Formyltetrahydrofolate (10-FTHF) is another important byproduct generated by folate metabolism, required for purine and thymidylate synthesis [317-320] (Figure 5).

2.3.2. Metabolism of Immune Cells:

Various immune cell types infiltrate the TME, and coordinate to recognize and eliminate cancer cells. The differentiation, proliferation and effector functions of tumor-infiltrated immune cells are regulated by several signals influenced by the metabolic activity in the TME [43, 187, 209, 213]. The metabolic characteristics of the TME play a crucial role in shaping the immune response against tumors. The metabolic cooperation and competition between tumor cells and immune cells can influence immune cell function and promote immune evasion by tumors [37, 38, 210-212]. Indeed, tumor cells exhibit high metabolic activity, and the surrounding cells in the TME are either engaged in metabolic competition or symbiosis with the tumor cells. This interplay between the different cell types in the TME leads to immune suppression due to immune cells exhaustion [38, 214-217]. Further, the hypoxic commitment in the TME creates a metabolic symbiosis between hypoxic and normoxic parts of TME [40]. Moreover, the distinctions between immune cell subtypes in energy and metabolic dependence for their activation and functionality, contribute to tumor immune escape [38, 39]. Illustratively, the hypoxic statement inhibits the anti-tumor activities of tumor-infiltrated immune cells such as M1 macrophages and cytotoxic T lymphocytes (CTLs) while promoting the activity of pro-tumoral immune suppressive cells such as Tregs, myeloid-derived suppressor cells (MDSCs), Tumor-associated macrophages (TAMs; M2) and tumor-associated neutrophils (TANs). These immune suppressive cells can utilize lactate produced by glycolysis to generate energetic molecules (ATPs) through OxPhos, leading to metabolic cooperation within the TME and sustaining metabolic adequacy of the tumor cells [39, 218, 219].

M1 macrophages primarily rely on glycolysis for ATP generation, while M2 macrophages utilize FAO and OxPhos for sustenance. This difference in metabolic dependence allows M2 macrophages to avoid competing with tumor cells for resources in a nutritionally challenged TME [62]. Additionally, lactate in the TME can induce the transformation of macrophages from an anti-tumor M1 phenotype to a pro-tumorigenic M2 phenotype through a pathway mediated by HIF1α. This process involves the upregulation of genes (e.g. VEGF) associated with M2-like macrophage functions [220-223]. M2 macrophages by secreting insulin-like growth factor 1 (IGF1), promote tissue regeneration and angiogenesis, potentially contributing to TME replenishment [224-226].

Similarly, T-regs preferentially utilize excess lactate by for oxidative metabolism compared to effector T cells [227]. Besides, quiescent T cells (Tregs and memory cells) prefer OxPhos and FAO for their metabolic needs [113, 228, 229]. Decreased glucose availability in TME impairs the anti-tumor function of CD8+ T (CTLs) cells which mainly rely on increased glycolysis upon activation while, in contrast, activated CD4+ T cells enhance both glycolysis and OxPhos [88, 230, 231]. Moreover, lactic acid production and consequent acidification in the TME can inhibit the proliferation and cytokine production of CTLs [232] and hinder the differentiation and activation of monocyte-derived DCs [233]. Additionally, lactate-mediated acidification and low pH in the TME can regulate macrophage polarization and induce arginase-I leading to arginine depletion leading to inhibit of the proliferation and activation of T cells [221, 234]. Furthermore, acidosis in the TME stimulates the activity of neutrophils while repressing the effector functions of NK cells and T cells [235].

In addition, amino acid metabolism, particularly arginine and tryptophan catabolism, is dysregulated in cancers [236, 237]. Increased activity of enzymes such as inducible nitric oxide synthase (iNOS) and arginase (ARG) in arginine metabolism can create toxic reactive nitrogen species that induce apoptosis in lymphocytes and T-cell tolerance in tumors. Altered arginine metabolism in the tumor can lead to local arginine deficiency, impairing protein synthesis in T cells and inhibiting cytokine production and effector function [238-242].

