12 Lipid Metabolism and Immune Checkpoints

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Abstract

Immune checkpoints are essential for the regulation of immune cell functions. Although the abrogation of immunosurveillance of tumor cells is known, the regulators of immune checkpoints are not clear. Lipid metabolism is one of the important metabolic activities in organisms. In lipid metabolism, a large number of metabolites produced can regulate the gene expression and activation of immune checkpoints through various pathways. In addition, increasing evidence has shown that lipid metabolism leads to transient generation or accumulation of toxic lipids that result in endoplasmic reticulum (ER) stress and then regulate the transcriptional and posttranscriptional modifications of immune checkpoints, including transcription, protein folding, phosphorylation, palmitoylation, etc. More importantly, the lipid metabolism can also affect exosome transportation of checkpoints and the degradation of checkpoints by affecting ubiquitination and lysosomal trafficking. In this chapter, we mainly empathize on the roles of lipid metabolism in the regulation of immune checkpoints, such as gene expression, activation, and degradation.

Keywords

Lipid metabolism · Immune checkpoints Ubiquitination · Lysosomal · Unfolded protein response

12.1 Introduction

The cellular metabolism is the basis of various physiological activities of cells. The increase in cellular metabolism allows rapid production of ATP and metabolic intermediates to meet the metabolic needs of proliferating cells [1]. Immune cells also show an increase in biosynthetic pathways, leading to the elevated production of macromolecules required for growth and proliferation [2]. Lipid accumulation and alterations resulted from lipid metabolism have been identified as the regulators of immune cell polarization [3, 4]. For instance, defective or aberrantly enhanced lipid metabolism in macrophages can result in several pathologies in the lung. More importantly, the metabolic patterns of some tissue-resident macrophages are lipid metabo-

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lism, including KCs (Kupffer cells) and AMs (Alveolar macrophages) [5, 6]. The correlation has led to much interest in understanding how the lipid impacts on immune polarization and how the immune polarization impacts on lipid metabolism.

The immune system shields the human body from pathogens and the development of malignancies. However, the immune system poses a severe and potentially lethal threat to the human body when overactive and/or abnormal silence. In terms of T lymphocytes, recognition by T cell receptor (TCR) of the specific antigen presented by the major histocompatibility complex (MHC) molecules provides the first signal for T cell activation [7]. The signal could stimulate the lymphocytes only briefly and requires co-stimulation to introduce T cells into full activation via CD28 molecule that recognizes its ligands (CD80 or CD86) on antigen-presenting cells (APCs) [8]. The process is additionally regulated by different cytokines acting on the T lymphocytes, as well as by the immune checkpoints. Intriguingly, several recent researches have shown that the aberrant expression of immune checkpoint proteins is closely correlated with tumor cell metabolic reprogramming. In this chapter, we mainly discuss that the crosstalk between these immune checkpoints and lipid metabolism may have a profound influence on the immune system.

12.2 Lipid Metabolism

Lipid metabolism is a range of important and complex biochemical reactions in the body. It refers to the process of digestion, absorption, synthesis and decomposition of lipids in the body with the help of various related enzymes to process the substances needed by the body to ensure various physiological functions and life activities. The primary function of lipid metabolism is to deliver lipids to peripheral tissues for use or to return lipids to the liver for recycling or clearance [3]. It is well known that there are three approaches of lipid metabolism, including exogenous, endogenous, and reverse transport [9]. Among three pathways, the exogenous pathway is used to process dietary lipids. The endogenous pathway refers to the processing of lipids synthesized in the liver, however, the process of removing lipids from tissues and returning to the liver is termed reverse transport [10].

In addition, lipid metabolism causes the generation of free fatty acids, which are subsequently absorbed by different cells. These fatty acids could be converted into numerous products in mitochondria that the cell can use to generate energy via fatty acid oxidation (FAO) [11]. However, different fatty acids enter the mitochondria in different ways. The short-chain fatty acids enter the mitochondria either via passive diffusion or via the carnitine shuttle, where medium/long-chain fatty acids are conjugated to carnitine via carnitine palmitoyl transferase 1A (CPT1A) and then transported into the mitochondria [12]. Of course, if the free intracellular lipids are not sufficient, the fatty acid synthesis (FAS) pathway can be activated in the cytoplasm to allow cells to generate fatty acids from precursors derived from other cell-intrinsic metabolic pathways, including the TCA cycle, glycolysis, and the pentose phosphate pathway [13].

In recent years, studies on the relationship between immune and lipid metabolism have shown that apolipoprotein A-I (apolipoprotein A-I, APOA-I), the main component protein of high-density lipoprotein (HDL), reduces the number of lymph node T cells and affects the metabolism of cholesterol in the cell, thereby promoting the inflammatory reaction of skin and lymph nodes [14]. Actually, recent studies have found that lipids play an important role in the development and differentiation of various immune cells and their typing functions.

12.3 Classification and Functions of Immune Checkpoints

12.3.1 The Classification of Immune Checkpoints

Immune checkpoints are co-inhibitory and co-stimulatory receptor molecules, mainly occurring on the surface of T lymphocytes and NK cells (Tables 12.1 and 12.2), but not exclusively, which can play a negative (inhibitory) or positive (stim-

Table 12.1 Classifications and function of stimulatory immune checkpoints

Immune checkpoint receptor	Ligand	Function
CTLA4	CD80 or CD86	Inhibiting T cell activation; Inducing Treg cell differentiation
PD-1	PD-L1 (CD274) or PD-L2 (CD273)	Inhibiting T cell proliferation and function; Enhancing IL-10 and TGF-β Secretion; Inhibiting IFN-γ production
LAG3	MHC class II/Lectins	Inducing the proliferation of Treg cell; Inhibiting the secretion of IFN-γ, IL-2, and TNF
TIGIT	CD155/ CD112	Inhibiting NK cell function; Inhibiting the co-stimulatory ability of dendritic cells; Enhancing IL-10 secretion
TIM3	Galectin 9/ PtdSer /HMGB1	Inhibiting M1 macrophage differentiation; Inhibiting Th1 and Th17 cell; Promoting Tregs cell and MDSCs cell proliferation
VISTA	VSIG-3	Inhibiting T cell functions; Decreasing the production of IFN-γ, TNF-α, and IL-17A
CEACAM1	CEACAM1	Inhibiting T cell and NK cell activity; Decreasing the secretion of IFN-γ, IL-2, and IL-4
BTLA	HVEM	Attenuating B cell function; Inhibiting the secretion of IL-12, TNF-α, and IFN-γ; Decreasing the proliferation of activated $CD4^+$ and $CD8^+$ T cell