Many tumors lacking argininosuccinate synthetase 1 depend on exogenous arginine for growth, which can be provided by tumor-associated myeloid cells (TAMCs) such as MDSCs, macrophages, monocytes, and neutrophils. MDSCs, in particular, express high levels of arginase-1 and sequester cysteine, leading to amino acid deprivation and inhibition of T cell activation [243-246]. In contrast to the activated effector T cells, nutrient-restrictive TME does not affect the immunosuppressive functions of T-regs, since T-regs preferentially utilize lipid beta-oxidation and have high levels of activated AMP-activated protein kinase (AMPK) [88, 247]. Further, the metabolic products of tumor cells such as lactate and kynurenine are utilized for T-reg differentiation [86, 248]. Depletion of tryptophan in the TME results in T cell apoptosis and anergy. Increased indoleamine 2,3-dioxygenase (IDO) enzyme activity in tumor cells leads to tryptophan depletion and the accumulation of kynurenine, which inhibits immune cell proliferation and activation. IDO expression can be upregulated by the interaction between cytotoxic T-lymphocyte antigen-4 (CTLA-4) on Tregs and CD80/CD86 on DCs, inducing tumor

antigen tolerance [249-251]. The depletion of tryptophan and the accumulation of kynurenine also affect other immune cells, such as NK cells, by inhibiting their cytotoxic activity [250, 252]. Under hypoxic conditions, HIF1α gets activated and increases the expression of several glycolysis-related enzymes such as lactate dehydrogenase (LDH), pyruvate dehydrogenase kinase-1 (PDK1) [253], glucose transporter-1 (Glut-1) and 6-phosphofructo-2kinase/fructose-2,6-biphosphatase-3 (PFKFB3) [254]. Moreover, HIF1α is associated with immune escape mechanisms such as shedding of cell surface immune checkpoint regulators like; MHC class-I chain-related (MIC) molecule-1 (MIC1) which leads to the resistance of tumor cells to NK cell recognition and attack [255, 256].

In addition to the direct effects of hypoxia on intratumoral immune cells, hypoxia induces adenosine production in tumors which is indicated as an important regulator for immune cell activities in hypoxic TME [257, 258] and inflammation [259-261]. Tumor-produced adenosine can be a potent factor that inhibits the functional activity of tumor-infiltrated immune cells and assist tumor growth by neo-angiogenesis [261, 262].

2.3.2.1. Succinate and regulation of immune cell functions:

The accumulation of oncometabolites, such as succinate, in TME brings about significant modifications in the metabolism of tumor-infiltrated immune cells. These modifications can affect the ability of immune cells to mount effective anti-cancer responses, thereby creating a less hostile environment for cancer cells [189-192].

Succinate has emerged as a crucial player in regulating immune cell function and response. It acts as both a signaling molecule and metabolic intermediate, exerting influence over various aspects of immune cell biology. Succinate plays a multifaceted role in immune cell biology, influencing inflammation, macrophage activation, metabolic reprogramming, immune cell migration, and epigenetic regulation [263, 264]. Additionally, succinate has been shown to promote the production of pro-inflammatory cytokines in immune cells, such as interleukin-1 beta (IL-1β). It can activate the NLRP3 inflammasome, a complex involved in the production of pro-inflammatory cytokines, thereby contributing to the initiation and amplification of inflammatory responses [265-269]. Furthermore, succinate plays a role in macrophage polarization and activation. High levels of succinate induce a pro-inflammatory phenotype in

macrophages, characterized by increased production of inflammatory mediators and enhanced antimicrobial activity. This metabolic reprogramming is associated with the activation of succinate receptor-1 (SUCNR1) and subsequent downstream signaling events [270, 271].

Succinate has also been implicated in regulating immune cell migration. It can act as a chemoattractant, attracting immune cells to sites of inflammation or tissue injury. Succinate-induced chemotaxis is mediated through the succinate receptor SUCNR1 and can influence the recruitment and positioning of immune cells within tissues [50, 272].

Moreover, succinate accumulation can impact epigenetic modifications in immune cells. It can inhibit enzymes involved in histone and DNA demethylation, leading to alterations in gene expression and immune cell function [273, 274].

2.3.2.2. Folate metabolism and immune cell functions:

Similarly to cancer cells, activated immune cells undergo rapid proliferation and require metabolic rewiring. This rewiring is facilitated by the **FOCM network**, which provides the necessary biosynthetic support. Therefore, folate metabolism in immune cells is indispensable for crucial processes such as DNA synthesis, nucleotide production, DNA methylation, and antioxidant defense. These processes are vital for immune cell growth, division, and optimal immune cell function [275]. However, the exact molecular mechanisms and interplay between folate metabolism and immune cell functionality and metabolism require further investigation.

The available evidence suggests that folate status plays a role in modulating immune functions and adequate folate intake through diet or supplementation is crucial for supporting immune health. Illustratively, research has indicated that both folate deficiency and excessive folate intake can increase susceptibility to infections [59, 62] and risk of cancers [276-278]. Therefore, maintaining a balanced and appropriate folate intake is important for optimal immune function. Furthermore, studies have shown that folate status is associated with various disorders characterized by elevated activation of immune cell responses, such as Th1-mediated responses observed in neurodegenerative disorders like Alzheimer's disease and autoimmune diseases like rheumatoid arthritis [279]. Impaired folate metabolism has also been linked to the development of atopic diseases, which involve an allergic Th2-type immune response [280].

Studies conducted *in vitro* have reported that folate deprivation can hinder the proliferation and nucleic acid biosynthesis of human CD8+T cells. Additionally, highly proliferative murine CD4+T cells increase the expression of folate receptor 4 (FR4 or FRδ), which is a high-affinity folate transporter, and is found to be associated with the effector memory traits of CD4+T cells [281]. Similarly, *in vivo* studies have shown that a folic acid deficient diet, blocking the FR4, or inhibiting folate metabolism through methotrexate treatment resulted in a significant decrease in colonic Foxp3+ FR4+ Treg cells [282]. Furthermore, vitamin B9 (folate) was found to be necessary for the survival of differentiated Treg cells but not for the differentiation of naïve T cells into Treg cells. In addition, mice fed with an FA-deficient diet exhibited a higher susceptibility to intestinal inflammation and failed to control ongoing immune responses [283].

Further, several studies have demonstrated that folate status and metabolism also influence the functions of macrophages and DCs. For example, folate deficiency impairs the maturation process and functions of bone marrow-derived DCs in mice, resulting in a reduction in the activity of dendritic cell-induced helper T cells [284].

Moreover, the expression of folate transporters also differs between macrophage subtypes. M1 macrophages display a higher expression of RFC, whereas FR and PCFT are preferentially expressed by anti-inflammatory and homeostatic M2 macrophages [285-288].

Studies have shown that folic acid can improve the inflammatory response in macrophages and monocytes [289, 290]. Additionally, folate deficiency has been found to enhance pro-inflammatory signals in the monocyte-macrophage lineage by epigenetic mechanisms, potentially exacerbating cardiovascular disease [291-294].

Therefore, folate status and intact FOCM could be critical for the balance of effective immune functions and might be implicated in several disorders characterized by aberrant immune cell activation.

2.4. Natural Killer (NK) cells and Cancer:

2.4.1. NK cell overview and Effector Functions:

NK cells are characterized as innate cytotoxic lymphocytes which have the antiviral and anticancer capacity for eradicating infected and transformed cells. NK cells are critical for the direct killing of cells identified as non-self and have the potential to destroy malignant cells with altered expression of major histocompatibility (MHC) antigens [8].

NK cells have also important roles in immune responses, directing and cooperating with the adaptive immune system. For instance, they can regulate and shape T-cell responses [4, 5] and they can promote the recruitment of conventional type-1 dendritic cells (cDCs), which is critical for antitumor immunity, into solid tumors through the release of CC-chemokine ligand 5 (CCL5), XC-chemokine ligand 1 (XCL1) and XCL2 [6]. Besides, it has been found that tumor-infiltrating NK cells regulate tumor-promoting inflammation in vivo through functional modification of neutrophils by a mechanism dependent on IFNγ and C-X-C motif chemokine receptor 3 (CXCR3) [7].

Indeed, NK cells resemble cytotoxic CD8+T lymphocytes in their ability to exert direct cytotoxic responses and produce pro-inflammatory cytokines [4]. NK cells become activated when infected or malignant cells upregulate stress-induced activating ligands or downregulate MHC class-I molecules. Upon activation, NK cells rapidly produce cytotoxic molecules and secrete pro-inflammatory cytokines and chemokines to kill targets or modulate immune responses [8].

Activation and function of NK cells are regulated through a combination of activating and inhibitory receptors, and stress-induced ligand receptors. These receptors detect changes in the patterns of expression of their ligands on the surface of a broad range of target cells, cancer cells and viral-infected cells [3, 9, 10]. Activating receptors, such as killer cell lectin-like receptor K1, NKG2D, DNAX accessory molecule-1 DNAM-1, and natural cytotoxicity receptors NCRs (NKp46, NKp44, NKp30), recognize stress-induced ligands expressed on the target cells but scarcely expressed on normal cells. These activating receptors detect changes in ligand expression patterns and trigger NK cell activation, cytotoxicity and target cell lysis [11, 12].

On the other hand, inhibitory receptors, including Killer cell immunoglobulin-like receptors KIRs, heterodimer CD94-Natural Killer Group 2A CD94-NKG2A, and immunoreceptor tyrosine-based inhibition motif (ITIM) domains (TIGIT) receptor, recognize self-proteins and transmit inhibitory signals that maintain tolerance to normal cells. KIRs and CD94-NKG2A specifically recognize self-MHC class I molecules, while TIGIT interacts with other self-molecules. These inhibitory receptors when engaged with their ligands suppress NK cell activation and inhibit NK cell cytotoxicity, cytokine production and target cell killing [10, 11, 12].