Table 12.2 Classifications and function of co-stimulatory immune checkpoints

Immune checkpoint receptor	Ligand	Function
CD28	B7 molecules: CD80 or CD86	Promoting the survival and proliferation of activated T cell; Decreasing the function and motility of Tregs cell
OX40	OX40L	Increasing the survival and expansion of effector and memory T cells; Increasing IL-2, IL-4, IL-5, IFN-γ secretion; Decreasing the immunosuppressive activity of Tregs cell
CD137 (4-1BB)	CD137L	Inducing the activation and survival of $CD8^+$ T cell; Increasing the secretion of IL-6 and IL-12 in DC cells; Enhancing the cytotoxic function of NK cell
GITR	GITRL	Promoting the proliferation and killing activity of activated T cells; Inhibiting Treg cell activity
ICOS	ICOSLG	Promoting B cell maturation and survival; Increasing IL-2, IL-4, IL-5, IFN-γ, and TNF-α secretion; Inhibiting the survival and proliferation of Treg cell
CD27	CD70	Promoting the proliferation of T cell; Promote B cell differentiation; Inducing the secretion of IL-2 and IFN-γ
HVEM	LIGHT	Inducing the secretion of IFN-γ in NK cell; Stimulating T cell proliferation and activation

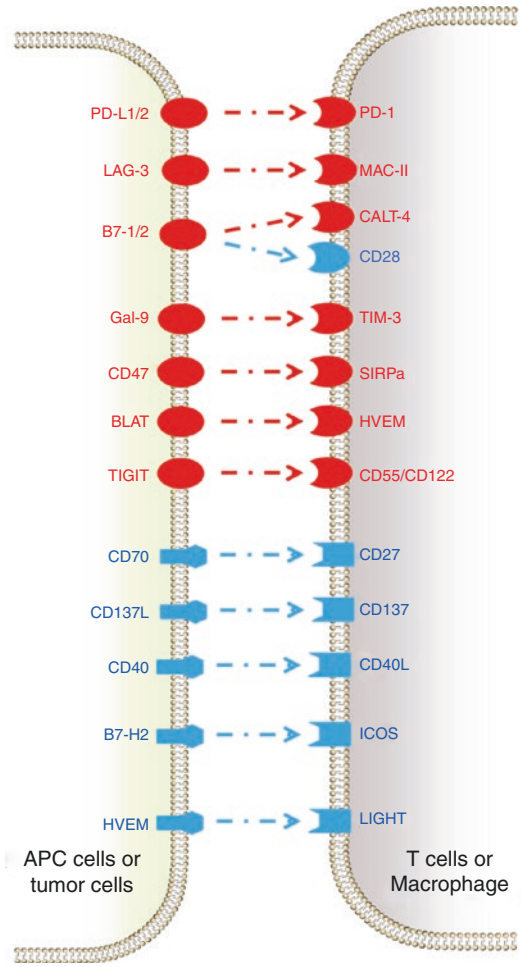

Fig. 12.1 Classification of inhibitory and stimulatory immune checkpoints in APCs, tumor cells, T cells, and macrophages. Red indicates inhibitory immune checkpoints; blue denotes stimulatory immune checkpoints

ulatory) role in the process of the lymphocyte activation after recognizing appropriate ligands on the antigen-presenting cells (APC) or the target cells (Fig. 12.1). We briefly introduce some typical immune checkpoints in the following sections.

12.3.2 Inhibitory Immune Checkpoints

12.3.2.1 PD-1

PD-1 (programmed death receptor 1), an important immunosuppressive molecule, belongs to the immunoglobulin superfamily. It is a membrane protein of 268 amino acid (aa) residues containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM) [15]. It was originally cloned from the apoptotic mouse T cell hybridoma 2B4.11. Immunomodulation with PD-1 as a target has important significance in tumors, infections, autoimmune diseases, and organ transplantation survival [16]. Actually, certain special types of malignant tumors express PD-1 in large amounts on the cell surface and evade the attack of immune cells by strongly suppressing the activation of immune cells. Intriguingly, PD-1 is expressed on the surface of activated T cells, B cells, and macrophages, indicating that PD-1 negatively regulates the immune response [17].

12.3.2.2 PD-L1

Programmed cell death 1 ligand 1 (PD-L1), also known as cluster of differentiation 274 (CD274) or B7 homolog (B7 homolog 1, B7-H1), is a protein encoded by the CD274 gene in humans [18]. PD-L1 is a type of transmembrane protein of 40 kDa, which is involved in the suppression of the immune system under certain special circumstances (such as pregnancy, tissue transplantation, autoimmune diseases, and certain diseases, e.g., hepatitis) [19]. PD-L1 is mainly expressed in T cells, natural killer cells, macrophages, myeloid dendritic cells, and B cells. In addition, PD-L1 expression on tumors cell is one of the mechanisms of immune evasion as it inhibits the functional activity of cytotoxic lymphocytes which will then not attack tumor cells. At present, more and more studies indicate that PD-L1 is important to maintain the tumor-associated biological features [20].

12.3.2.3 CTLA-4

Cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), also known as CD152, is a leukocyte differentiation antigen and a transmembrane receptor on T cells and shares the B7 molecular ligand with CD28 [21]. While CTLA-4 is combined with B7 molecule, it induces T cell anergy and participates in the negative regulation of

immune response [22]. In the cellular level, CTLA-4 is expressed on non-lymphoid cells including placental fibroblasts, cultured muscle cells, monocytes, and a variety of leukemia cells [22]. Previous studies showed that CTLA-4 expression varied remarkably among various cancer cells [23]. Also, CTLA-4 is a prognostic factor for survival in different cancers, but current data remain incomplete and inconclusive [24]. However, Donnem, T. and colleagues have reported that the CTLA-4 expression level of tumor cells in lymph nodes but not primary tumors was a negative predictor in NSCLC patients [25].

12.3.2.4 Tim-3

T cell immunoglobulin and mucin domain 3 (TIM-3) belongs to the TIM family, which mainly contains three members TIM-1, TIM-3, TIM-4 in humans and TIM-1 ~ TIM-8 in mice [26]. TIM-3 mainly expresses on $CD4^+$ T helper cells 1 (Th1) and $CD8^+$ T cytotoxicity 1 (Tc1) T cells that produce IFN-γ [27]. In addition to the expression on T cells, TIM-3 has been found on Treg cells and innate immune cells (DC cells, NK cells, and monocytes) [28]. Studies have shown that TIM-3 signaling is necessary to induce antigen-specific tolerance, and silencing TIM-3 promotes the progression of spontaneous autoimmunity. C-type lectin galectin-9 is a TIM-3 ligand [27]. This discovery consolidated TIM-3 inhibitory function. Recently, ceacam-1 in cell surface was identified as a novel ligand of TIM-3 [29]. Importantly, the negative regulatory function of TIM-3 is defective in the absence of ceacam-1, which indicates that the interaction between ceacam-1 and TIM-3 is required to obtain proper TIM-3 function [30].

12.3.3 Stimulatory Immune Checkpoints

12.3.3.1 4-1BB (CD137)

4-1BB (also called CD137 and OX40) is a member of the tumor necrosis factor (TNF) receptor family and is encoded by the tumor necrosis factor receptor superfamily member 9 (TNFRSF9) genes. Human 4-1BB is located on chromosome 1p36, with the full length of 255aa that contains a 17aa signal peptide, 169aa extracellular region, 27aa transmembrane region (pp. 187–213), and 42aa intracellular region. 4-1BB is an inducible co-stimulatory receptor expressed on activated $CD4^+$ and $CD8^+$ T cells, NKT, NK cells, DC cells, macrophages, eosinophils, neutrophils, cells, and Treg cells [31]. In addition to the constitutive expression of 4-1BB on APCs and $Foxp3^+$ Tregs, the expression of 4-1BB is induced on the surface after cell activation in most cases [32]. Due to its wide expression and the ability of 4-1BB to enhance strong and lasting immune effects, 4-1BB has become a clinical target for cancer immunotherapy [33].