The balance between activating and inhibitory signals received by NK cells determines their activity. If the activating signals dominate, NK cells become activated and can eliminate infected or abnormal cells. Conversely, if the inhibitory signals prevail, NK cell activation is suppressed, and healthy cells are spared from destruction. This delicate balance ensures that NK cells can selectively target cells requiring elimination while preserving normal tissue integrity. As such, the activation of NK cells occurs when tumor-expressed ligands engage activating receptors and there is a lack of co-engagement of inhibitory receptors. This activation leads to the killing of target cells by NK cells through the secretion of cytotoxic granules containing perforin and granzymes that induce apoptosis. NK cells can also eliminate target cells through the use of Fas ligand and tumor necrosis factor (TNF)-related apoptosis-inducing signals [11-13]. Cancerous or transformed cells often downregulate MHC class-I molecules, thereby evading recognition by CD8+ cytotoxic T cells, however; concomitantly this downregulation can induce NK cell activation through missing self-recognition [10]. Nonetheless, certain cancer cell types overexpress MHC class-I molecules which can inhibit NK cell activation by interacting with KIR receptors [14, 15].

In addition to these receptors, NK cells express cellular adhesion molecules and cytokine receptors, such as IL-12, IL-15, and IL-18 receptors which help regulate NK cell function and response to cytokine signals [12]. Furthermore, NK cells possess FcγRIIIa (CD16s) receptors, which bind to the Fc region of specific IgG antibodies bound to target cells. This interaction mediates antibody-dependent cellular cytotoxicity (ADCC), enabling NK cells to kill antibody-coated targets [10, 16]. Overall, the balance between activating and inhibitory signals received by NK cells determines their activation state and ability to eliminate abnormal cells while maintaining tolerance to normal cells.

2.4.2. Regulation of Immunometabolic Pathways in NK cells:

Resting NK cells have low basal metabolic rates of glycolysis and OxPhos, which are important for conserving the NK cell effector functions, besides stimulation for a defined period that impact the metabolic changes [25]. The rapid NK cell response changes extensively the metabolic program to meet the increased energy demands for their effector function [24].

Metabolic reprogramming plays a crucial role in regulating the function of natural killer (NK) cells. The activation, proliferation, and effector functions of NK cells are governed by various immunometabolic signaling pathways. These pathways include the mTOR pathway, AMPK pathway, PI3K-AKT pathway, and glycolytic pathway.

The metabolic rates of NK cells increased depending on the activation stimuli [26]. For instance, the inhibition of glycolysis and OxPhos impair IFNγ production at a short time of stimulation with cytokine (4 hrs with IL-15, IL-12+IL-15 or IL-12+IL-18) or by receptor ligation (e.g. 6 hrs of anti- NK1.1 in human or anti- Ly49D in mice). However, receptor stimulation is much more sensitive to metabolic inhibition than cytokine stimulation [25, 26]. Overnight stimulation of mouse or human NK cells with various cytokine combinations results in substantial increases in the rates of both glycolysis and OxPhos [24, 26, 31]. With high IL15 stimulation, NK cells elevate the activity of mTOR to boost bioenergetic metabolism, increase glucose uptake, and upregulate the expression of transferrin receptor CD71 and amino acid transporter CD98 [26]. Accordingly, impairment of glucose metabolism and disruption of mTOR signaling leads to diminished cytotoxic activity in NK cells [24]. Metabolic analysis of human blood NK cells shows that NK cells increase glycolysis and OxPhos rates after overnight stimulation with (IL2 or IL12+IL15) [31]; however, chronic exposure of human NK cells to IL15 in vitro results in reduced metabolic rates [295].

Upon activation, NK cells increase glucose uptake and flux through aerobic glycolysis, which is supported by increased expression of glycolytic enzymes and associated nutrient transporters. Moreover, activated NK cells increase OxPhos rates accompanied by increased mitochondrial mass (Figure 6). Then, the effector NK cells are rapidly ready to kill target cells and produce IFNγ [28, 29]. This metabolic reprogramming is likely to be activation-dependent. Cytokine-

stimulated NK cells can produce IFNγ independent of glycolysis or OxPhos, while activating-receptor-stimulated NK cells require OxPhos [25].