12.3.3.2 CD28

CD28 is a co-stimulatory molecule expressed on the surface of T lymphocytes and plays an important role in the activation of T cells. It binds to B7 molecules on APC (antigen-presenting cells), mediates T cell co-stimulation, and promotes survival, proliferation, and production of cytokines [34]. Although CD28 and CTLA-4 bind to the common ligands CD80 and CD86 expressed differently on T cells, their expression distribution is different: CD28 is expressed on the surface of inactivated and activated T cells while CTLA-4 is only expressed on activated T cells. It t indicates that CD28 enhances T cell response while CTLA-4 negatively regulates the activation process. Additionally, CD28 can upregulate lymphokine gene transcription, mRNA stability, and the longevity of the T cell response via binding CD80/CD86 on presenting cells and preventing non-responsiveness anergy to antigenic challenges.

12.3.3.3 GITR

Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), the 18th member of the tumor necrosis factor receptor superfamily (TNFRSF), was originally cloned by differential display following treatment of hybridoma cell line with dexamethasone in 1997. GITRL (also referred to as TNFSF18, AITRL), a type II transmembrane protein, is the specific activating ligand of GITR [35]. GITR is a co-

stimulatory checkpoint molecule that is involved in suppressing the inhibitory effect of Treg cells and promoting effector T cell survival. GITR is mainly enriched on Tregs and could be expressed quickly when Treg is stimulated. The expression of GITR is maintained at a relatively low level on the resting effector $CD4^+$ and $CD8^+$ T cells, but rapidly up-regulated when they are activated [36]. Moreover, it has also been reported that human GITR is expressed on DC, macrophages, and NK cells. Interestingly, GITR is also regulated by the CD28 signaling pathway in both conventional and regulatory T cells [37].

12.3.3.4 ICOS

ICOS, a type I transmembrane protein with a molecular weight of 55–60 kDa, is a homodimer composed of two subunits and is part of the immunoglobulin superfamily [38]. The binding of ICOS to ligand ICOSL can provoke a series of effects related to immune response. Similarly, ICOSL secreted by B cells can mediate the atypical NF-κB signaling pathway, thus regulating the differentiation of T cells [39]. In addition, ICOS/ICOSL signaling pathway can promote the expression of CD40L on T cells, and then, CD40 and CD40L can further prolong the survival of B cells [40]. ICOS/ICOSL signaling pathway can also promote the secretion of Th1 and Th2 related cytokines IFN-γ, TNF-α, IL-4, IL-5, and IL-10. ICOS also plays an important regulatory role in maintaining Treg balance. Some studies showed that ICOS/ICOSL signaling pathway can promote the proliferation and survival of Treg cells, and Treg cells cannot proliferate and survive when the ICOS/ICOSL signaling pathway is silenced.

12.4 The Functions of Immune Checkpoints

The activating process of T cells requires two signals: one is an antigen-specific signal to the T cell receptor (TCR) by specific antigens on major histocompatibility (MHC) molecules that are expressed on antigen-presenting cells (APCs) or target cells (Fig. 12.2a). The other signal is a stimulatory signal provided by B7 and other stimulatory molecules to assist in T cell activation and inhibitory molecules to hinder T cell signal transduction process, thus restraining T cell functions. The physiological function of immune checkpoints is to prevent harmful immune attacks against self-antigens by negatively regulating the effector immune cells. Blockade of inhibitory checkpoints or up-regulation of stimulatory checkpoints has been intensively studied in recent years as a strategy to enhance T cell infiltration and effector functions in cancer (Fig. 12.2b). Therefore, it is suggested that the immune checkpoints are the promising treatment targets in many diseases, including cancer, severe inflammation, etc.

At present, more and more immune checkpoints have been discovered, such as PD-1, PD-L1, LAG3, B7-H3 (CD276), and TIM-3 [8, 41, 42]. Both inhibitory immune checkpoints and stimulatory immune checkpoints have become prime targets of targeted drugs. Immune checkpoint proteins are abnormally expressed in different cancers, which are called cancer-associated immune checkpoint molecules, and play an essential role in the biological function of tumor cells [43–45]. Particularly, induction of epithelial-mesenchymal transition (EMT), acquisition of tumor initiation potential, unique metabolism network for the capacity to metastasize, anti-apoptotic and anti-tumor drugs, and higher proliferation requirements to promote tumor survival are closely related to abnormal expression of immune checkpoints [43, 46, 47]. Silencing inhibitory immune checkpoints can positively reverse T cell activation and prevent immune from escaping in the tumor microenvironment. For example, the binding of TIM-3 and Gal-9 constitute an autocrine loop to stimulate the activation of NF-κB and β-catenin signaling pathways to facilitate self-renewal and progression of human acute myelocytic leukemia (AML) [48]. Interestingly, the previous study pointed out that PD-L1 could induce EMT occurrence and enhance the stemness of renal cell carcinoma (RCC) through induction of sterol regulatory element-binding protein 1 (SREBP-1c), which is an important transcription factor in lipogenesis