Human NK cells in the blood can be divided into two subsets: $CD56^{bright}$ & $CD56^{dim}$, which differ not only in the CD56 expression levels but also in various metabolic parameters. Both of them produce cytokines (IFNγ) in response to cytokine stimulation and receptor ligation, respectively. The $CD56^{bright}$ cells exhibit lower cytotoxic activity than the $CD56^{dim}$ cells, albeit they are much more efficient in cytokines production (e.g. IFNγ, TNFa, IL-10). On the other hand, the $CD56^{dim}$ cells have higher cytolytic activity to target cells and can embody more perforin and granzymes than $CD56^{bright}$ cells [30-32]. Further, stimulated $CD56^{bright}$ NK cells show greater metabolic responses compared to $CD56^{dim}$ NK cells. Stimulation with IL2 and (IL12+ IL15), upregulates the expression of nutrient receptors, including the glucose transporter (Glut-1; SLC2A1), amino acid transporters (SLC1A5; ASCT2, SLC7A5 and SLC3A2 (CD98)) and the transferrin receptor (CD71) to a greater degree in $CD56^{bright}$ NK cells than in $CD56^{dim}$ NK cells [31, 32]. Further, analyzing the expression levels of nutrient transporters in cytokine-induced memory-like (CIML) NK cells which are IL-12/15/18-stimulated NK cells, showed elevated levels of CD98 and Glut-1 expression. Therefore, higher expression of CD98 and Glut-1 could be beneficial in supporting the functionality of CIML NK cells in nutrient-restricted environments. Differences between $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets were observed at day (0) of stimulation, but the expression of CD56 was modulated during the subsequent culture period, making it difficult to identify these subsets at day (7) [27].

Human NK cells exhibit a preference to upregulate glycolysis and/or OxPhos regarding different effector functions. Glycolysis and OxPhos were upregulated in response to anti-CD16 antibody or NKG2D ligand engagement, and inhibition of either glycolysis or OxPhos impaired NK cell production of IFNγ. However, inhibition of glycolysis but not OxPhos decreased NK cell killing and dampened NK cell degranulation and Fas ligand expression, suggesting that glycolysis is more critical for NKR-activated cell cytotoxicity [307]. Further, a study compared the metabolic requirements between human CD8+T cells and NK cells after stimulation with IL-12/IL-18; and found that glucose starvation did not decrease the percentage of NK cells that produce IFNγ in contrast with CD8+T cells. Surprisingly, it has been reported that human NK cells were relatively flexible in using different fuel sources (glucose, glutamine, fatty acid, or acetate) to

support IFNγ expression [308]. Further, glycolytic inhibition with 2-DG is dependent on the way of stimulation in cytokine-induced memory-like (CIML) NK cells and that differently affects distinct effector functions [309]. Moreover, metabolic signatures also differ between these two human NK cell subsets. Cytokine-activated $CD56^{bright}$ cells have higher rates of glucose uptake and glycolysis and greater activation of mTORC1 compared with $CD56^{dim}$ cells, associated with their higher expression of IFNγ. While CD56 dim cells, although more cytotoxically active, have lower metabolic requirements [31].

Tissue-resident NK cells also increase the expression of nutrient transporters (Glut-1, CD98, and CD71) after stimulation; however, the magnitude of these increases is less than it is for blood NK cells. After stimulation, $CD56^{bright}$ tissue-resident NK cells derived from livers and spleens have lower expression levels of Glut1 but higher levels of the amino acid transporter CD98 than $CD56^{bright}$ NK cells from peripheral blood [33].

Murine NK cells activated by IL2 and IL12 metabolize glucose primarily through aerobic glycolysis to pyruvate and then lactate in the cytosol. However, some pyruvate that enters the mitochondria is not metabolized through the TCA cycle, as is the case in other lymphocytes, instead, pyruvate is converted to mitochondrial citrate by the citrate–malate shuttle (CMS) to drive OxPhos and ATP production. Alternatively, the CMS generates NADH in the mitochondria, to fuel OxPhos and ATP synthesis via the export of mitochondrial citrate in exchange for cytosolic malate, and also provides cytosolic NAD+, which is the cofactor for the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), thus facilitating elevated rates of glycolysis. The CMS, unlike the TCA cycle, is fuelled exclusively by glucose [29] (Figure 6).