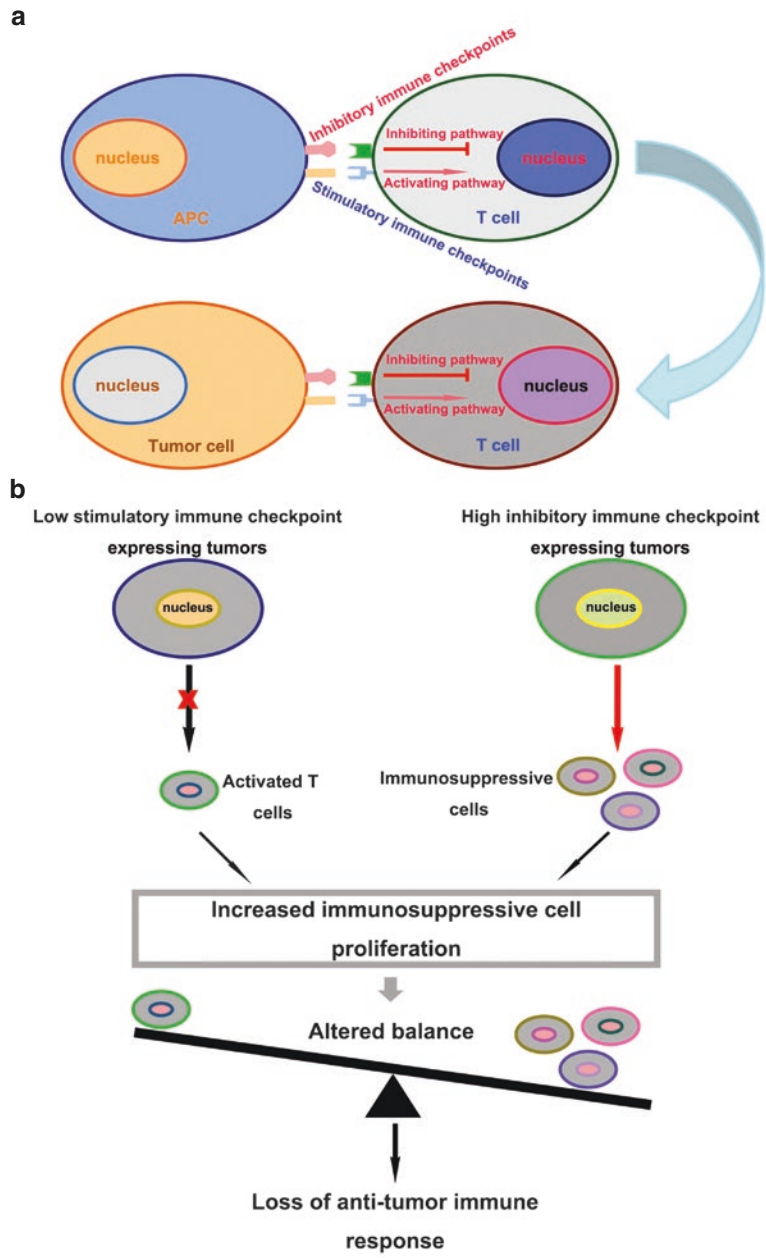

Fig. 12.2 (**a**) The functions of inhibitory and stimulatory immune checkpoints in APC cells and tumor cells. The stimulatory immune checkpoints in APC cells and tumor cells could induce the activation of T cells, whereas the inhibitory immune checkpoints suppress the process. (**b**) The differential expression of immune checkpoints in cancer cells. The tumor cells with high inhibitory and low stimulatory immune checkpoints will lead to immunosuppressive immune cell proliferation to inhibit the anti-tumor response

[49]. Subsequently, successive studies have found that PD-L1 was closely correlated with the tumor-initiating activities and participated in tumor cell resistance and anti-apoptotic response via regulating the PI3K/AKT signaling pathway [46, 50, 51].

In addition, activating stimulatory immune checkpoints can strengthen the effect of the immune response. The co-stimulatory receptor of T cells surface is a protein called CD70. Actually, CD70, an important member of the TNF superfamily, is found on activated dendritic cells, B cells, and NK cells and also a type II transmembrane receptor of stimulatory CD27/CD70 signaling pathway which play an important role in providing co-stimulation sig-

naling during the activation of functional lymphocytes [7]. In vitro and in vivo experiments indicated that monomeric CD70 expression inhibited the migration, invasion, and pulmonary metastasis of melanoma cells. However, silencing CD70 in primary glioblastoma multiforme (GBM) could inhibit monocyte-derived M2 macrophages migration, growth, and chemoattraction abilities and attenuate the expression of CD44 and sex-determining region Y-box2 (SOX2) [52]. However, the stimulation of CD137 induced by its ligand could prolong the survival of chronic lymphocytic leukemia (CLL), which was mediated by the nuclear translocation and activation of p52 (a non-canonical NF-κB factor) [53]. Similarly, the enhanced CD137/CD137L signaling opposed the cytotoxic effects of anticancer drugs, reduced the apoptotic DNA fragmentation, and stimulated doxorubicin-escaped leukemia cell proliferation [54]. Up to date, significant advances have been made in immunotherapy while the recent knowledge about the biological consequences of tumor-associated immune checkpoints becomes increasingly comprehensive. Therefore, targeting those checkpoint molecules on the basis of their roles in maintaining malignant traits in tumor cells may provide us with novel therapeutic approaches.

12.5 The Effect of Lipid Metabolism on Immune Checkpoints

Lipid metabolism has been implicated in immune response regulation. At present, Yang, et al. showed that cholesterol metabolism can modulate the anti-tumor activity of $CD8^+$ T cells. Inhibition of ACAT1 (acetyl-CoA acetyltransferase 1), a cholesterol esterification enzyme that converts free cholesterol to cholesteryl esters for storage, could increase membrane cholesterol levels in plasma, enhancing the effector function and proliferation of $CD8^+$ T cells [55]. In addition, induction of de novo fatty acid synthesis is essential for effector T cell proliferation and differentiation. Based on the fact that the immune checkpoints are essential for immune cell activation, we summarize the ways in which lipid metabolism regulates immune checkpoints in the following aspects.

12.5.1 Affecting the Chromosomal and Microsatellite Stability

Chromosomal instability (CIN) and microsatellite instability (MSI) are the most common form of genomic instability, which can enhance tumor heterogeneity, drug resistance, and immunity escape [56]. The most common characteristic of CIN is the aberrant chromosomal architecture, ranging from small insertions or deletions to large chromosomal alterations. Indeed, malignant tumors with CIN rapidly acquire somatic copy-number alterations (SCNAs) during proliferation, creating intratumor genetic heterogeneity within the population. The previous study has indicated that lipid metabolism is associated with genetic regulation and chromosomal stability [57]. For instance, the spindlin 1 (SPIN1) is involved in the process of spindle organization and chromosomal stability serving as an important player in carcinogenesis. Meanwhile, SPIN1 triggers lipid metabolism disorders and enhances the growth of liver cancer through SREBP1c-triggered FASN signaling pathway [58]. Fatty acid synthase (FASN), a key metabolic enzyme involved in de novo lipogenesis, could increase the formation of fatty acids and lipid droplets, and a previous study indicated that FASN knockdown diminished DNA damage [59]. The MSI-high tumors showed a consistently high frequency of FASN overexpression than MSI-low tumors [60].

In fact, CIN is mainly caused by impaired mitotic fidelity and leads to aneuploidy. CIN and aneuploidy are hallmarks of various cancers. The CIN tissues up-regulate different signaling pathways known to be activated in colon cancer, including lipid metabolism, notch signaling, insulin signaling, and PPAR pathways [61]. As inhibitory immune checkpoint expression becomes increasingly studied in the clinical settings, CIN and MSI tumors would be predicted to correlate with

immune checkpoint inhibition. Researchers have shown that MSI tumors have at least 20 times higher mutational burden or neoantigens that lead to enhanced immunotherapy responsiveness in these "hypermutator" phenotypes compared to microsatellite stable tumors (MST) [60]. The studies indicated that MSI tumors have 32% PD-L1 expression compared with 13% in MSS tumors. In addition, the CIN is associated with depressed tumor immunity via targeting CTLA-4, PD1, or PD-L1 [62]. More importantly, SCNAs status exhibits association with immune checkpoints inhibitor (ICI) response independent of tumor mutational burden (TMB), and combination of SCNAs and TMB has much higher prediction efficiency for ICI treatment according to independent clinical trials for metastatic melanoma treated with anti-PD1 and anti-CTLA4 [63].

12.5.2 Affecting the Transcriptional Activity of Immune Checkpoints

It is well known that transcription factors can bind to defined DNA sequences and/or protein chaperones shared with the gene, including immune checkpoints. Although more and more studies report that transcriptional regulation depends on oxidized forms of cholesterol, desmosterol, and elevated concentrations of D-glucose, the links between lipid metabolism and immune response are not very clear. A recent study indicated that the LXRs (liver X receptors), is the connection between lipid metabolism and immune regulation. LXRs are important transcription factors in macrophages that act as crucial mediators in cholesterol metabolism and modulate several anti-inflammatory pathways and are now recognized as "cholesterol sensors," as they induce a transcriptional program that regulates reverse cholesterol transport. Evident links between lipid metabolism and immunity are regulated through LXR transcriptional activity [64]. Therefore, the unique transcription factors can coordinate the gene expression of immune checkpoints, and thus a gene expression program. The transcription factors, such as STAT3, HIF-1α, and members of the AP-1 family, control the transcriptional activity of PD-L1 [65]. Actually, STAT3 not only promotes the breast stem cancer cells and cancer chemoresistance via regulating lipid metabolism [66], but also binds to the promoter region of PD-L1 to activate its transcription, and then inhibit the immune response [67]. As an important metabolic switch, HIF-1α could translocate to the nucleus where it binds specific hypoxia response element sequences under hypoxic conditions (low oxygen levels). It was recently discovered that the HIF-1α could directly bind to the promoter of PD-L1 to enhance the transcriptional activity [68]. A hypoxic environment leading to the accumulation of lactate and aberrant lipid metabolites may prevent cancer cells from cytotoxic T cells, and hence induce immunosuppression [69, 70]. Interestingly, as a key modulator of hepatic lipid metabolism, the activator protein 1 (AP-1) could be induced by CD28 and is involved in the transcriptional regulation of CD40L in T cells [71]. Additionally, AP-1 also binds to the promoter region of PD-L1 [72], indicating that AP-1 may orchestrate a regulatory transcription network that controls multiple immune checkpoints.

12.5.3 Affecting the Gene Expression of Immune Checkpoints

Multiple intercellular signaling pathways driven by lipid mediators (for example, leukotrienes and prostaglandins) are associated with the expression of cytokines, chemokines, growth factors, the extracellular matrix, and immune checkpoints [73–75]. For example, raptor-mTORC1 signals in Tregs cells augment lipid and cholesterol metabolism to allow cell proliferation and surface expression of important molecules mediating immune suppression, such as CTLA-4 and ICOS [76]. Lipids regulate Treg cell development and function via affecting the expression of the immune checkpoint. Prostaglandin E_2 (PGE_2) as a type of unsaturated fatty acids with physiological activity originated from lipid metabolism, can

directly or indirectly participate in the development of an immunosuppressive tumor milieu, promoting tumor growth, angiogenesis, and metastasis [77]. It has been reported that PGE_2 acts as an inducer of co-inhibitory marker expression, including TIM-3, PD-1, CTLA-4, and LAG-3 [78, 79]. This new study had reported that PGE_2 promoted the up-regulation of TIM-3 and PD-1 and consequent co-expression of TIM-3 and PD-1 on T cells [80]. Meanwhile, they also observed that PGE_2 decreased the expression of HLA-DR (MHC-II molecules) in $CD8^+$ T cells, but not significantly in $CD4^+$ T cells and the CD28 expression [81]. In addition, increased cholesterol concentration derived from lipid metabolism by tumor-infiltrating $CD8^+$ T cells was positively and progressively associated with up-regulated T cell expression of PD-1, 2B4, TIM-3, and LAG-3 [8].

The expression of immune checkpoints is not only regulated by lipid metabolites, but also by lipid chaperone proteins. Lipid chaperones are a group of molecules that coordinate the intracellular lipid response and are also closely related to metabolic, immune, and inflammatory pathways [82–84]. Like the metabolism of cholesterol and phospholipids, lipid chaperones have potential effects on the storage and transfer of fatty acids [85]. These lipid chaperones play an important role in lipid-mediated metabolic and immune response [86], and the alterations of lipid chaperone protein expression will cause changes in lipid metabolism, ultimately leading to the differential expression of the immune checkpoints. For example, fatty acid-binding proteins (FABPs) are a family of lipid chaperones required to facilitate uptake and intracellular lipid trafficking [87, 88]. Since FABP7 knockdown in the breast cancer cells leads to the alteration of PI (phosphatidylinositol) composition and the gene expressions of PD-1-related immune checkpoint pathway, targeting PUFA (polyunsaturated fatty acids) trafficking mediated by FABP7 is likely to enhance the effect of immune checkpoint inhibition [89, 90]. However, some scholars pointed out that it remains unclear whether FABP7 positively or negatively regulates the immune checkpoint pathways in the tumor microenvironment as PD-L1 was up-regulated in cancer cells upon FABP7 knockdown [89].

12.5.4 Affecting the Protein Folding of Immune Checkpoints

As well known, most proteins are glycoproteins, which are precisely modified in the endoplasmic reticulum (ER) with the help of a series of ER-resident chaperones and folding enzymes [91]. Dysfunctional lipid homeostasis could cause the transient generation or accumulation of toxic lipids that result in ER stress with inflammation, hepatocellular damage, and apoptosis. It is no doubt that ER stress activates the unfolded protein response (UPR), which is classically viewed as an adaptive pathway to maintain protein folding homeostasis [92, 93]. UPR is a series of signal transmission processes in which cells respond to protein folding errors. As a result, protein production slows down, unfolded proteins are degraded, and protein folding function is enhanced. However, recent studies have demonstrated that the UPR sensors reversely play a role in the regulation of lipid metabolism, and lipotoxicity can activate an ER stress response [92]. Therefore, evidence has indicated that lipid metabolism may play a role in the protein folding of immune checkpoints via regulating the UPR.

IRE1α, a key regulator of hepatic lipid homeostasis that represses hepatic lipid accumulation and maintains lipoprotein secretion, is the most conserved arm of the UPR [94]. In addition, experimental manipulation of XBP1 (X-box binding protein 1), a downstream transcription factor of IRE1α, highlighted a critical role of IRE1α in lipid metabolism [95]. Constitutive activation of the IRE1α/XBP1 pathway could promote the folding of immune checkpoints, such as PD-1 and CALT-4, through enhancing the UPR [96]. In addition, the PERK-eIF2α pathway also regulates hepatic lipid metabolism [97]. Among the downstream effectors of the PERK-eIF2α pathway, ATF4 is closely involved in lipid metabolism [92]. The

previous study has confirmed that the PERK-eIF2α-ATF4 signaling pathway activates the transcription of specific UPR target genes, such as CCAAT-enhancer-binding protein homologous protein (CHOP) and the growth arrest and DNA damage-inducible protein (GADD34) [98]. Interestingly, the FK506-binding protein 51 (FKBP51), a member of the FKBP family encoded by the FKBP5 gene, is closely associated with lipid metabolism [99]. More importantly, FKBP51 could promote the protein folding of PD-L1and thus results in the up-regulation of it [100].