Over and above; NK cells, unlike other lymphocytes, do not use glutaminolysis to drive OxPhos and NK cell effector functions mainly; cytotoxicity and IFNγ production [25]. However, glutamine withdrawal, but not the inhibition of glutaminolysis, results in the loss of cMyc protein, reduced cell growth and impaired NK cell responses [124]. Using fatty acids as a fuel by activated NK cells has not been studied well, however, it has been found that the blocking of FAO by etomoxir inhibitor did not affect IFNγ production by receptor-activated or cytokine-activated NK cells, suggesting that fatty acids are not an important fuel for NK cells

[25]. Moreover, the excess accumulation of fatty acids within NK cells is found to be detrimental to NK cell metabolism and function [296].

The regulation of NK cells metabolic response is mediated by the intracellular nutrient sensor, mTOR complexes. Both mTOR complexes, mTORC1 and mTORC2, are essential for NK cell activity, but they operate through different mechanisms. While mTORC1 is involved in early NK cell development, mTORC2 may function during terminal maturation, and their functions may be complementary rather than redundant [179, 180]. Specifically, mTORC1 sustains mTORC2 activity through IL-15 stimulation mediated by CD122 [180]. mTORC1 in NK cells is critical for regulating glucose uptake, glycolysis and the production of perforin and granzyme B [26].

The PI3K-AKT pathway, which is activated by cytokines and growth factors, plays a critical role in NK cell activation and function. Activation of this pathway enhances NK cell cytotoxicity and cytokine production, while its inhibition impairs NK cell function. The PI3K-PDPK1-AKT pathway, activated by high concentrations of IL-15, is involved in mTORC1 activation and controls various NK cell effector functions. High concentrations of IL15 and other cytokines (IL2, IL12, IL18) can be involved in the activation of mTORC1 via the PI3K-PDPK1-AKT pathway (PI3K (phosphatidylinositol 3-kinases), PDPK1 (Phosphoinositide Dependent Protein Kinase 1), AKT pathway)) [297, 298]. mTORC1 also enhances glycolysis by promoting transcription factor HIF1α and mitochondrial biogenesis through PPARγ co-activator 1α (PGC1α) and yin and yang 1 (YY1) [163].

The AMPK pathway is another nutrient sensor that facilitates FAO and OxPhos to generate energy during periods of low nutrient availability or metabolic stress. The specific role of AMPK in NK cell metabolism is not yet well understood. However, studies have shown that activating AMPK or inhibiting mTOR promotes mitophagy-dependent NK memory cells during the transition from contraction to the memory phase [311]. Current evidence suggests that both the AMPK and mTOR pathways can limit NK cell function. One study observed impaired function in senescent NK cells characterized by high expression of the inhibitory receptor Killer cell lectin-like receptor G1 (KLRG1) which correlated with spontaneous AMPK activity [312]. KLRG1 bright NK cells exhibit spontaneous activation of AMPK, and ligation of KLRG1 further activates AMPK, leading to negative regulation of NK cell proliferation, cytotoxicity and IFNγ production [313]. Further, transcription factors such as SREBP and cMyc are also implicated in the metabolic reprogramming of NK cells. SREBP which regulates *de novo* lipid

synthesis has been shown to be implicated in cytokine-induced metabolic reprogramming of NK cells. SREBP supports glycolysis and OxPhos by regulating key metabolic enzymes of the CMS, Acly (ATP citrate synthase) and Slc25a1 (mitochondrial citrate transporter). Hence, regulates the functional responses of NK cells. Moreover, SREBP inhibition prevented this phenotype and decreased NK cell cytotoxicity [29]. Additionally, cMyc controls the expression of glucose transporters and glycolytic enzymes, which are required to support increased metabolism during NK cell activation [124] (Figure 6).

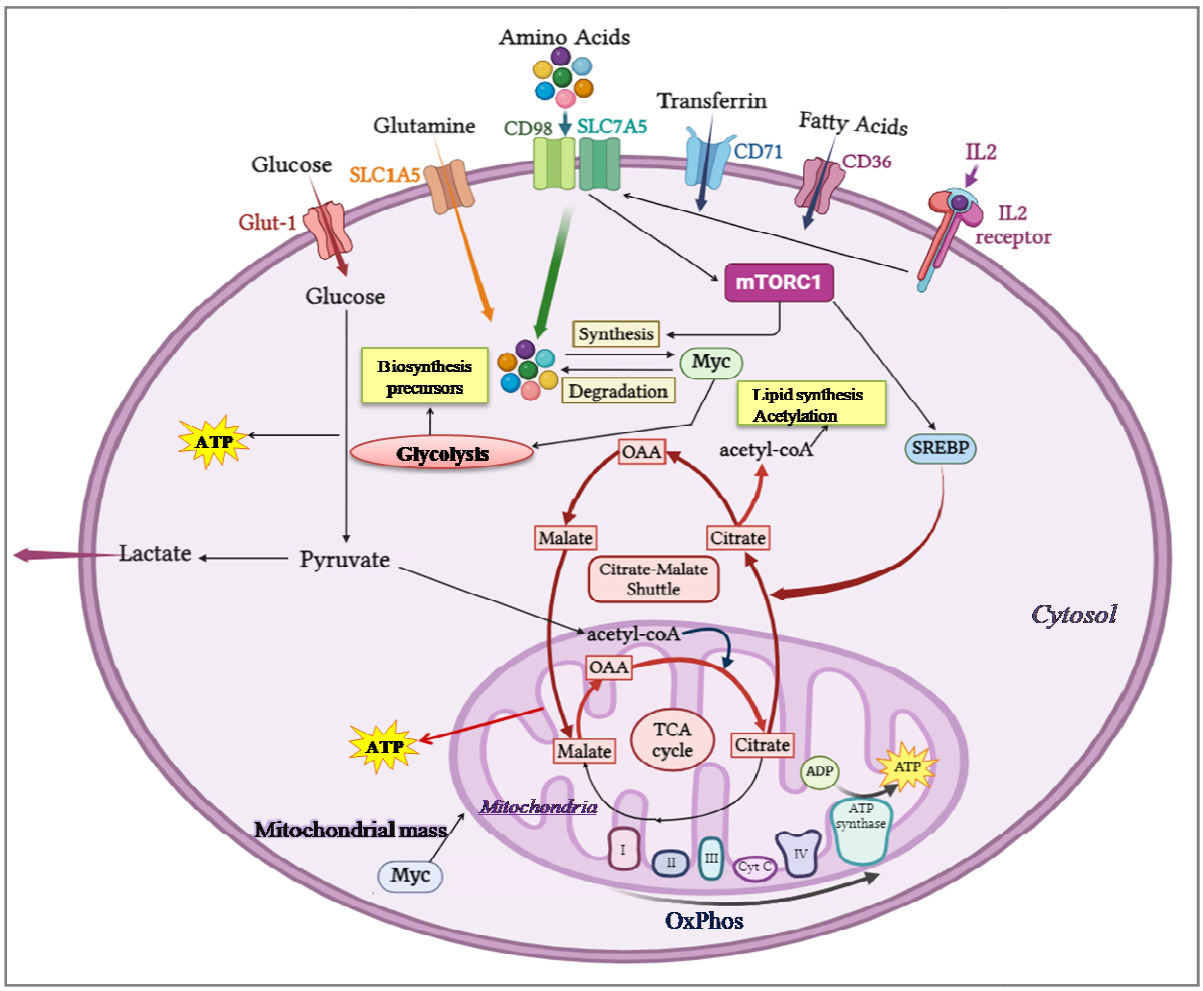


Figure (6): Immunometabolic Regulation of NK Cells. Adapted from [23].

Figure (6): Immunometabolic Regulation of NK Cells. Adapted from [23].

NK cells primarily rely on glucose as their main source of fuel. upon activation, NK cells increase the expression of various nutrient receptors, including Glut-1 (glucose receptor), CD71 (transferrin receptor), SLC7A5 (transporter of large neutral amino acids), SLC1A5 (glutamine transporter), and CD36 (free fatty acid transporter). Glucose is taken up into the NK cell cytoplasm through glucose transporters. Inside the cell, glucose is metabolized through glycolysis, resulting in the production of pyruvate. Pyruvate can take one of two paths: it can either be converted to lactate in the cytosol, which is then secreted by the cell, or it can enter the mitochondria. Within the mitochondria, pyruvate is initially converted to citrate as part of the normal TCA cycle. However, most of the citrate is then exported out of the mitochondria. The exported citrate is processed by an enzyme called ATP citrate lyase, which generates acetyl-CoA and oxaloacetate (OAA). Acetyl-CoA in the cytosol is utilized for lipid synthesis and acetylation reactions. The OAA generated is further metabolized to malate, which reenters the mitochondria, completing the cycle. This shuttling of citrate and malate between the cytosol and mitochondria is known as the Citrate-Malate Shuttle (CMS). The CMS can provide reducing equivalents that are required by the electron transport chain for the process of Oxidative Phosphorylation (OxPhos), leading to the efficient production of ATP. MYC and SREBP are key transcription factors involved in regulating metabolism in mouse NK cells. MYC is influenced by mTORC1-mediated translation and amino acid uptake through SLC7A5, while SREBP activity is primarily governed by mTORC1 but involves other mechanisms. These factors play crucial roles in shaping the metabolic characteristics of NK cells, including glucose metabolism and amino acid utilization. IL-2 and IL-12 stimulation greatly enhances the activity of SREBP in mouse NK cells. mTORC1 is the primary regulator of SREBP activation, additional mechanisms contribute to SREBP activity in cytokine-stimulated NK cells. MYC is vital for the expression of metabolic machinery in NK cells, while SREBP controls the metabolic configuration, specifically glucose metabolism through the CMS.