A large number of molecular chaperones in the ER play an active role to promote proper folding and protect proteins from aggregation [101–103]. E.g., Immunoglobulin binding protein (BiP, also known as glucose regulatory protein 78, GRP78), belongs to the heat shock protein 70 (Hsp70) family and is the most popular chaperones among ER residents [104]. BiP can directly interact with immature polypeptides by recognizing unexposed hydrophobic fragments. Once the unfolded, aggregate-prone substrate binds to BiP, it becomes soluble, triggering processes such as translocation, maturation, and ERAD (ER-related degradation) pathways, etc. Additionally, these studies extend the role of ER chaperone GRP78, controlling the unfolded protein response and thereby regulating the important function of lipid metabolism [105, 106]. Blockade of GRP78 causes the accumulation of cellular essential fatty acids, prompting that GRP78 regulates their uptake and/or catabolism. At the same time, GRP78 could bind to the SREBP (sterol regulatory element-binding transcription factor) complex preventing SCAP (SREBP cleavage-activating protein) translocation to the Golgi complex and activation. However, researchers also found that silencing GRP78 reduced the cellular SCAP protein levels, thereby inhibiting SREBP translocation to the Golgi complex and activation [107]. Therefore, the modification process of immune checkpoints and research related to the regulation of immune checkpoint proteins in ER seem to provide a promising prospect for exploring effective immunotherapy, which is worthy of further study.

12.5.5 Affecting the Activation of Immune Checkpoints

The activation of immune checkpoints is an extremely complex process. Lipid metabolism not only provides energy for the activation of immune checkpoints, but also affects the degree of activation of checkpoints through the activation of specific pathways by its products. For example, high-density lipoproteins (HDL) are important in cholesterol metabolism that carries cholesterol from surrounding tissues to the liver, which is then converted to bile acids or excreted directly through bile. The previous study has confirmed that HDL modulated TCR/CD28 activation by inducing sustained signaling through p-Lck, pERK, and p-Akt [108]. Additionally, cellular lipid accumulation could activate the expression of ATP binary cassette transporter A1 (ABCA1) to promote the lipid outflow via inhibiting the activation and expression of TIM-3 [109]. More importantly, researchers have confirmed that elevated cholesterol could inhibit the proliferation of breast cancer cells and promotes their invasion through the TIM-3 independent pathway [82, 110].

Fatty acids are a type of important metabolic intermediates of lipid metabolism because they can be used for lipid synthesis and protein modifications, and can also be degraded by mitochondrial β-oxidation to generate energy [111]. Additionally, fatty acid oxidation (FAO) allows fatty acids to enter the mitochondria and then convert into various products that the cell can use, such as acetyl-CoA, NADH, and $FADH_2$ [9]. Endogenous fatty acid generation is essential to maintain energy level after PD-1 activation [112]. In addition, short-chain fatty acids (SCFAs) are products of fat metabolism in the body [113]. Valproic acid (VPA), a short-chain fatty acid, could induce the activation of the interleukin-4 receptor-α (IL-4Rα)/PD-L1 and toll-like receptor 4 (TLR4) signaling pathways and inhibits the expression of retinoblastoma 1 (Rb1) in myeloid-derived suppressor cells (MDSCs) [114]. However, up to date, there are not many studies on the influence of lipid metabolism on the activation of immune checkpoints, and it is currently impossible to clearly explain the specific mechanisms.

12.5.6 Affecting the Phosphorylation of Immune Checkpoints

Phosphorylation is a well-studied post-translational modification (PTM) that can coordinate various cell activities, including cell growth, differentiation, and apoptosis. The process of protein phosphorylation often occurs on certain amino acids, namely, threonine, serine, and tyrosine. During the regulating process of immune checkpoints, phosphorylation plays a critical role. These immune checkpoints are transmembrane glycoproteins with inhibitory motifs based on intracellular tyrosine, which can be phosphorylated and transduce negative signals to inhibit the activation of receptors. For example, the binding of PD-L1 on cancer cells with PD-1 on T cells leads to phosphorylation of the immunoreceptor tyrosine-based switch motif, which then inhibits T cell receptor signaling, leading to T cell proliferation, cytokine production, and lysis. Cell function is inhibited [115]. In fact, PD-L1 is a typical membrane protein that transduces extracellular signals through its tyrosine kinase phosphorylation. Recent studies have shown that phosphorylation plays an important role in regulating the stability of PD-L1 protein. In addition, the TIM-3, CD137, LAG-3, CTLA-4, PD-1, and PD-L2 have the potential phosphorylation sites.

The study points out that lipid metabolism is under the control of hormones, transcription factors, secondary messengers, and posttranscriptional modifications [116]. Actually, protein phosphorylation is central to lipid metabolism and multiple phosphorylases are involved in lipid accumulation or hydrolysis. Similarly, the metabolites of lipid metabolism also affect the process of protein phosphorylation. For example, docosahexaenoic acid (DHA), an $n-3$ polyunsaturated fatty acid, could curtail ERK1/2 and Akt phosphorylation and down-regulate the Smad7 levels to up-regulate the expression of Foxp3, CTLA-4, TGF-beta, and IL-10. More importantly, the DHA also increases the expression of p27 (KIP1) mRNA, known to be involved in Treg cell unresponsiveness [117]. The previous study indicated a direct interaction of CTLA-4 with the phosphorylated form of T cell receptor (TCR)-zeta within the glycolipid-enriched micro-domains associated with the T cell signaling complex. In this research, the authors pointed out that CTLA-4 regulated the accumulation/retention of TCR-zeta in the signaling complex, as the lipid raft fractions from CTLA-4 KO T cells contained significantly higher amounts of the TCR components when compared to wild-type littermates [118].

Additionally, accumulation of lipid droplets accompanied by continuous activation of the peroxisome proliferator activates receptor alpha (PPARα) pathway and phosphorylated glycogen synthase kinase 3 beta (GSK-3β) [119]. Interestingly, GSK-3β can interact with PD-L1 and cause phosphorylation-dependent proteasomal degradation through the E3 ligase beta-TrCP. Subsequent studies showed that PD-L1 is phosphorylated by GSK3β at two sites of T180 and S184, which leads to ubiquitination and degradation of PD-L1 in the cytoplasm. It has been found that many types of immune checkpoint molecules can transduce extracellular signals into cells through phosphorylation of the cytoplasmic tail, thereby recruiting kinases and subsequent factors. Due to the effects of lipid metabolism on immune checkpoints, phosphorylation changes greatly, increasing the difficulty and complexity of researches. On the other hand, it also increases the value of developing effective and unique therapies.

12.5.7 Affecting the Palmitoylation of Immune Checkpoints

As one of the most important PTMs, protein lipidation, especially protein fatty acylation, is not only PTM, but also a co-translational modification. By linking different fatty acyl groups to a subset of proteins, the positioning, activation, interaction, and stability of a group of important proteins are greatly affected, leading to a series of cellular activities. There are two recognized forms of fat acylation: palmitoylation and myristoylation [120]. Due to the nature of the thioester

bond, palmitoylation is usually reversible. Protein palmitoylation is catalyzed by a class of palmitoyl acyltransferases containing Asp-His-His-Cys (DHHC) in the active center. Indeed, palmitoylation is usually essential for the delivery and localization of certain membrane proteins and the interaction with other proteins. More importantly, the process of depalmitoylation, that is, the separation of the palmitoyl group from the protein, may affect the transport, stability, and function of this protein in the opposite way to palmitoylation.

It is recognized that palmitoylation plays a key role in the regulation of immune checkpoints. According to the literature reports, the ligands and receptors of immune checkpoints are usually transmembrane proteins, which can be regulated by palmitoylation as a reversible lipid modification. Therefore, lipid metabolism may play an important role in regulating the palmitoylation of immune checkpoints. However, up to date, researches on palmitoylation of immune checkpoints are still at an early stage, but the researchers still revealed that PD-L1 has a palmitoyl modification in breast cancer cells [121]. PD-L1 is palmitoylated by covalently binding a palmitic acid (a 16 C saturate fatty acid) to the cysteine residue at 272 to maintain stability and accelerate the development of breast cancer. The recent study showed that high-fat diet (HFD) has been shown to increase the level of free fatty acids and is related to the activation of STAT3 and inflammation in animals via inducing the STAT3 palmitoylation. At the same time, they further found that HFD promoted the palmitoylation of STAT3 by up-regulating ZDHHC19, a palmitoyl acyltransferase. Interestingly, ZDHHC9 is associated with palmitoylation of PD-L1. Silencing of ZDHHC9 can eliminate the palmitoylation of PD-L1, and then reduces the cell surface distribution after INF-γ treatment, thereby making cancer cells sensitive to T cell killing and inhibiting tumor growth in mice [121].

In addition, PD-L1 is palmitoylated in its cytoplasmic domain, which stabilizes PD-L1 by blocking its ubiquitination, consequently suppressing PD-L1 degradation by lysosomes. Palmitoyl transferase ZDHHC3 (DHHC3) is also identified as the main acetyltransferase responsible for the palmitoylation of PD-L1. PD-L1 can be modified by palmitoylation catalyzed by DHHC3 enzyme, thereby inhibiting the ubiquitination modification of PD-L1 and enhancing the expression and function of PD-L1 [122]. In vivo and in vitro research models have consistently shown that targeting PD-L1 palmitoylation can inhibit the expression and function of PD-L1, thereby enhancing the killing effect of T cells on tumor cells. Therefore, the design of PD-L1 palmitoylation inhibitors can reduce the level of PD-L1 and enhance the immune clearance of tumor cells, which provides a new strategy for immunotherapy. Although more and more studies have shown that PD-L1 palmitoylation has a promising potential, researches on other immune checkpoints are still in progress.

12.5.8 Affecting the Exosomes Transport of Immune Checkpoints

Mammalian cells synthesize and release heterogeneous EVs. These heterogeneous EVs can usually be subdivided into exosomes (30–150 nm in diameter), microvesicles (MVs, or ectosomes or microparticles, 0.1–1.0 μm) and apoptotic bodies (0.8–5.0 μm), whose biogenesis, composition, and biological function are different from others [123]. Exosomes play a crucial role in distant cell–cell communication and transfer active forms of various biomolecules; the molecular composition of the exosomal cargo is a result of targeted selection and depends on the type of producer cells. Exosomes are spherical bodies surrounded by the lipid bilayer membrane, suggesting the important role of lipid metabolism in exosomes biogenesis. The exosome biogenesis is associated with changes in the endosomal membrane lipid composition, accompanied by lipid clustering into subdomains called lipid rafts, which mediate membrane invagination and vesicle formation. Importantly, recent study indicated that adipocytes do not only release the fatty acid

components of triglycerides, but also release triglycerides packaged in small particles. These lipid-filled particles called adipocyte exosomes (AdExo) are taken up by macrophages in adipose tissue [124]. It is suggested that cellular lipid metabolism could affect the function of immune cells via regulating the exosomes.

Actually, exosomes can carry a large number of active molecules, including lipid mediators or metabolites (such as eicosanoids), proteins, and nucleic acids that can regulate the phenotypes of cells. For instance, exosomes derived from tumor cells actively promote tumor progression and metastasis. Tumor cells evade immune surveillance by increasing the surface expression of PD-L1. PD-L1 interacts with PD-1 on T cells and promotes the dephosphorylation of T cell receptors and its co-receptor CD28 through SHP2 phosphatase, inhibiting antigen-driven T cell activation. However, not all cell membrane surfaces express PD-L1, but those cells that do not express PD-L1 can still evade surveillance by the immune system. Interestingly, PD-L1 exists on the surface of exosomes, and the level of exosomes PD-L1 is related to cancer progression and response to immunotherapy [125]. A study pointed out that inhibition of exosomal PD-L1 induces systemic anti-tumor immunity, even in a model of anti-PD-L1 antibody resistance. In addition to PD-L1 protein, PD-L1 mRNA can also be detected in exosomes. Researchers found that patients with periodontitis were enriched in PD-L1 mRNA exosomes than the control group. The exosomal PD-L1 mRNA in saliva correlates with the severity/stage of periodontitis and can be potentially distinguished from healthy periodontitis [126].

Importantly, tumor-derived exosomes (TEX) and their effects on immune cells in cancer are likely to be translatable, in part, to other pathological conditions. TEXs carrying and delivering various inhibitory ligands to immune cells in the tumor microenvironment and in the periphery represent one of many mechanisms that tumors use to engineer their escape from the host immune system. Besides the PD-L1, exosomes from plasma of human head and neck cancer are reported to carry various immune-inhibitory proteins, including PD-1, CTLA-4, CD39, TGF-β, CD73, and TRAIL [127]. The previous study found a high level of circulating exosomal Galectin-9 in the plasma of NSCLC patients compared to the healthy controls [128]. Meanwhile, the TIM-3 is highly expressed and associated with aggressive clinicopathological parameters such as larger tumor size, more metastasis, and advanced stages. Therefore, the combination of inhibition of exosome biogenesis and secretion with anti-immune checkpoint antibody therapy achieves a stronger tumor suppression effect.

12.5.9 Affecting the Degradation of Immune Checkpoints

Recent studies have revealed the importance of post-translational modifications in regulating the expression of immune checkpoints [70]. Glycosylation is a ubiquitous and highly conservative post-translational modification in eukaryotic cells. Canonical protein *N*-glycosylation includes two stages: [1] synthesis of lipid-linked oligosaccharide (LLO) donors and [2] transfer of carbohydrates to nascent polypeptides [129, 130]. It means that lipid metabolism is associated with protein glycosylation. It has been reported that glycosylation is closely correlated with immune activation, including antigen modification, presentation, and T cell priming [131]. A recent study showed that the *N*-glycosylation of PD-L1 has a significant impact on its stability and immune function [132]. In this study, the researchers found that the extensive unglycosylated N192, N200, and N219 domain of PD-L1 is targeted by glycogen synthase kinase 3β (GSK3β), then facilitating the proteasome-related degradation of PD-L1 via β-transducin repeats-containing proteins (β-TrCP) [132].