2.4.3. Immunometabolic Regulation of NK cells in TME:

The regulation of NK cell metabolism within the TME is a complex and dynamic process. In TME, various factors can influence the metabolic profile of NK cells, ultimately affecting their function and anti-tumor activity.

High rates of glycolysis exhibited in tumors hinder the function of tumor-infiltrating NK cells through the action of cancer-associated lactate dehydrogenase-A (LDHA). LDHA in tumors converts excess pyruvate and NADH into lactate and NAD+ to support tumor glycolysis. The high accumulation of lactic acid, generated by LDHA activity, in TME inhibits NK cell activity and diminishes IFNγ production. This inhibition occurs through the downregulation of the nuclear factor of activated T cells (NFAT) leading to tumor immune escape [299]. As tumor cells consume high amounts of glucose, the availability of glucose in TME decreases. Consequently, NK cells undergo metabolic reprogramming driven by IL15 and mTOR dependency instead of relying on activating receptors (such as KIR in humans, or Ly49D in mice) [300].

Besides, hypoxic TME induces NK cells to regulate their metabolism by overexpression of HIF-1α and HIF-2α [301, 302]. The overexpression of HIF-1α in hypoxic environments, which is partially dependent on mTOR signaling, leads to the downregulation of NK activating receptors NKp46, NKp30, NKp44, and NKG2D. However, the mechanisms by which hypoxia affects NK cells are still unclear [303, 304]. Moreover, cancer cells also release suppressor factors such as transforming growth factor-beta (TGF-β) which induces anergic phenotype in intratumoral NK cells [305].

TGF-β signaling, an immunosuppressive cytokine, has also been found to inhibit IL-15-induced NK cell effector functions by mTOR inhibition. This inhibition of mTOR by TGF-β dampened the upregulation of anaerobic glycolysis and OxPhos upon stimulation with IL-15 [314].

The dysregulated glycolysis observed in tumor-infiltrating NK cells has been found to be associated with the aberrant expression of fructose-1,6-biphosphatase 1 (FBP-1), an enzyme involved in gluconeogenesis, which is linked to NK cell antitumor dysfunction during tumor development [306].

2.4.4. Folate status and NK cells:

Some epidemiological and experimental studies have indicated an association between folate status and the function of NK cells. Excessive intake of folic acid (FA) in adults, which can occur through FA supplementation and fortification, has been found to elevate the concentration of folate and UMFA in serum and plasma. This elevation in UMFA levels was observed together with a reduction in the number and cytotoxicity of NK cells [65, 66]. Similar findings were observed in aged mice model where a high FA diet resulted in reduced NK cell cytotoxicity and a lower ratio of mature cytotoxic to naïve NK cells compared to a control group [64]. Additionally, in mice with malarial infection, a high FA diet was associated with decreased activity, numbers, and survival of NK cells, while no such effects were seen in mice fed a control diet [62]. However, some studies have not found significant differences in the cytolytic or cytotoxic activity of NK cells associated with high folate levels, either *in vitro* or in the serum of healthy individuals [67, 68]. On the other hand, a severe folate-deficient diet in rats was reported to impair NK cell cytotoxicity [310].

2.4.5. Succinate and NK cells:

The impact of succinate on NK cell functionality and metabolism has not been thoroughly investigated. However, *in silico* studies have explored an association between succinate receptor-1 (SUCNR1) expression and immune cell infiltration as well as tumor progression in different types of cancer [52, 53]. Bioinformatics analyses on renal cell carcinoma (RCC) revealed that high expression of SUCNR1 was associated with lymphocyte infiltration, including various subsets of NK cells and T cells. Despite this association with certain immune effector cells, the presence of SUCNR1 in RCC was linked to unfavorable outcomes [52].

Similarly, in ovarian cancer, SUCNR1 expression was correlated with tumor-infiltrating lymphocytes, T cell exhaustion, and the expression of genes related to cytokines and chemokines. Furthermore, it was suggested that SUCNR1 expression may regulate various immune-related pathways and can predict worse progression-free survival [53].

These studies suggested the potential involvement of SUCNR1 in immune responses and tumor progression in certain types of cancer. However, the specific impact of succinate on NK cell function and metabolism remains unclear and requires further investigation.