Molecules involved in ubiquitination and lysosomal transport control the immune system. In fact, ubiquitination is closely related to immune checkpoint degradation [97, 133]. A recent study showed that PD-1 on the surface of activated T cells undergoes internalization,

followed by ubiquitination and proteasomal degradation, and the Lys48-linked polyubiquitinated E3 ligase that mediates PD-1 is FBXO38 [134]. In addition, poly-ubiquitination of PD-L1 by the E3 ligases cullin-3 [133] and β-TrCP20 [132] promotes degradation of PD-L1, while COP9 signalosome 5 (CSN5) antagonizes this process [135], although the exact sites and types of ubiquitination remain to be further clarified. The previous studies have reported that CKLF-like MARVEL transmembrane domain-containing proteins CMTM6 and CMTM4 have been found to stabilize PD-L1 and their effects seem to involve not only ubiquitination-dependent degradation but also lysosome-dependent proteolysis [136].

Meanwhile, lysosomes are tightly associated with cell proliferation, cancer cell death, cancer therapy, drug resistance, and immune checkpoints [137]. At present, the lysosomal degradation of PD-L1 and PD1 is getting increasing attention. For instance, CMTM6 could reduce PD-L1 ubiquitination and increases its stability and function in protecting PD-L1 from lysosome-mediated degradation via regulating the LDL-uptake [135, 138]. It is suggested that cholesterol metabolism was closely associated with the degradation of PD-1 and PD-L1. Previous studies found that palmitoylation plays a crucial role in regulating the stability of the PD-L1 protein, which involves molecular masking of an intrinsic lysosomal sorting signal of PD-L1 [139]. In this study, they also found palmitoylation of proteins was positively correlated with the attachment of the 16-carbon fatty acid palmitate. Although it is evident that the transportation between ubiquitination and lysosome may control the fate of PD-L1 protein [140], the exact effects underlying degradation of other immune checkpoints remain unclear.

At present, more and more researches have pointed out that targeting these checkpoints reversely increased or decreased metabolism [112, 141–143], including glycolysis, oxidative phosphorylation, and lipid metabolism, etc. However, studies on lipid metabolism affecting the expression of immune checkpoints still have a long way to go, and further study is warranted.

12.6 Conclusion

Lipid metabolism is essential for the synthesis of some transcription factors [144, 145]. Surprisingly, the transcriptional control of immune checkpoint genes has not yet been fully studied. The transcriptional regulation of gene expression programs, including immune checkpoint genes, has been known in detail, but little is known about the post-transcription and activation processing of immune checkpoint genes. Lipid metabolism is involved in the energy supply of immune cells, which is the beginning of the immune response (Fig. 12.3). Aberrant lipid metabolism can activate multiple signaling pathways (e.g., PI3K/AKT and ERK/MAPK pathways) to induce immune repression through its metabolites or metabolic intermediates. Up to date, researchers have gathered and adduced pieces of evidence that the aberrant lipid metabolism creates the favorable immunosuppressive environment for diseases via regulating the immune checkpoints in the following aspects: (1) increases the expression of inhibitory immune checkpoints or decreases the expression of stimulatory immune checkpoints, (2) affects the corrected folding of immune checkpoints, (3) provides energy for activation of immune checkpoints, (4) affects the degradation of immune checkpoints. All these special attributes of lipid metabolism have to be considered in the context of immune physiology and the fragile balance between pro- and anti-immune activities of the components.

In this chapter, we summarized the role of lipid metabolism in many aspects of immune checkpoints. As a model of cell metabolism, lipid metabolism is deeply involved in gene expression and protein regulation during immune activities. However, it is clear that we have not yet fully understood. There are many unanswered questions in this field, and we still have a lot to learn about the fascinating role of lipid metabolism, especially in the transcriptional expression and post-translational modifications of immune checkpoints. Therefore, further study is necessary, so as to provide the evidence for testifying the role of lipid metabolism in the regulation of immune checkpoints.

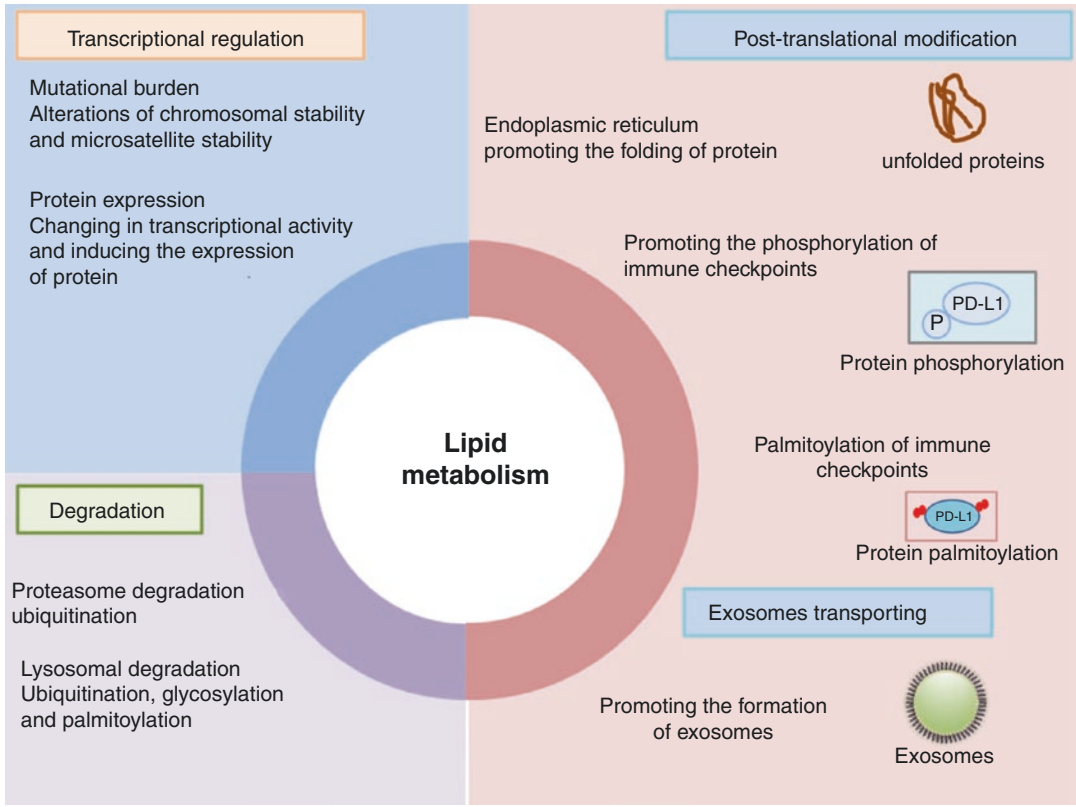

Fig. 12.3 The effect of lipid metabolism on the regulation of immune checkpoints. First, various metabolites produced by lipid metabolism can regulate gene expression of the immune checkpoints, such as affecting the stability of the genomic sets, acting on the promoter activity of the immune checkpoints, etc. Second, lipid metabolism can also regulate protein modifications and degradation of immune checkpoints. The unfolded immune checkpoints are accumulated in the ER and undergo UPR to promote their folding. Then they are phosphorylated, palmitoylation and glycosylation, and ultimately, they are ubiquitinated and degraded through proteasomal degradation and lysosomal degradation. Third, the lipid metabolism plays an important role in the formation of exosomes and then affects the transportation of immune checkpoints

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